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Differentiation of rum and Brazilian artisan cachaça via electrospray ionization mass spectrometry fingerprinting

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Rum and cachaça are sugarcane distillates produced on large scales and of similar composition, and their differentiation is currently a subject of commercial dispute and a challenging analytical task. We have investigated the ability of direct-infusion electrospray ionization mass spectrometry in the negative ion mode, i.e. ESI(−)-MS, to distinguish between samples of these distillates. ESI(−)-MS fingerprints were collected for some samples of Brazilian artisan cachaça, aged in two types of wooden casks, i. e. amburana (*Amburana cearensis*) and jequitibá (*Cariniana estrellensis*), and of commercial rum. The mass spectra were found to be very distinctive, showing sets of diagnostic ions for each type of sample, i. e. (1) cachaça aged in amburana (*m/z* 271, 313, 377) and jequitibá (*m/z* 171, 255, 455) casks; and (2) commercial rum (*m/z* 89, 97, 179, 255, 283). When applied to the ESI(−)-MS data, principal component analysis and hierarchical cluster analysis split rum and cachaça samples into well-defined groups. Moreover, the two types of cachaça samples aged in wooden casks of amburana or jequitibá were also split into two distinct groups. Direct-infusion ESI(−)-MS can therefore be potentially applied to the rapid, simple, and accurate differentiation of these commercially important sugarcane distillates. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: cachaça and rum; electrospray ionization mass spectrometry fingerprinting; principal component analysis; hierarchical cluster analysis

INTRODUCTION

Cachaça, the most typical Brazilian alcoholic beverage, is the third largest consumed distilled drink in the world. This distillate is produced mostly from the fermentation of sugarcane (*Saccharum officinarum*), and the finest and highest-quality artisan cachaça is made exclusively in copper distillers and aged for at least 18 months in wooden casks. The production of the finest artisan cachaça is now close to 180 million liters per year. Rum is produced from the same raw material, but its fermentation mostly uses molasses instead of sugarcane. Molasses, the sweet and sticky residue that remains after sugarcane juice is boiled and the crystallized sugar is extracted, is over 50% sugar, but it also contains significant amounts of minerals and other trace elements, which contribute to the final rum flavor. However, the Alcohol and Tobacco Tax and Trade Bureau (TTB), the American agency that regulates the production, labeling, importation, and marketing of alcoholic beverages within the United States under the Federal Alcohol Administration Act (FAA), has classified cachaça either as a rum or as a distilled-spirit specialty product. This agency has also recommended that the name 'cachaça' may appear only as additional information on the bottle label.[1] Because cachaça export has increased by a large extent recently, the recognition of cachaça as a typical beverage from Brazil (and thus distinct from rum) has been a central aspiration of the Brazilian producers.

Electrospray ionization (ESI)[2] has greatly extended the applicability of mass spectrometry (MS) for the structural analysis of a great variety of molecular species, allowing gentle transfer of ions from solution to the gas phase. ESI-MS with direct sample injection has also been demonstrated to be a powerful technique for fast fingerprint characterization of complex chemical mixtures such as beer,[3] wine,[4] propolis,[5] whisky,[6] crude petroleum oils,[7] vegetable oils,[8] and plant extracts.[9]

Chemometric methods,[10,11] such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), have been successfully applied to chemical composition information to perform exploratory analysis of beverage samples.[12–16] For instance, Herranz and coworkers[17–19] characterized samples of whisky, rum, and gin by using

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ethyl acetate or terpene contents as variables. Seeber and coworkers[20] also used multivariate analysis to distinguish between different vintages of Chardonnay musts and wines using amino acids, volatile organic compounds, and metal contents as variables. Spectrometric methods have also been used to generate fingerprints of alcoholic drinks.[21,22] For instance, pyrolysis mass spectrometry was successfully used to characterize 33 certified wine samples. PCA treatment on these data grouped the samples according to their geographic regions, whereas no correlation was found with the grape species.[23]

Franco and coworkers[24] have also succeeded in differentiating cachaça and rum by using a set of analytical techniques: UV-vis spectroscopy, atomic absorption, and gas and liquid chromatography. The combined data were treated by means of the PCA and HCA methods and two distinct groups for cachaça and rum samples were observed.

In this paper we describe the use of direct-infusion ESI-MS in the negative mode to discriminate among samples of Brazilian artisan cachaça, aged in casks of amburana (*Amburana cearensis*) and jequitibá (*Cariniana estrellensis*), and commercial rum. The ESI-MS technique is shown to be much faster (each analysis requires about 1 min), simpler (no need for sample preparation such as derivatization and preconcentration steps), and equally reliable in comparison with the multitechnique methodology described by Franco and coworkers.[24] The ESI(−)-MS fingerprints were also treated by the PCA and HCA chemometric methodologies, which provided extra and significant sets of information.

EXPERIMENTAL

Samples

Eight commercial samples of rum (r1–r8), initially stored in glass bottles, were purchased from local liquor stores. These rum samples were from five different countries: Jamaica, the Dominican Republic, France, Cuba and Brazil. The 30 samples of Brazilian artisan cachaça were supplied directly by several qualified producers. Among these samples, 9 (a1–a9) were aged in amburana casks, whereas 16 (j1–j16) in jequitibá casks, with aging times varying from 18 to 24 months. Furthermore, four blend samples (b1–b4) were also collected, which were produced by mixing both types of cachaça in varied proportions (the Brazilian producers usually use this method to adjust the color of the final product). We note that these casks (of amburana and jequitibá) are the most widely used by the small Brazilian producers for the aging process, but are not commonly employed by rum manufacturers. All the cachaça samples were produced by using copper distillers with no addition of caramel. This procedure is sometimes illegally used to standardize the final color of cachaça.

Mass spectrometry

The mass spectra were acquired with a Q-TOF mass spectrometer (Micromass, Manchester, UK). The general conditions were as follows: source temperature, 80 °C; capillary voltage, 2.1 kV; cone voltage of 40 V. Prior to the ESI-MS analysis, 250 μl of an aqueous solution of ammonium hydroxide 0.1% (v : v) was added to 1 ml of each sample and the mixture vigorously stirred for 15 s. Sample introduction was by a syringe pump (Harvard Apparatus, Pump 11) at a flow rate of 10 μl min^{-1}, pumped through an uncoated fused silica capillary.

Mass spectra were scanned from $50 < m/z < 500$. Tandem ESI-MS/MS spectra were collected upon CID with argon under a collision energy of 15–20 eV of the mass-selected ion of interest. Mass selection was performed by the first r.f./d.c. quadrupole using a unit m/z window, and collisions were performed in the r.f.-only quadrupole collision cell, followed by mass scanning of the product ions by the orthogonal-reflectron time-of-flight (o-ToF) analyzer.

ESI-MS data handling and chemometric treatment

All mass spectra were accumulated over 60 s, centered, aligned and handled using the MassLynx 3.5 software (Waters, Manchester, UK). Correlation-optimized warping (COW) was used as a preprocessing tool in order to obtain precise alignment of the normalized MS data.[25] To remove the noise signals, only ions with a relative abundance higher than 10% were included in the final data matrix. Multivariate analyses by PCA and HCA were performed by running the software MATLAB, version 6.1. The experimental data were compiled to generate a final matrix of 37 objects (samples) and 84 variables (mass-to-charge ratio values with their respective relative intensities). These data were previously mean-centered and autoscaled to variance 1, aimed at ensuring that all variables contributed equally to the model, independent of the scale in which they were measured.

The central idea of PCA is to reduce the dimensionality of a data set, explaining the variance structure. This is achieved by linear transformation of the original data set of variables into a smaller number of uncorrelated significant principal components (PCs). Geometrically, this transformation represents the rotation of the original coordinate system, and the direction of the maximum residual variance is given by the first PC axis. The second PC, orthogonal to the first one, has the second maximum variance, and so on. In this way, projections preserving maximum amounts of statistical information can be visualized in order to display a more detailed study of the data structure.

HCA is a qualitative pattern classification method based on creating tree-structured clusters (dendrogram) of data objects (samples), according to the distances between their profiles. HCA begins by finding the closest pair of cases and combines them to form a cluster. The algorithm proceeds one step at a time, joining pairs of cases, pairs of clusters, or a case with a cluster, until all the cases are in one cluster. The steps are displayed as a dendrogram, which represents the similarity groupings between objects (samples). The method is hierarchical because once two cases are joined in a cluster they remain joined. The K-means nearest group method was used to generate the dendrogram. The K-means algorithm assigns each point to the cluster whose center (also called centroid) is the nearest. The center is the average of all the points in the cluster, i.e. its coordinates are the arithmetic mean for each dimension separately over all the points in the cluster.

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RESULTS AND DISCUSSION

Figure 1 shows the ESI(−)-MS fingerprints of two typical samples of cachaça aged in casks of amburana (Fig. 1(a)) and jequitibá (Fig. 1(b)), and for a blend sample aged both in amburana and jequitibá casks (Fig. 1(c)). The other cachaça samples (see 'Experimental') provided fingerprints (not shown) quite similar to one of those displayed in Fig. 1 as directly related to their type. These mass spectra are very characteristic of each sample and the anions detected are most likely the deprotonated forms of the acidic components such as carboxylic acids, aliphatic alcohols,[26] and phenols.[27,28] These components were probably extracted from the wooden casks during the aging process. For instance, a visual inspection of these mass spectra reveals that the ions of m/z 271, 313, and 377 are the most characteristic (diagnostic) for samples aged in amburana casks, whereas the ions of m/z 171, 255, and 455 are diagnostic for those aged in jequitibá casks. In Fig. 1(c) we note that ESI(−)-MS is even able to identify blended cachaça, that is, those produced by mixing the genuine samples.

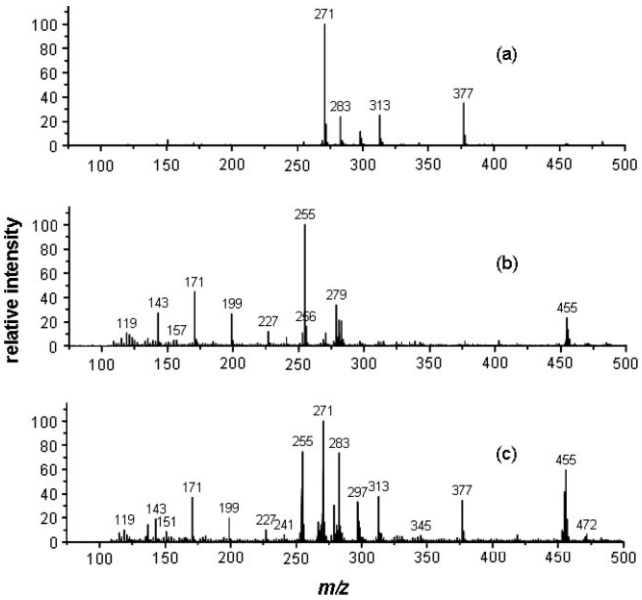

Figure 1. ESI(−)mass spectra of three typical samples of cachaça aged in different wooden casks: (a) amburana; (b) jequitibá and (c) both amburana and jequitibá casks.

Figure 2 shows the ESI(−)-MS fingerprints of rum samples produced in Brazil (a), Cuba (b), and France (c). The other five samples showed similar fingerprints (not shown), again directly related to their origins. Regardless of the geographical origin, common ions are always detected such as those of m/z 89, 97, 179, 255, and 283, whereas the Cuban rum shows also prominent ions of m/z 179 and 341 (Fig. 2(b)). As evidenced by the comparison with the ESI(−)-MS/MS of the authentic ion, (Fig. 3), obtained from an aqueous solution of glucose, the anion of m/z 179 is likely that of deprotonated glucose, whereas the anion of m/z 341 is likely related to deprotonated saccharose (ESI(−)-MS/MS not shown). All rum samples come out of the still as clear, colorless spirits, and cask-aging as well as the addition of caramel can determine their final color. Since caramel is burnt sugar, the detection of anions of m/z 179 and 341 in the rum fingerprints is likely related to the caramel addition, as was also observed for caramel beer[3] and counterfeit samples of whisky.[6] In fact, the Cuban rum was the one displaying the darkest caramel color among the other

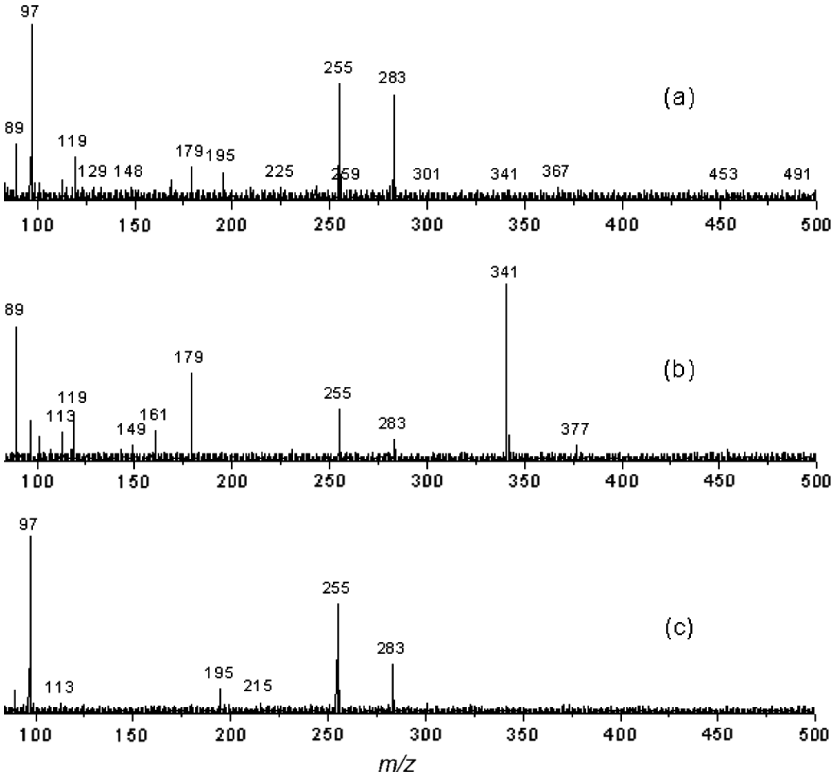

Figure 2. ESI(−)-MS of three samples of rum from different countries: (a) Brazil; (b) Cuba; and (c) France.

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samples. Finally, note that the anion of m/z 283 is detected in the cachaça (aged in both amburana and jequitibá casks) and rum fingerprints (Figs 1 and 2) and therefore cannot be considered as diagnostic anions to be used to distinguish between these samples. Analogously, the ion of m/z 255, detected only in the ESI(−)-MS of the rum and 'jequitibá cachaça' samples, can only be regarded as a diagnostic anion to differentiate them from the 'amburana cachaça' samples.

Multivariate data analysis

To test the statistical relevance of cachaça and rum differentiation by ESI(−)-MS fingerprinting, the data were analyzed first by the PCA method with four PCs (nearly 92% of the total variance). An examination of the PC loadings (Table 1) shows that no more than ten variables (ions of m/z 89, 97, 171, 179, 255, 271, 283, 313, 377, 455) are the most important (possess the highest values) in explaining the entire set of data. Only the variables with loading values higher than 0.2 in at least one of the first three PCs were considered for selection. It was observed that PC1, which in fact discriminates among the cachaça samples aged in different types of wooden casks (see scores plot, Fig. 4), is strongly correlated with the ions of m/z 89, 97 (characteristic of the rum samples), 255 (detected in the ESI(−)-MS of rum as well as cachaça samples aged in jequitibá casks), 313, 377, and mainly 271 (diagnostic for the cachaça samples aged in amburana casks). The component PC2, which is also responsible for the differentiation of the cachaça samples (Fig. 4), is mostly correlated to the ions of m/z 89, 97 (rum samples), 171, 455 (characteristic of the cachaça samples aged in jequitibá), and mainly 255 (characteristic of the rum and 'jequitibá cachaça' samples). Finally, the component PC3, which really promotes the formation of two distinct groups of rum and cachaça samples (Fig. 4), is typically associated with the ions of 89, 97 (main variables, characteristic of the rum samples), 179 (glucose), and 283 (detected in the ESI(−)-MS of both the cachaça and rum samples). Note that the ion of m/z 283, which has a negligible contribution to PC1 and PC2 (Table 1), plays a minor role in the separation of both types of cachaça samples because it appears simultaneously in both sets of ESI(−)mass spectra. The ion of m/z 179, which is observed only in the ESI(−)-MS of the rum samples, has also a small contribution on both PC1 and PC2 (Table 1) and therefore on the separation of the cachaça samples.

The score plot was built (Fig. 4) by using a matrix comprising these ten selected variables (the use of these selected variables instead of the raw data allowed a better differentiation among the cachaça and rum samples). In this plot, PC1, PC2, and PC3 account for 45, 26, and 18% of the total variance, respectively. Separation of cachaça and rum samples into two well-defined groups is evident. Note that the cachaça samples aged in the amburana and jequitibá casks also noticeably form distinct groups. The four blend samples (see 'Experimental') fail to fit properly in any of these two main clusters and appear to form two extra distinct groups.

The HCA method was also applied to the ESI(−)-MS data as seen in the dendrogram of Fig. 5. Two clusters were formed at a distance to K-nearest group of nearly 140, the first one comprising the rum samples, whereas the second consisting

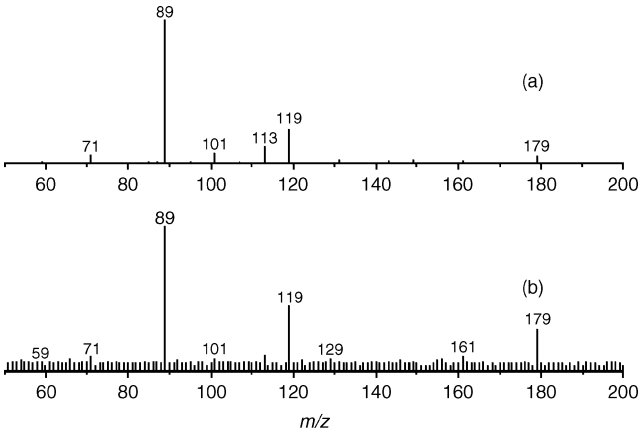

Figure 3. ESI(−)-MS/MS of the anion of *m/z* 179: (a) detected in the ESI(−)-MS of the Cuban rum; (b) from an aqueous/ethanol solution of glucose.

Table 1. Loadings for the first three PCs with the *m/z* values (variables) acquired from the ESI(−)mass spectra of the rum and cachaça samples. The most important loading (with values higher than ±0.2) for each component are shown in bold

m/z Values (variables)	PC1	PC2	PC3
89	**−0.225**	**−0.275**	**0.35438**
97	**−0.36**	**−0.396**	**−0.37172**
171	−0.044	**0.2865**	0.00977
179	−0.126	−0.137	**0.29263**
255	**−0.272**	**0.5721**	−0.15791
271	**0.684**	−0.088	0.00379
283	0.1795	0.1226	**−0.24549**
313	**0.2066**	−0.024	−0.00416
377	**0.2667**	−0.04	0.03368
455	−0.036	**0.212**	0.03357

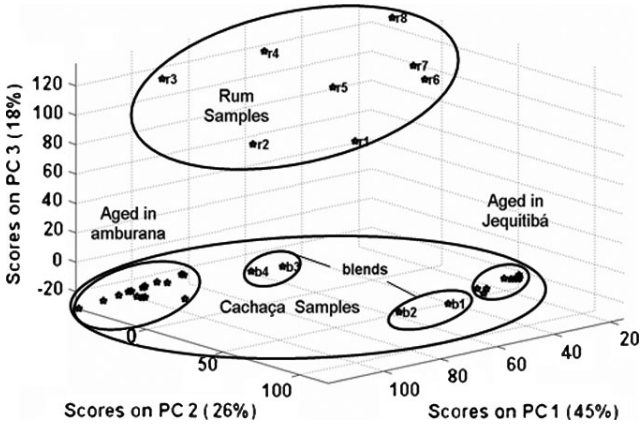

Figure 4. PCA score plot of cachaça and rum samples obtained from the ESI(−)-MS data.

of cachaça samples. The cachaça cluster is divided into two distinct subclusters at a distance of about 100. This behavior, as observed in the scores plot of Fig. 4, is related to the two different wooden casks (jequitibá and amburana) in which the cachaça samples were matured. The HCA methodology

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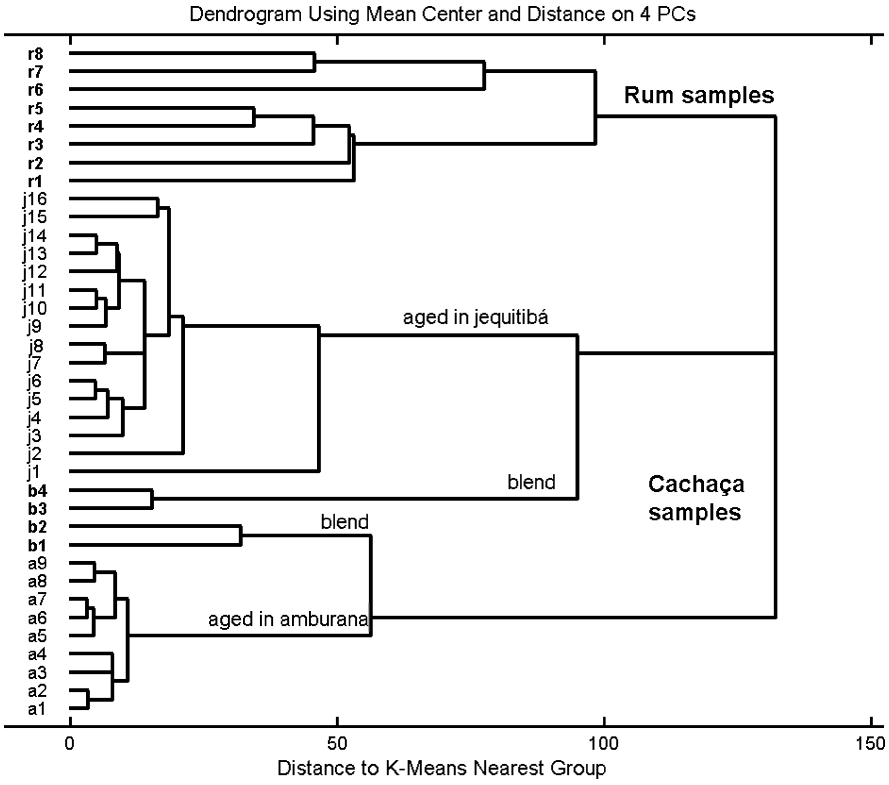

Figure 5. Dendrogram for cachaça and rum samples obtained from the ESI(−)-MS data. This dendrogram was built by using mean center and distances on four PCs.

was also able to distinguish the blend samples in two distinct groups: two of these samples (b3 and b4) were grouped adjacent to the 'jequitibá cluster', whereas the other two (b1 and b2) nearby the 'amburana samples'. This trend is probably related to the different proportions of the unmixed cachaças (aged in amburana or jequitibá casks) used to prepare such samples. For instance, blend samples with a higher content of 'amburana cachaça' tend to group with the 'amburana samples' and vice versa. For the rum cluster, however, no similar trends were observed that could justify the appearance of two distinct subclusters at a distance of around 80 (Fig. 5).

CONCLUSIONS

We demonstrated that a rapid differentiation of samples of rum and artisan cachaça could be achieved by analyzing the corresponding ESI-MS fingerprints. Furthermore, it can be envisaged that such methodology can be applied to distinguish between cachaça samples aged in different types of wood casks. Other related possibilities, currently under study in our laboratory, involve a rapid and reliable ESI-MS differentiation among industrial and artisan cachaça samples as well as the detection of adulterations by the addition of caramel and other substances such as dyes.

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REFERENCES

1. http://www.ttb.gov/press/fy05press/011905cachaca.htm. [accessed 2007].
2. Gaskell SJ. Electrospray: principles and practice. *J. Mass Spectrom.* 1997; **32**: 677.
3. Araujo AS, da Rocha LL, Tomazela DM, Sawaya ACHF, Almeida RR, Catharino RR, Eberlin MN. Electrospray ionization mass spectrometry fingerprinting of beer. *Analyst* 2005; **130**: 884.
4. Cooper HJ, Marshall AG. Electrospray ionization Fourier transform mass spectrometric analysis of wine. *J. Agric. Food Chem.* 2001; **49**: 5710.
5. Sawaya ACHF, Tomazela DM, Cunha IBS, Bankova VS, Marcucci MC, Custodio AR, Eberlin MN. Electrospray ionization mass spectrometry fingerprinting of propolis. *Analyst* 2004; **129**: 739.
6. Moller JKS, Catharino RR, Eberlin MN. Electrospray ionization mass spectrometry fingerprinting of whisky: immediate proof of origin and authenticity. *Analyst* 2005; **130**: 890.
7. Rodgers RP, Schaub TM, Marshall AG. Petroleomics: MS returns to its roots. *Anal. Chem.* 2005; **77**: 20a.
8. Wu ZG, Rodgers RP, Marshall AG. Characterization of vegetable oils: Detailed compositional fingerprints derived from electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *J. Agric. Food Chem.* 2004; **52**: 5322.
9. Mauri P, Pietta P. Electrospray characterization of selected medicinal plant extracts. *J. Pharm. Biomed. Anal.* 2000; **23**: 61.
10. Rodionova OY, Pomerantsev AL. Chemometrics: achievements and prospects. *Russ. Chem. Rev.* 2006; **75**: 271.
11. Cazar RA. An exercise on chemometrics for a quantitative analysis course. *J. Chem. Educ.* 2003; **80**: 1026.
12. Arozarena I, Casp A, Marin R, Navarro M. Multivariate differentiation of Spanish red wines according to region and variety. *J. Sci. Food Agric.* 2000; **80**: 1909.
13. Navarro M, Arozarena I, Marin R, Casp A. Characterisation and classification of Spanish white wines by multivariate data analysis. *J. Int. Sci. Vigne Vin* 2001; **35**: 107.
14. Gonzalez-Arjona D, Gonzalez-Gallero V, Pablos F, Gonzalez AG. Authentication and differentiation of Irish whiskeys by higher-alcohol congener analysis. *Anal. Chim. Acta* 1999; **381**: 257.

15. Lee KYM, Paterson A, Birkmyre L, Piggott JR. Headspace congeners of blended Scotch whiskies of different product categories from SPME analysis. *J. Inst. Brew.* 2001; **107**: 315.
16. da Costa RS, Santos SRB, Almeida LF, Nascimento ECL, Pontes MJC, Lima RAC, Simoes SS, Araujo MCU. A novel strategy to verification of adulteration in alcoholic beverages based on Schlieren effect measurements and chemometric techniques. *Microchem. J.* 2004; **78**: 27.
17. Herranz A, Delaserna P, Barro C, Martin PJ, Cabezudo MD. Application of the statistical multivariate-analysis to the differentiation of whiskies of different brands. *Food Chem.* 1989; **31**: 73.
18. Herranz A, Delaserna P, Barro C, Martinalvarez PJ. Multivariate statistical-methods applied to the differentiation of rum brands. *J. Sci. Food Agric.* 1990; **51**: 555.
19. Martinalvarez PJ, Herranz A. Application of multivariate statistical-methods to the differentiation of gin brands. *J. Sci. Food Agric.* 1991; **57**: 263.
20. Seeber R, Sferlazzo G, Leardi R, Serra AD, Versini G. Multivariate data-analysis in classification of musts and wines of the same variety according to vintage year. *J. Agric. Food Chem.* 1991; **39**: 1764.
21. Faria JB, Cardello HMAB, Boscolo M, Isique WD, Odello L, Franco DW. Evaluation of Brazilian woods as an alternative to oak for cachacas aging. *Eur. Food Res. Technol.* 2003; **218**: 83.
22. Cuadrado MU, de Castro MDL, Gomez-Nieto MA. Study of spectral analytical data using fingerprints and scaled similarity measurements. *Anal. Bioanal. Chem.* 2005; **381**: 953.
23. Montanarella L, Bassani MR, Breas O. Chemometric classification of some European wines using pyrolysis mass spectrometry. *Rapid Commun. Mass Spectrom.* 1995; **9**: 1589.
24. Cardoso DR, Andrade-Sobrinho LG, Leite-Neto AF, Reche RV, Isique WD, Ferreira MMC, Lima-Neto BS, Franco DW. Comparison between cachaca and rum using pattern recognition methods. *J. Agric. Food Chem.* 2004; **52**: 3429.
25. Nielsen NPV, Carstensen JM, Smedsgaard J. Aligning of single and multiple wavelength chromatographic profiles for chemometric data analysis using correlation optimized warping. *J. Chromatogr., A* 1998; **805**: 17.
26. Boscolo M, Bezerra CWB, Cardoso DR, Neto BSL, Franco DW. Identification and dosage by HRGC of minor alcohols and esters in Brazilian sugar-cane spirit. *J. Braz. Chem. Soc.* 2000; **11**: 86.
27. Cardoso DR, Bettin SM, Reche RV, Lima-Neto BS, Franco DW. HPLC-DAD analysis of ketones as their 2,4-dinitrophenylhydrazones in Brazilian sugar-cane spirits and rum. *J. Food Compos. Anal.* 2003; **16**: 563.
28. do Nascimento RJ, Cardoso DR, De Keukeleire D, Lima-Neto BD, Franco DW. Quantitative HPLC analysis of acids in Brazilian cachaças and various spirits using fluorescence detection of their 9-anthrylmethyl esters. *J. Agric. Food Chem.* 2000; **48**: 6070.