Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 569–574
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Simultaneous determination of aging markers in sugar cane spirits

Francisco Wendel Batista de Aquino [a], Sueli Rodrigues [a,*], Ronaldo Ferreira do Nascimento [b], Antônio Renato Soares Casimiro [a]

[a] Departamento de Tecnologia de Alimentos, Laboratório de Biotecnologia DETAL/UFC, Universidade Federal do Ceará, Av Mister Hull, 2977 bloco 858, Campus do Pici, CEP 60356-000 Fortaleza, CE, Brazil
[b] Departamento de Físicoquímica, Laboratório de Cromatografia DFQ/UFC, Universidade Federal do Ceará, Av Mister Hull, 2977 Bloco 940, Campus do Pici, CEP 60356-000 Fortaleza, CE, Brazil

Received 11 February 2005; received in revised form 14 July 2005; accepted 30 July 2005

Abstract

In this work a method for simultaneous determination of 10 representative compounds in sugar cane spirits is reported. The low molecular weight phenolic compounds: gallic acid; vanillic acid; syringic acid; vanilin; syringaldehyde; coniferaldehyde; sinapaldehyde and coumarin; and the furanic aldehydes: 5-hydroxymethyl-furfural and furfural were simultaneously quantified by high performance liquid chromatography with UV detection. These compounds, together with acids, aldehydes and tannins are responsible for the organoleptic properties of aged beverages and can assest product quality, as well as be used as aging indicators. Determination of this group of compounds is important, as they are characteristic of types and styles of aged beverages.

Keywords: Aged beverages; Sugar cane spirits; Phenolic compounds; Aging markers

1. Introduction

Sugar cane spirit, or cachaça, is a typical Brazilian distilled beverage. As some distilled beverages, sugar cane spirits are aged in wooden barrels for a given period of time. Barrels used as aging barrels are thermally treated and during the aging process a series of compounds are extracted from the wood by the spirit influencing the beverage organoleptic characteristic (Reazin, 1981).

The compounds responsible for taste, aroma and flavor of an aged alcoholic beverage are: acids, aldehydes, tannins and other compounds referred as low molecular weight phenolic compounds (Bozhinov, 1994; Mangas, Rodriguez, & Moreno, 1996). Some of them are used as markers or aging indicators, since their quantification during the aging process can be used to estimate the time required to age a distilled beverage (Jaganathan & Dugar, 1999; Lo Coco, Valentini, Novelli, & Ceccon, 1995).

Lignin hydrolysis is the major chemical process that occurs during aging in wooden barrels (Martinez, Mir, Serrana, & Marinez, 1993) and is the process from which several phenolic compounds are extracted. Oxidation of these compounds yields aldehydes, acids with radicals and aromatic agents such as vanillin and syringaldehyde (Gimenez, Lopez, Villalon, Quesada, & Lopez, 1996; Granados et al., 2002). Furanic aldehydes are another group of compounds that are extracted from the wooden barrels but do not directly affect the final organoleptic characteristic of the beverage (Quesada, Villalón, Lopez, & Martinez, 1996).

As a general rule, syringaldehyde and vanillin are the predominant compounds in aged alcoholic beverages. However, other compounds as syringic acid, sinapic

* Corresponding author. Tel.: +55 85 32889656/40089656; fax: +55 85 32889751/34583407.
E-mail address: sueli@ufc.br (S. Rodrigues).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.07.034

acid, vanillic acid and ferulic acid, as well as their esters can also be found (Yokoya, 1995). Some studies indicate that low molecular weight phenolic compounds present antioxidant, anti free radical, anti inflammatory and anti carcinogenic properties (Goldberg, Hoffman, Yang, & Soleas, 1999; Salagoityaguste, Tricard, & Susdraud, 1987). Coumarins are also present in aged alcoholic beverages, and lately have drawn attention because their presence indicates long periods of storage inside the barrels (Caldeira, Canas, Costa, Carvalho, & Belchior, 1999; Canas, Grazina, Belchior, Spranger, & Souza, 2000; Izquierdo, Granados, Mir, & Martinez, 2000; Lake, 1999; Puech, Rabier, & Moutounet, 1988; Vivas, Bougeois, & Vitry, 1998). Coumarins such as esculine, umbeliferone, escopoletin, 4-methyl-umbeliferone and coumarin (1,2-benzopyrone) are most studied as markers in aged beverages (Puech et al., 1988).

Although the most traditional wood used to age alcoholic beverages is oak, in Brazil, other woods, especially Balm and local woods, are often employed making the phenolic profile of Brazilian aged sugar cane spirits a special field of investigation.

In this work, a high performance liquid chromatographic method was developed for the simultaneous determination of 10 representative compounds in aged sugar cane spirits. Gallic acid; 5-hydroxymethyl-furfural (5-HMF); furfural; vanillic acid; syringic acid; vanilin; syringaldehyde; coniferaldehyde; sinapaldehyde and coumarin were simultaneously quantified. Although the presence of these compounds in sugar cane spirits is known, a method for their simultaneous and direct quantification in these beverages is not available. These compounds (except furfural) are absent in fresh distilled sugar cane spirit and can be considered as typical components of aged sugar cane spirits. A detailed understanding of aging markers content in sugar cane distilled spirits should significantly contribute to improve quality control.

The method was validated and applied to samples of aged sugar cane spirits from several small producers from Ceará State (Northeast Brazil), where different kinds of woods are employed in beverage aging. The method presented herein has all the requirements to be applied as a standard protocol and allow for direct determination of the compounds of interest.

2. Materials and methods

2.1. Reagents and standards

Sugar cane spirits from Ceará State (Brazil) were used. The samples were collected by laboratory members and stored at 4 °C prior to analysis. Standards of gallic acid; 5-hydroxymethyl-furfural (5-HMF); furfural; vanillic acid; syringic acid; vanilin; syringaldehyde; coniferaldehyde; sinapaldehyde and coumarin were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Acros Organics (New Jersey, USA). All the chemicals were HPLC grade.

Methanol HPLC grade from Tedia Company Inc. (Fairfield, OH, USA), ultra high-purified water obtained by reverse osmosis system filtered in a 0.45 μm ultrafiltration system supplied by Fresenius Kabi Brasil (Aquiraz, CE, Brazil) and acetic acid from Merck (Darmstadt, Germany) were used as solvent for chromatography.

2.2. Sample preparation and analysis

The analytes were quantified using the external standard method. Calibration curves were built diluting stock solutions containing 1000 ppm of each standard diluted in ethanol 50% pH 4.5. Calibration curves were obtained by linear regression using the StarCrom integration software (Varian) considering a minimum correlation coefficient of 0.995. A total of 28 samples of sugar cane spirits from small producers were analyzed. The results were compared to the analysis of three export grade sugar cane spirits aged in oak and balm barrels.

The samples and standards were filtered in a 0.45 μm nylon membrane and directly injected into the chromatographic system (Quesada et al., 2002). Injections were done in triplicate and the analytes identities were confirmed by retention time and by spiking the sample with the standards. A maximum deviation of ±5% was considered.

2.3. Apparatus

A Varian Pro Star HPLC system with two high-pressure pumps model Pro-Star 210, a column oven Timberline model 101, a double channel UV-detector Pro-Star model 345 with programmable wavelength variation and a Rheodyne injector loop of 20 μl was used. The separations were done using a Merck LiChrospher 100 RP-18 endcapped column (250 mm × 4 mm) with 5 μm spherical particles.

2.4. HPLC conditions

The elution solvents used were: 2% acetic acid in water (phase A) and 70% methanol and 2% acetic acid in water (phase B). The samples were eluted according to the gradient presented in Table 1. Wavelength variation was used to improve the detector response (Table 1). Flow rate was 1.25 ml/min and run time was 60 min. The run was performed at 40 °C. The injection volume of the sample was 20 μl. Identification of the compounds was achieved by comparing their retention time with those of the authentic compounds (Table 2). Quantification was done using external standards (0.50–10.00

Table 1
HPLC conditions

Time (min)	Solvent A (%, v/v)	Solvent B (%, v/v/v)	Wavelength (nm)
0.00	100	0	271
3.00	100	0	271
6.00	100	0	280
25.00	60	40	345
28.00	60	40	320
35.00	60	40	271
43.00	40	60	271
50.00	0	100	271
55.00	100	0	271
60.00	100	0	271

Solvent A: water:acetic acid, 98:2% (v/v).
Solvent B: methanol:water:acetic acid, 70:28:2% (v/v/v).

Table 2
Retention times (RT), parameters and correlation coefficients (r^2) of the calibration plots

Compound	RT (min)	a	b	r^2
Gallic acid	4.291	1.4940×10^4	6.3028×10^2	0.9996
5-HMF	8.588	4.8828×10^4	1.7143×10^3	0.9986
Furfural	12.646	4.7143×10^4	-6.6039×10^3	0.9962
Vanillic acid	17.225	1.0183×10^4	1.3096×10^3	0.9987
Syringic acid	19.763	1.6971×10^4	-2.0611×10^3	0.9960
Vanillin	20.823	2.5201×10^4	1.0032×10^3	0.9967
Syringaldehyde	22.961	1.1172×10^4	3.5534×10^3	0.9963
Coniferaldehyde	27.111	8.6760×10^4	7.0057×10^4	0.9988
Sinapaldehyde	27.853	4.2771×10^4	2.5107×10^4	0.9956
Coumarin	29.988	2.1135×10^4	2.6712×10^4	0.9995

ppm). The data was acquired and handled by Star Chromatography Workstation 5.51 software. Calculations were accomplished at 5% confidence level.

3. Results and discussion

3.1. Method validation

3.1.1. Wavelength choice

The analytes were grouped according to their maximum absorbance after the identification of the wavelength response of each compound as well as its intensity and the evaluation of the peak shape. Four wavelengths were chosen according to the data presented in Table 1.

3.1.2. Separating condition

The best separating condition was obtained after several tests where mobile phase, elution gradient and column temperature were evaluated. The best HPLC condition found was: column temperature of 40 °C, total flux of 1.25, two different mobile phases (A and B) and the elution gradient presented in Table 1. Wavelength variation was employed to improve the sensitivity of the method by monitoring the compounds at the maximum absorbance wavelength.

3.1.3. Calibration curve

Quantifications were done by the external standard method and the calibration curves were built through linear regression of the data obtained for the mean peak area of each analyte after triplicate injection of mixed solutions containing 0.25, 0.50, 0.75, 1.00, 5.00, 7.00 and 10.00 ppm of each standard. Table 2 presents the calibration curves parameters and the correlation coefficients of the calibration plots (r^2) for each standard. Calibration plots are expressed as linear regression equations ($y = a + bx$), where y is the peak area and x is the analyte concentration (ppm).

3.1.4. Selectivity

Chromatograms with optimized resolution peaks were obtained under the optimized separating conditions (Fig. 1). The method has presented satisfactory selectivity since no interference in the analysis was caused by the presence of other compounds.

3.1.5. Detection and quantification limits

The linear range for most compounds was 0.25–10.00 ppm. Gallic acid, vanillic acid, syringic acid and syringaldehyde presented lower quantification limit of 0.50 ppm. The estimated detection limits were obtained after successive dilutions (1:1 ratio) of a 10.00 ppm standard mixture. Dilutions were carried out until the signal-to-noise ratio was 3:1, for peak heights in mV (Nascimento, Rodrigues, Neto, & Franco, 1998; Nascimento, Marques, Neto, De Keukeleire, & Franco, 1997). Quantification limits were obtained using the same procedure but dilutions were carried out until the signal-to-noise ratio was 5:1. Table 3 presents the detection limits (DL) and quantification limits (QL) for each compound.

3.1.6. Precision

The precision study was done by repeating 10 consecutive injections of a 5 ppm standard solution of each analyte. The results were submitted to a statistical evaluation and the method precision was established as presented in Table 4. The precision of the method was characterized by the relative standard deviations (RSD) that range from 2.10% (furfural) to 7.10% (gallic acid) with an average value of 4.60%, which are satisfactory for analysis.

3.1.7. Accuracy

Analytical accuracy was evaluated by spiking a previously analyzed sample with the standards. The recovery level was determined according to Eq. (1) and the results are presented in Table 5.

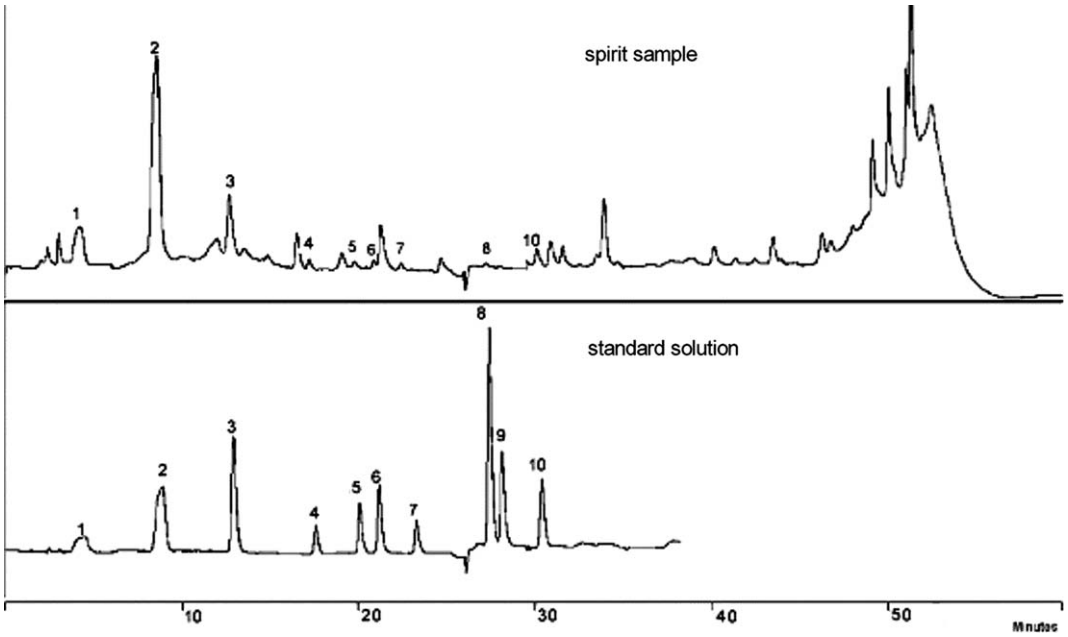

Fig. 1. Chromatograms obtained using a 5 ppm standard solution and an aged sugar cane spirit sample: (1) gallic acid; (2) 5-HMF; (3) furfural; (4) vanillic acid; (5) syringic acid; (6) vanillin; (7) syringaldehyde; (8) coniferaldehyde; (9) sinapaldehyde, (10) Coumarin.

Table 3
Detection limits (DL) and quantification limits (QL) for each compound

Compound	DL (ppb)	QL (ppm)
Gallic acid	120	0.50
5-HMF	80	0.25
Furfural	80	0.25
Vanillic acid	120	0.50
Syringic acid	120	0.50
Vanillin	80	0.25
Syringaldehyde	120	0.50
Coniferaldehyde	30	0.12
Sinapaldehyde	80	0.25
Coumarin	80	0.25

Table 4
Repeatability of the quantitative analysis

Compound	Mean^n (ppm)	SD	RSD (%)
Gallic acid	5.04	0.35	7.01
5-HMF	5.02	0.13	2.54
Furfural	5.05	0.11	2.10
Vanillic acid	5.01	0.19	3.81
Syringic acid	5.03	0.22	4.36
Vanillin	5.14	0.22	4.27
Syringaldehyde	4.98	0.14	2.84
Coniferaldehyde	4.95	0.11	2.14
Sinapaldehyde	4.96	0.19	3.85
Coumarin	5.03	0.21	4.08

n = average of 10 analyses; SD, standard deviation; RSD relative standard deviation.

Table 5
Accuracy by recovery investigation

Compound	Concentration (ppm)			Recovery (%)
	Sample	Added	Found	
Gallic acid	1.27	5.00	6.09	97.13
5-HMF	1.04	5.00	6.12	101.32
Furfural	7.12	5.00	11.97	98.76
Vanillic acid	2.1	5.0	7.56	98.05
Syringic acid	3.7	5.00	8.45	97.46
Vanillin	3.53	5.00	8.38	98.24
Syringaldehyde	8.18	5.00	13.41	101.74
Coniferaldehyde	3.55	5.00	8.50	99.41
Sinapaldehyde	9.03	5.00	14.27	101.71
Coumarin	0.47	5.00	5.62	102.74

$$\text{Recovery}\,(\%) = \left(\frac{\text{measured concentration}}{\text{expected concentration}}\right) \times 100 \qquad (1)$$

According to Table 5 the recovery ranged from 97.13% to 102.74%, which can be considered satisfactory taken into account the non-homogeneity of the samples (Swartz & Krull, 1997).

3.2. Determination of aging markers in aged sugar cane spirits from Ceará State (Brazil)

Table 6 presents the phenolic contents of the analyzed aged sugar cane spirits. Except for 5-HMF, the average content of aging markers found in the aged spirits from small producers is in agreement with the average content found for aged spirits of export grade products. The level of 5-HMF can be attributed to the use of very old

Table 6
Average values of aging markers content in aged sugar cane spirits

Compound	Concentration* (ppm)	
	Small producers	Export product
Gallic acid	0.6293 ± 0.0315	0.1528 ± 0.0076
5-HMF	3.1110 ± 0.1556	0.8235 ± 0.0412
Furfural	1.0968 ± 0.0548	0.7727 ± 0.0386
Vanillic acid	0.9021 ± 0.0451	0.9942 ± 0.0497
Syringic acid	2.1016 ± 0.1051	1.9411 ± 0.0971
Vanillin	1.3193 ± 0.0660	1.5412 ± 0.0771
Syringaldehyde	5.2442 ± 0.2622	6.2653 ± 0.3133
Coniferaldehyde	0.5080 ± 0.0254	1.2535 ± 0.0627
Sinapaldehyde	1.4666 ± 0.0733	1.2356 ± 0.0618
Coumarin	0.0953 ± 0.0048	0.0429 ± 0.0021

Average values ± SD (small producers $n = 28$ and export product $n = 3$).

barrels or to the use of barrels without any treatment before re-utilization.

4. Conclusion

This work has presented a method developed for simultaneous analysis of 10 representative aged sugar cane spirits aging markers through HPLC analysis. The method was validated and applied to a set of 28 sugar cane spirits from small producers from Ceará state (Brazil). The results showed satisfactory agreement between the spirits aged in barrels made from local woods (spirits from the small producers) and the spirits aged in oak and balm barrels (export grade products) for all compounds excepting 5-HMF, indicating an inadequate re-utilization of the barrel.

The developed method has presented all necessary requirements (linearity, precision and accuracy) for its application as a standard protocol to quantify the analyzed compounds in aged sugar cane spirits. The aging markers contents in aged distilled beverage are important and can be considered a good parameter to assest the authenticity and quality of these beverages. These compounds have been studied in wines, whisky and other distilled beverages but few studies have been published on sugar cane spirits.

Acknowledgement

The authors thank Fundação Cearence de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) for the financial support.

References

Bozhinov, A. (1994). Study of the processes occurring during ageing of wine distillate. *Khranitelna Promishlenost, 43*(3), 25–27.

Caldeira, I., Canas, S., Costa, S., Carvalho, E., & Belchior, A. P. (1999). Formação de uma câmara de prova organoléptica de aguardentes velhas e selecção de descritores sensoriais. *Ciência e Tecnologia Vitivinícola, 14*, 21–30.

Canas, S., Grazina, N., Belchior, A. P., Spranger, M. I., & Souza, R. B. (2000). Modelisation of heat treatment of portuguese oak wood (*Quercus pyrenaica* L.). analysis of the behaviour of low molecular weight phenolic compouds. *Ciência e Tecnologia Vitivinícola, 15*, 75–94.

Gimenez, R., Lopez, H., Villalon, M., Quesada, J., & Lopez, M. (1996). Influence of wood heat treatment temperature and maceration time in vanillin, syringaldehyde and gallic acid contents in oak wood and wine spirit mixture. *American Journal of Enology and Viticulture, 47*, 441–446.

Goldberg, D. M., Hoffman, B., Yang, J., & Soleas, G. J. (1999). Phenolic constituents, furans, and total oxidant status of distilled spirits. *Journal of Agricultural and Food Chemistry, 47*, 3978–3985.

Granados, Q. J., Guérvos, J. J. M., López, M. J. O., Penãlver, J. G., Herrera, M. O., Herrera, R. B., et al. (2002). Application of artificial techniques to samples of rum and comparison with traditionally aged rums by analysis with artificial neural nets. *Journal of Agricultural and Food Chemistry, 50*, 1470–1477.

Izquierdo, M. E. F., Granados, J. Q., Mir, V. M., & Martinez, M. C. L. (2000). Comparison of methods for determining coumarins in distilled beverages. *Food Chemistry, 70*, 251–258.

Jaganathan, J., & Dugar, S. M. J. (1999). Authentication of straight whiskey by determination of the ratio of furfural to 5-hydroxymethyl-2-furaldehyde. *Journal of AOAC International, 82*(24), 997–1001.

Lake, B. G. (1999). Coumarin metabolism, toxicity and carcinofenicity: Relevance for human risk assessment. *Food Chemistry Toxicology, 37*, 423–453.

Lo Coco, F., Valentini, C., Novelli, V., & Ceccon, L. (1995). Liquid chromatograph determination of 2-furaldehyde and 5- hydroxymethyl-2-furaldehyde in beer. *Analytica Chimica Acta, 306*, 57–64.

Mangas, J., Rodriguez, R4., & Moreno, J. (1996). Evolution of aromatic and furanic congeners in the maturation of cider brandy: A contribution to its characterization. *Journal of Agricultural and Food Chemistry, 44*, 3303–3307.

Martinez, R. G., Mir, M. V., Serrana, H. L. G., & Marinez, M. C. L. (1993). Simultaneous determination of vanillin and syringaldehyde using high performance liquid chromatography. Application to the static and soleras aged brandies. *Journal of Liquid Chromatography, 16*, 4079–4094.

Nascimento, R. F., Rodrigues, D. C., Neto, B. S. L., & Franco, D. W. (1998). Determination of fatty acids in Brazilian cane sugar spirits and other alcoholic beverages by HRGC-SPE. *Chromatographia Wiesbaden, 48*, 866–871.

Nascimento, R. F, Marques, J. C., Neto, B. S. L., De Keukeleire, D., & Franco, D. (1997). Qualitative and quantitative high-performance liquid chromatography analysis of aldehydes in Brazilian sugar cane spirits and other distilled beverages. *Journal of Chromatography A, 782*, 13–23.

Puech, J. L., Rabier, P., & Moutounet, M. (1988). Preparative separation by high-performance liquid chromatography of an extract of oak wood and determination of the composition of each fraction. *Journal of Chromatography A, 457*, 431–436.

Quesada, J., Villalón, M., Lopez, H., & Martinez, C. L. (1996). Influence of aging factors on furanic aldehyde contents of matured brandies: aging markers. *Journal of Agricultural and Food Chemistry, 44*, 1378–1381.

Quesada, J. G., Guervós, J. J. M., López, M. J. O., Peñalver, J. G., Herrera, M. O., Herrera, R. B., et al. (2002). Application of artificial techniques to samples of rum and comparison with traditionally aged rums by analysis with artificial neural networks. *Journal of Agricultural and Food Science, 20*, 1470–1477.

Reazin, G. H. (1981). Chemical mechanisms of whisky maturation. *American Journal of Enology and Viticulture, 32*, 283–289.

Salagoityaguste, M. H., Tricard, C., & Susdraud, P. (1987). Simultaneos determination of aromatic-aldehydes and coumarins by high performance liquid chromatography – applicaton to wines and brandies stored in oak barrels. *Journal of Chromatography, 392*, 379–387.

Swartz, M. E., & Krull, I. S. (1997). *Analytical methods development and validation*. Marcel Dekker, Inc.

Vivas, N., Bougeois, G., & Vitry, C. (1998). Development of analytical method for the detection of specific biomarkers of wines aging in barrels. *Analysis, 26*, 88–92.

Yokoya, F. (1995). Série Fermentações Industriais 2, Fabricação de aguardente de cana. Fundação Tropical de Pesquisas e Tecnologia André Tosello, Campinas, SP, Brazil.