Journal of Food and Drug Analysis

Volume 13 | Issue 2 | Article 1

Using 13C/12C isotopic ratio analysis to differentiate between rice spirits made from rice and cane molasses

Journal of Food and Drug Analysis, Vol. 13, No. 2, 2005, Pages 159-162 藥物食品分析 第十三卷 第二期

Using $^{13}C/^{12}C$ Isotopic Ratio Analysis to Differentiate between Rice Spirits Made from Rice and Cane Molasses

YUNG-HUA RAU, GUEY-PYNG LIN, WEI-SHAN CHANG, SHIH-SHUN WEN AND WEIGUANG FU*

Food Industry Research and Development Institute, Analysis Research and Service Center, 331, Shi-Pin Rd., Hsin-Chu City 300, Taiwan, R.O.C.

(Received: November 16, 2004; Accepted: February 22, 2005)

ABSTRACT

In this paper, we used automated ^{15}N ^{13}C analysis MS (ANCA-MS) and stable carbon isotope ratio analysis to analyze samples of rice- and cane molasses-based spirits and their constituent principal ingredients. This methodology was developed to distinguish, in a scientifically accurate manner, rice spirits made of rice from that made of cane molasses. Spirits with different mixed ratios of rice and cane molasses were also prepared for a detection capability test. Series dilution for spirit samples was performed to assess operational consistency. Test results demonstrated that $^{13}C/^{12}C$ values, as measured by ANCA-MS, can be used as reliable benchmark to distinguish between the two types of rice spirits. It is recommended that test samples should be distillated or diluted to an equivalent level of ethanol prior to measuring $^{13}C/^{12}C$ values. Data established in this paper can provide criteria necessary to monitor rice spirits sold in the marketplace.

Key words : rice spirit, stable carbon isotopic ratio, cane molasses

INTRODUCTION

Rice spirit, most commonly fermented either from rice or cane molasses, is an important component of the typical Chinese diet. The spirit fermented from rice has its own special flavor and aroma and can be used directly in the preparation of herbal medicine and functional foods. The spirit fermented from cane molasses is sold as "rice aroma spirit", with its flavor enhanced by added rice flavoring. Rice spirits are normally slightly salted (0.5%) following the fermentation process and have an ethanol content that typically ranges from 15% to 60%. While a price difference may be expected to exist between the two types of rice spirits due to cost differences between the two principal raw materials, it is not the case in the current market. Therefore, in order to differentiate between final products made from rice and molasses, it is important to develop an effective testing methodology to ensure effective product monitoring.

Stable carbon isotope ratio analysis (SCIRA) has been used successfully for many years in the monitoring of processed fruit and vegetable products. SCIRA compares carbon isotope ratio values in samples for different photosynthesis cycles of the plants, with different ratios indicating samples were produced by different photosynthesis cycles. Plants such as rice, with a Calvin-Benson photosynthetic cycle (C_3), are reported to have $^{13}C/^{12}C$ values ranging from -21 $^0/_{00}$ to -32 $^0/_{00}$. Plants such as sugar cane, with a Hatch-Slack photosynthetic cycle (C_4), are reported to have values ranging from -12 $^0/_{00}$ to -19 $^0/_{00}$[1-3]. In addition to the dual-inlet isotope ratio mass spectrometer (IRMS), AOAC 978.17 specifies automated ^{15}N ^{13}C analysis MS (ANCA-MS) as the alternate instrument permitted for use in fast detection of stable carbon isotope ratios[4]. As ANCA-MS is fast and easy to perform, the authors believe it to be an effective tool to measure the $^{13}C/^{12}C$ values of any liquid or solid. Since SCIRA's successful use in distinguishing between cane and corn-based alcohol in sake samples[5], stable carbon isotope ratios have been employed to detect adjunct ingredients in other alcoholic beverages such as wine[6], brandy[7-9], whiskey[10-11] and beer[12]. It was also concluded by Brooks[12] that the stable carbon isotope ratio of an alcoholic beverage is an indicator of the quantity of C_3 and C_4 ingredients used in its production. For this paper, we have assumed that spirits made from rice or cane molasses can also be differentiated using ANCA-MS.

The major goal of this research is to use ANCA-MS to differentiate between rice spirits made of rice from that made of cane molasses. The sample preparation process is discussed below. Rice, cane molasses, and rice-based and cane molasses-based spirit samples, collected from a rice spirit production facility, were analyzed to assess the potential for differentiation.

MATERIALS AND METHODS

I. *Samples*

Actual rice and rice spirit samples (hereafter, "the samples") were prepared and provided by Dr. Fu-Lin Lee from the Bioresources Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI). The rice spirit sample provided was fermented exclusively from rice, without adulteration. The

* Author for correspondence. Tel: +886-3-5223191; Fax: +886-3-5214016; E-mail: fwg@firdi.org.tw

cane molasses and cane molasses spirit samples were collected from the manufacturer. The cane molasses spirits were fermented from molasses of a specification identical to that of our cane molasses sample.

II. *Samples Preparation and Measurement for* $^{13}C/^{12}C(\delta\,^0/_{00})$

The rice sample was homogenized and passed through a 100-150 mesh strainer prior to measurement. Spirit samples were distillated before analysis in order to prevent interference from potential adulterates, such as salt. Samples (25 mL) were placed for 5 min in a flask for distillation using a Buchi Distillation Unit B-323. The sample was then placed in a 25 mL volumetric flask, into which distilled water was added to bring the volume up to 25 mL. Ethanol content and $^{13}C/^{12}C$ values were analyzed to determine whether any difference could be detected between distilled and non-distilled rice spirit samples. Pee Dee Bee Belemintella american fossil limestone from the National Institute of Standards and Technology in Gaithersburg, Maryland, USA was used as a reference standard. All samples were placed into tin capsules and run for $^{13}C/^{12}C$ in triplicate. An ANCA 20-20-IRMS on-line Mass Spectrometer (Europa Scientific, UK) was used for sample measurement. Ethanol content was determined by gas chromatographic method using flame ionization detection based on AOAC 984.14[13].

III. *Test of Detection Capability*

We mixed our rice spirit sample with cane molasses spirit sample in different ratios (by volume) to evaluate detection capability. A linear regression curve was plotted that compared isotope ratio values of mixed spirits against mixed levels.

IV. *Test of Consistency*

We performed series dilutions on the samples to confirm operational consistency. Undistilled cane molasses spirit with an ethanol content of 85% was diluted with distilled water to provide test samples of 34% and 17% ethanol content. Rice spirit with an ethanol content of 40% was first distillated and concentrated to achieve an ethanol content of 68% and then diluted with distilled water to provide test samples of 34% and 17% ethanol content.

RESULTS AND DISCUSSION

I. *Study on the Distillation Process*

The $^{13}C/^{12}C$ values and ethanol contents of rice spirit sample were measured before and after the distillation process. Measurements (Table 1) showing no significant difference between pre- and post-distillation stable carbon isotope and ethanol content values between spirit samples, which were fermented from rice, distillated and prepared without adding any additives or salts, indicate that the distillation process does not alter either. In other words, stable carbon isotope values do not change through the distillation process as long as ethanol content remains stable.

Table 1. Stable carbon isotope values and ethanol contents of rice spirits before and after distillation

	Stable carbon isotope value ($^0/_{00}$)[a]	Ethanol content (%)[b]
Rice spirit before distillation	-24.01±1.37	20.0±0.5
Rice spirit after distillation	-24.53±1.89	20.2±0.5

[a]Triplicate analyses.
[b]Duplicate analyses.

Table 2. Stable carbon isotope values for cane molasses spirits of different ethanol contents

Ethanol content ($^v/_v$)	Stable carbon isotope value ($^0/_{00}$)
Cane molasses spirit	
85%(without distillation)	-14.83
34%(diluted from 85%)	-15.90
17%(diluted from 85%)	-16.52

Table 3. Stable carbon isotope values for rice spirits of different ethanol contents

Ethanol content ($^v/_v$)	Stable carbon isotope value ($^0/_{00}$)
Rice spirit	
40%(without distillation)	-25.02
68%(distillated)	-24.37
34%(diluted from 68%)	-25.02
17%(diluted from 68%)	-25.82

Table 4. Stable carbon isotope values of rice, cane molasses and their fermented spirits

	Stable carbon isotope value ($^0/_{00}$)		Ethanol content (%)
	Raw material[a]	Spirits[a]	Spirits[a]
Rice	-26.90±0.02	-27.27±2.26	>30
Cane molasses	-11.98±0.80	-11.85±1.85	>60

[a]More than triplicate analyses.

According to Brookes *et al.*[4], stable carbon isotope values should remain constant in samples measured using ANCA-MS, even if in the presence of different ethanol content levels. However, in our work to develop a science-based product monitoring procedure, stable carbon isotope ratio tests done on series dilutions of rice spirit samples showed disparities. Data in Table 2 and Table 3 show that the lower the ethanol content in rice spirit, the lower the stable isotope values in spirits with or without distillation. When a sample was distillated to higher content of ethanol, pre- and post-distillation values showed significant differences. Martin *et al.*[5] also include a distillation process before sample testing, in which samples are all distillated to a 95% (v/v) ethanol content. Distillation was also recommended by Bauer-Christoph *et al.*[14] to give samples an ethanol content greater than 90% in order to prevent evaporation and subsequent fractionation problems.

Variations detected may be due to CO_2 dissolved in spirits. As rice spirits are sold at different ethanol content levels, we suggest that, for product monitoring and comparison purposes, samples be distillated or diluted to a uniform ethanol content level prior to conducting ANCA-MS measurements.

II. *Stable Carbon Isotope Value Differences between Raw Materials and Spirit Products*

Our findings indicate no significant difference in stable carbon isotope values between raw materials and their associated spirit products (Table 4). The greater range of variability in spirit product $^{13}C/^{12}C$ values (versus that for its associated raw material) is attributed to the variable ethanol content level in processed spirits. On the other hand, significant differences do exist between rice and cane molasses-based rice spirits and between their associated raw materials. Our findings conform to those reported in previous papers that recommend the adoption of $^{13}C/^{12}C$ values to differentiate between C_3 and C_4 plant products. For purposes of product monitoring and quality control, we further concluded that the stable carbon isotope value of the relevant raw material is a reliable predictor of the stable carbon isotope value of the associated final rice spirit product. Therefore, stable isotope values derived from raw material samples taken at the factory can be used as a valid baseline against which to check rice spirit products collected in the marketplace.

Using a linear regression curve to measure the stable carbon isotope values of rice and cane molasses spirits of different ratios

Spirits of different mixed ratios of rice and cane molasses were prepared for the detection capability test. We assumed that if the stable isotope values of the raw materials were measured, the values of their fermented products could also be reasonably predicted (an important topic deserving follow-on study is the investigation of rice-cane molasses spirit mixtures). Data in Figure 1 show that the plot of stable carbon isotope values in different mixed levels of rice and cane molasses spirits with a final ethanol content in the ranges of 34-35% and 14-17% have correlation coefficients greater than 0.96. Excellent linearity is shown when rice and cane molasses spirits are mixed in differing levels.

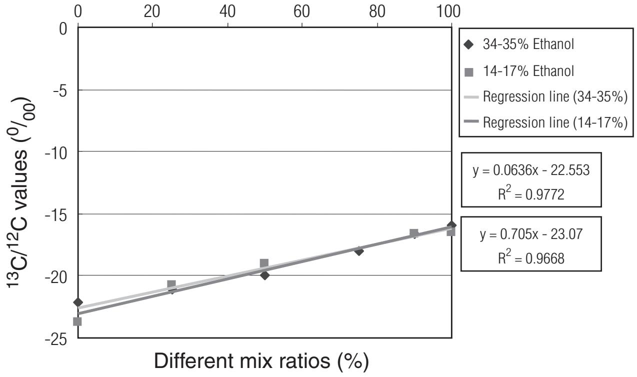

Figure 1. The $^{13}C/^{12}C$ values of rice and cane molasses spirits mixed in different ratio.

CONCLUSIONS

In conclusion, using ANCA-MS to measure the stable carbon isotope value of rice spirit samples can be an effective method by which to differentiate between rice-based and cane molasses-based rice spirits. Prior to measuring the $^{13}C/^{12}C$ value by ANCA-MS, it is recommended that test samples be distillated or diluted to achieve an equivalent ethanol content.

ACKNOWLEDGEMENTS

The following individuals are gratefully acknowledged for their support of this research: Dr. Fu-Lin Lee from BCRC, FIRDI and Mr. S.C Lee from the Institute of Nuclear Energy Research. We also thank Drs. Ming-Sai Liu, Wei-Hsien Chang and Mr. Chi-Chung Chou for their technical discussions. This work was supported by grants from the Department of National Treasury, Ministry of Finance.

REFERENCES

1. Calvin, M. and Bassham, J. A. 1962. The Photosynthesis of Carbon Compounds. W. A. Benjamin. New York, U. S. A.
2. Smith, B. N. and Epstein, S. 1971. Plant Physiol. 47: 380-384.
3. Ro β mann, A., Schmidt, H. L., Reniero, F., Versini, G., Moussa, I. and Merle, M. H. 1996. Stable carbon isotope content in ethanol of EC data bank wines from Italy, France and Germany. Z. Lebensm. Unters. Forsh. A. 203: 293-301.
4. Brookes, S. T., Barrie, A. and Davies, J. E. 1991. A rapid $^{13}C/^{12}C$ test for determination of corn syrups in honey. J. AOAC Int. 74: 627-629.
5. Martin, G. E., Burggraff, J. M., Alfonso, F. C. and Figert, D. M. 1983. Determination of authenticity of sake by carbon isotope ratio analysis. J. AOAC Int. 66: 1405-1408.
6. Weber, D., Rossmann, A., Schwarz, S. and Schmidt, H. L. 1997. Correlations of carbon isotope ratios of wine ingredients for the improved detection of adulterations I. Organic acids and ethanol. Z. Lebensm. Unters. Forsh. A 205: 158-164.
7. Simpkins, W. A. and Rigby, D. 1982. Detection of the illicit extension of potable spirituous liquors using $^{13}C:^{12}C$ ratios. J. Sci. Food Agric. 33: 898-903.
8. Pissinato, L., Martinelli, L. A., Victoria, R. L. and Camargo, P. B. 1999. Using stable carbon isotopic

analyses to access the botanical origin of ethanol in Brazilian brandies. Food Res. Int. 32: 665-668

9. Hogben, R. and Mular, M. 1976. Major congeners of Australian and imported brandies and other spirits as indicators of authenticity. J. Sci. Food Agric. 27: 1108-1114.
10. Simpkins, W. A. and Rigby, D. 1982. Detection of the illicit extension of potable spirituous liquors using ^{13}C:^{12}C ratios. J. Sci. Food Agric. 33: 898-903.
11. Parker, I. G., Kelly, S. D., Sharman, M., Dennis, M. J. and Howie, D. 1998. 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. Food Chem. 63: 423-428.
12. Brooks, J. R., Buchmann, N., Phillips, Sue., Ehleringer, B., Evans, R. D., Lott, M., Martinell, L. A., Pockman, W. T., Sandquist, D., Sparks, J. P., Sperry, L., Williams, D. and Ehleringer, J. R. 2002. Heavy and Light Beer: A carbon isotope approach to detect C4 carbon in beers of different origins, styles, and prices. J. Agric. Food Chem. 50: 6413-6418.
13. Official Mithods of Analysis. 2002. 17th ed. AOAC, Arlington, VA, sec 984.14.
14. Bauer-Christoph, C., Wachter, H., Christoph, N., Robmann, A. and Adam, L. 1997. Assignment of raw material and authentication of spirits by gas chromatography, hydrogen- and carbon-isotope ratio measurements. Z Lebensm Unters Forsch A 204: 445-452.