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ScienceDirect
Food Research International 40 (2007) 324–331
FOOD RESEARCH INTERNATIONAL
www.elsevier.com/locate/foodres

Helping to authenticate sparkling drinks with $^{13}C/^{12}C$ of CO_2 by gas chromatography-isotope ratio mass spectrometry

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Received 3 April 2006; accepted 2 October 2006

Abstract

We applied direct injections of headspace gas into a Gas Chromatography – Combustion – Isotope Ratio Mass Spectrometry (GC-C-IRMS) system to measure $^{13}C/^{12}C$ isotopic ratio of carbon dioxide from the headspace of several sparkling and soft-drinks. This straightforward technique, which consists in an automated analysis of the headspace sample with no prior purification steps, improves the determination of $\delta^{13}C$ values of CO_2 gas as a food probe authenticity. However, for an ultimate test, combination with other isotopic data is required.

Keywords: Food authentication; Stable isotope ratio methods in food; Carbon dioxide; Ethanol; $\delta^{13}C$; GC-C-IRMS, IRMS; Sparkling wine; Beer; Carbonated water; Carbonated drinks; Multi isotopic analysis; ^{2}H-SNIF-NMR

1. Introduction

Sparkling drinks are among the most marketed foodstuff in the world. Carbon dioxide is a "magic" ingredient which gives drinks a sourer and a more pleasant taste. Bubbles, either naturally present or added, characterize several beverage products: wine, beer, water, cider, hydromel, soft-drinks, etc. Some aspects of CO_2 in beverages, such as pureness grade, concentration and origin, are regulated in *European Union* (EU) regulations.

1.1. Stable isotope ratio methods in food

In the last 20 years, the stable isotope measurements have demonstrated their convenience as a tool in the fight against fraud in the food products industry (Rossmann, 2001). These methods are based on stable isotope ratio measurements ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) from a product or of a specific component such as an ingredient or a target molecule of the product. The determinations can be carried out using nuclear magnetic resonance (NMR) and/or isotope ratio mass spectrometry (IRMS) techniques and they can provide information on the botanical and geographical origins.

Over the last 15 years, the EU and the *Organisation* (former *Office*) *International de la Vigne et du Vin* (OIV) have adopted analyses of $^{2}H/^{1}H$, $^{13}C/^{12}C$ and $^{18}O/^{16}O$ in their regulations as official methods to assure the authenticity of wine (Calderone, Guillou, & Naulet, 2003; E.C. Regulations No. 2676/90, No. 822/97, No. 440/2003; OIV – Résolution OENO 17/2001). In the same way,

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doi:10.1016/j.foodres.2006.10.001

industrial production has implemented the use of routinely isotope ratio analyses also to assess the origin of flavors and aromas, honeys (Association of Official Analytical Communities methods), fruit juices (Comité Européen de Normalisation methods) and sometimes of the residual sugar. Focusing only on the naturally distributed $^{13}C/^{12}C$ ratio, a wide range of $\delta^{13}C$ values is observable depending on botanic, geological and industrial origins of CO_2 (see in Section 3), thus the headspace CO_2 of a drink can be an interesting probe of its authenticity, especially if in combination with isotopic data of other components, such as ethanol in fermented drinks.

1.2. Rapid measurement of the ^{13}C content of CO_2

The aim of this study is to illustrate possible applications of a previously validated technique to analyze the $^{13}C/^{12}C$ ratio in natural abundances of CO_2 (Calderone, Naulet, Guillou, Reniero, & Blanch Cortes, 2005). We used a slightly modified continuous flow gas chromatography-combustion system coupled to IRMS (GC-C-IRMS) to characterize beverages and origin of CO_2. This technique overcomes technical difficulties associated with sample collection and purification occurring when traditional procedures are employed. The GC-C-IRMS is a relatively young technique (being developed in the 1980s) that allows on-line measurements of carbon-13 and nitrogen-15 content of many matrices in different fields (Meier-Augenstein, 1999). For $^{13}C/^{12}C$ measurements, yielding information about the ^{13}C abundance of each isolated molecule, CO_2 is the gas compatible with the IRMS system.

For low volume sample analyses, GC-C-IRMS techniques for $^{13}C/^{12}C$ ratio measurements of CO_2 gas are already applied in environmental studies at natural abundance level and in the medical field (e.g. *^{13}C-Urea Breath Test*) using ^{13}C-enriched products (Lamrini, Lacan, Francina, Guilluy, & Brazier, 1995; Mortazavi & Chanton, 2002; Prosser, Brookes, Linton, & Preston, 1991). In the current work, our objective is to demonstrate that this tool can be applied for the analysis of various kinds of sparkling beverages, in order to verify their compliance with relevant regulations and labeling.

Previously described methods are based on the isolation of pure carbon dioxide in wines, before to injection into the mass spectrometer (Dunbar, 1982; González-Martin, González-Pérez, & Marqués-Macías, 1997; Guilluy et al., 1992). These methods required several steps for purifying headspace gas from water and ethanol (whose mass 46 is interfering in analysis with isotopomers of CO_2) including cryogenic distillation procedures and cold traps and the use of the dual inlet mode. A continuous flow method was also described, but it still required a dedicated on-line system with a cryogenic trap and a vacuum line (Boner & Förstel, 2001).

The GC-C-IRMS method applied in this paper offers clear advantages of rapidity and repeatability (SD $<$ 0.1‰). Furthermore, the present method can be easily implemented in enforcement laboratories, considering also the increasing diffusion of the GC-C-IRMS technique.

1.3. Some economical and production aspects

Sparkling and semi-sparkling wines – whose category includes French Champagne, Italian Spumante and Spanish Cava: approximately 742 million bottles of these wines have been produced in 2002 (Internet survey: http://www.darapri.it/immagini/nuove_mie/spumante/datistatistici.htm) – are traditionally produced by a second fermentation that originates from added sugar to already fermented wine, directly in bottles (*Champenois* or *Champagne* method) or in tanks (*Charmat Process*). An industrial gasification is possible, the *carbonation*, by direct injection of "food grade" CO_2, but EU legislation forbids the use of any exogenous carbonic anhydride in semi-sparkling and sparkling quality wines (E.C. Regulation No. 1493/1999).

A survey showed that beer is one of mostly consumed beverage all over the world: 123.4 billion liters were produced in 1998 in the 68 countries under study, about 30 billion liters in the EU in 2000, with consumption peak of 159 liters per person per year in Czech Republic.

Although among the EU and the *European Economic Area* (EEA) countries there is no unique law that regulates production of beer, European national regulations are mostly modeled on the old German *Reinheitsgebot* (originally enacted in 1516) or "*Germany's Beer Purity Law*" (BGBI, 1993) that imposed strict brewing standards. Despite the countless varieties of this beverage, beer is defined all over the world essentially as the fermentation product of musts prepared of malted barley, yeasts (*Saccharomyces carlsbergensis* or *S. cerevisiae*), water and hops, as flavoring agent. Malted barley can be substituted with other cereals (wheat, rice or corn) in variable percentage, depending on the specific national regulations.

Other minor fermented beverages are cider and perry (or Perry pear cider), derived, respectively, from apple and pear juice, and hydromel which is obtained from fermentation of honey in water. Cider is principally produced in specific regions of England, France and Spain, in Scandinavian countries and in the USA, where today it is mostly a non-alcoholic beverage. National legislations can differ, however, European policies regarding quality products tend to protect geographical origin, traditional recipes and production methods of agricultural products (E.C. Regulations No. 2081/92 and 2082/92). In agreement with these principles, only those ciders, perries and hydromels not added with extraneous carbon dioxide or sugars (added to promote a second fermentation) can be considered as genuine, whereas carbonation is permitted in the USA for these types of products.

Italy is the largest bottled water consumer in Western Europe, with total volume sales of 10.2 billion liters in 2003 and perhaps the largest consumer in the World, with 177 liters per person of annual consumption, whereas a