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Traditional balsamic vinegar and balsamic vinegar of Modena analyzed by nuclear magnetic resonance spectroscopy coupled with multivariate data analysis

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ABSTRACT

Balsamic vinegar of Modena (BVM) and traditional balsamic vinegar of Modena (TBVM) are highly appreciated typical Italian products. The quality control and authentication assurance of both these balsamic vinegars are very important topics. In the recent years, the interest to develop new and standardized analytical procedures, able to further enhance the quality and commercial value of these typical and unique products and to preserve them from possible sophistications and adulterations, is increased. In this work, 76 samples of both BVM and TBVM were analyzed by ^{1}H NMR spectroscopy coupled with multivariate data analysis. The spectral data were analyzed by principal component analysis (PCA), general discriminant analysis (GDA) and classification tree analysis (CTA). The best and very promising model was obtained by a GDA which shows 98.6% of total variance explained by the first canonical function and a predictive capacity of 98.4% with a good separation between clusters. The signals of 5-HMF, α-glucopyranose, malic acid, succinic and acetic acids and the signal at 3.3 ppm were found to be the most statistically significant variables.

1. Introduction

The use of high resolution NMR in food authenticity is a subject of great interest and in recent years a widespread diffusion of this technique has been observed. In particular, the usability of this technique as a fingerprint analysis tool coupled with multivariate data analysis was widely discussed (Bertelli, Papotti, Bortolotti, Marcazzan, & Plessi, 2012; Bertelli et al., 2010; Koda, Furihata, Wei, Miyakawa, & Tanokura, 2012; Krishnan, Kruger, & Ratcliffe, 2005; Lopez-Rituerto et al., 2012; Mannina et al., 2012).

Balsamic vinegar of Modena (BVM) and traditional balsamic vinegar of Modena (TBVM) are considered typical and well known Italian products, highly appreciated all over the world. Although BVM and TBVM have some common characteristics, they are different products. TBVM is a Protected Designation of Origin (PDO) product (Reg. CE n. 813/2000 April 17, 2000), owing to its typical production procedure and the well-defined area of origin. Nowadays, local vinegar houses, often founded by small family-run business, produce the TVBM, according to the ancient methods of production, whose origins are to be found in the Modenese traditions. Long fermentation and aging procedures, which require expertise and caution in respect of the maturation state of the product, contribute to develop the unique and unmistakable characteristics that we recognize today in this very valuable product. BVM has recently obtained the registration with Protected Geographical Indication (PGI) status, granted by the European Union (Reg. CE n. 583/2009 July 3rd, 2009). They are both obtained from the alcoholic and acetic fermentation of cooked and concentrated grape musts, and this is the main characteristic that distinguishes balsamic vinegars from other vinegars, which are generally produced from alcoholic solution. TBVM and BVM mainly differ in the aging process and the production procedures. TBVM is aged in characteristic wooden barrels and may be found on the market in two different products according to the aging process: old (>12 and <25 years) and extra old (>25 years). During the production, the inoculation of colonies of acetic bacteria is allowed, while the use of any extra additive is forbidden. BVM is a cheaper product, with maturation in wooden barrels from two months up to 3 years, and it

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is allowed to add vinegar obtained by wine acetification (10% v/v minimum) and caramel (2% v/v maximum) for color correction (Decreto Ministeriale, 3 Dicembre 1965). Normally, the quality control and authentication assurance of both these balsamic vinegars was performed by means of sensorial analysis and by very simple chemical–physical property determinations, like total acidity, density and dry matter. Here arises the interest to develop new and standardized analytical procedures, able to further enhance the quality and commercial value of these typical and unique products and to preserve them from possible sophistications and adulterations. These new approaches, coupled with chemometric analysis, may provide helpful classification models for authentication and commercial quality characterization.

Several analytical studies focused their attention on the balsamic vinegars characterization and, some of them aim to investigate the aging process and the related changes. (Antonelli, Chinnici, & Masino, 2004; Chiavaro, Caligiani, & Palla, 1998; Cocchi, Lambertini, Mancini, Marchetti, & Ulrici, 2002; Cocchi et al., 2006; Del Signore, Stancher, & Calabrese, 2000; Gullo, Caggia, De Vero & Giudici, 2006; Plessi, Bertelli, & Miglietta, 2006; Plessi, Monzani, & Coppini, 1989; Theobald, Muller, & Anklam, 1998). However, they are often time consuming approaches, not compatible with routine analyses. Among many advantages that the ^{1}H NMR spectroscopy offers, it may simultaneously determine the different metabolites of vinegar in few minutes, as required for food authenticity and quality control. To our knowledge, only few studies have been carried out on Traditional Balsamic Vinegar of Modena (TBVM) and Balsamic Vinegar of Modena (BVM), regarding their quality evaluation and valorization, using NMR (Cirlini, Caligiani, & Palla, 2009; Consonni, Cagliani, Benevelli, et al., 2008; Consonni, Cagliani, Rinaldini, & Incerti, 2008; Consonni & Cagliani, 2007). Here a characterization of both BVM and TBVM using high-resolution ^{1}H NMR spectroscopy coupled with multivariate statistical data analysis is presented. Besides, we aim to build a discriminant model able to characterize TBVM according to the aging process, which is actually the most required information for quality assessment, and, nowadays, no objective analytical techniques have been officially defined. The particular climatic characteristics, the soil and the grape varieties typically grown in Modena strongly contribute to make the TBVM a unique and unmistakable product. These characteristics and the particular production procedures used by the local producers, which follow the Modenese traditions of cooking musts and drawing and topping up procedures among the wooden casks, make it is difficult to obtain statistical models which are representative of the intrinsic variability and peculiarities of TBVM. In this work, we applied supervised pattern recognition procedures, never used before, to a very significant number of samples, that is non-obvoius, also considering the high price of the samples.

2. Materials and methods

2.1. Materials and sample preparation

A total of 76 samples of both TBVM and BVM have been analyzed. Among them, 23 were extra old (>25 years of aging) TBVM, 17 were old (>12 and <25 years of aging) TBVM and 36 were BVM of unknown aging (Table 1). All the TBVM and several BVM were provided by local vinegar houses, while the other BVM were purchased on the market. All TBVMs and BVMs are labeled as PDO and PGI products respectively. Samples were prepared by dissolving 0.1 g exactly weighed of vinegar in 500 μL of dimethyl sulphoxide-d_6 (DMSO-d_6) (Sigma–Aldrich, Milan, Italy), and transferred into the Wilmad NMR tube (5 mm, Ultra-Imperial grade, 7 in. L, 526-PP, Sigma–Aldrich, Milan, Italy). Twenty μL of tetramethylsilane (TMS) was added as reference compound. Standard compounds for metabolite assignments were from Sigma–Aldrich (Milan, Italy).

2.2. Physical and chemical determinations

Undiluted samples were used for °Brix measures, which were carried out with refractometer. Total acidity (g/100 mL of acetic acid) was determined by the titration with sodium hydroxide 0.5 M using the method reported in the Resolution OIV-OENO 52-2000. *R ratio*, which is the rate between °Brix and Total Acidity, and indicates the balance among sweet and sour tones, correlating in this way the density with the acidity (Gullo & De Vero, 2004, pp. 93–107; Satrioni, 2010), was also calculated for TBVM samples. This parameter is often used as a tool to correctly conduct vinegar houses and, in this work, to identify possible outliers among the samples, before performing NMR analysis. All the determinations were performed in triplicate.

2.3. NMR spectroscopy

To characterize samples ^{1}H NMR, ^{13}C NMR, two-dimensional ^{1}H–^{13}C heteronuclear multiple-bond correlation (HMBC) and

Table 1
Values of °Brix and *R ratio* in Traditional Balsamic Vinegar of Modena (TBVM) and °Brix in Balsamic Vinegar of Modena (BVM) samples ($n = 3$).

TBVM samples	Type[a]	°Brix	*R ratio*[b]	BVM samples	Brix
1	Old	65 ± 0.2	10.50 ± 0.2	41	32 ± 0.1
2	Old	67 ± 0.2	12.91 ± 0.4	42	22 ± 0.2
3	Old	61.5 ± 0.3	9.70 ± 0.1	43	15.2 ± 50.1
4	Old	69.5 ± 0.1	11.94 ± 0.2	44	26.5 ± 0.2
5	Old	68.2 ± 0.1	9.50 ± 0.5	45	24 ± 0.1
6	Extra old	71 ± 0.2	10.88 ± 0.4	46	19 ± 0.1
7	Extra old	71.75 ± 0.1	11.00 ± 0.3	47	27 ± 0.2
8	Extra old	71 ± 0.3	11.14 ± 0.5	48	23.5 ± 0.1
9	Extra old	73 ± 0.2	11.09 ± 0.6	49	15.3 ± 0.1
10	Extra old	70.5 ± 0.1	12.56 ± 0.4	50	20 ± 0.4
11	Old	68.5 ± 0.2	11.01 ± 0.2	51	19.5 ± 0.2
12	Extra old	73 ± 0.4	9.92 ± 0.1	52	19 ± 0.1
13	Extra old	74 ± 0.1	12.07 ± 0.1	53	29.5 ± 0.2
14	Old	70 ± 0.1	11.59 ± 0.7	54	38.5 ± 0.1
15	Extra old	72 ± 0.1	9.28 ± 0.2	55	32 ± 0.2
16	Extra old	71 ± 0.1	11.75 ± 0.2	56	36.5 ± 0.1
17	Old	60.5 ± 0.2	8.75 ± 0.6	57	44.5 ± 0.2
18	Old	65.5 ± 0.2	9.86 ± 0.2	58	40 ± 0.1
19	Old	68 ± 0.2	10.33 ± 0.2	59	28.5 ± 0.2
20	Extra old	72 ± 0.1	12.16 ± 0.1	60	39 ± 0.2
21	Extra old	72.5 ± 0.1	10.63 ± 0.1	61	38.5 ± 0.1
22	Extra old	70 ± 0.1	10.08 ± 0.1	62	53.5 ± 0.1
23	Extra old	73 ± 0.2	10.08 ± 0.	63	38.5 ± 0.1
24	Extra old	72.5 ± 0.1	10.54 ± 0.2	64	42.5 ± 0.1
25	Extra old	71.8 ± 0.2	11.21 ± 0.3	65	36 ± 0.1
26	Old	65 ± 0.1	9.88 ± 0.2	66	18 ± 0.1
27	Old	63 ± 0.1	9.36 ± 0.1	67	36 ± 0.2
28	Old	64 ± 0.1	10.24 ± 0.2	68	31 ± 0.1
29	Old	63.5 ± 0.2	9.92 ± 0.3	69	29 ± 0.1
30	Old	65 ± 0.1	10.25 ± 0.4	70	30 ± 0.2
31	Extra old	71.5 ± 0.2	10.87 ± 0.5	71	38.5 ± 0.2
32	Extra old	71 ± 0.1	9.89 ± 0.1	72	53 ± 0.3
33	Extra old	73.5 ± 0.1	11.38 ± 0.2	73	19 ± 0.1
34	Extra old	72 ± 0.2	11.41 ± 0.2	74	38 ± 0.2
35	Extra old	71 ± 0.3	10.64 ± 0.1	75	25 ± 0.1
36	Extra old	72.5 ± 0.2	11.49 ± 0.2	76	30 ± 0.1
37	Extra old	70.5 ± 0.3	10.72 ± 0.1		
38	Extra old	71 ± 0.1	8.75 ± 0.3		
39	Old	60.8 ± 0.2	12.90 ± 0.2		
40	Old	62 ± 0.1	9.92 ± 0.1		

[a] Age is indicated only for known aging process samples. Extra old >25 years; old >12 and <25 years. For BVM the aging is unknown, however it is < 3 years.
[b] °Brix/Total acidity (g/100 ml).