Representative Champagne Wine Extracts for Gas Chromatography Olfactometry Analysis

Christine Priser,*,† Patrick X. Etiévant,† Sophie Nicklaus,‡ and Olivier Brun‡

Laboratoire de Recherches sur les Arômes, INRA, 17 rue Sully, 21034 Dijon Cedex, France, and Mumm - Perrier-Jouët, Vignobles et Recherches, 11 avenue de Champagne, 51206 Epernay Cedex, France

Gas chromatography olfactometry (GCO) analysis has proved to be a powerful method to determine key compounds of food aroma, but up to now few papers involving this technique in wine aroma studies were published. An accurate representation of the olfactory quality of the extracts is necessary to ensure the reliability of the conclusions resulting from such analyses. Three commercial champagne wines were used for this study. The champagne wine extracts were obtained from three different extraction methods: adsorption on XAD resins, solvation in dichloromethane, and extraction by demixing ethanol. Thus the extracts were tested by a panel trained on champagne wines, using a profile descriptive method. The comparison of the odor profiles of the extracts outlined the extract obtained by demixing as the closest to the wine profile.

Keywords: *Aroma extraction; sensory analysis; gas chromatography olfactometry; champagne wine*

INTRODUCTION

Gas chromatography olfactometry (GCO) analysis was proved to be a powerful way to determine key compounds of food aroma. However, the results of a GCO analysis were considered to be more valid when the odor of the extracts was proved close to that of the food submitted to extraction (Etiévant et al., 1994). This similarity in odor is not obvious since, according to the method chosen, different classes of compounds are preferentially extracted. Therefore prior to GCO analysis a systematical sensory comparison of the food to the corresponding extract should be made. Abbott et al. (1993) and Moio et al. (1995) used this approach on beer and wine, respectively. Both authors used sensory analysis and GCO detection to confirm the olfactory representativeness of their extracts. Abbott et al. chose a method involving an adsorption on a mixture of XAD resins. Moio et al. demonstrated that the odor from an extraction with dichloromethane without the concentration step was more similar to the odor of the original wine.

Because of the undesirable odor of dichloromethane and its anesthetic effects on olfactory receptors, the similarity between the extracts and the wine was established by GCO detection. The aim of this study was to compare the olfactory quality of different types of extracts obtained from three champagne wines and to choose the best quality extract for GCO analysis. Three types of extracts were selected: the solvent and the resin extracts already tested on beer and wine and a demixing extract used on wine and described by Singleton (1961).

MATERIALS AND METHODS

Champagne Wines. Three commercial champagne wines, one vintage from 1985 (A), one from 1989 (B), and one nonvintage (C), were stored in a cellar for a maximum of 6 months before use.

Analytical Reagents. The different XAD resins were purchased from Fluka (Buchs, Switzerland). A mixture containing equal weights of three wet resins (XAD_2, XAD_7, and XAD_{16}) was washed in a Soxhlet apparatus with methanol for 12 h. The resins were then rinsed with water (10 × 50 mL) before use. All reagents used were HPLC grade, and water was purified with a milli-Q system (Millipore, SA, Saint-Quentin, France). Smelling strips were purchased from Granger-Veyron (Privas, France).

Methods. *Solvent Extraction.* The volatile constituents of the three champagne wines were isolated as described by Moio et al. (1995). Wine (200 mL), CH_2Cl_2 (20 mL), and sodium chloride (50 g) were poured in a flask (600 mL) cooled with melting crushed ice and magnetically stirred at 200 rpm for 2 h. The wine/CH_2Cl_2 emulsion formed during stirring was separated from the aqueous layer and frozen at −20 °C. The flask was then allowed to reach room temperature, and the CH_2Cl_2 solution, progressively separated from the remaining wine, was transferred without concentration into a small vial and stored at −20 °C.

Resin Extraction. The volatile compounds from the three champagne wines were extracted using the following procedure. Ten grams of the cleaned mixture of resins was placed in a flask (650 mL) with wine (200 mL), sodium chloride (50 g), and diluted hydrochloric acid (3.2%, w/v, 4 mL) and magnetically stirred at 200 rpm for 2 h. The mixture was then poured into a glass column (i.d. = 11 mm) stoppered with glass wool. Complete transfer of the resins was achieved by rinsing the flask with water (3 × 10 mL). Residual water was removed from the column with nitrogen, and the volatile compounds were eluted stepwise with absolute ethanol (10 × 2 mL, 5 min between each addition) in a cooled flask. The final aliquot of ethanol was eluted under a flow of nitrogen. The ethanolic solution was stored at −20 °C until analysis. A blank sample was prepared by substituting water for wine.

Wine Demixing. Wine (200 mL), ammonium sulfate (100 g), and absolute ethanol (11 mL) were magnetically stirred in a flask (600 mL) for 30 min at 25 °C. The solution was then decanted in a separating funnel and the ethanolic phase transferred into a small vial and stored at −20 °C. The percentage of ethanol was measured with a Salleron−Dujardin ebulliometer, and the dry matter was estimated from the dry residue of 1 g of the extract after 15 h at 115 °C.

Sensory Analysis. *Panels.* Two panels were employed for this study. The first panel used a quantitative descriptive method to describe the three champagne wines and their three corresponding extracts (solvent, resins, and demixing extracts). This panel was composed of 17 subjects (4 women, 13 men)

* Author to whom correspondence should be addressed (fax, 33 3 80 63 32 27; e-mail, etievant@aligote.dijon.inra.fr).

† INRA.

‡ Mumm - Perrier-Jouët.

S0021-8561(97)00123-4 CCC: $14.00

from the expert panel of Mumm - Perrier-Jouët Co. They have been trained for 1.5 years to perform profile descriptive analysis on champagne wines. The second panel was employed to compare the odor of the demixing extract before and after distillation. This panel was composed of 20 flavor chemists (15 women, 5 men) from our laboratory of INRA, and the odor differences among the extracts were assessed by a two out of five test (AFNOR, 1983).

Sample Presentation. Champagne wine samples (10 °C, 70 mL) were presented to the Mumm - Perrier-Jouët Co.'s panel in coded glasses. Wine samples were assessed for odor and flavor, in isolated booths. Wine extracts were presented to the two panels on smelling strips. The strips were dipped in the extracts and then introduced after 6 min (time necessary for solvent evaporation) in a flask hermetically closed with a cap. For the sensory evaluation, panelists had to open the coded flasks. Extracts were assessed for odor only.

Profile Descriptive Analysis of Champagne Wine and Extracts. A list of 16 consensus descriptors previously established by the Mumm - Perrier-Jouët Co.'s panel was used to describe the odors of the three wines and their corresponding extracts. Champagne wine and extracts were assessed successively during separate sessions and within each session; the order of sample evaluation was randomized over all the subjects. Each sample was presented with two replicates. Panelists were asked to rate the intensity of each descriptor on an unstructured scale of 100 mm anchored at the left end with low intense and at the right end with high intense. All the data were analyzed by FIZZ software (Biosystem, Dijon, France) and SAS software (SAS Institute Inc., 1985 SAS/STAT).

Two Out of Five Test Realized with Demixing Extracts before and after Distillation. Because of the impossibility to inject directly the demixing extracts in a gas chromatograph, a distillation of these extracts was done to eliminate the nonvolatile residue. The assessment of the potential differences between the extracts before and after distillation was realized by the INRA panelists using a two out of five test on one champagne extract. The champagne wine C demixing extract (10 mL) was submitted to two vacuum distillations as described by Guichard and Issanchou (1983). The first distillation lasted 1.5 h at 5 Pa, and the residue was further extracted during a second distillation on a cold finger cooled with liquid nitrogen for 3.5 h at 5×10^{-2} Pa. After the previous distillation, the compounds on the cold finger were rinsed with the distillate obtained from the first distillation and stored at −20 °C before use. For the two out of five test, five coded flasks were presented to the INRA panelists. Three flasks contained the demixing extract, and the two others contained the distilled demixing extract. The order of sample presentation was randomized over all the subjects. Panelists were asked to find the group of the two different samples from the three others. All the data were analyzed by BINRISKS SAS macro (Schlich, 1993).

Gas Chromatography Olfactometry. The analysis was carried out using a Hewlett-Packard 5890 chromatograph equipped with an on-column injector (J&W Scientific Inc.), a flame ionization detector, a sniffing port, and a DB-Wax fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.5 μm) (J&W Scientific Inc.). Humid air was added in the sniffing port at 100 mL min^{-1}, resulting in a 1/50 dilution ratio of the effluent. The hydrogen carrier gas velocity was 50 cm s^{-1}, and the temperature of the injector and detector was 250 °C. The oven temperature was programmed from 40 to 240 °C at 5 °C min^{-1}.

RESULTS AND DISCUSSION

Profile Descriptive Analysis. Before testing the significance of the differences in the odor of extracts from different products, it is necessary to know if a difference actually exists between the products. To evaluate the differences between the three wines, a two-way (champagne wine, panelist) analysis of variance with interaction was done on each of the 16 descriptors.

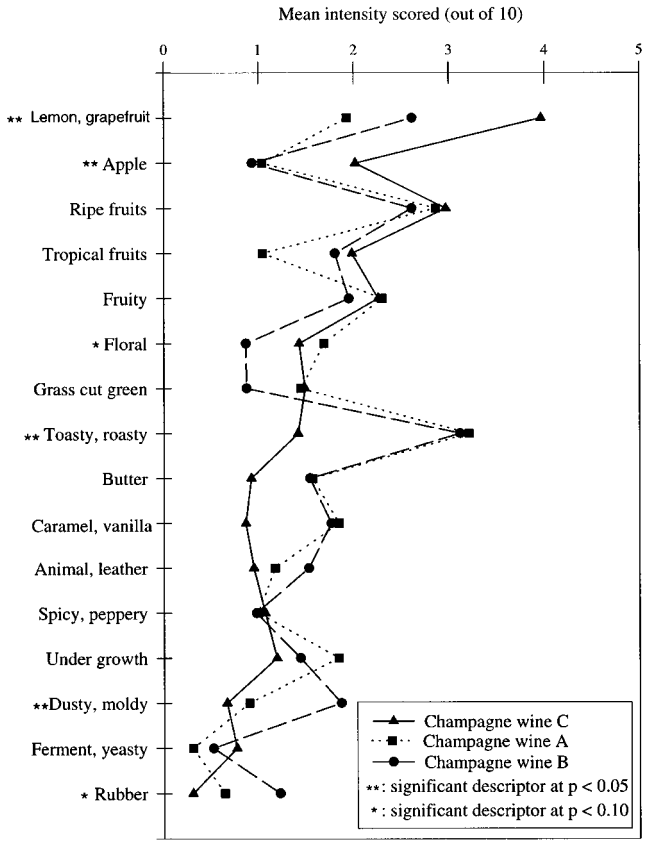

Figure 1. Sensory profile of champagne wines A, B, C.

The results (Figure 1) showed that four descriptors, lemon, apple, toasty, and dusty, were the descriptors on which the panelists could differentiate the wines at a significance level of 5% and two descriptors, floral and rubber, at a significance level of 10%. Therefore we can conclude that the profiles of the three wines were significantly different. Following the previous statement, the similarity of the extracts with the corresponding wines was performed with three two-way analyses of variance with interaction [product (Pr), panelist (Pa), product*panelist (Pr*Pa)]. The results, reported in Table 1, indicate that for each wine three to five descriptors had a significant product effect (Table 1, column Pr). Most of these descriptors were already found significant by the panel when they evaluated the three champagne wines (Figure 1). The profile of one of the three wines (wine B) with the profiles of its corresponding extracts is illustrated in Figure 2.

As often in sensory analysis, the panelist effect was significant for all the discriminant descriptors, indicating a different use of the scoring scale. About the interaction, we can observe that the panelists agreed when they scored 9 of the 13 descriptors. Concerning the four remaining descriptors, for which the panelists disagreed, we tested again the significance of the product effect with a new Fisher value calculated by using the interaction Pr*Pa instead of the residue (Lundhal and McDaniel, 1993). The results of this test (Table 1, column Pr/Pr*Pa) confirmed the product effect for the rubber descriptor in champagne wine B. The product effect was no more significant for the three others descriptors. A Dunnett's test (O'Mahony, 1986) was realized in order to compare each extract to the corresponding wine. This test allows to declare for the 10 discriminant descriptors whether an extract is similar or not to the wine. The results are also given

Table 1. Evaluation of the Difference between Champagne Wines and Extracts[a]

champagne wines	significant descriptors	results of the three two-way analyses of variance				Dunnett's test: type of extract scored similarly to the wine
		Pr	Pr/Pr*Pa	Pa	Pr*Pa	
A	toasty, roasty	*		***	NS	demixing and solvent
	ripe fruits	*	NS	***	*	
	floral	*		**	NS	all the three extracts
	apple	**	NS	***	**	
	dusty, moldy	**		**	NS	demixing and resins
B	toasty, roasty	***		***	NS	demixing and solvent
	dusty, moldy	**		**	NS	none
	tropical fruits	*		***	NS	demixing and resins
	rubber	***	*	*	**	none
	floral	*		***	NS	demixing and solvent
C	lemon, grapefruit	**		***	NS	demixing
	fruity	**	NS	***	***	
	apple	*		***	NS	demixing and resins

[a] NS, not significant; *significance, $p < 0.05$; **significance, $p < 0.01$; ***significance, $p < 0.001$.

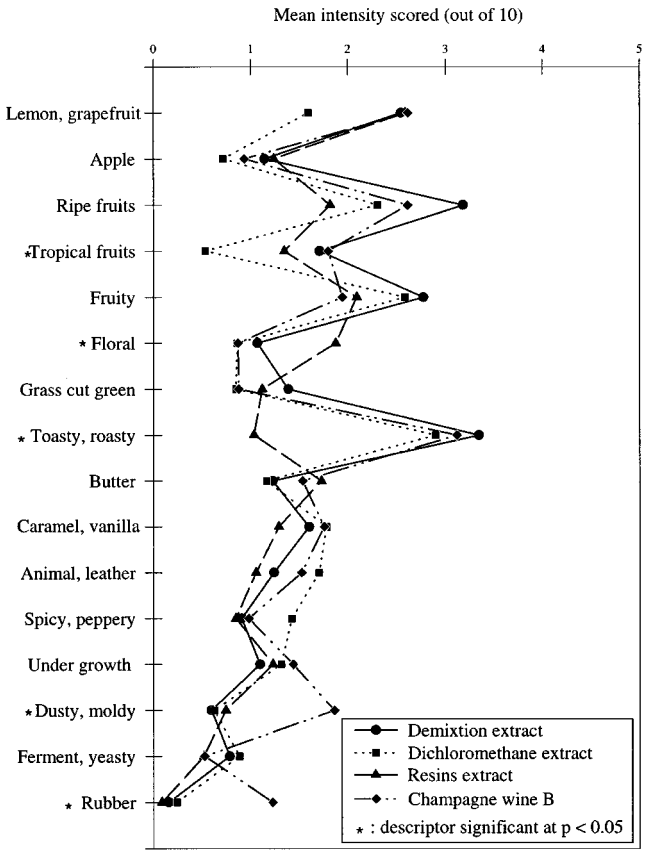

Figure 2. Sensory profile of champagne wine B and its corresponding extracts.

in Table 1. For champagne A, the case of the floral descriptor is particular. The analysis of variance showed that there was a significant difference between the four products, but the Dunnett's test showed that the difference was not between the extract and the wine but between the extracts themselves. According to these data, the Dunnett's test gave 8 times out of 10 the demixing extract as mingled with the wine, against 4 times for the resin extract and 4 times for the solvent extract. Therefore the profiles of the extracts obtained by demixing were judged similar to the profile of champagne wines A and C for all the descriptors. The demixing extract of champagne wine B was judged similar to this wine for 14 descriptors out of 16. In fact, the dusty and the rubber notes were not well recovered in any of our extracts. These results also show that the scores obtained by sniffing the extracts on strips after evaporation of the solvent were not very different from the scores obtained on the wine itself, thus validating the procedure chosen to prepare and assess the samples. We concluded from this experiment that demixing, with the target to perform GCO analysis, was the best method among those tested to extract flavor active compounds in the three champagne wines studied.

Vacuum Distillation of the Extracts. However, attempt to use it for this purpose showed that this extract contains high amounts of water (44%) and 2% of nonvolatile residue which is hardly compatible with an on-column injection because of possible formation of artifacts. In order to evaluate the effects of the presence of nonvolatile residue, the three types of extracts of the wine A were injected and the odors of compounds eluted were detected by GCO. As expected, most descriptors were found common to the three extracts. However, the presence of many burnt descriptors in the profile of the demixing extract seemed suspicious. After the injection of 2 μL of demixing extract, dark spots, probably due to nonvolatile products, were moreover observed on the first 60 cm of the precolumn. In order to solve the problem due to the presence of nonvolatile products, a vacuum distillation was applied to the demixing extract of champagne wine C. A sensory analysis was then realized to test the effect of the distillation on the sensory characteristics of the extract. A two out of five test was applied to an extract obtained from demixing against the same extract submitted to distillation. The data analysis allowed to consider the type 1 risk (conclusion of a difference for similar samples) and type 2 risk (conclusion of a similarity for different samples). Since we could not take the risk to use a distillate whose aroma was different from that of the demixing extract, we have chosen to set the risk 2 at a low level, 5%, and to set the risk 1 at 10%. For power computation, it is necessary to declare the magnitude of a difference which should make the test significant. In difference testing, it remains to define which percentage of true distinguishers is important enough for declaring the product as being different. In our case we have decided to set up this latter parameter as 37.5%. Since only five positive responses among 20 were obtained and with the setting of the parameters above, the BINRISKS macro indicated that the two products can not be considered as different. Therefore, we can conclude that at this probability level the distillation does not change the flavor characteristics of the extract.

As a conclusion, extracts obtained by vacuum distillation of champagne wine demixing extracts seem more suitable for olfactometry analysis because the sensory

profiles of the demixing extracts were closest to the wine compared to a dichloromethane extract or a resins extract; moreover they were compatible with a GC analysis using an on-column injection. GCO analyses are currently performed on extracts obtained with the distillation demixing method in order to point out the flavor active constituents of champagne wine and those responsible for flavor differences between the three champagne wines.

ABBREVIATIONS USED

Pr, product; Pa, panelist; Pr*Pa, product*panelist; NS, not significant; *p*, significance level.

ACKNOWLEDGMENT

We thank Sylvie Issanchou, Isabelle Lesschaeve, and Pascal Schlich for very valuable and helpful discussions about the statistical interpretation of data.

LITERATURE CITED

Abbott, N.; Etiévant, P.; Langlois, D.; Lesschaeve, I.; Issanchou, S. Evaluation of the representativeness of the odor of beer extracts prior to analysis by GC eluate sniffing *J. Agric. Food Chem*. **1993**, *41*, 777−780.

AFNOR. Analyse sensorielle. Méthodologie. Directives générales. Norme V 09-001, 1983.

Etiévant, P.; Moio, L.; Guichard, E.; Langlois, D.; Lesschaeve, I.; Schlich, P.; Chambellant, E. Aroma Extract Dilution Analysis (AEDA) and the Representativeness of the Odour of Food Extracts. In *Proceedings of the 7th Weurman Flavour Research Symposium, Noordwijkerhout, Trends in Flavour Research*; Maarse, H., Van Der Heij, D. G., Eds.; Elsevier: Amsterdam, 1994.

Guichard, E.; Issanchou, S. Extraction of volatile compounds from raspberry by different methods. Application of principal component analysis to gas chromatographic data. *Sci. Aliments* **1983**, 3, 427−438.

Lundhal, D. S.; McDaniel, M. R. The panelist effect - fixed or random? *J. Sens. Stud.*, **1988**, *3*,113−121.

Moio, L.; Chambellant, E.; Lesschaeve, I.; Issanchou, S.; Schlich, P.; Etiévant, P. Production of representative wine extracts for chemical and olfactory analysis. *Ital. J. Food Sci*. **1995**, *3*, 265−278.

O'Mahony, M. *Sensory evaluation of food-Statistical Methods and Procedures*; Marcel Dekker Inc.: New York, Basel, 1986.

Schlich, P. Risk tables for discrimination tests. *Food Qual. Pref.* **1993**, *4*, 141−151.

Singleton, V. L. An extraction technique for recovery of flavors, pigments, and other constituents from wines and other solutions. *Am. J. Enol. Vitic.* **1961**, *12*, 1−8.

Received for review January 12, 1997. Accepted June 6, 1997.[⊗] This work was supported by the Mumm - Perrier-Jouët Co. and by the Conseil Régional de la Bourgogne.

JF970123B

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.