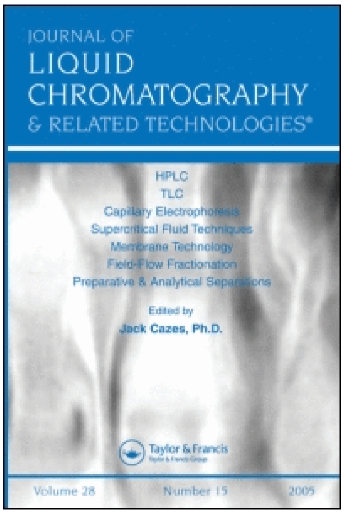

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Simultaneous Determination of Vanillin and Syringaldehyde Using High Performance Liquid Chromatography. Application to Static and Soleras Aged Brandies

R. Gimenez Martinez[a]; M. Ytllalon Mir[a]; H. Lopez Ga De La Serrana[a]; M. C. Lopez Martinez[a]; M. Olalla Herrera[a]
[a] Department of Nutrition and Bromatology Faculty of Pharmacy, University of Granada, Granada, Spain

To cite this Article Martinez, R. Gimenez , Mir, M. Ytllalon , De La Serrana, H. Lopez Ga , Martinez, M. C. Lopez and Herrera, M. Olalla(1993) 'Simultaneous Determination of Vanillin and Syringaldehyde Using High Performance Liquid Chromatography. Application to Static and Soleras Aged Brandies', Journal of Liquid Chromatography & Related Technologies, 16: 18, 4079 — 4094

To link to this Article: DOI: 10.1080/10826079308019688
URL: http://dx.doi.org/10.1080/10826079308019688

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(18), 4079-4094 (1993)

SIMULTANEOUS DETERMINATION OF VANILLIN AND SYRINGALDEHYDE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. APPLICATION TO STATIC AND SOLERAS AGED BRANDIES

R. GIMENEZ MARTINEZ, M. VILLALON MIR, H. LOPEZ G^{a} DE LA SERRANA, M. C. LOPEZ MARTINEZ, AND M. OLALLA HERRERA

Department of Nutrition and Bromatology
Faculty of Pharmacy
University of Granada
18012 Granada, Spain

ABSTRACT

A technique for the simultaneous determination of vanillin and syringaldehyde using HPLC was developed and applied to the analysis of commercial brandies, aged either along the traditional static system or along the soleras system, in an attempt to establish the influence the aging system might have on the quality of the brandies. The separation and quantification of these aldehydes were carried out by direct injection of the samples into the chromatograph. The precision obtained under this method was expressed as a percentage of recovery, vanillin presenting 99.803% and syringaldehyde 99.009%

INTRODUCTION

Vanillin and syringaldehyde are two aromatic aldehydes present in the chemical composition of spirits aged in oak barrels (1).

By definition, brandy is "the compound obtained from wine distillates, spirits or wine holands, and must be stored under suitable atmospheric conditions, in oak containers sufficient time to acquire the organoleptic features peculiar to each production system" (2).

The procedure whereby the brandy is stored in oak barrels is commonly known as "MATURING" or "AGING". It allows the organoleptic qualities of the brandy to develop and improve (3).

Traditionally, two aging systems have been followed:

a) THE TRADITIONAL STATIC SYSTEM

b) THE DYNAMIC SOLERA SYSTEM

In the former, also known as the TRADITIONAL STATIC SYSTEM, the brandy remains static in the aging container, and is neither mixed nor combined with other brandies throughout the aging process. The characteristics peculiar to brandy are produced depending on the time spent in storage (4). This aging process is followed in countries with a high tradition of vitiviniculture such as France, Italy and the north of Spain.

In the SOLERAS SYSTEM, also known as the dynamic system, the aged brandy is partially extracted or withdrawn from each one of the large barrels which form the fixed scale, and the replacement or "rocío", is done with brandy from another stage of aging (5). This is the system traditionally followed in the south of Spain (Jerez de la Frontera, Puerto de Santamaria and Sanlucar de Barrameda) and is sometimes preceded by the static system.

In the course of maturing the brandy, whether by one aging process or by the other, some of the constituents of the oak wood are extracted, as is the case of lignin (6).

It is generally accepted that following the extraction, a series of degradations are experienced which lead to the formation of diverse compounds including the aromatic aldehydes vanillin and syringaldehyde which stand out and contribute to the aroma of the aged alcohols. The mechanisms that condition these processes are not well-known, but may be of a biochemical type (biodegradation of the lignin), a chemical type (hydroalcoholisis and acidosis) and a physical type (hydrothermolisis when the staves are bound to give the barrel it's shape or direct pyrolysis during the burning process). In any case, these compounds are produced when the ethanol reacts with the lignin of the barrel, favoured by an acid pH and by the appropriate burning of the inner face of the staves (7). If the burning process is not sufficient, no changes take place in the structure of the lignin, as no extraction occurs; on the contrary, if the pyrolysis is carried out at too high temperatures, a very strong thermodegradation occurs. However, the structure is modified, producing less reactive compounds, which is associated with a decrease in the concentration of extractable compounds (8).

On numerous occasions authors such as Bricourt, J., Alibert, G. et al. (10) studied the separation of aromatic aldehydes in spirits using fine layer and column chromatographic techniques however, these techniques are not sufficiently accurate for an adequate quantitive analysis.

Other authors (11) carried out the quantification of vanillin and syringaldehyde using spectrophotometric techniques with Ultraviolet absorption, prior treatment of the sample with hydrogen peroxide. However, this method is a long and complicated process.

More recently, high performance liquid chromatography on silica type C_{18} column with a detector for the absorption of Ultraviolet, has allowed both the separation and accurate quantification of these aldehydes in brandies aged in oak barrels. The only inconvenience is that prior extraction and separation of the aromatic aldehydes using various solvents is necessary and somewhat complicates the application.

The aim of the present study was to develop an analytical technique for the direct and simultaneous determination of vanillin and syringaldehyde using high performance liquid chromatography, where no sample preparation was needed before injecting it into the chromatograph.

The second part of the present study involved the application of this HPLC technique to the determination of both aldehydes in commercial brandies aged along different systems. The aim was to establish the influence the aging process might have on the concentrations of vanillin and syringaldehyde.

MATERIAL AND METHODS

1.- Solutions standard

A standard solution containing 5 g/l of vanillin and 5 g/l of syringaldehyde in ethanol 40º G.L. was used, from which hydroalcoholic solutions were prepared 40% v/v of increasing concentrations ranging between 0.5 mg/l and 80 mg/l for vanillin and 0.25 mg/l and 80 mg/l for syringaldehyde. The solutions were degassed in an ultrasonic bath, then directly injected into the chromatograph, prior filtration through Millipore filters (0.45 mm pore diametre) under the following conditions.

2.- Experimental conditions

- Liquid chromatograph: HPLC instrumentation consisted of a Konik model KNK-500A chromatograph with

a Konik injector model 7125. The chromatograph was equipped with a pump thus allowing the use of gradient elution. The separation was based on a distribution mechanism in inverse phases, with detection by absorption in the U.V.

-Mobile phase: The separation required the use of a gradient elution comprising two solvents: A (bidistilled water containing 0.15% of trifluroacetic acid) and B (bidistilled water containing 70% of methanol and 0.15% of trifluoroacetic acid) with a flow rate of 1 ml/min. All the solvents used were analytically pure.

A correct separation was obtained with the following elution gradient:

t (min)	A (%)	B (%)
0	100	0
5	95	5
10	75	25
18	70	30
33	60	40
53	0	100

- *Detector: Absorption spectrophotometer in the ultraviolet at 280 nm.*
- *Temperature: Room temperature*
- *Volume injected: 10 µl*
- *Column: The column used was a Lichrospher Merk CH-18, 10 cms long and with an internal diameter of 4.7 mm.*

A.- Proposed Chromatographic Method

1.- Reproducibility of chromatographic method

Reproducibility studies confirmed the simultaneous determination of vanillin and syringaldehyde. Ten

TABLE 1: Study of Reproducibility in the Determination of Vanillin and Syringaldehyde by HPLC.

	Vanillin (15 mg/l) Area	Syringald (15 mg/l) Area
	316760	140940
	312080	140450
	313930	140730
	313590	140210
	315280	142500
	315820	140480
	313350	140090
	318540	140750
	310085	143910
	314350	142890
Mean Area	314378	141295
R.E.	0,54	0,66

injections of a hydroalcoholic mixture at 40% v/v containing 15 mg/l of each aldehyde were carried out. This mixture was injected directly into the chromatograph under identical experimental conditions. The results obtained appear in Table 1.

Figure 1 corresponds to the chromatogram of this synthetic solution in aromatic aldehydes. The precision of this method for each aldehyde is expressed by the relative error (R.E.) calculated from Student's factor $t_{0.05}$.

$$R.E. = \frac{Sd \times t}{\text{mean peak area}} \times 100$$

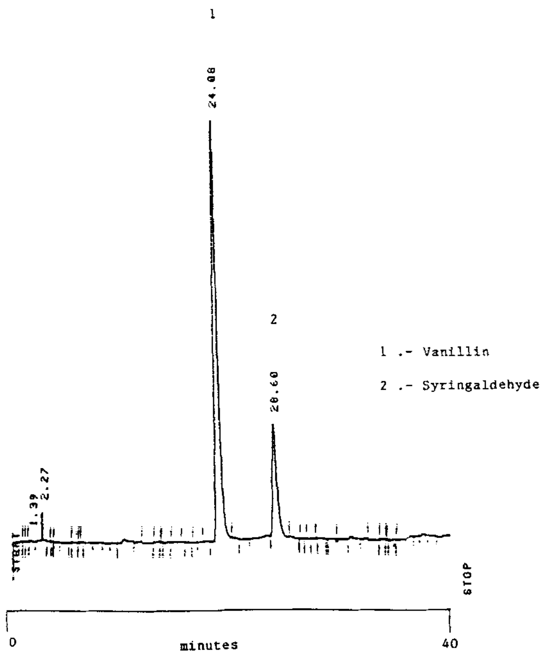

FIGURE 1: Chromatogram of a Mixture of Vanillin (15 mg/l) and Syringaldehyde (15 mg/l).

2.- Calibration curves

To determine the intervals in which the analytical method presented linear proportionality between the phenolic aldehydes' concentrations and the peak area found, the solutions standard previously outlined was used with increasing concentrations of both aldehydes and directly injected into the chromatograph. Figure 2 reproduces the calibration curves obtained for vanillin and syringaldehyde.

It was possible to obtain a response factor (R.F.) for each aldehyde from the intervals where linearity

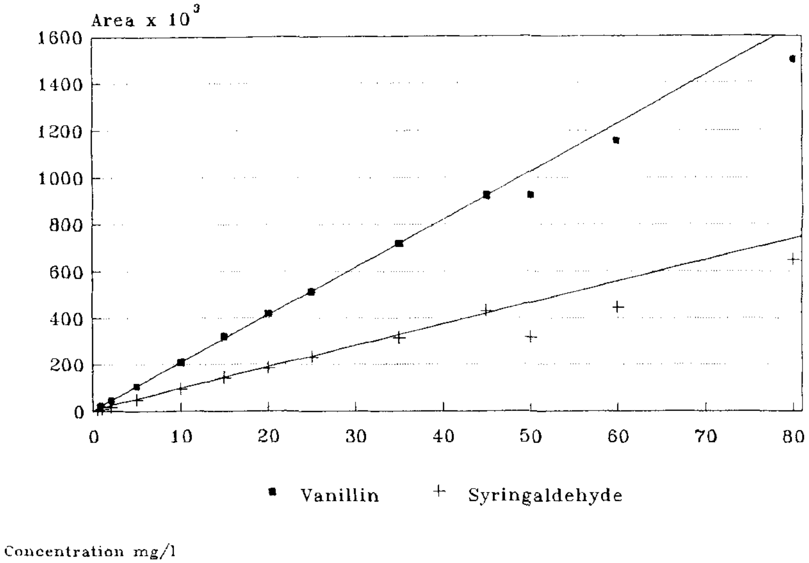

FIGURE 2: Calibration curves of Vanillin and Syringaldehyde.

existed:

$$\text{Absolute R.F.} = \frac{\text{Concentration}}{\text{Peak area}}$$

Neither changes in quantities of the components present nor the absence of one of the components was seen to affect this response factor.

The calibration curves show that vanillin presents linearity between 0.8 mg/l and 45 mg/l and syringaldehyde presents linearity between 0.5 mg/l and 45 mg/l (Table 2).

Linear proportionality between the peak area of each aldehyde and its corresponding concentration did not exist outside these intervals.

TABLE 2: Determination of the Average Response Factor from the Solutions Standard of Increasing Concentrations.

Vanillin (mg/l)	Area x 10^4	Reponse Factors	Syringaldehyde (mg/l)	Area x 10^4	Reponse Factors
0.8	1.6736	4.78 X 10^{-5}	0.5	0.4672	1.07 X 10^{-4}
1	2.0790	4.81 X 10^{-5}	1	0.9505	1.05 X 10^{-4}
2	4.1395	4.80 X 10^{-5}	2	1.8385	1.08 X 10^{-4}
5	10.5125	4.76 X 10^{-5}	5	4.7429	1.05 X 10^{-4}
10	20.8768	4.79 X 10^{-5}	10	9.2532	1.08 X 10^{-4}
15	31.8150	4.71 X 10^{-5}	15	14.0495	1.07 X 10^{-4}
20	42.0088	4.77 X 10^{-5}	20	18.5185	1.08 X 10^{-4}
25	51.3315	4.87 X 10^{-5}	25	23.2145	1.08 X 10^{-4}
35	71.8820	4.87 X 10^{-5}	35	31.4470	1.10 X 10^{-4}
45	93.3609	4.82 X 10^{-5}	45	43.0290	1.05 X 10^{-4}
Mean Reponse Factor 4.79 X 10^{-5}			Mean Reponse Factor 1.07 X 10^{-4}		

Table 2 shows the response factors obtained for each hydroalcoholic solution in the concentration intervals where linearity existed together with the mean response factor for each aldehyde. The mean response factor for vanillin was 4.79 x 10^{-5} and syringaldehyde presented a mean response factor of 1.07 x 10^{-4}.

*3.- **Accuracy confirmation***

To verify the accuracy of the analytical technique by HPLC, the statistical calculation proposed by Martin, A. et al. (12) was applied to a hydroalcoholic solutions standard at 40% v/v which contained vanillin and syringaldehyde, 10 g/l concentrations of each aldehyde. The accuracy of this method was studied from 10 injections of this solution under identical conditions.

TABLE 3: Confirmation of Accuracy in the Analytical Technique by HPLC in the Simultaneous Determination of Vanillin and Syringaldehyde.

	Vanillin	Syringaldehyde
Real Amount (mg/l)	10	10
Amount Found (mg/l)	9.9803	9.9009
Recovery %	99.803	99.009
Standard deviation (Sd)	0.0196	0.0275
Coefficient variance (C.V.)	0.1971	0.2776
Relative error (E.R.)	0.4459 %	0.6279 %
Standard error (S_m)	$6.22 \cdot 10^{-3}$	$8.69 \cdot 10^{-3}$
$X_m \pm S_m \cdot t$	99.883 ± 0.0141	99.009 ± 0.0196

The accuracy was expressed as a percentage of recovery and the results found appear in Table 3. The concentration found for each aldehyde represents the mean of the factors obtained in the 10 determinations. These concentrations were calculated from the peak area of each aldehyde in the chromatogram and the response factor of each previously calculated from the equation:

Concentration (i) mg/l=peak area (i) x absolute RF (i)

The results found confirm the accuracy presented by this HPLC method of phenolic aldehyde determination with *recovery percentages that range from 99.803% for vanillin to 99.009% for syringaldehyde. Standard deviation (Sd), relative error (E.R.) and coefficient variance on the mean value (C.V.) are other representative parameters used to determine accuracy (Table 3).*

B.- <u>Application of HPLC Technique to the Determination of Vanillin and Syringaldehyde in Commercial Brandies</u>

Samples of commercial brandies produced both in Spain and in other EEC countries were analyzed. Their origin and authenticity were guaranteed by the quality of the establishments where they were bought. The aging processes varied, but the brandies chosen for this study had been aged along either the traditional system or the dinamic system, as these are two of the most widely used aging systems.

The concentrations of vanillin and syringaldehyde in the brandies aged along the traditional static system appear in Figure 3. Figure 4 represents the content of both aldehydes in the soleras brandies.

<u>DISCUSSION AND RESULTS</u>

The technique proposed enabled a comparative study of the traditionally aged and the soleras aged brandies, once the concentrations of vanillin and syringaldehyde were known.

In the samples analyzed, the content of vanillin varied from 0 to 7.024 mg/l, for the brandies aged along the soleras system, the mean concentration was 1.963 mg/l; and the concentration of vanillin in the traditionally aged brandies ranged from 0.804 mg/l to 8.173 mg/l with a mean of 2.94 mg/l.

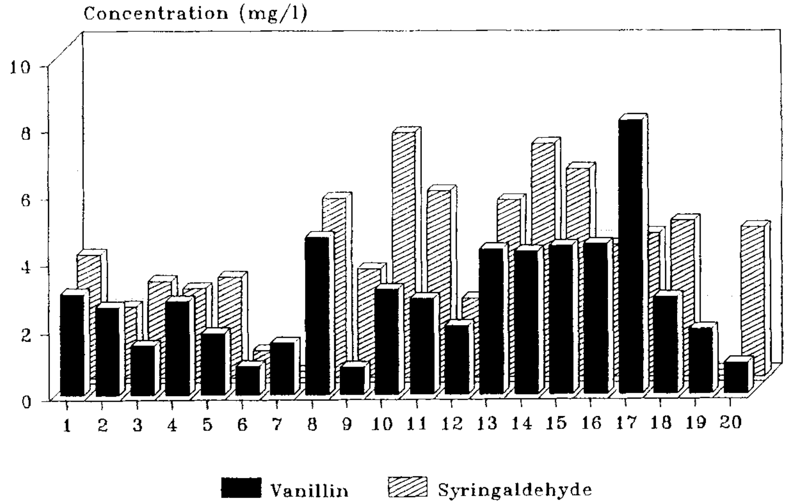

FIGURE 3: Vanillin and Syringaldehyde in Commercial Samples of Traditionally aged Brandies.

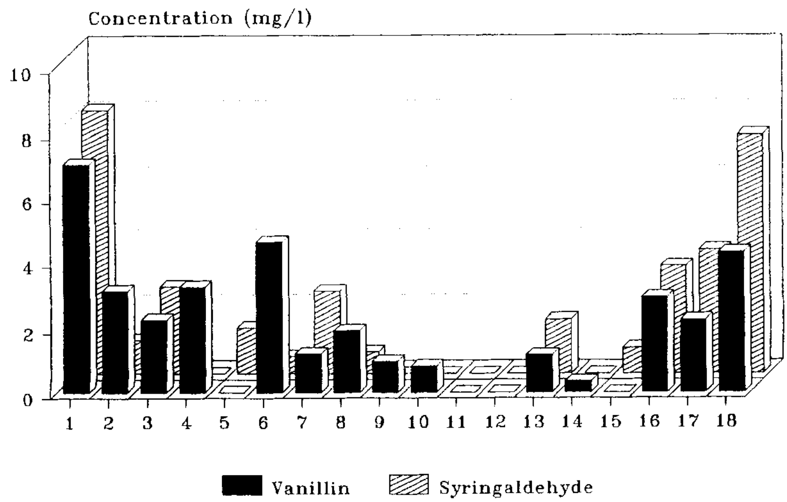

FIGURE 4: Vanillin and Syringaldehyde in Commercial Samples of Soleras aged brandies.

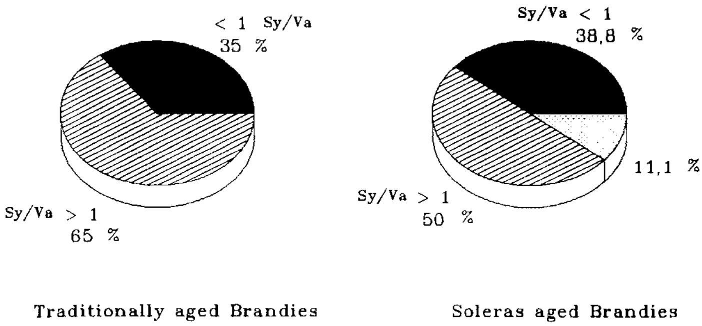

FIGURE 5: Syringaldehyde/Vanillin. Ratio in Commercial Brandies.

The values of syringaldehyde detected varied from 0 to 7.358 mg/l for brandies aged along the soleras system, with a mean value of 1.868 mg/l, and 0 to 7.296 mg/l for the ones aged along the traditional system, the mean value being 3.724 mg/l.

The concentrations of vanillin and syringaldehyde were appreciated to be higher in the brandies aged traditionally than in the ones aged along the soleras system.

On the other hand, the ratio Syringaldehyde/Vanillin fluctuated between 0 and 2.08 for the soleras brandies and the ratio Sy/Va was above unity in 50% of the cases and less than one in 38.8%. It was impossible to establish the ratio in the rest of the brandies aged along this system as these aldehydes were not detected (samples nº 11 and 12).

The Sy/Va ratio for the traditionally aged brandies ranged from 0 to 4.85; it was below unity in only 35% of the drinks made along this system and above unity in 65%.

Studying this ratio could serve as a guide in detecting the possible fraudulent addition of vanillin to these drinks with the aim of strengthening their aroma and favouring the "aged" character, as was suggested by Villalón, M. et al. (14) when determining the aromatic aldehydes in different brandies stored in oak barrels. The aforementioned author believes that when the value of this ratio is less than unity, one can assume that the brandy has experienced an addition of vanillin with the aim of reducing the time spent in the barrels and thus lower the product's price. In the present case, the cheapest brandies presented ratio values which were less than 1, possibly conditioned by the shorter time spent in barrels, because as the aging time increases the brandy becomes more expensive commercially.

Apart from economic considerations, it also proves interesting to point out that the extraction of syringaldehyde from the brandy is seen to be favoured, when compared with vanillin, by the aging time and by the more or less intense burning of the staves during the binding process (15). This fact may condition, to a great extent, the higher percentage of traditionally aged brandies presenting ratio values above unity as these brandies should spend a minimum of 3 years in oak barrels (16) as suppose to the soleras brandies whose aging process usually lasts six months.

CONCLUSIONS

1.- High performance liquid chromatography has proved to be a rapid and accurate technique in the simultaneous determination of the aromatic aldehydes Vanillin and Syringaldehyde in samples of commercial brandy.

2.- These aldehydes, which are present in brandies, can be analyzed efficiently by direct injection into the liquid chromatograph.

3.- The concentrations of vanillin and syringaldehyde are generally higher in the traditionally aged brandies than in those aged along the soleras system. This may be due to the fact that the latter spend less time in barrels.

4.-The ratio between the concentrations of vanillin and syringaldehyde may reveal the possible fraudulent addition of vanillin.

- *If the ratio Sy/Va is less than 1, it may indicate the addition of vanillin.*
- *If the ratio Sy/Va is greater than 1, it may indicate that the vanillin present in the brandy is the result of the aging process in oak wood.*

REFERENCES

1.- Puech, J.L.; Leanté, R.; Clot, G.; Nomdedeu, L; Mondies, H. Evolution de divers constituants volatils et phénoliques des eaux-de-vie de Cognac au cours de leur vieillissement. Sciences des Aliments, 4, 65-80, 1984.

2.- B.O.E. Reglamentación Especial Para la Elaboración, Circulación y Comercio del Brandy. Nº 218, 11-Septiembre-1974.

3.- Puech,J.L.; Jouret,C. Dosage des aldéhydes aromatiques des eaux-de-vie conservées en fûts de chêne: Détection d'aulteration. Am.Fals. Exp. Chim. 75, Nº 805, 81-90, 1982.

4.- Lafon,J. Problèmes actuels de technologie et de vieillissement des eaux-de-vie de vin et de marc de raisin. Bulletin de l'O.I.V., Nº 482, 340-360, 1971.

5.- B.O.J.A. Reglamento de la Denominación Específica Brandy de Jerez. Nº 35, 5-mayo-1989.

6.- Puech,J.L.; Moutounet,M. Phenolic Compounds in an Ethanol-Water Extract of Oak Wood and in a Brandy. Lebensm.- Wiss. u.-Technol., 25, 350-352, 1992.

7.- Salagoity,M.H. La scopoletine: Un marqueur de la conservation en fûts de chêne. Connaissance de la Vigne et du Vin, 101-112, 1987.

8.- Chatonnet, P.; Bodron,J.N.; Pons,M. Incidence du traitement thermique du bois de chêne sur sa composition chimique. 2ª Partie: évolution de certains composés en fonction de l'intensité de brûlage. Connaissance de la Vigne et du Vin, 23, nº 4, 223-250, 1989.

9.- Bricout,J. Analyse des constituants dérivés du chêne dans les vieilles des eaux-de-vie d'Armagnac. Ann. Technol. Agric. 20, 217-223, 1979.

10.- Alibert,G.; Puech, J.L. Separatión et dosage automatique des aldéhydes benzoïques et cinnamiques par chromatographie en phase liquide. Journal of Chromatography, nº 124, 369-375, 1976.

11.- Villalon,M.; Montilla,J.; García-Vilanova,R.; Determinación espectrofotométrica de vainillina y syringaldehído en ron. Anales de Bromatoligía, XXXIX-2, 271-279, 1987.

12.- Martín,A.; Luna del Castillo,J. Bioestadística para las Ciencias de la Salud. Ed. Noima, 3ª ed., Madrid, 1990.

13.- Villalón, M.; López Gª,H.; López Martínez,M.C. Etude des constituants des rhums par chromatographie en phase gazeuse: Contrôle de qualité. Analisis, v.16, nº6, 341-345, 1988.

14.- Mersal,F.; Serra,Ch. Etude par phase gazeuse des substances volatiles issues du bois de chêne. Connaissance Vigne Vin, 21, nº1, 71-80, 1987.

15.- Hugh,J; El Vino. Atlas Mundial de Vinos y Licores, Ed. Blume, 3ª ed., Barcelona, 1886.

Received: April 11, 1993
Accepted: April 25, 1993