

OBTAINING AND CHARACTERIZATION OF VANILLA ESSENCE FROM MADAGASCAR'S BOURBON PODS

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Abstract

The paper presents the steps of extracting vanillin from pods of the Bourbon vanilla species in Madagascar. Subsequently, the obtained vanilla extract containing vanillin was characterized in terms of chemical and spectrophotometric properties. The methods of analysis used were UV-VIS spectrophotometry, FT-IR spectrophotometry, mass spectrometry and nuclear magnetic resonance by ¹³C-NMR and ¹H-NMR techniques. In the last part of the paper some applications of the obtained vanilla essence are presented.

Introduction

Vanilla planifolia is part of the Orchidaceae family, being a hanging and perennial plant, specific and native to tropical rainforest in Mexico but it grows also in Madagascar, Tahiti, Indonesia, Seychelles and the Philippines [1-3]. *Vanilla planifolia* is the main orchid species used to produce vanilla [4]. Vanilla is a tropical perennial plant with very long leaves and stalks, with a height of 10-15 m. It blooms 2-3 years after planting, many years in a row, and from yellow-green flowers, with a diameter of 5 cm, which lasts only one day and must be manually pollinated. After pollination, vanilla pods are formed [5-8]. The vanillin concentration of the fruit is between 1.3-3%. "Bourbon" vanilla produced in Madagascar, contains beside vanilline, cinnamic acid and esters; p-cresol and methyl ethyl; guaiacol; benzoyl benzoate and caprylate capronate [2]. Vanillin can be synthesized using as raw material eugenol or coniferin. Eugenol is a source of cheaper raw material for vanillin synthesis that is found to be 70-90% in the ethereal oil obtained by the water-vapor distillation of *Eugenia caryophyllata* as a matter much more accessible than coniferin [9-11]. The use of vanillin in the perfumer and food industries was a natural consequence of its discovery in vanilla seeds, much used in the flavoring of various foods. Vanilla is one of the most popular flavors, the most precious spice in the world, not only because it is expensive but also because it offers a special flavor [12-14]. Commercial vanilla extracts are obtained by macerating a portion of vanilla seeds in ten parts of 40-50% ethyl alcohol. Although vanillin is the active principle of vanilla seeds, the total vanilla extract flavor is not only due to the presence of vanillin, but also to other ingredients, especially some little known residual materials, which have a significant contribution to the quality of vanilla flavor [15]. Incorporating the vanillin into ethyl cellulose plastics ensures their stability at exposure to the atmosphere [6, 16].

Experimental

The vanilla pods are ripped and then cut into 4-5 cm pieces. This operation is important because the pods must be perfectly covered with alcohol and the bottle should be shaken daily in the first month. The vanilla pods thus prepared are placed in a glass container, over which alcohol is added, then the bottle is hermetically sealed and stored in a dark place. In the first

month, you need to shake the bottle well, at least once a day. After the first month, the bottle can be shaken just once a week. After 2 months, the essence can be used, but the final flavor develops only 9 weeks after the start of the extraction. With the passage of time the essence turns dark until it becomes almost black. To remove any vegetal residue in the essence, it is filtered through a paper filter. The essence doesn't alter its properties through time, it can be used indefinitely. Figure 1 shows the transformation of vanilla extract during 9 weeks.

On the first day, only a transparent solution with pods and a strong odor of alcohol is noticed. After the first week after the daily shake of the extract a slight coloration is observed, the pods begin to decompose into small black particles, the smell being similar with the one from the first day. After two weeks, a slight color appeared, the pods begin to decompose into small black particles, the odor remaining the same. Only after 4 weeks the vanilla smell appears, and the color darkens. After six weeks, the color darkens even more and the vanilla smell intensifies. After 9 weeks, the final coloring is formed and the vanilla essence is obtained. The solution is filtered and it can undergo characterization.



Figure 1. Steps of obtaining vanillin essences

The UV spectra of the synthesized acetylsalicylic acid samples were recorded on Lambda 25 Perkin Elmer spectrophotometer after dissolving of sample in 0.1 N NaOH. The FTIR spectra of the synthesized acetylsalicylic acid were acquired using a Spectrum BX II (Perkin Elmer) spectrometer on 1% KBr pellets. Mass spectra were recorded on a R.F. Quadrupolar Mass Spectrometer. NMR spectra were recorded on a Bruker Fourier 300 FT-NMR apparatus against the line of the solvent (CDCl_3). Chemical shifts are expressed in ppm.

Results and discussion

After obtaining the vanilla essence it was subjected to spectrophotometric analyzes. The vanilla essence was diluted with distilled water in a ratio of 1: 10000, then the spectra was recorded using the UV-VIZ molecular UV spectrophotometer in the ultraviolet range between 200-400 nm. In the UV-VIS spectrum (Fig. 2 left), the maximum of absorbance was observed at 348 nm, characteristic of vanillin [17].

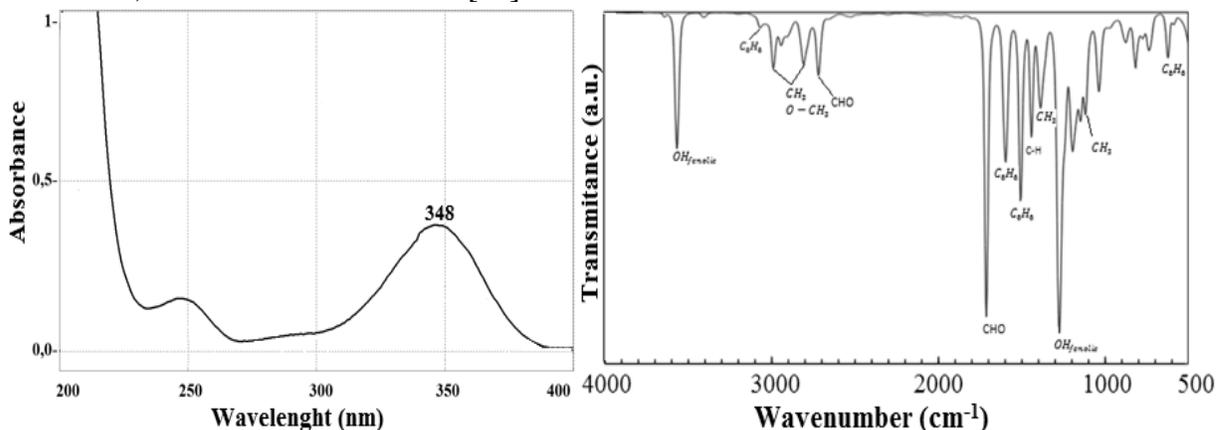


Fig. 2. The UV spectrum (left) and the FT-IR spectrum (right) of the obtained vanillin

In Figure 2 (right), the FT-IR spectrum of vanilla essence is shown. The presence of functional groups in the vanillin structure is attributed to the frequency bands of the C = O bond in the aldehyde group at 1720 cm^{-1} and 1700 cm^{-1} respectively [18]. The hydroxy-phenolic group shows a sharp frequency band at 3620 cm^{-1} and intense deformation band at 1260 cm^{-1} [18]. The methoxy group (OCH_3) can be identified by the asymmetric vibration band C-H at 2980 cm^{-1} and symmetrical from 2870 cm^{-1} , vibration band of valence bond at 1380 cm^{-1} of the C-O, and less intense at 1045 cm^{-1} attributed to the vibration band of the C-H bond [18]. The aromatic nucleus is visible in the FT-IR spectrum by the vibration band C-H of the aromatic nucleus at 3010 cm^{-1} , 1600 cm^{-1} , at 1506 cm^{-1} more intense; and the broad and weak in intensity bands at 950 cm^{-1} and 700 cm^{-1} [18]. Substitution 1,2,4 characteristic to vanillin is attributed to deformation vibration out-of-plane of the C-H bond from the ortho-disubstituted benzene nucleus at $860\text{-}900\text{ cm}^{-1}$ [18].

Figure 3 shows the MS spectrum of vanilla essence. It is observed that the molecular peak overlaps the base peak, and the molecular weight 155, which corresponds to vanillin, can also be deduced from the spectrum.

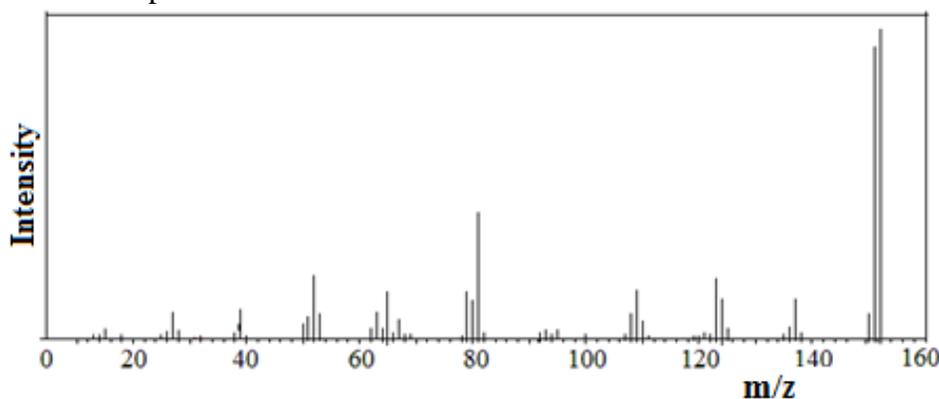


Figure 3. MS spectrum of vanilla essence.

Figure 4 shows the ^1H -NMR spectrum for the vanillin essence, the hydrogen line in the CHO group of the H singlet is assigned at 9.8 ppm, the very strong singlet of the hydrogen in the methoxy group (ether) is at 3.9 ppm. From a qualitative point of view, OCH_3 contains three H atoms for which the OCH_3 singlet line is three times larger than the CHO and OH singlets. The H_3 singlet line of the hydroxy group would normally be in the range of 0.7-5.5 ppm; but since it binds to the aromatic nucleus it moves to 6.6 ppm. On the aromatic nucleus, we have three unsaturated hydrogen atoms (one singlet and two doublets). The doublets have two electronegative groups in the neighborhood. Although the two groups (substance of order I and substance of order II) are different in the H-NMR spectrum, only one signal is observed which includes both doublets. The hydrogen singlet on the aromatic nucleus located between two active functional groups has a greater chemical shift than the other two doublets on the aromatic nucleus [18].

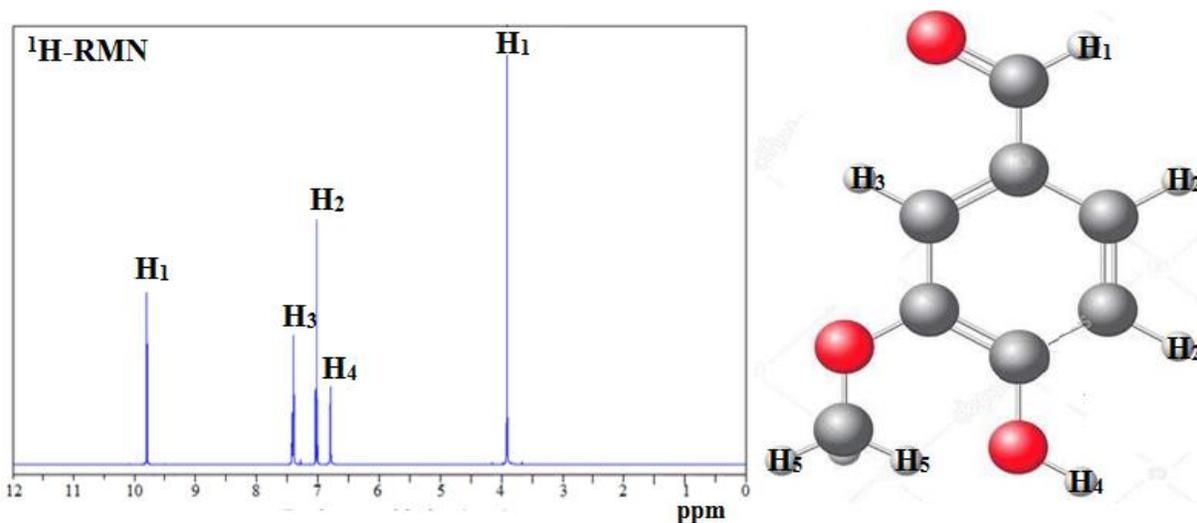


Figure 4. ^1H -NMR spectrum of vanillin essence

Figure 5 shows the ^{13}C -NMR spectrum of vanillin essence. The largest chemical shift is due to the carbon atom of the carbonyl group at 192 ppm, and the lowest chemical shift of 55 ppm is attributed to the carbon of the methoxy group. Carbon atoms appear in descending order of their reactivity in pair with the decreasing chemical shifts. The C_5 and C_2 carbon atoms bond to reactive groups and have greater chemical displacement than C_7 and C_6 carbon atoms which are further away from the reactive groups but are larger than C_3 and C_4 [18].

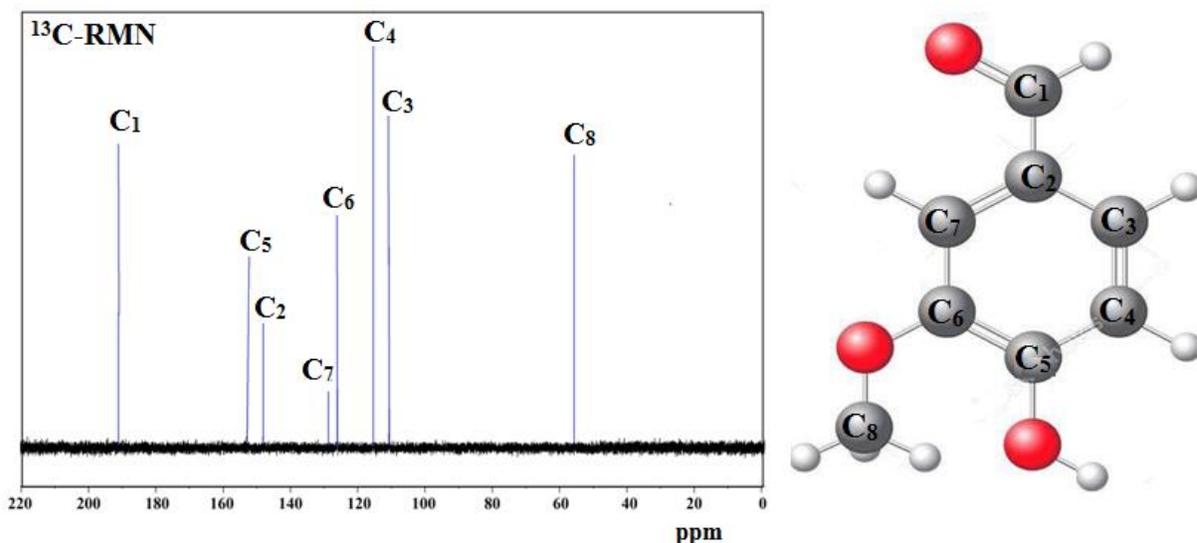


Figure 5. ^{13}C -NMR spectrum of vanillin essence

Vanilla extract can be used as an aromatic ingredient for food, alcoholic beverages (liqueurs, creams) and soft drinks, dairy products (ice cream and yoghurt), sweets, pastries and confectionery (cakes, puddings, creams).

Conclusion

House made vanilla was obtained by extraction after 9 weeks, during which, differences in the color of extracts were found. The vanillin in the vanilla extract (vanilla essence) was identified by UV-VIZ spectroscopy by the maximum absorption band at 348 nm and by FT-IR by the presence of the benzyl, carbonyl, hydroxy and methoxy-specific bands.

Also, mass spectra overlap with those of pure vanillin, identifying the molecular weight of vanillin of 155. In the vanilla essence obtained, more intense H-NMR lines are found compared with the pure vanillin. The C-NMR lines of pure vanillin essence are overlapping in most cases with the vanilla essence lines.

References

- [1] E. Pooper, L. Roman, R. Crăciuneanu, *Chimie analitică cantitativă*, Editura Didactică și Pedagogică București, 1965.
- [2] V. E. Ceausescu, G. Radoias, T. Cadariu, *Odorante și aromatizante-Chimie tehnologie și aplicații*, Editura Tehnică, București, 1988.
- [3] L. Cao, K. Hu, *Detarminarea vanilinei din extractul de vanilie sintetică*, Institutul Pharmacol, Academia Chemică, Shanghai, R.P. Chineza, 1983, 38.
- [4] L. Savici, *Aparate de analiză fizico-chimică*, Editura Tehnică, București, 1980.
- [5] W. Brockel, I. Gatfield, *Vanillin*, Contact, 4, 1997, 16.
- [6] T. Dippong, *Obținerea, caracterizarea și utilizările aditivilor alimentari*, Editura Risoprint, Cluj-Napoca, 2016.
- [7] R. Kumar, P. K. Sharma, P. S. Mishra, *A Review on the Vanillin derivatives showing various Biological activities*, International Journal of PharmTech Research, 4 (2012) 266.
- [8] Z. Wong, K. Chen, J. Li, *Formation of vanillin and syringaldehyde in an oxygen delignification process*, BioResources 5(3) (2010) 1509.

- [9] J. Kerler, R. Verpoorte, Vanilla Production: Technological, Chemical, and Biosynthetic Aspects, *Food Reviews International*, 17 (2) (2001) 119.
- [10] K.J. Edgar, S.N. Falling, Synthesis of vanillin. *J.Org.Chem*, 55 (1990) 5287.
- [11] T. Li, J. P. N. Rosazza, Biocatalytic Synthesis of Vanillin, *Appl Environ Microbial*, 66(2) (2000) 684.
- [12] M. Mitrescu, *Chimie analitică cantitativă*, Editura Institutului Politehnic Timișoara, 1955
- [13] P Suppakul, T. Jinkarn, Antimicrobial effects of vanillin coated solution for coating paperboard intended for packaging bakery products, *J. Food Ag-Ind.* 2 (2009) 138.
- [14] A.I. Badea ,Aplicații ale spectrometriei UV-VIS în chimia analitică, Ed.Didactică și Pedagogică R.A., București, 2006.
- [15] S. Oprea, *Tehnologie Chimie Organică - Îndrumar de laborator*, I.P.I, Iași, 1986.
- [16] B. Toulemande, I. Horman, H. Egli, Diferențierea între vanilina sintetică și cea naturală, doar folosind deuteriu NMR. *Hel. Chem. Acta*, 66(7) (1983) 2342.
- [17] T. Dippong, C. Mihali, *Tehnici avansate de analiză instrumentală utilizate în industria alimentară chimie și mediu*, Editura Risoprint, Cluj-Napoca, 2017
- [18] A.T.Balaban, M. Banciu, I Pogany, *Aplicații ale metodelor fizice în chimia organică*, Editura științifică și enciclopedică, București, 1983