

# Characterization of Patchouli and Vanillin Industrial Wastes as a strategy for their valorization

Lígia L. Pimentel<sup>a</sup>, Susana S. M. P. Vidigal<sup>a</sup>, Francisca S. Teixeira, Paula T. Costa, Manuela E. Pintado, and Luís M. Rodríguez-Alcalá\*

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

<sup>a</sup> both authors contributed equally

\*contacting author: [lalcala@ucp.pt](mailto:lalcala@ucp.pt)



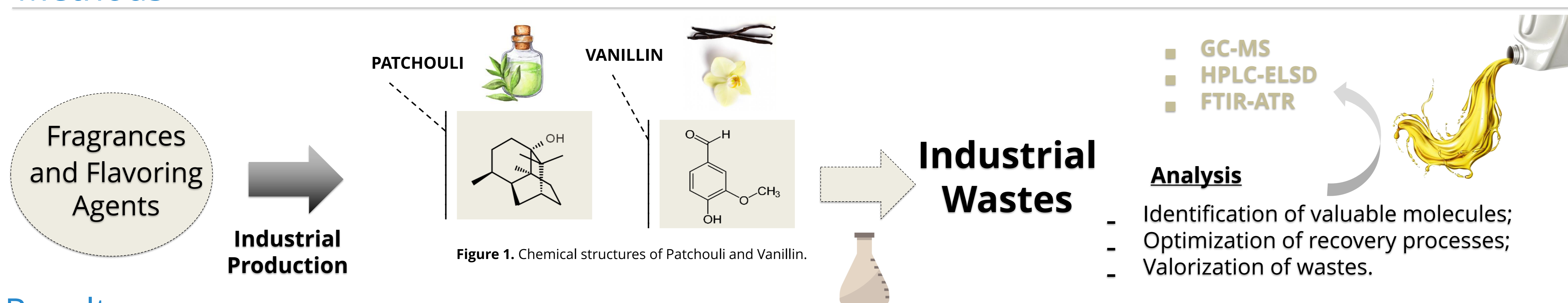
## Introduction

Patchouli is a cyclic terpene alcohol used as a fragrance ingredient (i.e. sweet, herbaceous and spicy) [1] that has been demonstrated to exert low acute dermal toxicity [2-3]. On the other hand, The aromatic aldehyde Vanillin is one of the most widely used flavoring agents and it is also an important antifoaming ingredient [4]. Several studies report fragrances/flavoring production by steam distillation extraction or by the usage of supercritical CO<sub>2</sub> as well as microwave assisted extraction methods from plants. Moreover, recent investigation suggests that synthesis of these compounds by genetically modified microorganism ensures its reliable and sustainable supply [5].

## Objectives

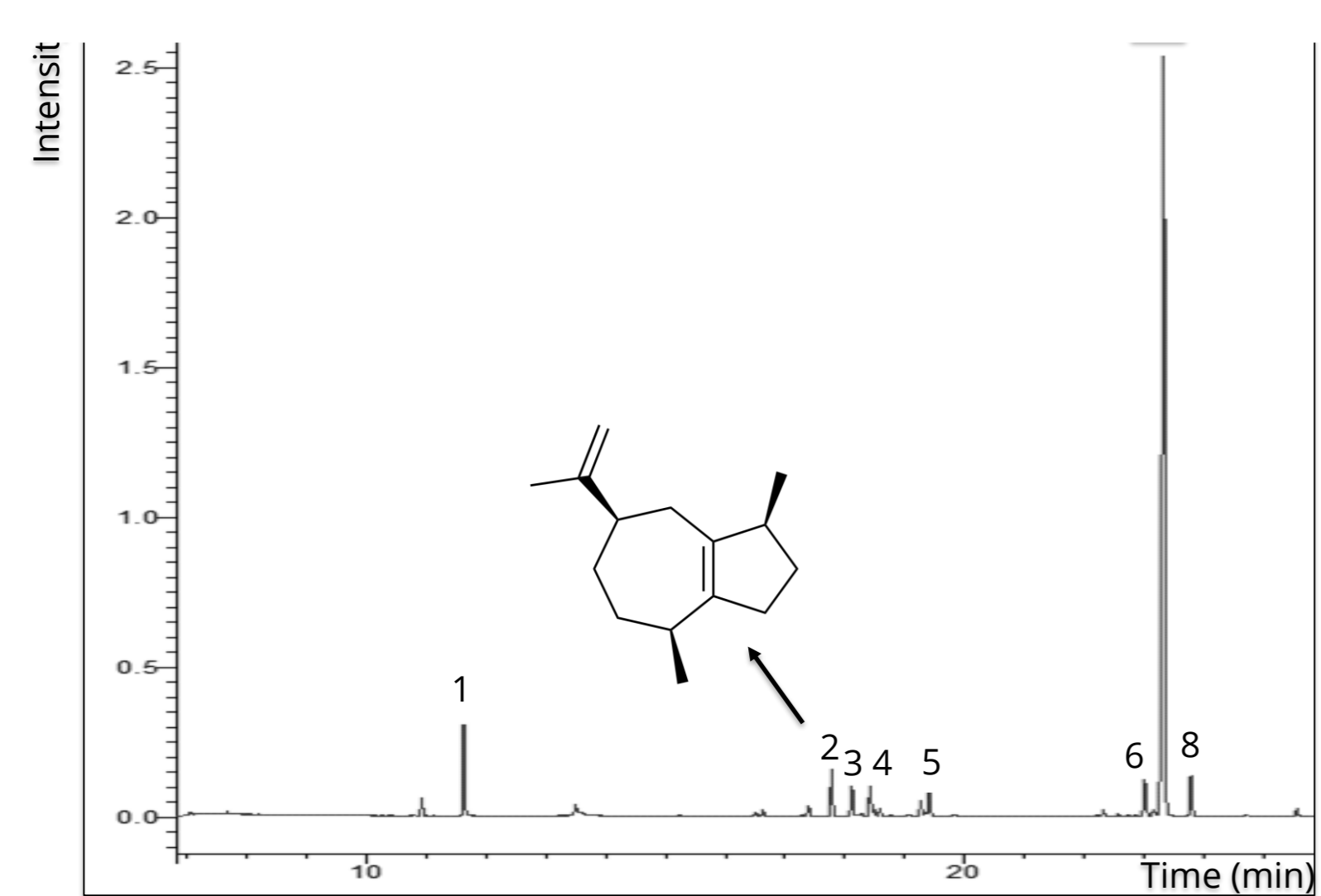
The chemical composition of patchouli essential oil is complex with more than 24 sesquiterpenes and its hydroxylated derivatives, being the (-)-patchoulol the main compound. Other products such as  $\alpha$ -bulnesene and  $\beta$ -guaiene are considered as side-products of the metabolic engineered pathway of terpenes [5]. Also, the production of Vanillin can be achieved by natural extraction from *Vanilla planifolia*, chemical synthesis and by biotechnological fermentation procedures [6]. This research work aims to characterize fermentation by-products of the production of Patchouli and Vanillin for their valorization after profiling through high performance liquid chromatography using an evaporative light scattering detector (HPLC-ELSD) and gas chromatography triple quadrupole (GC-QqQ).

## Methods



## Results

### GC-MS Analysis of Patchouli Production Waste



**Figure 1.** GC-MS Chromatogram of Patchouli Production Waste.

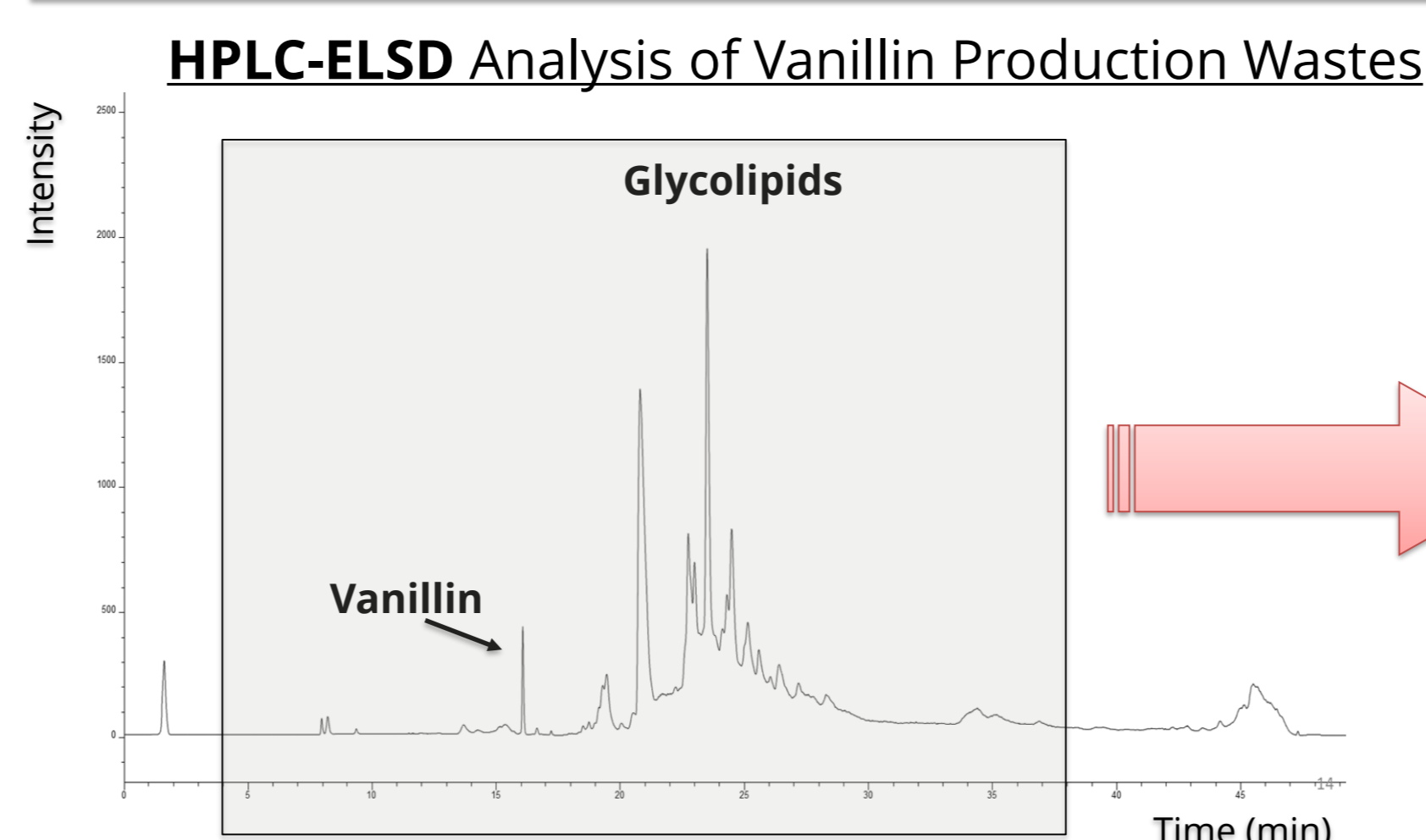
**Table 1.** GC-MS detected compounds in Patchouli biowaste

Peak	Compound	Concentration (g/kg)
1	Dodecane – Internal Standard	-
2	$\alpha$ -Guaiene	0.85 $\pm$ 0.06
3	Seychellene	0.75 $\pm$ 0.04
4	$\alpha$ -Patchoulene	0.82 $\pm$ 0.03
5	$\alpha$ -Bulnesene	0.98 $\pm$ 0.02
6	Pogostole	0.63 $\pm$ 0.08
7	Patchouli alcohol	25.38 $\pm$ 1.12
8	2-butyl-5-methyl-3-(2-methylprop-2-enyl)cyclohexanone	1.03 $\pm$ 0.07

#### Patchouli alcohol

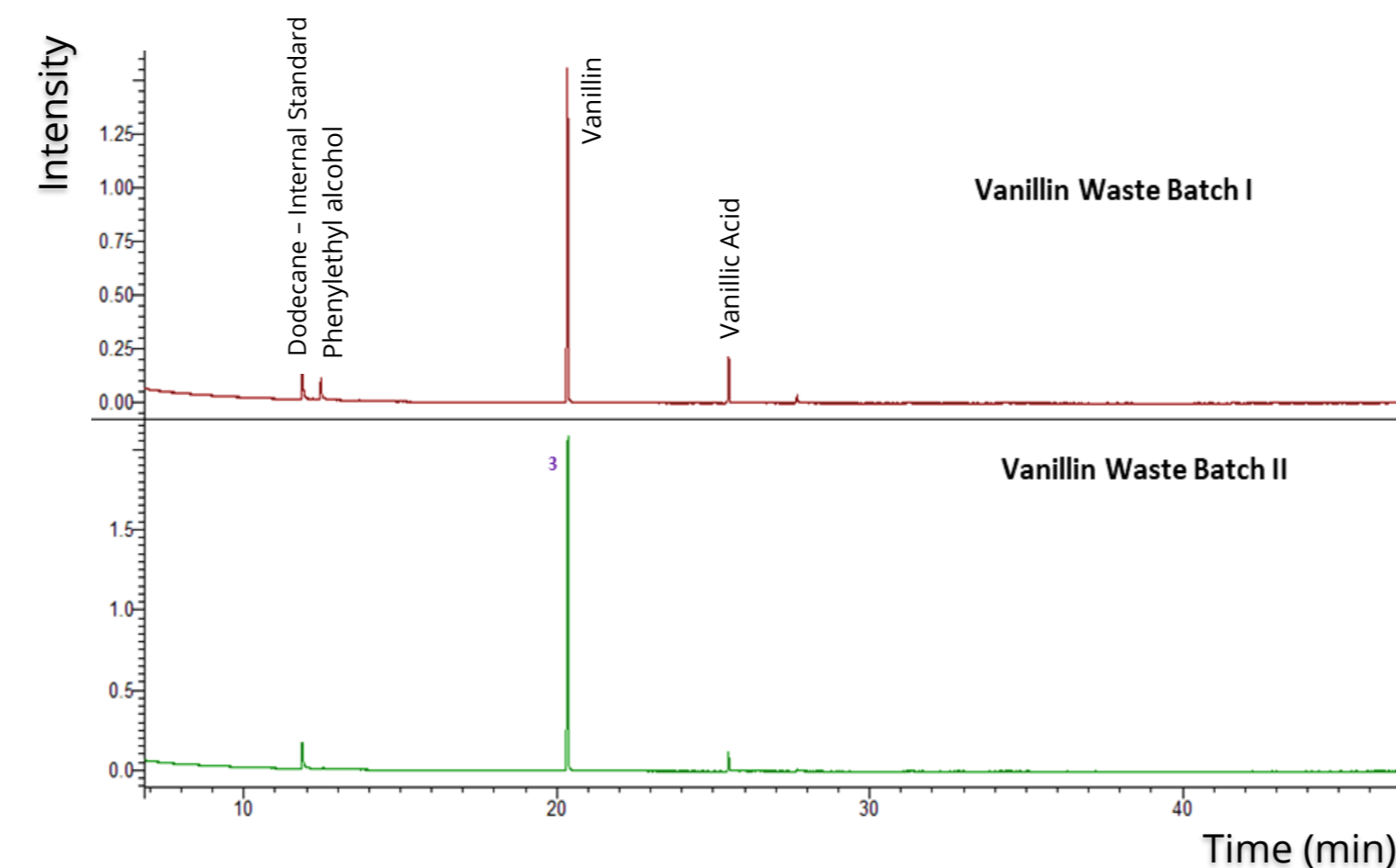
In addition to Patchouli alcohol, other compounds were detected by GC-MS analysis. The presence of  $\alpha$ -Guaiene, Seychellene,  $\alpha$ -Bulnesene (Figure 1 and Table 1) and other molecules classified as **Sesquiterpenes that are associated to fragrance and flavoring properties.**

**Vanillin**  
In addition to Vanillin, other compounds were detected after performing HPLC-ELSD (Figure 2) and GC-MS analyses (Figure 3 and 4) in Vanillin biowastes. By HPLC-ELSD **glycolipids** were identified while by using GC-MS **vanillic acid** and **vanillin alcohol** were detected.



**Figure 2.** HPLC-ELSD Chromatogram of Vanillin Production Wastes.

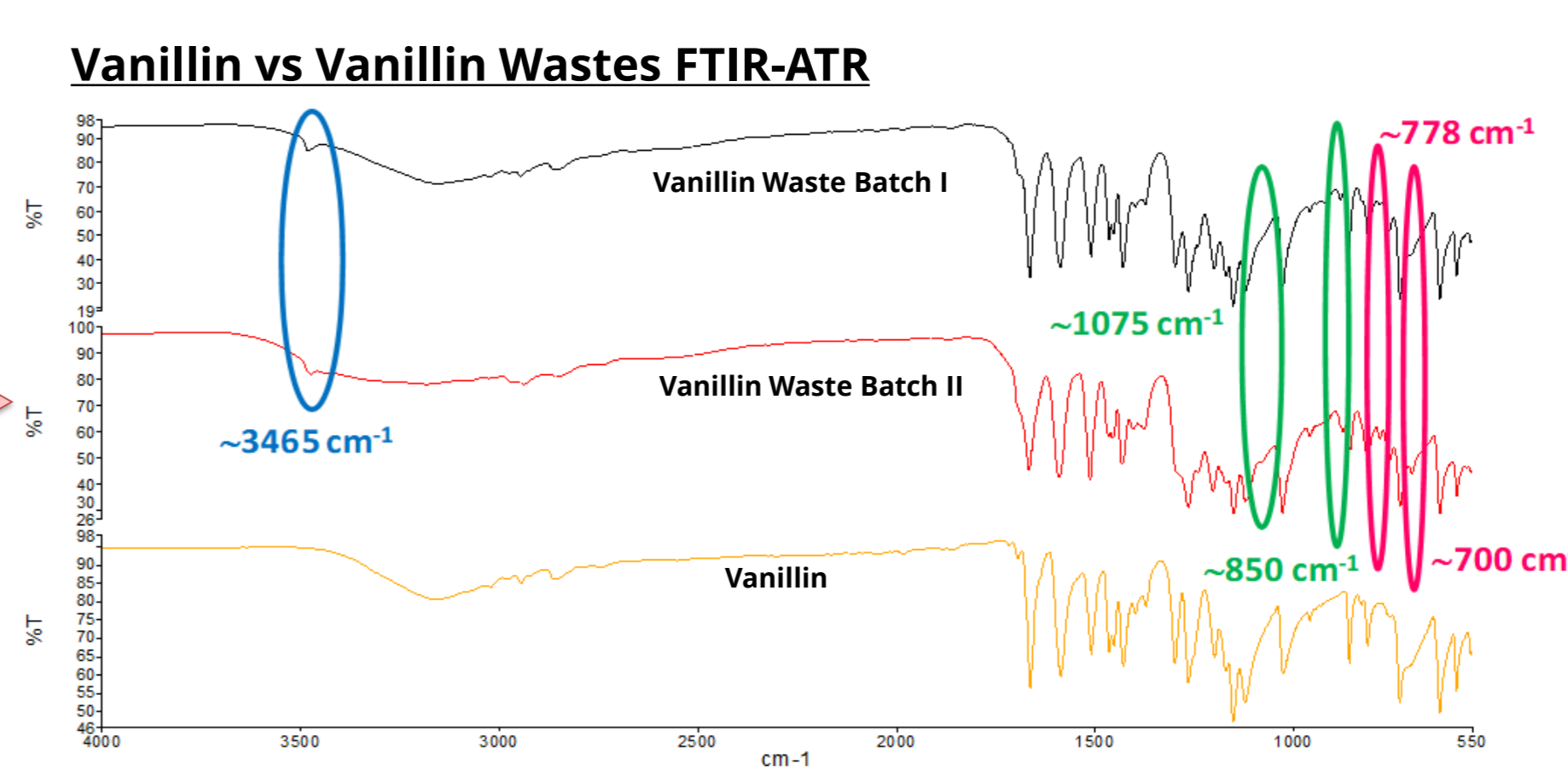
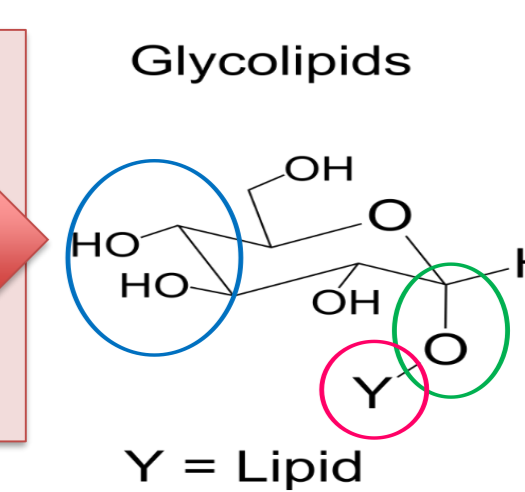
### GC-MS Analysis of Vanillin Production Wastes



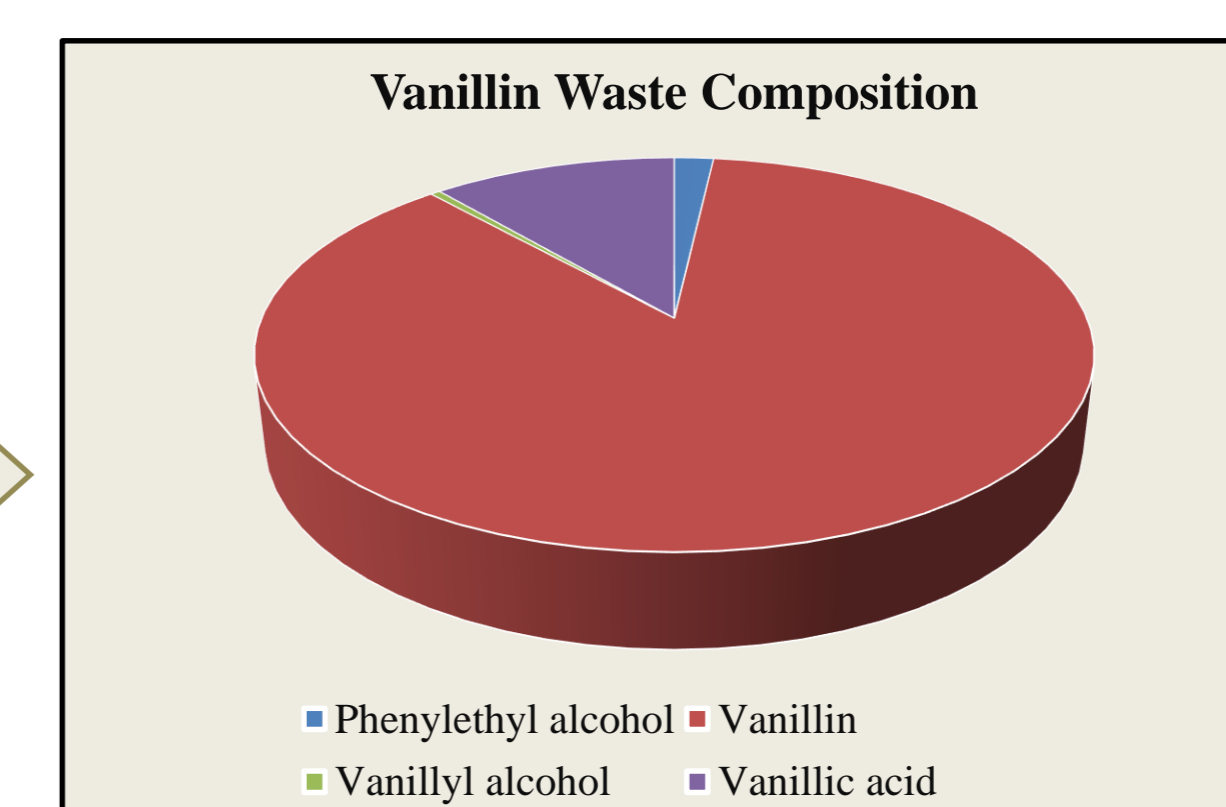
**Figure 3.** GC-MS Chromatogram of Vanillin Production Waste.

**Possible correspondence between FTIR bands and glycolipids groups**

- Dimeric -OH stretch;
- C-O Polysaccharides Glycosidic linkage;
- -CH<sub>2</sub> wagging and C-H out-of-plane bending of long-chain aliphatic.



**Figure 5.** FTIR-ATR spectra of vanillin and vanillin wastes.



**Figure 4.** Diagram of the composition (%) performed by GC-MS of Vanillin Wastes.

## Conclusions

The industrial by-products derived from the production of Patchouli and Vanillin contained high-value molecules such as Patchouli alcohol,  $\alpha$ -Guaiene,  $\alpha$ -Bulnesene as well as Vanillin, Vanillic Acid and glucosides of Vanillin. Regarding these industrial processes, analytical methods that ensure the chemical composition are fundamental in order to assess wastes valorization. Future assessment of its bioactivity will provide better information on the application of these compounds to turn waste into a resource.

## References

1. Bhatia SP et al. Food Chem. Toxicol., **2008**, 46, S255-S256. doi: 10.1016/j.fct.2008.06.069.
2. Belsito D. et al. Food Chem. Toxicol., **2008**, 46, S1-S71. doi: 10.1016/j.fct.2008.06.085.
3. Soon Hong Soh et al. J. Clean Prod., **2021**. doi:10.1016/j.jclepro.2021.126661.
4. Banerjee G, Chattopadhyay P. J. Sci. Food Agric., **2019**, 99(2), 499-506. doi: 10.1002/jsfa.9303.
5. Francisco Aguilar et al. ACS Omega, **2020**, 50, 32436-32446. doi: 10.1021/acsomega.0c04590
6. Kaur B, Chakraborty D. Appl. Biochem. Biotechnol., **2013**, 169,1353-72. doi: 10.1007/s12010-012-0066-1.

## Acknowledgements

This research was funded by Amyris Bio Products Portugal Unipessoal Lda and Escola Superior de Biotecnologia – Universidade Católica Portuguesa through Alchemy project- Capturing high value from industrial fermentation bioproducts project UIDB/50016/2020ucts (POCI-01-0247-FEDER-027578). We would also like to thank the scientific collaboration under the Fundação para a Ciência e a Tecnologia (FCT) under the project UID/Multi/50016/2019.