Study and Characterization of Vanilla Pods *Tahitensis* Species for Characterization for Future Application in Products Derived from Vanilla *in natura*

Emanuele Santana Bispo dos Santos[1*], Thâmilla Thalline Batista de Oliveira[1], Ingrid Lessa Leal[1], Aderbal de Castro[2,3], Eliete Costa Alves[1], Tatiana Barreto Rocha Nery[1], Ana Lucia Barbosa de Souza[1]

[1]SENAI CIMATEC University Center; [2]State University of Southwest Bahia, Brazil; Vanilla Brasil Company, Brazil

The growing search for natural and healthier foods is a determining factor for studying and applying new routes for developing sustainable products. Therefore, this work aims to study and characterize the *V.tahitensis* vanilla bean for future application *in natura* vanilla extract. The physical-chemical and centesimal characterizations were carried out according to the methods described in the Adolfo Lutz Institute manual. The results obtained from the analyses were auspicious, and the intention is to develop the vanilla extract through a removal method that presents the best percentage of vanillin to be applied in the market.

Keywords: Vanilla Pods. Natural. Centesimal. Physical-Chemistry. Characterization.

Introduction

Vanilla is a plant belonging to the *Orchidaceae* family; it originates from a flower, the orchid. Among its species, the most famous are *Vanilla planifolia* and *Vanilla tahitiensis*. On the world stage, vanilla pods are produced in tropical and subtropical regions, mainly in India, Indonesia, Mexico, Uganda, and Congo. Madagascar is currently the world's largest producer. In Brazil, Vanilla is cultivated south of the state of Bahia [1].

After harvest in the field, the pods go through a long process of beneficiation, drying, and cure to obtain the characteristic aroma, with vanillin primarily responsible for vanilla's aromatic composition, where its content varies according to the species and origin of the pod. Among the products derived from vanilla, one of the most prominent in the market is the commercial extract. The global vanilla extract market was valued at US$5.26 billion in 2021 and is projected to grow from US$4.94 billion in 2022 to US$6.30 billion by 2029 [1,2]. Commercial vanilla extract can be obtained in several ways. One is the percolation method, which consists of a circulating mixture of ethanol and water containing 35-50% alcohol for 30 days with constant agitation. Another known extraction technique is Soxhlet, in which an organic solvent, usually hexane, is used in an extraction chamber coupled to a condenser, with a temperature close to the solvent's boiling point and a time varying from 3 to 8 hours. Another method used is ultrasound, which consists of immersion in an ultrasonic bath at a frequency of 60khz and ethanol/water in different proportions as solvent [3-5]

Aiming to develop sustainable practices, mainly about sustainable production and consumption, companies have sought to create strategies and technological routes for sustainable development. Therefore, seeking to meet the Sustainable Development Goals (SDGs) of the United Nations (UN) is a significant differential, as it helps guarantee sustainable food production systems, in addition to helping maintain ecosystems [6]

Faced with the demand for healthier foods and natural sources to produce safer and more attractive products, the present work aims to develop a study and characterization of vanilla pods of the *tahitensis* species to carry out future application in products derived from natural vanilla based on analysis for centesimal and physical-chemical characterization, in addition to evaluating global market.

Received on 15 September 2023; revised 28 November 2023.
Address for correspondence: Emanuele Santana Bispo dos Santos. Rua Portugal, number 48, Vila Canária, Salvador, Bahia, Brazil. Zipcode: 41390-080.

Materials and Methods

Vanilla pods (*V. tahitensis*), *in natura*, and dehydrated after the extraction process, and the extract were supplied by Vanilla Brasil. Vanilla pods were received and underwent a pre-treatment stage, which consisted of drying the beans in a dehydrator (Figure 1A and 1B) for 36 hours at 40 °C. After drying, the beans were ground with a blender until reaching a granulometry of 28 mesh. After the pre-treatment, the samples were stored in glass jars at room temperature. Subsequently, physical-chemical characterization and centesimal composition analyses were carried out. The procedure performed with vanilla pods *in natura* and dehydrated after the extraction process (Figure 2).

The centesimal and physical-chemical characterization of the samples will be carried out following the methodologies described in the manual of physical-chemical methods for food analysis from the Institute Adolfo Lutz. [7] Some of the analyses will be carried out with equipment available in the laboratory (Table 1).

As for the analysis of vanillin determination in the vanilla extract, by the thin layer chromatography method, chromatoplates of aluminum, and hexane and ethyl acetate 1:1 were used. The extract, previously diluted in three concentrations (100, 50, and 25%), was applied to the chromatoplate approximately 0.5 cm from its lower base. Then, the TLC was introduced into a glass containing hexane and ethyl acetate, igniting and dragging the less adsorbed compounds into the stationary phase, separating them from the more adsorbed compounds. Then, the plate was dried with a hot air dryer, and the chromatoplates were submitted to the development process using UV (254 nm) and sublimated iodine. [8] In addition to the characterization, a market study

Figure 1. Vanilla pods *in natura* (a) Vanilla pods dried after the extraction process (b).

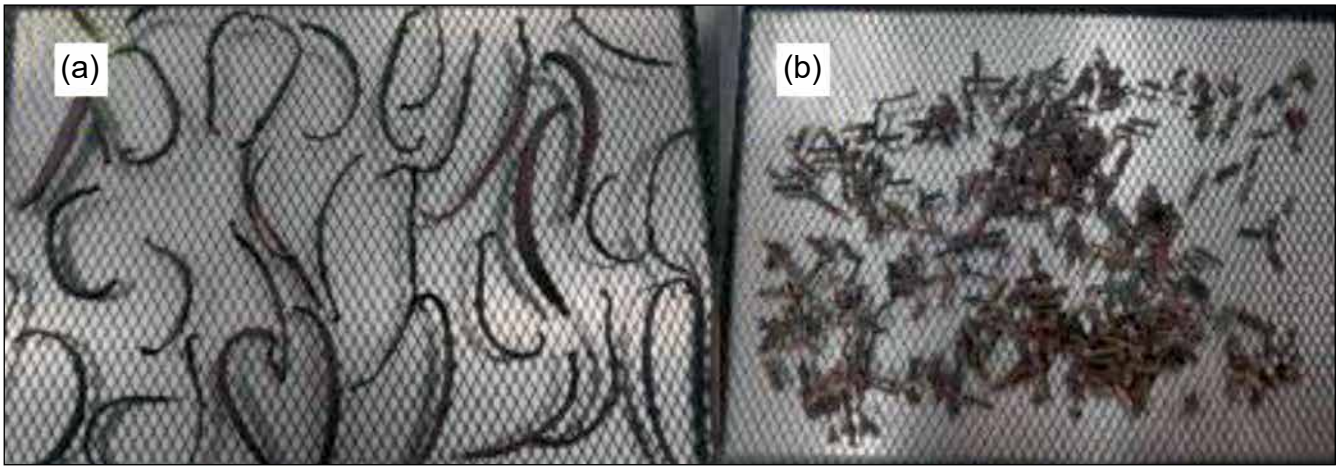


Figure 2. The procedure was carried out with vanilla pods.

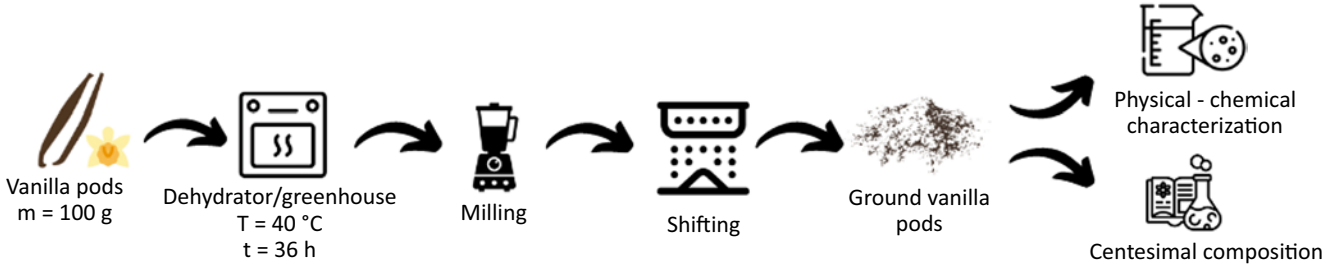


Table 1. Methods for physical-chemical characterization and centesimal composition.

Analyses	Experimental Methods
Physical-Chemical Characterization	
pH	pH content in the Bel Engineering PHS3BW digital pH meter
Total soluble solids (°Brix)	Content of soluble solids in the portable digital refractometer (SPINLAB/SPIN-104-D)
Water activity	Water Activity Content (LabMaster-Aw TECNAL)
Humidity	Humidity Content (Shimadzu Moc 120 H)
Color	Color Content in the KONICA MINOLTA Handheld Colorimeter (CR-400)
Centesimal Characterization	
Ashes	Total ash content by muffle incineration at 550 °C [7]
Lipids	Total lipid content by Bligh Dyer [7]
Proteins	Protein content by Kjeldahl digestion method [7]
Carbohydrates	Total carbohydrate content will be calculated by the difference: 100 - (% moisture + % ash + % protein + % lipids)

was carried out using the Mordor Intelligence platform to obtain an overview of the vanilla market. In addition to checking the leading players and the main trends in the market.

Results and Discussion

The vanilla pod samples, *in natura* and after the extraction process, after a pre-treatment step, were assigned to conduct the analyses to obtain results regarding physical-chemical characteristics (Table 2) and centesimal composition (Table 3).

According to the results obtained from the physical-chemical analyses, the pH values are very close, which indicates that the beans maintained their acidity after the extraction process; these values are above 4.5, that is, they have low acidity, and there may be a proliferation of fungi, yeasts, and pathogenic bacteria.[9] As for the brix degree, the values are very consistent, in which there is a reduction in the total soluble solids of the pods after extraction, which means that part of these components were consumed in the process. The water activity is a parameter that relates to the water availability of the product, with values found between 0.4 and 0.7, values that configure the low and medium aw, that is, the beans are in a qualitatively acceptable range, indicating that the samples have a satisfactory level of perishability [10]. Regarding moisture, pods *in natura* are higher than post-extraction beans, as the pods used in the extraction undergo a pre-treatment, in which the raw material is dried before being introduced into the process. As for color, the values of L* (Brightness) are below 100, compared to white, which has a brightness of 100. As for chromatic coordinates, a*(red/green coordinate) and b* (yellow/blue coordinate), the a* and b* of the beans are positive, indicating a region close to red and yellow, respectively. [11]

Regarding the results of the centesimal composition, the ash and lipid content of both broad beans are very close, with a slight variation. As for protein content, fava beans *in natura* contain a smaller amount; however, in the study by Barrientos and colleagues [12], the percentage found in the bean of the species V. Planifolia was 4.58, that is, very close to the value obtained in

Table 2. Results of physical-chemical characterization.

Vanilla pods *in natura*		**Vanilla pods dried after the extraction process**	
pH	4.527 ± 0.006	pH	4.790 ± 0.036
Total soluble solids (°Brix)	2.300 ± 0.001	Total soluble solids (°Brix)	0.767 ± 0.058
Water activity	0.721 ± 0.002	Water activity	0.414 ± 0.006
Humidity (%)	37.713 ± 0.150	Humidity (%)	7.763 ± 0.365
Color	L*= 36.323 ± 0.218 a*= 17.833 ± 0.172 b*= 6.143 ± 0.208	Color	L*= 26.673 ± 0.025 a*= 12.380 ± 0.173 b*= 5.243 ± 0.085

Table 3. Results of centesimal composition.

Vanilla Pods *in natura*		**Vanilla Pods Dried After the Extraction Process**	
Ashes (%)	5.495 ± 0.112	Ashes (%)	5.935 ± 0.070
Lipids by Bligh Dyer (%)	50.701 ± 0.618	Lipids by Bligh Dyer (%)	50.184 ± 1.143
Proteins (%)	4.359 ± 0.117	Proteins (%)	8.087 ± 0.179
Carbohydrates (%)	5.7	Carbohydrates (%)	28.2

the present work. As for the carbohydrate content, found by calculating the difference, it is noticed that the vanilla pod *in natura* has a lower content; this can be explained due to the significant difference between the values obtained for moisture of the pods. As for the results of the analysis of vanillin determination by the TLC method (Figure 3), we observed that this method presented initially positive results for high concentrations of vanillin (from 0.1 %). The commercial vanilla extract and the standard vanillin solution showed the same retention time. However, regarding spot intensity, the commercial alcoholic extract was visible, while the dot of the other standard extract did not even appear on the plate. Therefore, carrying out new tests to validate the results is interesting.

According to the market analysis conducted on Mordor Intelligence, the major players in the market are Mc Cormick, Sensient, Nielsen-Massey, Symrise, and Givaudan (Figure 4), with Mc Cormick & Company Inc. being the leader in the field, as it sought to create more efficient market strategies to expand its products and gain more visibility.

These companies gain prominence in the market, as they have natural vanilla-based products such as vanilla extract and paste. In addition to assessing market players, the survey analyzed how the demand for natural foods is going (Figure 5), with a growing consumer consensus in perceiving food products as healthy if derived from natural ingredients, such as vanilla.

After the analysis, we observed that the trend towards natural products is growing, both in the food market and in the cosmetics and personal care sector, making it increasingly important for companies to consider the inclusion of natural ingredients in their products.

Conclusion

Due to the results presented, the physical-chemical characterization and the centesimal

Figure 3. Results of the analysis of the vanillin content of vanilla by the TLC method.

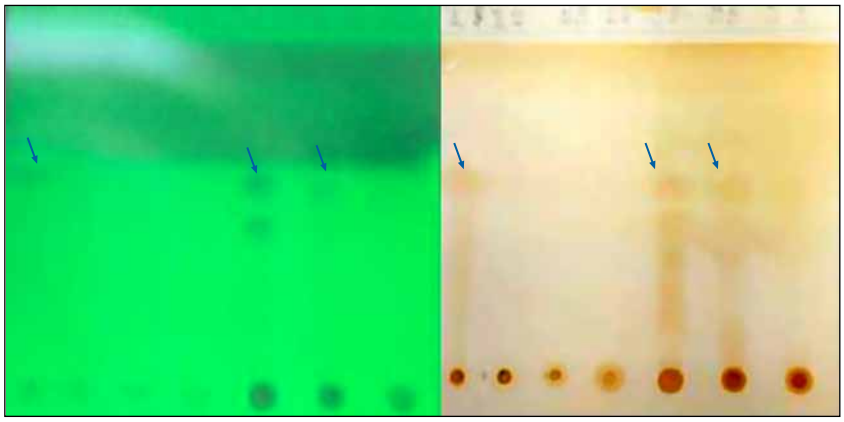
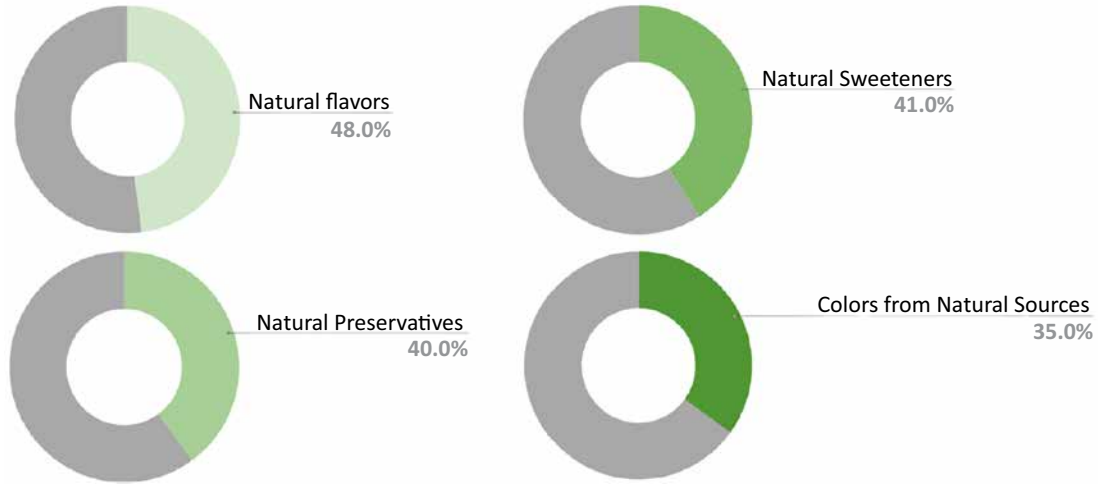


Figure 4. Significant players in vanilla's products.

Source: Mordor Intelligence (2020).

Figure 5. Vanilla Market: Consumer Preference for Food Products in %, US, 2021.



Source: Adapted from Mordor Intelligence (2020).

composition of the vanilla pods *in natura* and after the extraction process, presented auspicious results, where it was possible to perceive essential points and the differences in the characteristics between the pods. From the results of the determination of vanillin in the commercial extract, it is interesting to carry out new tests to validate the results and use a more robust analysis such as High-Performance Liquid Chromatography (HPLC). Thus, from these results, it is intended to develop the vanilla extract with an exciting percentage of vanillin to be applied in the market, testing three extraction methods, with modifications in the process variables such as temperature, time, solvent, and equipment, to obtain the product through an optimized process.

Acknowledgments

We thank the University Center SENAI CIMATEC for the opportunity and all the space available to carry out the project's activities. In addition, thank Vanilla Brasil company for all the support and teaching.

References

1. Messias KLS. Baunilha, difícil resistir aos seus encantos. Food Ingredients Brazil. Nº 19. 2011. Available at: < https://revista-fi.com/upload_arquivos/201606/20160600124460014672017 53.pdf >. Accessed on: June 29, 2023.
2. Mercado de Baunilha - Crescimento, Tendências e Previsões. Mordor Intelligence. Available at: < https://www.mordorintelligence.com/pt/industry-reports/global-vanilla-industry >. Accessed on: June 29, 2023.
3. Sinha AK, Sharma UK, Sharma N. A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and others constituents. International Journal of Food Sciences and Nutrition 2008;59(4):299–326.
4. Tolentino SS et al. Extração de Lípidios: Método Soxhlet. UNICRUZ. 2015.
5. Sharma A, Verma SC, Saxena N et al. Microwave and ultrasound assisted extraction of vanillin and its quantification by high performance liquid chromatography in *vanilla planifolia*. Journal of Separation Science 2006;29:613–619.
6. Nações Unidas Brasil. Sobre o nosso trabalho para alcançar os Objetivos de Desenvolvimento Sustentável no Brasil. Available at:< https://brasil.un.org/pt-br/sdgs > Accessed on: July 6, 2023.
7. Instituto Adolfo Lutz. 1ª Edição Digital. Métodos físicos-quimicos para análise de Alimentos, 2008.
8. Brondani PB. Cromatografia em Camada Delgada (CCD). 2019. Available at:< https://patyqmc.paginas.ufsc.br/files/2019/07/Cromatografia-de-Camada-Delgada.pdf> Accessed on: June 29, 2023.
9. A importância do pH dos alimentos. Terra Food Tech. Available at:< https://www.terrafoodtech.com/pt-pt/a-importancia-do-ph-dos-alimentos/> Accessed on: June 29, 2023.
10. Water Activity (aw) in Foods. FDA U.S Food & Drug Administration. 2014. Available at:< https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/water-activity-aw-foods#:~:text=The%20water%20activity%20(a%20w)%20of,distilled%20water%20under%20identical%20conditions. > Accessed on: June 29, 2023.
11. Beetsma J. The CIELAB L*a*b* System – the Method to Quantify Colors of Coatings. 2020. Available at:< https://www.ulprospector.com/knowledge/10780/pc-the-cielab-lab-system-the-method-to-quantify-colors-of-coatings/ > Accessed on: July 6, 2023.
12. Barrientos AP et al. Chemical and microstructural characterization of vanilla waste compounds (*Vanilla planifolia*, Jackson) Using Eco-Friendly Technology. Waste and Biomass Valorization 2022;13:271–286.