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Scientific article

Quality of green and cured vanilla (*Vanilla planifolia* Jacks. ex Andrews) fruit in relation to its age at harvest

Calidad de fruto verde y beneficiado de vainilla (*Vanilla planifolia* Jacks. ex Andrews) con relación a su edad a la cosecha

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Abstract: The fruit maturity index of *Vanilla planifolia* Jacks. ex Andrews is considered important to obtain high vanilla quality. In Mexico, the harvest is carried out when the distal part of the fruit turns yellow, but there is no evidence of the benefits of this practice. This research aimed to evaluate the quality of vanilla fruit at 224, 252 and 273 days after pollination, and its relationship with the aroma profile of cured vanilla. *Vanilla planifolia* flowers were manually pollinated in a commercial plantation and the fruit were harvested on each corresponding date. The evaluated variables were moisture, dry matter and content of sugars (glucose, fructose and sucrose), and in the cured fruit they were vanillin, *p*-hydroxybenzoic acid, vanillic acid and *p*-hydroxybenzaldehyde. The results showed that the dry matter content and the sugar concentration of the green fruit of 252 days were significantly lower (14.92 and 10.6 %, respectively) than in the rest of the treatments. Likewise, in cured fruit of 252 days, the content of *p*-hydroxybenzoic acid, vanillic acid and *p*-hydroxybenzaldehyde was significantly lower (103, 855 and 1434 $mg \cdot kg^{-1}$, respectively) than those of 224 days of age (148, 1132 and 2035 $mg \cdot kg^{-1}$, respectively). The fruit of 252 days had a lower quality, possibly because their harvest coincided with the coldest month, which could affect the accumulation of dry matter and aromatic compounds.

Keywords: *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxybenzaldehyde, vanillin, harvest index.

Resumen: El índice de madurez del fruto de *Vanilla planifolia* Jacks. ex Andrews se considera importante para obtener vainilla de calidad. En México, la cosecha se realiza cuando la parte distal del fruto se torna amarilla, pero no hay evidencia de los beneficios de esta práctica. El objetivo de este trabajo fue evaluar la calidad del fruto de vainilla a los 224, 252 y 273 días de edad, y su relación con el perfil de aroma de la vainilla beneficiada. Las flores de *Vanilla planifolia* se polinizaron manualmente en una plantación comercial y los frutos se cosecharon en cada fecha correspondiente. Las variables evaluadas fueron humedad, materia seca y contenido de azúcares (glucosa, fructosa y sacarosa), y en fruto beneficiado fueron vainillina, ácido *p*-hidroxibenzoico, ácido vainillínico y *p*-

hidroxibenzaldehído. Los resultados mostraron que el contenido de materia seca y la concentración de azúcares de los frutos verdes de 252 días fueron significativamente menor (14.92 y 10.6 %, respectivamente) que el resto de los tratamientos. Asimismo, en frutos beneficiados de 252 días, el contenido de ácido *p*-hidroxibenzoico, ácido vainillínico y *p*-hidroxibenzaldehído fue significativamente menor (103, 855 y 1434 mg $\cdot kg^{-1}$, respectivamente) que los de 224 días de edad (148, 1132 y 2035 mg $\cdot kg^{-1}$, respectivamente). Los frutos de 252 días tuvieron una calidad inferior, posiblemente porque su cosecha coincidió con el mes más frío, lo cual pudo afectar la acumulación de materia seca y de compuestos aromáticos.

Palabras clave: ácido p-hidroxibenzoico, ácido vainillínico, p-hidroxibenzaldehído, vainillina, índice de cosecha.

Introduction

Vanilla *(Vanilla planifolia* Jacks. ex Andrews) is one of the three species of the genus *Vanilla* that are grown commercially, accounting for 98 % of the world's commercial production, and is the most important due to the organoleptic properties of its fruit (Besse et al., 2004).

Vanilla fruit when harvested lacks aroma, so it must undergo curing to acquire aroma, brightness, color and texture. In general, this process consists of four steps: scalding, sunning/sweating, drying and conditioning (Odoux & Grisoni, 2011). According to Dunphy and Bala (2011), the main elements that define the quality of the cured fruit are: a) the fruit's genetic profile, b) geographical origin, soil, climate and growing conditions (irrigation and nutrition), c) stage of maturity at harvest, d) conditions of the curing process, and e) balance between the aromatic compounds, specifically between the content of vanillin and that of minor compounds (*p*-hydroxybenzoic acid, vanillic acid and *p*-hydroxybenzaldehyde) (van Dyk, Barry-McGlasson, Williams, & Gair, 2010). The combination of these elements helps forge the vanilla's organoleptic characteristics.

The vanilla's green fruit contain different precursors of the aroma; glucovanillin is one of the most important and it accumulates in the fruit from 15 to 30 weeks after pollination (Havkin-Frenkel, Podstolski, Witkowska, Molecki, & Mikolajczyk, 1999). Traditionally, producers carry out the harvest when the distal part of the fruit turns yellow, which they consider as an adequate harvest index. However, there are no studies that show that the quality of the fruit harvested under this index is superior, nor has the influence of age at harvest on the organoleptic quality of cured vanilla fruit been proven.

Therefore, this research aimed to evaluate the quality of vanilla fruit at 224, 252 and 273 days of age (32, 36 and 39 weeks, respectively), and its relationship with the aroma profile of cured vanilla.

Materials and methods

The commercial vanilla plantation studied in this work is located in the town of Puntilla Aldama, belonging to the municipality of San Rafael, Veracruz, Mexico (20° 14' 4.49'' North latitude and 96° 54' 13.75'' West longitude, at 12 masl). The average temperature of this locality is 22.5 °C and its relative humidity is 90 % (Servicio Meteorológico Nacional [SMN], 2017). The crop was established in the year 2000 under the shade mesh system with living tutors of Malabar chestnut (*Pachira aquatica*) and flame coral tree (*Erythrina coralloides*), in a luvisol-type soil. Among the main management activities, frequent pruning was done to the tutor to control the percentage of shade and the channeling of the vanilla on the tutor's branches, limiting the height of the binomial to two meters. Additionally, temperature data were taken from the Martinez de la Torre weather station (DGE)-VER, located 24.8 km from the plantation.

During the flowering period, between April and May 2015, one or two flowers per healthy *V. planifolia* plant were selected and manually pollinated, giving a total of 100 flowers. The three treatments were defined by the age of the fruit at harvest, namely 224, 252 and 273 days after pollination (dap) (32, 36 and 39 weeks, respectively), for which a completely randomized design was established.

The harvest was carried out during December 2015 and January-February 2016. At 273 dap, most of the fruit showed yellowing in the distal part (Figure 1). On the other hand, vanilla fruit were collected as a reference, where the color change in the distal part of the fruit was taken as the harvest index.

Figure 1
Fruit of *Vanilla planifolia* Jacks. ex Andrews harvested at a) 224, b) 252 and c) 273 days after pollination.

For each harvest date 30 fruit were taken for curing, and 10 more to perform quality evaluations in green state. The average weight of each green fruit was 9 to 15 g, with a size of 15 to 20 cm. The curing process of all the fruit, including the reference ones, was carried out in the "Beneficio la Alternativa" in the town of Primero de Mayo, Papantla, Veracruz.

Curing begins with the killing of the vanilla fruit (immersion in 70 °C water for 5 to 6 seconds). Afterwards, the fruit are accommodated in wooden boxes and covered to maintain the high temperature (45 to 65 °C) for 12 h; this activity is called sweating and provides the conditions for the production of aromatic compounds. After the sweating, the fruit are exposed to the sun until reaching 45 °C, and again they are placed in the drawers so that they sweat. This cyclic operation is repeated between 20 and 30 times, until obtaining an entire cured fruit without physical damage that is flexible, reddish brown to bright dark brown, with 25 to

30 % moisture and a pleasant aroma. Finally, the cured vanilla is stored in plastic bags until marketing (Mariezcurrena, Zavaleta, Waliszewski, & Sánchez, 2008).

Variables evaluated

The variables evaluated in all the fruit were: dry matter (%), moisture (%) and total soluble sugars (%; glucose, fructose and sucrose); additionally, in cured fruit, the concentration of the four main aromatic compounds (*p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, vanillin and vanillic acid) was measured, expressed as mg $\cdot$kg^{-1}.

Dry matter (DM) and moisture (M): 500 mg of fruit were weighed and placed in trays inside an oven (Lab-Line Imperial®, AM, Inc, USA) with forced air at 80 °C for 48 h, until reaching constant weight. Subsequently, with the moisture percentage, the DM content was calculated.

Total soluble sugars (TSS): They were quantified with the methodology of Mustafa, Mustafa, Mustafa, and Mhemet (2003), with some modifications. A portion of the middle part of each fruit was taken, finely ground with a loading mill (25,000-rpm Krups Gx4100), 100 mg were weighed and 3 mL of 80 % (v/v) ethanol were added; the sample was incubated in a water bath at 80 °C for 10 min. This process was repeated successively five times, and the obtained extracts were placed in an oven at 55 °C for 24 h until dry. Subsequently, the residue was resuspended with 2 mL of distilled water. This sample was stored at -20 °C until analysis.

To quantify the sugars, 1 mL of each extract was taken and filtered in cleaning cartridges (Chromabond C18ec, 3 mL $\cdot$500 mg^{-1}, 60Å, 45 µm). For this, the cartridge was conditioned with 6 mL of methanol and then 6 mL of HPLC-grade water; subsequently, 1 mL of sample was passed through it and a washing was performed with 3 mL of HPLC-grade water to ensure the elution of all sugars. Both filtrates were mixed and brought to a volume of 5 mL; from here, 1 mL was taken and filtered with an acrodisk (Titan, 0.45 µm). The filtrate was placed in a vial and analyzed by HPLC (High Performance Liquid Chromatography system, Series 200, Perkin Elmer™) with an autosampler and refractive index detector. A Pinnacle II Amino column (Restek™, 150 x 4.6 mm, 5 mm) was used, and the mobile phase was an acetonitrile:water (80:20, v/v) solution with a 14-min run time.

For the calibration curves, 0.05 g of fructose, glucose and sucrose at 99.5 % (all from Sigma-Aldrich, USA) were diluted separately in 10 mL of methanol:water (1:9, v/v) and the corresponding dilutions (0.15 to 5 mg $\cdot$mL^{-1}) were performed. Chromatograph conditions were 35 °C, flow of 1 mL $\cdot$min^{-1} and injection volume of 10 µL. The results were reported as a percentage of sugars on a dry basis.

Aromatic compounds: The *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, vanillic acid and vanillin were quantified according to the method of Cicchetti and Chaintreau (2009), with some modifications. Then 0.05 g of milled sample were weighed and 18 µL of ethanol-distilled water (1:1) solution (HPLC-grade anhydrous absolute ethyl alcohol) were added; this solution was prepared 24 h beforehand and kept refrigerated (4 °C). Subsequently, the mixture was stirred for 30 min on a digital hotplate (6 stir, Thermo Scientific™, Cimarec™, USA) and again refrigerated for 24 h. After this time, the sample was stirred for 5 min and 1 mL was filtered with an acrodisc (Titan, 0.45 µm). The filtrate was placed in a 2-mL vial and taken to HPLC with a UV detector at 254 nm. For the analysis, a Silica C18 column (ACE®, 5 µm, 240 x 4.6 mm) was used, with a 20-min run time and a 10-µL injection volume.

For quantification, standard solutions of *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde and vanillic acid (4-hydroxy-3-methoxybenzoic acid) were prepared from 0.1 to 10 µg ·mL^{-1}, and vanillin (3 methoxy-4-hydroxybenzaldehyde) (Sigma-Aldrich®, USA) from 0.5 to 45 µg ·mL^{-1}.

Statistical analysis

With the data obtained, an analysis of variance and Tukey's range test ($P \leq 0.05$) were performed. Additionally, a Pearson correlation analysis was carried out between the green fruit DM and the four aromatic compounds. For all analyses the Statistical Analysis System package (SAS Institute, 2002) was used.

Results and discussion

Vanilla fruit reaches its final size between 10 and 15 weeks after pollination, and no significant changes in its appearance are observed until the yellowing and dehiscence of the distal part begin (van Dyk et al., 2014). Therefore, producers cannot standardize a harvest index, not until they observe the change to yellow, which causes the aromatic quality of the cured vanilla to be very variable.

The DM content and moisture showed notable differences in green fruit; for example, the DM content was significantly higher ($P \leq 0.05$) in fruit of 224 and 273 days compared to those of 252 dap (Table 1). It is interesting to note that the DM content in the producer's reference fruit is similar to that of 224 days. Although the age of the fruit could be considered as a reliable parameter to determine a harvest index, climate changes in the area can affect DM reserves, which may explain, in part, why producers traditionally avoid harvesting in January.

Table 1
Percentage of dry matter (DM) and moisture (M) in green and cured fruit of *Vanilla planifolia* Jacks. ex Andrews of different ages.

Fruit age (days after pollination)/ Edad de fruto (días después de la polinización)	Green fruit/Fruto verde		Cured fruit/Fruto beneficiado	
	MS	H	MS	H
	(%)			
224	16.84 a[z]	83.15 b	71.88 a	28.05 a
252	14.92 b	85.07 a	72.23 a	27.76 a
273	17.86 a	82.14 b	70.37 a	29.63 a
R[1]	16.71 a	83.27 b	71.95 a	28.12 a
CV (%)	8.77	1.74	1.81	4.58
LSD/DMSH	1.27	1.27	2.35	2.35

According to the SMN (2017), during the harvest period of fruit of 224 days, the average temperature was 21.2 °C (December 2015), while when the fruit of 252 days were harvested, the temperature dropped drastically to 17 °C (January 2016), and for February the temperature increased again. In addition, during this period there was little precipitation, with a monthly average, from December to February, of 68.4 and 95.0 mm for the years 2013 to 2015, respectively, which could cause stress in the fruit, by reducing its reserves.

Van Dyk et al. (2014) evaluated vanilla fruit and found that at 105 dap the DM content was 10 % and the vanillin content 0 %, while at 280 dap the values were 18 % and 1.5 %, respectively. The above suggests that at a higher DM content there will be more reserves that will contribute to the aroma and flavor development of the cured fruit. It is important to note that even though the moisture content is reduced by more than 50 % from green to cured fruit, the differences that exist between the DM content and moisture among fruit ages at harvest disappear in the cured vanilla.

The purpose of curing is to create conditions for the substrate-enzyme interaction for the biosynthesis of vanillin and other aromatic compounds, as well as the dehydration of the fruit as a method of conserving and retaining the aromatic compounds formed (Frenkel, Ranadive, Vázquez, & Havkin-Frenkel, 2011). According to NOM-182-SCFI-2011 (Secretaría de Economía, 2011), the moisture of the cured vanilla should be between 25 and 38 %, a range in which the evaluated treatments are found, without significant statistical difference ($P > 0.05$) (Table 1).

Generally, carbohydrates are the most abundant constituents in fruits after water. In green vanilla, the fruit of 224 dap had a significantly higher concentration (66 %, $P \leq 0.05$) of these compounds, compared to those of 252 dap, while this difference was lower in the fruit of 273 dap and the producer's reference ones (Table 2). The highest coefficients of variation were reported in the fructose and glucose content of green fruit, perhaps due to the combination of the high variability between the fruit and the low concentration of sugars. The rest of the variables had a coefficient of variation of less than 20 %, which indicates the reliability of the experimental data.

Table 2

Content of fructose, glucose, sucrose and total soluble sugars in green and cured fruit of *Vanilla planifolia* Jacks. ex Andrews of different ages.

Fruit age (days after pollination)/ Edad del frutos (días después de la polinización)	Green fruit/Fruto verde				Cured fruit/Fruto beneficiado			
	F[1]	G	S	TSS/AST	F	G	S	TSS/AST
	(%)							
224	0.81 a[z]	2.27 a	12.93 a	16.02 a	3.04 a	9.17 a	7.04 a	19.26 a
252	0.55 bc	0.93 c	9.12 b	10.60 d	2.77 a	8.91 a	7.13 a	18.82 a
273	0.48 c	0.98 c	13.19 a	14.67 b	2.14 a	8.06 a	6.62 a	16.85 b
R	0.66 ab	1.34 b	9.75 b	11.76 c	1.16 b	6.43 b	5.31 b	12.91 c
CV (%)	27.00	21.95	10.69	8.41	13.06	9.28	9.62	6.04
LSD/DMSH	0.14	0.26	1.05	0.98	0.53	1.36	1.13	1.85

It is possible that the drop in temperature and the low rainfall, during the harvest at 252 dap, have influenced the reduction of sugar reserves as a result of stress. Despite these differences, it is notable that the sucrose content, in green fruit, represented, in the three stages of maturity, more than 80 % of the total sugars, followed by glucose and fructose (Table 2). These results coincide with those reported by Palama et al. (2009), who found that three-month-old vanilla fruit accumulate more glucose, while eight-month-old ones contain more sucrose, as a reserve substrate.

In cured fruit, a significant decrease in sucrose content can be observed, with the consequent increase in fructose and glucose, without significant statistical differences ($P > 0.05$) attributable to the age of the fruit (Table 2). This is because during curing there are different enzymatic and non-enzymatic reactions, accelerated by the high humidity and temperature (45 to 65 °C). These reactions catalyze the hydrolysis and conversion of glucose and fructose. In addition, it is important to consider that the green fruit has certain starch reserves, which also contribute to increasing glucose and TSS (Havkin-Frenkel et al., 2004; Röling et al., 2001). On the other hand, the reference fruit (without age control) are those that have a significantly lower sugar content compared to the cured fruit (Table 2).

Although the aroma is the most important parameter in the marketing of cured vanilla, it is not the decisive factor to evaluate the quality. The acceptance and price of vanilla depends on the aromatic balance, size, appearance, color and moisture of the fruit (Secretaría de Economía, 2011; Sinha, Sharma & Sharma, 2008). Its aroma is a mixture of more than 200 volatile compounds, which include hydrocarbons, alcohols, aldehydes, ketones, esters, lactones, acids, terpenoids, ethers, and phenolic and carbonyl compounds. Of these compounds, vanillin (3-methoxy-4-hydroxybenzaldehyde), as the most abundant compound, *p*-hydroxybenzaldehyde, vanillic acid (4-hydroxy-3-methoxybenzoic acid) and *p*-hydroxybenzoic acid are recognized as indicators of commercial quality due to their high concentrations and qualitative and quantitative participation in the aroma, since they represent 97 % of the total (Kumar-Keekan et al., 2010; Odoux & Grisoni, 2011).

The aroma profile, of the four most abundant compounds in cured fruit, showed significant statistical differences ($P \leq 0.05$), with the exception of vanillin. The cured fruit of 252 dap had the lowest concentrations of *p*-hydroxybenzoic acid, vanillic acid and *p*-hydroxybenzaldehyde (Table 3), which may be the consequence of a lower DM content (Table 1) and which reflects a relationship with the minor compounds.

Table 3

Concentration of the aromatic compounds in cured fruit of *Vanilla planifolia* Jacks. ex Andrews of different ages.

Fruit age (days after pollination)/ Edad de fruto (días después de la polinización)	C1[1]	C2	C3	C4	∑CM/C4 (%)
	(mg·kg $^{-1}$ DM) / (mg·kg $^{-1}$ MS)				
224	148.53 b[z]	1,132.45 a	2,035.60 a	22,746 ab	14.0 b
252	103.03 c	855.77 b	1,434.60 b	22,231 ab	10.4 c
273	135.43 bc	1,297.50 a	1,980.00 a	25,043 a	13.2 b
R	226.74 a	1,197.44 a	2,130.60 a	20,978 b	16.6 a
CV (%)	14.55	12.2	9.7	9.9	9.2
LSD/DMSH	40.41	247.8	333.4	4090.20	0.01
NOM-182-SCFI-2011	58-100	411 – 861	219 - 498	Minimum /Mínimo 20,000	
Toth, Lee, Havkin-Frenkel, Belanger, and Hartman (2011)	218-255	887-1315	635-1549	9296-22,757	

It is notable that the intervals established in NOM-182-SCFI-2011 (Table 3) are not similar to the data obtained, this discrepancy being more evident in *p*-hydroxybenzaldehyde, with values up to five times higher than those established in the standard. In the same way, Toth et al. (2011) reported high variability in the content of minor compounds in Mexican vanilla and attributed it to the fact they are transition compounds. The above does not occur with vanillin because it is a final compound of biosynthesis, so its content is not very variable.

The analysis between the DM of the green fruit and the aroma compounds showed a significant correlation between DM and *p*-hydroxybenzaldehyde ($P \leq 0.05$), and a highly significant one ($P \leq 0.0001$) between the DM and the vanillin concentration (Table 4). Similarly, in vanilla fruit harvested in the previous cycle (year 2014), a significant correlation ($P \leq 0.01$) of 0.783 was observed between the DM content and vanillin (data not reported), which supports the argument that DM accumulation can be proposed as a reliable harvest index, rather than changes in fruit color or phenological age. In this way, producers could record the DM of the fruit before 32 weeks, to ensure a higher vanillin content and better aromatic balance due to the accumulation of minor compounds (van Dyk et al., 2014).

Table 4

Pearson correlation matrix of dry matter and content of aromatic compounds in cured vanilla fruit.

Compound/ Compuesto	DM[1]/MS[1]	C1	C2	C3
C1	0.005 ns	-	-	-
C2	0.384 ns	0.376 ns	-	-
C3	0.500*	0.564*	0.650*	-
C4	0.773***	-0.264 ns	0.243 ns	0.350 ns

Conclusions

Although curing defines the final quality of vanilla, the results obtained show that the percentage dry matter has a high correlation with the content of vanillin, the main compound of the aroma. In addition, the lower content of dry matter and total soluble sugars in green fruit influences the concentration of minor compounds (*p*-hydroxybenzoic acid, vanillic acid and *p*-hydroxybenzaldehyde), which was significantly lower in 252-day-old fruit, harvested when the temperature dropped; the above resulted in cured vanilla having a lower aromatic balance.

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References

Besse, P., Da Silva, D., Bory, S., Grisoni, M., Le Bellec, F., & Duval, M. F. (2004). Rapd genetic diversity in cultivated vanilla: *Vanilla planifolia*, and relationships with *V. tahitensis* and *V. pompona*. *Plant Science*, *167*(2), 379-385. doi: 10.1016/j.plantsci.2004.04.007

Cicchetti, E., & Chaintreau, A. (2009). Comparison of extraction techniques and modeling of accelerated solvent extraction for the authentication of natural Vanilla flavors. *Journal of Separation Science*, *32*(11), 1957-64. doi: 10.1002/jssc.200800650

Dunphy, P., & Bala, K. (2011). Green vanilla bean quality. *Perfumer & Flavorist*, *36*, 38-46. Retrieved from https://www.perfumerflavorist.com/fragrance/rawmaterials/natural/116757729.html

Frenkel, C., Ranadive, A. S., Vázquez, J. T., & Havkin-Frenkel, D. (2011). Curing of vanilla. *In*: Havkin-Frenkel, D., & Belanger, F. C. (Eds), *Handbook of vanilla science and technology* (pp. 79-106). USA: Wiley-Blackwell Pub.

Havkin-Frenkel, D., French, J. C., Graft, N. M., Joel, D. J., Park, F. E., & Frenkel, C. (2004). Interrelation of curing and botany in vanilla (*Vanilla planifolia*) bean. *Acta Horticulturae*, *629*, 93-102. doi: 10.17660/ActaHortic.2004.629.12

Havkin-Frenkel, D., Podstolski, A., Witkowska, E., Molecki, P., & Mikolajczyk, M. (1999) Vanillin biosynthetic pathways, an overview, *In*: Fu, T. J., Singh, G., & Curtis, W. R. (Eds), *Plant cell and tissue culture for the production of food ingredients* (pp. 35-43). New York, USA: Kluwer Acad. Press / Plenum Publ.

Kumar-Keekan, K., Anantha-Kumar, A., Ahmad, R., Adhikari, S., Variyar, P. S., & Sharma, A. (2010). Effect of gamma-radiation on major aroma compounds and vanillin glucoside of cured vanilla beans (*Vanilla planifolia*). *Food Chemistry*, *122*(3), 841-845. doi: 10.1016/j.foodchem.2010.03.006

Mariezcurrena, M. D., Zavaleta, H. A., Waliszewski, K. N., & Sánchez, V. (2008). The effect of killing conditions on the structural changes in vanilla (*Vanilla planifolia* Andrews) pods during the curing process. *International Journal of Food Science & Technology*, *43*(8), 1111-1365. doi: 10.1111/j.1365-2621.2007.01691.x

Mustafa, K., Mustafa, E., Mustafa, K. U., & Mehmet, A. (2003) Comparison of different extraction and detection methods for sugar using amino-bonded phase HPLC. *Journal of Chromatographic Science*, *41*(6), 331-333. doi: 10.1093/chromsci/41.6.331

Odoux, E., & Grisoni, M. (2011). Vanilla. *Medicinal and Aromatic Plants-Industrial Profiles*. USA: Taylor and Francis Group.

Palama, T. L., Khatib, A., Choi, Y. H., Payet, B., Fock, I., Verpoorte, R., & Kodja, H. (2009). Metabolic changes in different developmental stages of *Vanilla planifolia* pods. *Food Chemistry*, *57*(17), 7651- 7658. doi: 10.1021/jf901508f

Röling, W. F. M., Kerler, J., Braster, M., Apriyantono, A., Stam, H., & van Verseveld, H. M. (2001). Microorganisms with a taste for vanilla: Microbial ecology of traditional Indonesian curing. *Applied and Environmental Microbiology*, *67*(5), 1995-2003. doi: 10.1128/AEM.67.5.1995-2003.2001

Secretaría de Economía. (2011). *Norma Oficial Mexicana (NOM-182-SCFI-2011), Vainilla de Papantla, extractos y derivados-especificaciones, información comercial y métodos de ensayo*. México: Author.

Servicio Meteorológico Nacional (SMN). (2017). Base de datos climática nacional. Sistema de información climática computadorizada (CLICOM). Retrieved June, 2017 from Retrieved June, 2017 from http ://clicom-mex.cicese.mx/mapa.html

Sinha, A. K., Sharma, U. K., & Sharma, N. (2008) A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and others constituents. *International Journal of Food Science and Nutrition*, *59*(4), 299-326. doi: 10.1080/09687630701539350

Statistical Analysis System (SAS Institute). (2002). *SAS/STAT user's guide version 9.0*. Cary: Author.

Toth, S., Lee, K. J., Havkin-Frenkel, D., Belanger, F. C., & Hartman, T. G. (2011). Volatile compounds in vanilla. *In*: Havkin-Frenkel, D., &

Belanger, F. C. (Eds), *Handbook of vanilla science and technology* (pp. 183-219). USA: Wiley-Blackwell Pub .

van Dyk, S, Barry-McGlasson, W., Williams, M., & Gair, C. (2010). Influence of curing procedures on sensory quality of vanilla beans. *Fruits*, *65*(6), 387-399. doi: 10.1051/fruits/2010033

van Dyk, S., Holford, P., Subedi, P., Walsh, K., Williams, M., & McGlasson, B. (2014). Determining the harvest maturity of vanilla beans. *Scientia Horticulturae*, *168*, 249-257. doi: 10.1016/j.scienta.2014.02.002

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