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Influence of Temperature and Sweating Duration on The Quality of Vanilla (Vanilla planifolia Andrews)

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ABSTRACT

Indonesia has significant potential for vanilla production. However, in terms of quality, Indonesian dried vanilla still has a low standard, one of the factors being the suboptimal post-harvest technology, particularly in the sweating process. This research aims to determine the optimal temperature and sweating duration to achieve the best vanilla quality in accordance with the Indonesian National Standard (SNI). After the harvesting process, the vanilla pods were treated by soaking them in warm water at a temperature of 65°C for 3 minutes. After the vanilla pods were wilted, they were wrapped using a combination of a towel cloth-black cloth-burlap. They were then sweated in an incubator at 40°C with 70% RH and 45°C with 70% RH for 2, 4, and 6 days. The observed vanilla quality parameters include weight loss, color, hedonic tests (aroma and color), vanillin content based on the methods of SNI 01-0010-2002 and ash content based on the methods of AOAC. The result shows that the optimal temperature and duration for sweating in an incubator are 40°C and 4 days.

1. INTRODUCTION

Vanilla comes from the seeds of the vanilla fruit, which originates from the vanilla plant (*Vanilla planifolia* Andrews) and belongs to the orchid family. These vanilla seeds have a rich, sweet flavor and a distinctive aroma (Yeh *et al.*, 2021). Vanilla production in Indonesia reaches 2,306 tons per year, supplying 30.3% of the total global production (FAO, 2020). Although Indonesia is one of the leading vanilla producers, it ranks seventh in global vanilla exports, contributing approximately 2.63% to the total world vanilla exports (Kementerian Keuangan, 2023). However, UN Comtrade (2022) recorded a decline in vanilla export value of Indonesia, from US\$ 90,578,913 in 2017 to US\$ 69,609,691 in 2019. According to Fitriani (2023), Nnanyange (2023), Ravier *et al.* (2024), and Lee *et al.* (2024) the low quality of vanilla is caused by several factors, such as agroclimate, cultivation technology, harvesting, and post-harvest technology, particularly the suboptimal sweating process.

Vanilla sweating is a crucial stage in developing the vanilla's unique aroma and flavor. The goal is to induce enzymatic reactions in the vanilla pods, leading to the formation of vanillin and causing the pods to change color to brown and produce oil. A common method used by farmers in the vanilla postharvest process involves wilting the vanilla pods by soaking them in warm water, followed by sweating by placing the soaked vanilla in a styrofoam box wrapped in black cloth for 48 hours. Several studies have been conducted related to the vanilla sweating process, such as Fitriani (2023) conducted a sweating process using a combination of towel, black cloth, and burlap for 48 hours, which resulted in a vanillin content of 0.501%. Meanwhile, after drying with Greenhouse Effect (GHE) Solar Dryer combined with an additional four nights of sweating, the vanillin content increased to 1.377%. In contrast, Reri's (2018) study, where vanilla pods weighing >15 grams were sweated using a combination of black cotton fabric, black

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flannel, and black plastic for 24 hours and then dried at 65°C for 9-12 days, resulted in vanillin content of only around 0.8-0.9%. In these studies, it can be observed that the vanillin content produced after both the sweating and drying processes remained relatively low. This may have occurred due to the lack of proper temperature and relative humidity (RH) control during sweating, which is important as it can affect the final results and quality of the vanilla produced. Therefore, it is necessary to assess sweating methods with controlled temperature and RH to improve the quality of the resulting vanilla. The objective of this research is to determine the appropriate temperature and duration of sweating to improve the quality of vanilla in terms of weight loss, vanillin content, ash content, color, and aroma. This research is expected to provide information as a reference for vanilla farmers, collectors, and processors to carry out proper vanilla curing, ensuring that the post-harvest process is conducted accurately. This, in turn, aims to improve the quality of vanilla pods and add value for vanilla processors or farmers in Indonesia.

2. MATERIALS AND METHODS

2.1. Research Location and Time

This research was conducted from March to July 2024. Vanilla samples were collected from Sinogo, Pagerharjo, Samigaluh, Kulon Progo Regency, Special Region of Yogyakarta, with an optimum harvest age (8-9 months) and pod length of approximately 15 cm. The sweating process, color measurement, weight loss, and hedonic tests (color and aroma) of the vanilla were carried out at the Food and Agricultural Products Processing Engineering Laboratory, Department of Mechanical and Biosystem Engineering, IPB University, Bogor. Ash content was determined at the Center for Research on Biological Resources and Biotechnology, while vanillin content were determined at the Goods Quality Testing Center, Ciracas, East Jakarta.

2.2. Materials and Equipment

The other materials used in this research include 70% alcohol, distilled water, vacuum sealer bags, labels, measuring plastic, small towels, burlap sacks, black cotton cloth, gloves, and cloths. The equipment used in this research includes an incubator (Eyela KCL-2000W) with the serial number 11001572, AC220V, 50 Hz, and 1.5 kVA; a chroma meter; a vacuum sealer; spray bottles; a thermometer; a stove; a pot; a strainer; an analytical balance type PA224C Ohaus; and a styrofoam box measuring 47 x 31 x 28 cm.

2.3. Research Procedure

The first step in postharvest processing of vanilla was harvesting, where it was essential to pay attention to the characteristics and length of the pods that were suitable for harvesting according to the SNI (Indonesian National Standard) to ensure good final physicochemical quality. After harvesting, the vanilla pods killed by soaking them in warm water at 65°C for 3 minutes. Then, the vanilla pods were wrapped with a combination of a towel cloth-black cloth-burlap and sweated using an incubator at 40°C with 70% RH and 45°C with 70% RH for 2, 4, and 6 days. After that, the vanilla's physicochemical properties were analyzed, including color, hedonic test (color and aroma), weight loss, vanillin, and ash content.

2.4. Research Design

The research design used was a Factorial Completely Randomized Design (CRD) with 2 factors, and data collection was carried out in 3 replications for each treatment combination. The first factor was the sweating temperature treatment (T) with 2 levels: 40°C RH 70% (T1) and 45°C RH 70% (T2). The second factor was the sweating duration time (D) with 3 levels: 2 days (D1), 4 days (D2), and 6 days (D3). A control treatment was also applied for comparison, where the vanilla pods were sweated using the conventional method practiced by farmers. After being wilted, the pods were wrapped with a combination of towel cloth-black cloth-burlap, then placed in an airtight container (a styrofoam box measuring 47 x 31 x 28 cm) and sweated for 2 days. To observe the effects of the treatments, analysis of variance (ANOVA) was conducted at a 5% significance level, and if differences were found, they will be followed by Duncan's Multiple Range Test (DMRT) using statistical analysis software Minitab 19 and the Statistical Analysis System (SAS).

2.5. Quality Observation

2.5.1. Physical Characterization of Vanilla

2.5.1.1. Weight Loss

Weight loss measurement was conducted by calculating the percentage of weight reduction of the sample during the sweating process. The weight measurement was carried out using a digital scale. Weight loss was calculated using Equation (1).

$$W_l = \frac{W_0 - W_1}{W_0} \times 100\% \tag{1}$$

where W_l is the weight loss (%), W_0 is the initial weight (g), and W_1 is the final weight after the process (g).

2.5.1.2. Color

Color was measured using a chroma meter with the model CR-410 Head, display range Y: 0.01% to 160.00% (reflectance), battery performance approximately 800 measurements, repeatability within ΔE^* ab 0.07 standard deviation, inter-instrument agreement ΔE^* ab: within 0.8, observer 2°, illuminant *C, D65, and using $L^*a^*b^*$ system. The L value indicates the level of brightness, ranging from 0 (dark/black) to 100 (light/white). The parameter a refers to the color range from green (negative a) to red (positive a), while the parameter b measures the color from blue (negative b) to yellow (positive b). The analysis procedure involved cutting the sample into several parts, which were then arranged in a test sample container that matched the chromameter measurement area. Subsequently, the analysis was performed with three repeated measurements. Before used, the chromameter was calibrated with the white color standard provided with the device (Fitriani, 2023).

2.5.2. Hedonic Test

Hedonic test was conducted by 25 semi-trained panelists who were asked to evaluate the available vanilla samples based on visual inspection and by smelling the product (subjective assessment). The hedonic test scores used a scale of 1 to 5, ranging from "strongly dislike" to "strongly like". Panelists tended to give higher hedonic scores when the vanilla pods appeared darker brown and oily, accompanied by a strong and pleasant characteristic vanilla aroma. The obtained scores were then analyzed statistically using ANOVA, and further testing could be carried out using the DMRT (Garnida, 2020).

2.5.3. Chemical Characterization

2.5.3.1. Vanillin Content

The vanillin content was determined in accordance with the AOAC (1981) method through the following steps.

a) Sample preparation

Vanilla pods were cut and weighed to 5 grams using an analytical balance before being soaked. In the first soaking, the vanilla pods were soaked overnight in 35 ml of alcohol in a 100 ml Erlenmeyer flask, which was then sealed. The alcohol used in the first soaking was then filtered through filter paper, rinsed with 5 ml of alcohol, and transferred into a 100 ml volumetric flask. This filtered alcohol was reserved for use during the second soaking. The vanilla pods, which had been crushed using a mortar, were then soaked again overnight in another 35 ml of alcohol. The alcohol from the second soaking was combined with the alcohol from the first soaking by passing it through the same filter paper used earlier. Subsequently, the vanilla pods were rinsed with alcohol, and the rinsing alcohol was used to adjust the volume of the soaking alcohol to 100 ml, resulting in the sample solution.

b) Preparation of vanillin standard

A total of 0.10 grams of vanillin was weighed using an analytical balance, then dissolved in 5 ml of 95% p.a. alcohol in a 100 ml volumetric flask. The solution was then diluted with distilled water to the mark. This solution is referred to as Solution A. Then, from Solution A, 5 ml, 10 ml, and 15 ml were pipetted and each was diluted with distilled water to a final volume of 250 ml. This process produced three types of Solution B. From each Solution B, 10 ml was

transferred into a 100 ml volumetric flask. To each flask, 2 ml of 0.10 N NaOH solution was added, followed by dilution with distilled water to the mark. This resulted in solutions with approximate concentrations of 2 ppm, 4 ppm, and 6 ppm, respectively. Next, the blank solution was prepared following the same steps as above, except without the addition of 2 ml of 0.10 N NaOH.

c) Determination of absorbance

A total of 10 ml of the sample solution was taken and diluted with distilled water to the mark. The resulting solution is referred to as Solution 1. From Solution 1, 10 ml was pipetted and further diluted with distilled water to a total volume of 250 ml. In another 250 ml volumetric flask, 10 ml of Solution 1 was taken. Then, 2 ml of 0.1 N NaOH solution was added, and the mixture was diluted with distilled water to the mark. The absorbance of the solution was measured at a wavelength of 348 nm using the blank solution as a reference. The vanillin content could be calculated using the formula in Equation (2).

Vanillin content (% d.b.) =
$$\frac{X \times 5}{M} \times \frac{100}{100-H}$$
 (2)

where X is concentration of the solution (ppm), M is mass of the sample (g), and H is moisture content of vanilla (%).

2.5.3.2. Ash Content

The steps for determining the ash content of a product sample according to AOAC (2005) were as follows: the crucible was placed in an oven at 105°C for 1 hour, then cooled in a desiccator for 15 minutes and weighed. A total of 1.5–2 grams of the sample was added to the crucible, which was then placed in a muffle furnace at 600°C for 3 hours. The sample was cooled outside the furnace until it reached a temperature of approximately 120°C, then placed in a desiccator. Finally, the crucible and ash were weighed until a constant weight was obtained. The ash content was then calculated using the formula in Equation (3).

$$Ash\ content = \frac{ash\ weight}{sample\ weight} \times 100\% \tag{3}$$

3. RESULTS AND DISCUSSION

3.1. Physical Characterization of Vanilla

The following are the results of the physico-chemical measurements of vanilla after the sweating process.

Table 1. Results of physico-chemical measurements of vanilla after sweating

Parameter	T_1D_1	T_2D_1	T_1D_2	T_2D_2	T_1D_3	T_2D_3
Weight loss (%)	6.85 ± 0.21^{d}	7.53 ± 0.41^{d}	11.94 ± 0.13^{c}	19.42 ± 0.91^{b}	20.89 ± 0.61^{b}	31.51 ± 1.10^{a}
Color L*	25.70 ± 1.55^{bc}	29.35 ± 2.04^a	25.22 ± 1.13^{bc}	28.48 ± 0.68^{ab}	22.68 ± 0.67^{c}	28.09 ± 0.34^{ab}
a*	4.79 ± 0.24^{ab}	4.49 ± 0.21^{ab}	5.02 ± 0.60^a	4.79 ± 0.23^{ab}	4.05 ± 0.40^b	5.18 ± 0.18^a
b^*	1.62 ± 0.38^{bc}	$0.35\pm0.65^{\rm c}$	1.93 ± 0.32^{bc}	$4.72\pm1.74^{\rm a}$	1.73 ± 1.05^{bc}	2.98 ± 0.10^{ab}
Vanillin content (%)	1.40 ± 0.02^a	1.47 ± 0.05^a	$1.40\pm0.05^{\rm a}$	$1.41\pm0.13^{\rm a}$	1.22 ± 0.01^{b}	0.96 ± 0.03^{c}
Ash content (%)	1.10 ± 0^{c}	1.55 ± 0.02^{b}	1.09 ± 0.02^{c}	1.72 ± 0.31^{b}	1.40 ± 0.03^{bc}	2.09 ± 0.02^a

3.1.1. Weight Loss

Results from analysis of variance (ANOVA) reveal that single factors temperature (T) and sweating duration (D) as well as their interaction are very significant for the weight loss of the vanilla pods with significance values (*p*-value) of 0.000. The effect of treatment on the weight loss is presented in Figure 1. Vanilla pods sweated at a temperature of 45°C result in significant weight loss compared to those sweated at 40°C and no treatment (control). This occur because at 45°C, the water in the pod tissues evaporates more quickly than that at 40°C. This faster evaporation leads to greater weight loss, as most of the vanilla pods consist of water. Additionally, higher temperatures may cause some organic components in the vanilla pods, such as sugars, organic acids, and aromatic compounds, to degrade or convert into simpler forms or gases. This degradation process can also contribute to the reduction in the total mass of the pods.

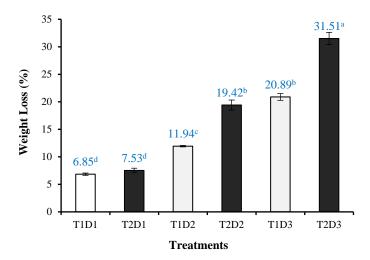


Figure 1. Comparison of weight loss at different treatments

This is consistent with research by Warji et al. (2023) that the high weight loss occurs due to the increased enzyme activity that degrades the components within the vanilla. Additionally, the longer the sweating time, the greater the weight loss of the vanilla due to the evaporation of free water from the material. Table 1 also shows that the weight loss in vanilla pods under the control treatment is relatively low compared to the other two treatments. This is because the sweating temperature used in the control treatment was only adjusted to the room temperature of the laboratory during the study.

3.1.2. Color

Figure 2 shows visual appearance of vanilla pods resulted from different treatments as well as control (no treatment). The results of color measurements for vanilla pods are summarized in Table 2. The analysis of variance (ANOVA) revealed that interaction of temperature (T) and sweating duration (D) is not significant (p-value = 0.300), but single factors temperature (T) and sweating duration (D) are significant to the color lightness (L^*) value of vanilla pods with p-value 0.000 and 0.028, respectively. The results of the L^* value measurements is presented in Table 2. From Table 2 and Figure 3, it can be seen that vanilla pods sweated at 40°C have more intense and oilier surface color. That may occur because, at 40°C, pigment degradation happens more slowly compared to higher temperatures, so the color of the pods remains more intense. Additionally, vanilla pods contain essential oils responsible for their aroma and quality. At 40°C, these essential oils tend to remain trapped within and on the surface of the pods due to slower evaporation. In contrast, at 45°C, these oils evaporate more quickly, making the surface of the pods appear less oily. Furthermore from Figure 2, vanilla pods in the control treatment still appear yellowish-green on day 2 of sweating. That indicates that the control treatment is less effective in changing the vanilla pods color to a dark brown or black.



Figure 2. Visual appearance of vanilla pods: (a) T1D1, (b) T1D2, (c) T1D3, (d) T2D1, (e) T2D2, (f) T2D3, and (g) Control

Table 2. Effect of treatments on the color lightness (L^*) of vanilla pods.

	D1 (2 days)	D1 (4 days)	D1 (6 days)	Average
T1 (40°C)	25.70	25.22	22.68	24.53 B
T2 (45°C)	29.35	28.48	28.09	28.64 A
Average	27.53 A	26.85 AB	25.39 B	

3.2. Hedonic Test for Color and Aroma

Table 3 shows the hedonic test scores for parameters color of vanilla pods and aroma as a result of temperature and sweating duration treatments. The analysis of variance (ANOVA) results showed that interaction of temperature (T) and sweating duration (D) as well as both single factors influence significantly on the hedonic score of vanilla pod color with *p*-value of 0.000. Referring to Table 3, it can be seen that vanilla pods sweated at 40°C (T1) have a higher average hedonic score compared to vanilla pods sweated at 45°C (T2) or under the control treatment. That indicates that the color of vanilla pods sweated at 40°C is preferred by panelists more than those sweated at 45°C or under the control treatment. At 40°C, both enzymatic and non-enzymatic processes involved in color darkening occur effectively. The formation of melanin pigments, which contribute to the desired brown color, happens more uniformly, resulting in a more intense color favored by the panelists. Furthermore, as shown in Figure 3, vanilla pods under the control treatment still appear yellowish-green on day 2 of sweating. That makes the hedonic score of the control treatment lower than the other two treatments.

The interaction of temperature (T) and sweating duration (D) and both single factors also significantly affect the hedonic score for aroma attribute. The analysis of variance (ANOVA) results show that the hedonic score for aroma of vanilla pods differed significantly between treatments with p-value of 0.000. Referring to Table 3, it can be seen that vanilla pods sweated at both 40°C and 45°C are most favored for their aroma by panelists on day 4 of sweating. On day 4 of sweating, vanilla pods sweated at 45°C are preferred for their aroma by panelists more than those sweated at 40°C. That may be due to the faster and more intense evaporation of volatile compounds contributing to the vanilla aroma at 45°C. The quicker evaporation can enhance the aroma intensity in a short period, resulting in a strong and preferred aroma. Additionally, the Maillard reaction, which is the reaction between amino acids and sugars, occurs more quickly at higher temperatures. This reaction often contributes to a richer and more complex aroma, which is favored by panelists. According to Little (2015), Adawiyah et al. (2020), and Antonio-Gutiérrez et al. (2023) during the sweating and drying processes, vanilla beans experience an increase in flavor and complex aroma as a result of the natural enzymatic action of β-glucosidase on glycosides. In this study, the complex aroma was described by the panelists as sweet, alcoholic, plant-like, and woody. As stated by Havkin-Frenkel & Belanger (2018) and Manyatsi et al. (2024), the conditioned pods produce improved flavors of sweet, flowery, smoked, spicy, sweet, and prune-to-raisin vanilla beans. In addition, Brunschwig et al. (2015) states that Vanilla planifolia showed stronger phenolic, woody, smoky notes due to guaiacol, creosol and phenol, which were found to be biomarkers of the species.

Table 3. Hedonic test of color and aroma of vanilla pods

Parameter	T_1D_1	T_2D_1	T_1D_2	T_2D_2	T_1D_3	T_2D_3
Hedonic test of color	3.72 ± 1.01^{e}	3.39 ± 0.59^{c}	4.12 ± 0.72^{d}	3.77 ± 0.69^{b}	$4.20\pm0.85^{\rm f}$	3.99 ± 0.85^a
	(Like)	(Like)	(Like)	(Like)	(Like)	(Like)
Hedonic test of aroma	3.00 ± 1.07^c	$2.91\pm0.99^{\rm d}$	3.12 ± 0.91^b	3.36 ± 1.10^a	2.12 ± 1.19^e	3.13 ± 1.22^{b}
	(Neutral)	(Neutral)	(Neutral)	(Neutral)	(Dislike)	(Strongly Dislike)

3.3. Chemical Characterization

3.3.1. Vanillin Content

High-quality vanilla pods contain vanillin of 1.00% to 2.25% according to Indonesian National Standard (BSN, 1990). Figure 6 shows effect of treatments on the vanillin content of vanilla pods. Based on the analysis of variance, sweating temperature did not have a significant effect on vanillin content (*p*-value 0.061), whereas the sweating duration and its interaction with temperature have a significant effect on the vanillin content of the vanilla pods with *p*-value of 0.000

and 0.001, respectively. As seen in Table 4 and Figure 3, vanilla pods on day 2 of sweating have the highest vanillin content, whether sweated at 40°C or 45°C. This is due to the peak of enzymatic activity producing vanillin, minimal degradation, and the preservation of compound stability Cai et al. (2019) and Pardío et al. (2018) stated that vanillin content increases at each stage, starting from after harvesting, killing, sweating, and drying, at 0.03%, 0.05%, 2.30%, and 2.69%, respectively. Delgado et al. (2021) in da Silva's et al. (2023) research stated that oxidative and hydrolytic reactions may occur during the sweating period. Therefore, Cai et al. (2019) concluded that vanillin formed through the hydrolysis of glucovanillin or the oxidation of vanillyl alcohol. However, over time, factors such as compound degradation, evaporation, and decreased enzymatic activity lead to a reduction in vanillin content on days 4 and 6. Vanilla pods sweated at 45°C for 2 days have a vanillin content of 1.47%, which is close to the quality level II of 1.50%. At 45°C, the enzymatic reactions converting vanillin precursors, such as glucovanillin, into vanillin occur more rapidly. The higher temperature increases enzyme activity, resulting in more vanillin being produced in a shorter period. Conversely, vanilla pods under the control treatment showed relatively low vanillin levels compared to the other two treatments on the second day of sweating. This may be due to the lack of temperature and RH control, causing significant variability in environmental conditions during sweating. Temperature and humidity fluctuations can disrupt the enzymatic processes crucial for vanillin formation. If temperature and humidity change drastically, the enzymes responsible for converting precursors into vanillin may not function optimally, leading to reduced vanillin production. According to Cai et al. (2019) the decrease in vanillin content may also be due to the oxidation or reduction of glucovanillin or vanillin into other compounds, such as vanillyl alcohol. Additionally, enzyme activity and quantity also affect the release of vanillin from glucovanillin.

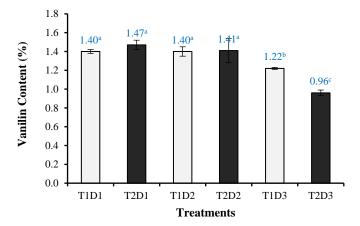


Figure 3. Vanillin content of vanilla pods at different treatments

3.3.2. Ash Content

Table 4 shows the effect of treatment on the ash content of vanilla pods. The ash content is lower than the maximum ash content for vanilla according to Indonesian National Standard (SNI) which is between 8% and 10% (BSN, 1990). Based on the analysis of variance, both treatment factors, namely temperature (T) and sweating duration (D) significantly affected the ash content of vanilla with a significance value (p-value) of 0.000. However, the interaction of the two factors did not significantly affect the ash content with a p-value of 0.290. As seen in Table 4, vanilla pods with the lowest ash content are those sweated at 40°C on days 2 and 4, with ash contents of 1.1% and 1.09%, respectively. That occurs because at 40°C, water evaporation from the vanilla pods is slower and more controlled than that at temperature of 45°C. That allows for better mineral retention within the pods, resulting in a lower mineral content that can convert to ash after combustion. Additionally, sweating at 40°C tends to maintain the structural integrity of the vanilla pod tissue throughout the sweating process. This more stable structure reduces the likelihood of tissue damage or degradation, which can release more inorganic materials, contributing to higher ash content at 45°C. According to the statement by Ndumuye *et al.* (2022) ash content reflects the amount of mineral material that does not volatilize, so a higher ash content indicates a higher mineral content in the material.

Table 4. Effect of treatments on the ash content (%) of vanilla pods.

	D1 (2 days)	D1 (4 days)	D1 (6 days)	Average
T1 (40°C)	1.1	1.09	1.40	1.20 B
T2 (45°C)	1.55	1.72	2.09	1.79 A
Average	1.33 b	1.40 b	1.75 a	

4. CONCLUSION

Based on the results of the analysis of variance, concluded that sweating temperature does not significantly affect the vanillin content of the vanilla pods, but it does significantly impact weight loss, the color (L^* value) of the vanilla pods, hedonic tests (color and aroma), and ash content. Meanwhile, sweating duration significantly affects all testing indicators, including weight loss, the color (L^* value) of the vanilla pods, hedonic tests (color and aroma), vanillin content, and ash content. The optimal method for sweating vanilla pods is using an incubator at 40°C for 4 days, as it resulted in a vanillin content (1.40%) and ash content (1.09%) that meet SNI standards, the lowest weight loss (11.94%), the best brightness level (L^* value of 25.22), and was supported by the presence of a deep color, an oily surface, and greater preference by the panelists. It is necessary to examine the impact of sweating duration on the third day, as well as the effect of the wrapping color and the light exposure on the samples during the sweating process, on the final quality of the vanilla pods.

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