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THE EFFECT OF VANILLIN ON THE OXIDATIVE STABILITY OF PALM OLEIN

(Mengkaji Kesan Penambahan Vanilin terhadap Kestabilan Pengoksidaan Olein Sawit)

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Abstract

The existence of unsaturated fatty acids in palm olein contributes to the oil's degree unsaturation and reduces the oil's oxidative stability when it is exposed to atmospheric oxygen and heat. The purpose of this research was to study the effect of vanillin on improving the oxidative stability of palm olein. 1% of vanillin was incorporated into palm olein and the sample was heated at 90 °C in an oven for 200 hours. Samples were collected at 0 hours, 50 hours, 100 hours, 150 hours and 200 hours heating prior to analysis using a thermal oxidative stability test including iodine value test, peroxide value test, Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analysis (TGA). The results obtained were compared with the result of palm olein without antioxidant (blank). The results showed that the presence of vanillin in palm olein reduced the autoxidation process and increased the oxidative stability of palm olein by 16.10%, 46.51% and 25.00% based on the peroxide value, absorbance of the infrared spectrum and thermogravimetric sample's weight gain per cent, respectively.

Keywords: oxidation, oxidative stability, palm olein, vanillin

Abstrak

Kewujudan asid lemak tak tepu dalam olein sawit menyumbang kepada darjah ketaktepuan minyak dan mengurangkan kestabilan pengoksidaan minyak apabila terdedah kepada oksigen dan haba atmosfera. Kajian ini bertujuan mengkaji kesan penambahan vanilin dalam meningkatkan kestabilan pengoksidaan olein sawit. Sampel olein sawit yang mengandungi 1% vanilin dipanaskan pada suhu 90 °C selama 200 jam. Sampel pada masa 0 jam, 50 jam, 100 jam, 150 jam dan 200 jam setelah dipanaskan telah dianalisis melalui ujian kestabilan pengoksidaan termal iaitu ujian nilai peroksida, spektroskopi inframerah transformasi Fourier (FTIR) dan analisis termogravimetri (TGA). Keputusan yang diperoleh dibandingkan dengan olein sawit tanpa antioksida. Hasil menunjukkan kehadiran vanilin dalam olein sawit mengurangkan proses pengautooksidaan dan meningkatkan kestabilan pengoksidaan olein sawit sebanyak 16.10%, 46.51% dan 25.00%, masing-masing berdasarkan nilai peroksida, keserapan pada spektrum inframerah dan peratus peningkatan berat sampel termogravimetri.

Kata kunci: pengoksidaan, kestabilan pengoksidaan, olein sawit, vanillin

Introduction

The oxidative stability of oil gives an indication of the oil's resistance to oxidation during processing and storage [1]. It is one of the important measures to determine the shelf life and quality of the oil. The oxidative stability of fats, oils and lipid-based foods will be affected by the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins and microorganisms [2, 3]. The catalytic system will act as an initiator that leads to the

oxidation process, resulting in the formation of low-molecular-weight off-flavour compounds that cause the oil to be rancid. Being used in daily life as an ingredient in food preparation and cooking, palm oil may have been subjected to high-temperature cooking such as in deep-fat frying. During this process, oxygen that existed in the deep-fat frying will react with the oil through thermal oxidation and leads to the formation of off-flavour, toxic and hazardous compounds that contribute to the degradation of palm oil and a decrease in its oxidative stability [4]. Thermal oxidation has a similar chemical mechanism to autoxidation [3, 4]. It proceeds via a free radical chain reaction that will be accelerated at a higher temperature for which the mechanism is shown in equation 1 below [1]:

Initiation	RH	$\rightarrow$	R• + H•		
Propagation	R• + $^3O_2$	$\rightarrow$	ROO•	(peroxide formation)	
	ROO• + RH	$\rightarrow$	ROOH + R•	(hydroperoxide formation)	
Termination	ROO• + R•	$\rightarrow$	ROOR		
	R• + R•	$\rightarrow$	RR		(1)

Autoxidation of oil requires the fatty acids and acylglycerols to be in radical forms [1]. In the presence of an initiator such as heat, the unsaturated lipid molecules lose a hydrogen atom and produce lipid alkyl radicals [3]. The lipid alkyl radicals react with atmospheric oxygen, $^3O_2$, and produce lipid peroxy radicals, which act as the chain carriers of a rapidly progressing reaction by attacking a new lipid molecule, leading to the formation of lipid hydroperoxides [1, 3]. Oxygen availability and temperature will determine the rate of formation of the lipid peroxy radicals and lipid hydroperoxides [1]. The primary oxidation products, lipid hydroperoxides, are stable at room temperature [1]. Nevertheless, at an elevated temperature or in the presence of metal, the lipid hydroperoxides will be broken down into alkoxy radicals, which will then form secondary oxidation products such as aldehydes, ketones, acids, esters, alcohols and low-molecular-weight hydrocarbons on further reaction [1]. Palm oil is composed of mixtures of triacylglycerols (TAGs) as the main constituent where each of the glycerol molecules is esterified with three fatty acids [5, 6]. The major components of fatty acids present in palm oil are palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2) [5]. To protect these fatty acids from being oxidized by free radicals, vitamin E, which is a major antioxidant in palm oil, plays an important role [5–7]. However, it was shown that the vitamin E in edible palm olein could be decomposed during a high-heat cooking process such as deep-fat frying [7]. Prior to this, the incorporation of another type of natural antioxidant in palm olein is really important to improve the oxidative stability of the oil towards oxidation processes, thus enhancing the nutritional value, organoleptic properties and sensory quality of the oil.

In this study, vanillin, with the IUPAC name of 4-hydroxy-3-methoxybenzaldehyde, which is the main component of natural vanilla that can be extracted from beans or pods of a tropical climbing orchid called *Vanilla planifolia*, *Vanilla tahitensis* or *Vanilla pompona* [8–12] was added to palm olein to slow down the oil's degradation and to increase its oxidative stability. Instead of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) that have an adverse effect on human health, a naturally occurring antioxidant is preferable to be used as an antioxidant in palm olein because it is non-toxic and readily accepted by the body [13, 14]. The aims of this research were to study the effect of vanillin on the oxidative stability of palm olein using peroxide value test and thermogravimetric analysis (TGA) in an oxygen atmosphere and to characterize the oxidative stability of palm olein using Fourier transform infrared (FTIR) spectroscopy.

Materials and Methods

Chemicals

The chemicals used in this study include vanillin (QReC), cyclohexane (Merck), Wijs solution (QReC), acetic acid (Bendosen), chloroform (R&M), sodium thiosulfate (Bendosen), palm olein, starch (QReC) and distilled water.

Sample preparation

1% of vanillin was added to 50 g of palm olein using a weight to weight ratio. The palm olein that contained vanillin (POV) was pre-heated to 90 °C while stirring to dissolve the vanillin. Palm olein without the addition of vanillin (PO) was used as the reference. The samples of PO and POV were then heated at 90 °C in an oven. Samples were collected at 0, 50, 100, 150 and 200 hours heating for analysis.

Starch indicator preparation
0.5 g of starch was mixed with enough cold water to make a thin paste, followed by the addition of 50 mL of boiling distilled water. The solution was stirred using a magnetic stirrer to dissolve the starch with rotation of speed 300 rpm. The starch solution was prepared each time before each analysis to ensure the effectiveness of the starch solution as an indicator.

Peroxide value (PV) test
30 mL of acetic acid–chloroform solution in the ratio of 3:2 was added to 5.0 g of sample in an Erlenmeyer flask. The flask was shaken to dissolve the sample. Then, 0.5 mL of saturated potassium iodide solution was added. The solution was shaken again for 1 minute before the addition of 30 mL distilled water. The solution was titrated with 0.1 N sodium thiosulfate solution until the yellow colour of the solution faded. 10 drops of starch indicator were added to the solution and the titration was continued until the blue colour of the solution disappeared. The test was done in triplicate to avoid any error that occurred during the titration. The above steps of the PV test were repeated for the blank determination without the presence of PO and POV. The PV was calculated using equation 2 below [15]:

$$\text{PV } (m_{equiv} \text{ peroxide/1000 g oil}) = \frac{(S-B) \times N \times 1000}{W} \quad (2)$$

S = volume of sodium thiosulfate used for sample's titration, mL; B = volume of sodium thiosulfate used for blank's titration, mL; N = normality of sodium thiosulfate, N; W = weight of sample used, g. Tukey's test was used in validating the significant effect of adding vanillin to the PO based on the PV test.

Fourier transform infrared (FTIR) spectroscopy
The oil sample was also characterized using an FTIR spectrometer (Perkin Elmer FTIR Frontier 96255) to determine the functional groups of compounds present in the sample, specifically the primary oxidation product. The characterization was done using the potassium bromide, KBr pellet method. First, the KBr pellet was prepared. The oil sample was then thoroughly spread on the prepared KBr pellet. The spectrum of the sample was recorded from 4000 cm^{-1} to 400 cm^{-1}. The FTIR characterization was conducted at each selected time for each sample of PO and POV [16, 17].

Thermogravimetric analysis in an oxygen atmosphere
The analysis was performed using a Hitachi STA7200 model TGA-DSC instrument. First, POV was prepared and weighed in an alumina cell. The cell was placed in the furnace at room temperature. After 2 minutes, the temperature of the furnace was increased from 30 °C to 70 °C. The sample was continuously heated from 70 °C to 210 °C with the temperature increment of 2 °C per minute. The sample was purged with oxygen at a rate of 50 cm^3 per minute during the analysis. The steps were repeated for the sample of PO [18].

Results and Discussion

Peroxide value test
The PV test was performed to determine the hydroperoxide concentration in the oil samples. This is one of the most common methods used to indicate the initial stages of oxidation [19]. The PV of the original palm olein was directly proportional to the hours of heating [20]. The longer the oil is heated, the greater the number of hydroperoxides generated as a result of oxidation of the unsaturated fatty acids in the sample. Figure 1 shows the variation of PV with different times of heating for both PO and POV, while Table 1 shows the PV of PO and POV after heating at 90 °C for different selected times.

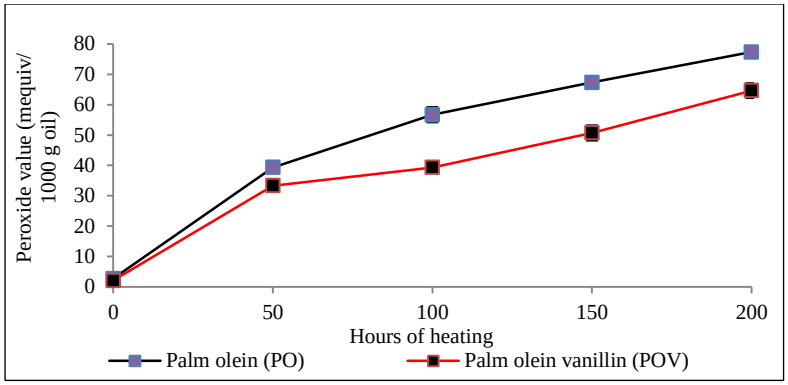

Figure 1. Variation of PV with different times of heating for PO and POV

Table 1. The PV of PO and POV after heating at 90 °C for different selected times

Hours of Heating	PV (mequiv peroxide/1000 g oil)				
	0 hours	50 hours	100 hours	150 hours	200 hours
PO	2.73 ± 0.94	39.35 ± 1.63	56.69 ± 2.49	67.31 ± 1.65	77.32 ± 1.65
POV	2.07 ± 0.94	33.34 ± 1.63	39.34 ± 1.63	50.69 ± 2.51	64.65 ± 2.49

The initial PV of PO at 0 hours was 2.73 mequiv peroxide/1000 g oil compared with the initial PV of POV, which was 2.07 mequiv peroxide/1000 g oil. The initial PV of PO was slightly higher than the initial PV of POV because the PO sample had been oxidized. Hydroperoxide had already started to form in PO although the sample was not yet thermally treated at 0 hours. The PV increased to 39.35 mequiv peroxide/1000 g oil, 56.69 mequiv peroxide/1000 g oil, 67.31 mequiv peroxide/1000 g oil and 77.32 mequiv peroxide/1000 g oil at 50 hours, 100 hours, 150 hours and 200 hours, respectively for PO and rose to 33.34 mequiv peroxide/1000 g oil at 50 hours, 39.34 mequiv peroxide/1000 g oil at 100 hours, 50.69 mequiv peroxide/1000 g oil at 150 hours and finally to 64.65 mequiv peroxide/1000 g oil at 200 hours for POV. The oxidation of PO and POV increased their PV due to the generation of primary oxidation product, hydroperoxide, as a result of heating in the presence of atmospheric oxygen. From 0 hours to 50 hours, the PV showed a relatively small difference between PO and POV and when the time passed from 50 hours to 200 hours, the PV of POV started to deviate to a smaller extent than the PO, as can be seen in Table 2 and Figure 2. The addition of vanillin to palm olein has a significant effect ($p \leq 0.05$) in reducing the rate of oxidation as well as the formation of hydroperoxide in the sample, which led to a lower PV. Vanillin efficiently worked as an antioxidant. It acted as a hydrogen donor to the free radicals that formed during the autoxidation, which by this way could prevent the radicals from attacking a new lipid molecule that resulted in generation of more hydroperoxide. The incorporation of vanillin into palm olein reduced the hydroperoxide formation by 16.10%, while the remaining 83.89% of the oil was being oxidized.

Table 2. PO's and POV's absorbance in between the region of 3600 and 3250 cm^{-1} for every selected hours of heating

Hours of Heating	Absorbance				
	0 hour	50 hours	100 hours	150 hours	200 hours
PO	0.04	0.06	0.09	0.18	0.23
POV	0.03	0.04	0.06	0.10	0.13

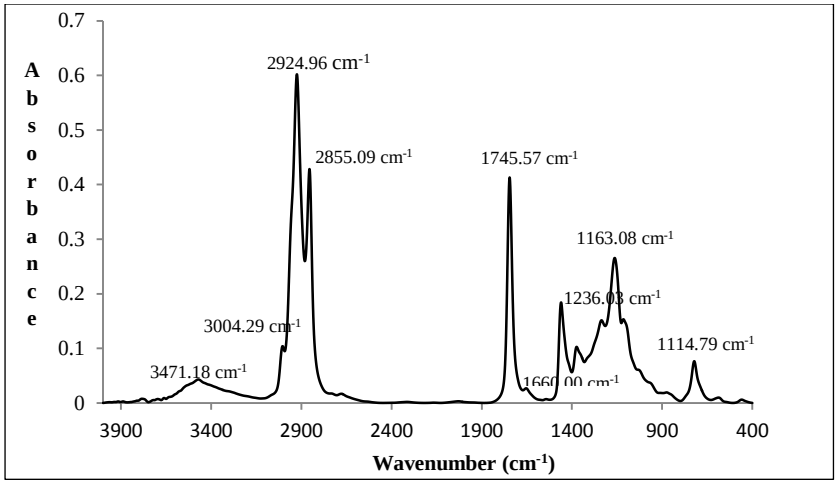

Figure 2. The assigned functional groups of the infrared spectrum of palm olein incorporated with vanillin, before heating (0 hours)

Fourier transform infrared spectroscopy (FTIR) analysis

Palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2) contributed in a large proportion in the composition of palm olein. All of these fatty acids consisted of O–H, C=C, C=O, C–O, aliphatic C–H and olefinic C–H functional groups in their structure. The differences among these fatty acids are in the length of the carbon chain and also the number of double bonds that are present in the compound. As for vanillin, the functional groups that exist in the compound include O–H, C=C, C=O, C–O, aliphatic C–H and olefinic C–H. The expected functional groups mentioned above could be confirmed using FTIR spectroscopy. Figure 2 shows the assigned functional groups of the infrared (IR) spectrum of palm olein incorporated with vanillin at 0 hours, before heat was applied. From the spectrum, there was IR absorption of the O–H stretching vibration at frequency 3471.18 cm^{-1} [21, 22] due to the presence of the carboxylic acid functional group in the fatty acids chain and phenolic O–H group in vanillin. The C=C stretching vibration absorbed at frequency 1660.00 cm^{-1} [23] due to the presence of unsaturated fatty acids and the aromatic ring of vanillin, C=O stretching vibration at frequency 1745.57 cm^{-1} arose from the existence of the carboxylic acid functional group in the fatty acid's chain and the aldehyde functional group in vanillin and C–O stretching vibration at frequency 1236.03 cm^{-1}, 1163.08 cm^{-1} and 1114.79 cm^{-1} [22] were due to the presence of the carboxylic acid functional group in the fatty acid's chain, phenolic O–H group and ether functional group in vanillin. There were also peaks that existed at the frequencies 2924.96 cm^{-1} and 2855.09 cm^{-1}, indicating the functional group of aliphatic C–H stretching vibration. The olefinic C–H stretching vibration functional group gave rise to the band at frequency 3004.29 cm^{-1} [24]. Both the aliphatic and olefinic C–H stretching vibration functional groups exist in the structure of free fatty acids and vanillin.

Although infrared spectroscopy was able to analyse most of the functional groups of the oxidation products formed, however, in this research, the use of this technique has focussed on the detection of primary oxidation products of hydroperoxides and the changes that occurred specifically in the band in the region of 3600–3250 cm^{-1}. Figure 3 shows the infrared spectrum of PO and POV from 0 hours to 200 hours of heating in the region of the O–H stretching vibration (3600–3250 cm^{-1}), indicating the presence of hydroperoxides. Based on the figure, the peak intensity of O–H stretching vibration for the sample of PO was higher than that of POV for every selected time of heating. This was due to the antioxidant activity of vanillin in the POV sample, which retards the formation of hydroperoxide during the oxidation process. The concentration of hydroperoxide in the sample increased with the increase in the time of heating because the heat that is applied acted as an initiator for the oxidation reaction and will be accompanied by an increase in the absorbance in the infrared spectrum because the peak intensity is directly proportional to the concentration of the respective functional groups, as stated by the Beer–Lambert law equation, $A = \varepsilon bc$ [22]. The hydroperoxide band shifted towards a smaller wavenumber and became more intense, broader and wider [22–24].

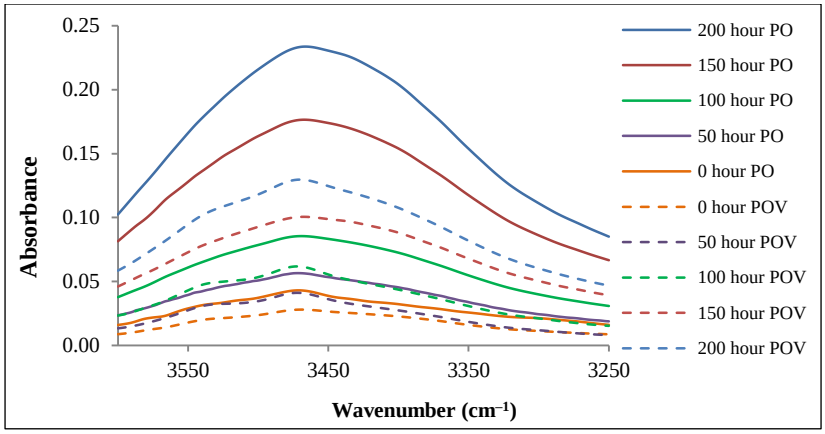

Figure 3. Palm olein (PO) and palm olein vanillin (POV) spectra from 0 hours to 200 hours of heating in the region of the O–H stretching vibration (3600–3250 cm^{-1}), indicating the presence of hydroperoxides

The tabulated absorbance of the O–H stretching vibration in the region 3600–3250 cm^{-1} is shown in Table 2 and the increasing trend of the absorbance throughout the heating can be seen in Figure 4. The absorbance of the O–H stretching vibration of PO increased from 0.04 at 0 hours to 0.06, 0.09, 0.18 and 0.23 at 50 hours, 100 hours, 150 hours and 200 hours, respectively. The initial absorbance of the O–H stretching vibration of POV was 0.03 at 0 hours, slightly lower than the PO due to the low concentration of hydroperoxide present in POV in the initial state. The value increased to 0.04 at 50 hours, 0.06 at 100 hours, 0.10 at 150 hours and finally to 0.13 at 200 hours for POV. Only a small difference could be observed in the absorbance values of PO and POV from 0 hours to 100 hours. However, a significant difference in the absorbance values between these two samples can be seen from 100 hours to 200 hours. The absence of vanillin in the PO sample encouraged the oxidation reaction and the formation of hydroperoxide, especially at elevated temperature. The large amount of hydroperoxide produced in PO sharply increased the intensity of absorption of the O–H stretching vibration between 100 hours and 200 hours of heating. The presence of vanillin in PO successfully inhibited the formation of hydroperoxide by 46.51%. The results obtained from this FTIR spectroscopic analysis correlated to the results of the PV test.

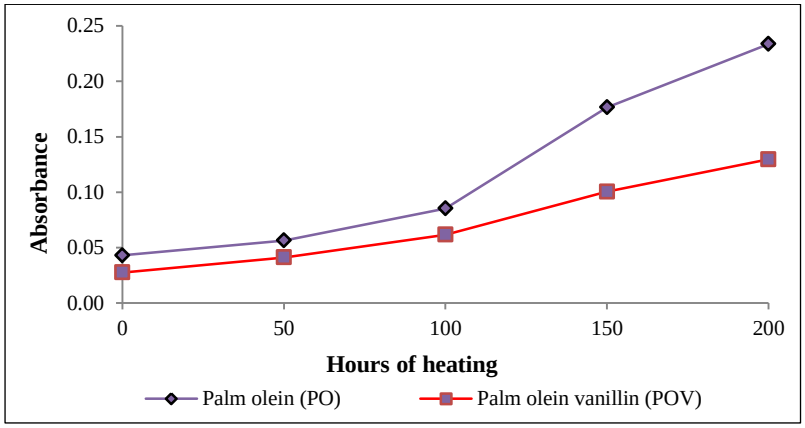

Figure 4. Variation of absorbance with different times of heating for PO and POV

Thermogravimetric analysis

Figures 5 and 6 show the thermogravimetric (TG) curves of PO and POV, respectively, whereas Table 4 shows the initiation (T_i) and final (T_f) oxidation temperatures of PO and POV. T_i is the temperature at which the oxidation rate increases quickly while T_f is the point at which the sample gains maximum weight [25]. The increase in temperature

after T_f results in a loss of the sample's weight until the run is completed [25].

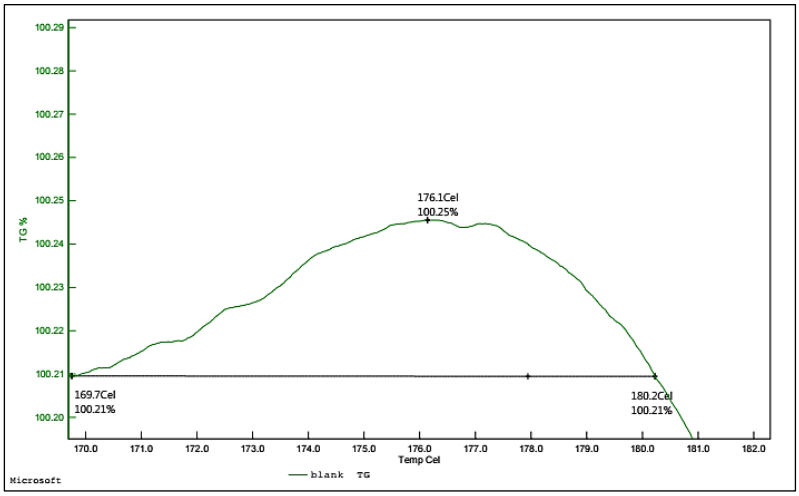

Figure 5. The thermogravimetric curve of PO

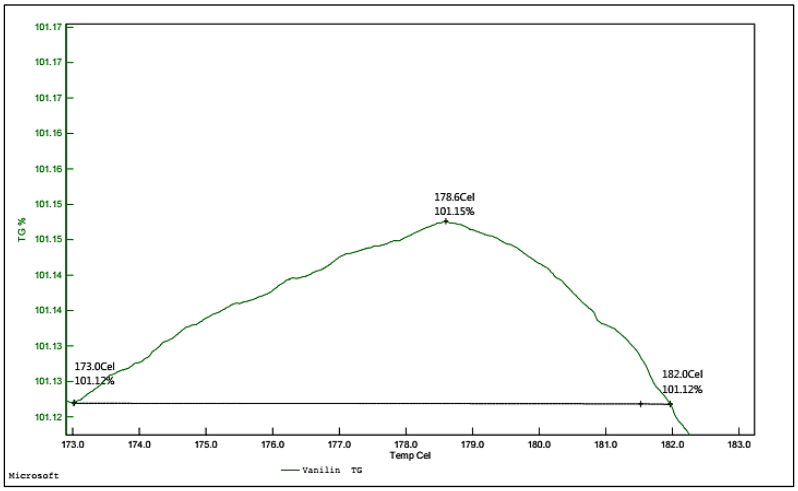

Figure 6. The thermogravimetric curve of POV

Table 4. The initial and final oxidation temperature (T_i and T_f) of PO and POV

Sample	T_i (°C)	T_f (°C)	Sample's Weight Gained, Δ (%)
PO	169.7	176.1	0.04
POV	173.0	178.6	0.03

Based on Figures 5 and 6, it can be seen that the PO sample started to be oxidized at a lower temperature than POV because there was no antioxidant in the PO sample that could help in delaying the oxidation process. The absence of natural antioxidant contributed to the difference in T_i [26]. The T_i of PO was 169.7 °C and the T_i of POV was 173.0 °C. The heat and oxygen that are present during the analysis acted as an initiator for the oxidation reactions of

PO and POV. The TG curve of PO increased until it reached T_f of 176.1 °C and the TG curve of POV increased to the T_f of 178.6 °C due to the gain in the sample's weight because of the formation of hydroperoxides from the oxygen uptake at the beginning of oxidation [26, 27]. The weight increased from 100.21% to 100.25% for PO and from 101.12% to 101.15% for POV with the samples' weight gained percentages of 0.04% and 0.03%, respectively. This showed that the maximum number of hydroperoxides was formed in PO at a lower T_f than in POV. The TG curves started to drop after T_f because the hydroperoxides previously formed were degraded into secondary oxidation products that were volatile [26, 27]. The volatility of the secondary oxidation products reduced the mass of PO and POV samples until the end of the analysis. The existence of vanillin in PO effectively increased the oxidative stability of PO and successfully reduced the deterioration of PO due to oxidation by 25.00% according to the TGA.

Conclusion

It can be concluded that vanillin, a naturally occurring antioxidant, inhibited the autoxidation process and increased the oxidative stability of PO by 16.10%, 46.51% and 25.00% based on the PV, absorbance of the infrared spectrum and the sample's weight gain per cent, respectively. Vanillin can be incorporated into PO as an alternative to a synthetic antioxidant. It is recommended that the incorporation of another antioxidant into PO be tested in the future to identify the antioxidant that has the optimum activity to improve the oil's oxidative stability. Other than that, vanillin could also be used as an antioxidant in other vegetable oils because it has the ability to slow down the oxidation process of oil due to the presence of the phenolic O–H group that it possesses. This condition enables the compound to act as a hydrogen donor to retard the free radical chain reaction during the initial stages of oxidation.

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