EFFECT OF OXYGEN AVAILABILITY ON β-IONONE FORMATION AS A DEGRADATION PROCESS OF CAROTENOIDS FROM CRUDE PALM OIL BY THERMAL PROCESS

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ABSTRACT

This study aimed to observe the effect of oxygen levels during the carotenoid degradation process to produce β-ionone, several parameters have been pointed, including carotenoid levels in CPO before and after the degradation process using a spectrophotometer, the color of the CPO solution before and after degradation using the color flex hunter lab and the β-ionone levels resulting from the degradation of Carotenoids by gas chromatography using a mass spectrometry detector. As the result, it can be stated that the presence of oxygen has influenced the degradation of carotenoids in CPO. The greater the amount of oxygen, the more carotenoids are degraded. The most excellent degradation process was found to be 80%. However, β-ionone was only detected in the air flow treatment of 20 L/h with a value of 0.44 mg/L. The results of this research have shown that the amount of oxygen dramatically influences the formation of β-ionone when the carotenoid degradation process is carried out under hot conditions. This information can be used to develop β-ionone formation technology for future industrial-scale application.

Keywords: aromatic compound, β-carotene, flow through system, Rancimat, thermal process

تأثير توفر الأوكسجين في تكوين β-أيونون كعملية تحلّل للكاروتينات من زيت النخيل الخام بالمعالجة الحرارية

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المستخلص

هدفت هذه الدراسة إلى ملاحظة تأثير مستويات الأوكسجين اثناء عملية تحلّل الكاروتينات لإنتاج مركب β-أيونون. وقد تم تحديد عدة مؤشرات، شملت: مستويات الكاروتينات في زيت النخيل الخام (CPO) قبل وبعد عملية التحلّل باستخدام جهاز المطياف الضوئي، ولون محلول زيت النخيل الخام قبل وبعد التحلّل باستخدام جهاز Color Flex Hunter Lab ، فضلاً عن قياس مستويات β-أيونون الناتجة عن تحلّل الكاروتينات بواسطة الكروماتوغرافيا الغازية المزودة بكاشف مطيافية الكتلة. وأظهرت النتائج أن وجود الأوكسجين قد أثّر بوضوح في تحلّل الكاروتينات في زيت النخيل الخام، إذ كلما زادت كمية الأوكسجين زاد معدل تحلّل الكاروتينات، وقد بلغت أعلى نسبة تحلّل 80%. ومع ذلك، لم يُكشف عن مركب β-أيونون إلا في معاملة تدفق الهواء بمعدل 20 لتر/ساعة، حيث بلغت قيمته 0.44 ملغم/لتر. وتبيّن من نتائج البحث أن كمية الأوكسجين تؤثر بشكل كبير في تكوين β-أيونون عند إجراء عملية تحلّل الكاروتينات تحت ظروف حرارية. ويمكن الاستفادة من هذه النتائج في تطوير تقانات إنتاج β-أيونون للتطبيقات الصناعية المستقبلية على نطاق واسع.

الكلمات المفتاحية: المركبات العطرية، بيتا كاروتين، نظام التدفق المستمر، جهاز Rancimat، المعالجة الحرارية

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INTRODUCTION

The need for rose aroma is very high at this time. The aroma of roses is one of the aromas that people like because of its specific smell (10 ,11). However, getting long-lasting roses in today's advanced era takes much work. Several things are the basis for this difficulty, including the rare number of existing rose plantations. This rare condition is because roses are categorized as decorative plants (16). Roses are a type of national cut flower that is popular in Indonesia. The appeal of cut roses is, among other things, due to the beauty of their very varied shapes and colors with relatively affordable and stable prices, in addition to the long freshness of the flowers. Roses can be planted at any time, according to market needs, with a production period of around 3 - 5 years and relatively high profits as decorative plants. Because of this, only a few farmers cultivate roses for the sake of the aromatic industry. Apart from that, the aromatic yield of roses and the essential oil content in a plant are minimal, around 1-3%. Therefore, more is needed if it is supplied from roses to meet the industrial needs for rose aroma. The aroma of roses is a combination of aromatic chemical components, where the main component is the compound β ionone (3,13). Industrially, the β-ionone component is made by chemical synthesis using petroleum as raw materials. Because the aroma of roses is mainly used for the aromatic needs of products related to humans, it is essential to have the chemicals including rose aroma in the natural category (18). For this reason, producing natural β-ionone compounds can be the first step. Naturally, β-ionone is the result of the degradation of carotenoid compounds, where this degradation process can be triggered by several things such as heat, acid, and oxygen(6). Studies to make β-ionone from carotenoids began in 19th century(15). However, in early research, this study was only used to study the degradation reaction process of carotenoids to become β ionone. The degradation process that has been developed can occur chemically by conditioning environmental conditions, but some also occur with the help of microorganisms to produce fermentation reactions. However, applying all these reaction processes has not yet reached the upscale or industrial stage.Industrially, chemical degradation processes can be developed because they are easy and fast. Several chemical procedures have been developed, including the heat degradation process. In this process, β-ionone compounds have been produced from the degradation of carotenoid compounds from carrot extract, sweet potato, and palm oil samples (2,21). This research has shown that the heat process has reduced carotenoids by up to 95% and produced significant amounts of β ionone. However, the existing thermal degradation processes are carried out under aerobic conditions or with an uncontrolled abundance of oxygen. No research has regulated the amount of oxygen present in a carotenoid degradation reaction to β ionone. Oxygen in a degradation reaction, which is an oxidation reaction, is essential. Oxygen is an element that can disintegrate macromolecules where oxygen can penetrate the oxidation substrate. Heat conditions function as a catalyst (24). Therefore, it is necessary to study the effect of oxygen content on the degradation process of carotenoid compounds to produce β ionone. In this research, samples of crude palm oil were used as raw materials rich in carotenoids used as substrates to produce β ionone.

MATERIALS AND METHODS

This research was carried out at the Laboratory of Technology and Process Engineering for Agricultural Products, the Laboratory of Chemistry, Biochemistry of Agricultural Products and Food Nutrition, Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, and the Animal Biotechnology Laboratory, Faculty of Animal Husbandry, Andalas University, Padang. The research was carried out from June 2023 to October 2024. The raw material used was CPO that obtained from PT Padang Raya Cakrawala at Teluk Bayur, West Sumatra. The chemicals for the saponification process were potassium hydroxide (KOH), ethanol, and hexane. The materials used in the carotene degradation process are glycerin and ethanol pro analysis. Furthermore, the β-ionone standard from Sigma Aldrich was used to determine the β-ionone content from carotenoid degradation products from CPO. The tools used for the CPO saponification process were a water bath, Erlenmeyer flask and separating funnel. The apparatus used in the carotene degradation process were analytical balance, volumetric flask. erlenmeyer flask, beaker glass, ultrasonic bath, rotary vacuum evaporator (B-one), desiccator, oxidation equipment Rancimat®, spectrophotometer (Shimadzu UV1800), GC-MS (Shimadzu QP 2010), and hunter Lab color flex and others supported apparatus. This research was conducted experimentally with four treatments and three replications. The treatments in this study were the amount of air of 8 L/h, 12 L/h, 16 L/h and 20 L/h. The obtained data will be calculated statistically by using Microsoft Excel.

Preliminary preparation: CPO (Crude Palm Oil) obtained from PT Padang Raya Cakrawala was prepared before degradation. CPO must be saponified to remove the existing fat or oil fractions[12]. Five grams of CPO were put into a 250 ml Erlenmeyer, added with 1 gram of KOH and 50 ml of ethanol. The mixture was heated in a water bath at 70 ºC for 30 minutes. The saponification process removes colorless substances containing fat and other impurities. The saponification results were transferred into a separating funnel and cooled to room temperature. A 110 ml of hexane in 100 ml of distilled water was added to the separating funnel, shaken slowly, and left for 30 minutes. The solution was allowed to stand until two layers were formed: the water layer at the bottom containing the saponified fraction, and the hexane layer at the top containing the unsaponified fraction. The bottom part is discarded, and the top part, which is the unsaponifiable fraction, is taken and put in the bottle. Hexane was separated from the unsaponified fraction using a rotary evaporator at a temperature of 45 °C and pressure of 200 mbar to obtain a solvent-free unsaponified fraction, namely carotenoid compounds.

Degradation procedure: Carotene degradation was carried out using the Rancimat®. One gram of saponified carotenoid sample was added to 4 grams of glycerin into the rancimat tube. Then, fill the container bottle with 20 mL of ethanol. Degradation was carried out using Rancimat® at a temperature of 140 °C with air flow concentrations starting from 8 L/h, 12 L/h, 16 L/h and 20 L/h for 2 h. The degradation products captured by ethanol were analyzed using GC-MS. The scheme of degradation process was described on Figure 1.

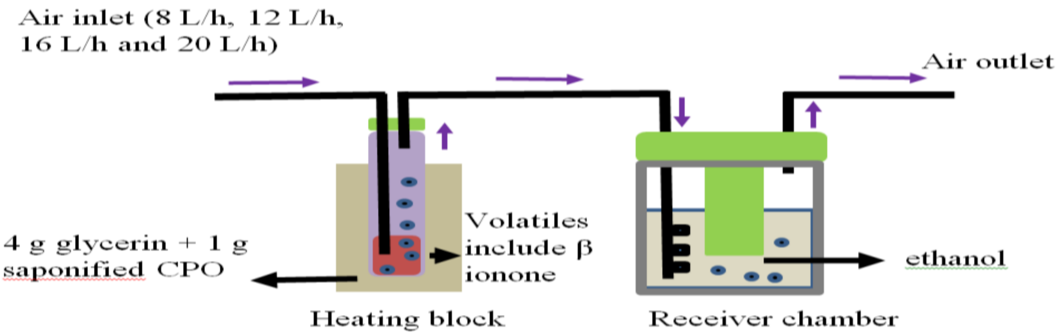


Fig 1. The Scheme of degradation process of carotenoid in Rancimat® apparatus

Observation parameters

Determination of carotenoid content by spectrophotometer: A total of 1 g of CPO sample before and after degradation was weighed and then 5 mL of hexane was added. The mixture was then put into a reaction tube and placed in an ultrasonic bath for 15 minutes. Then the homogenized mixture was transferred into a centrifuge tube and centrifuged at a speed of 3000 rpm for 10 minutes (20). The resulting supernatant was then transferred into a 25 mL volumetric flask and stamped with hexane. The solution was the dilute 10 times for obtaining the proper absorbance. The analysis for the final solution was conducted by spectrum analysis and photometric analysis. The spectrum analysis was done by the observation on UV-Vis wavelength and the photometric analysis was performed by measurement at the wave length of 446 nm. For photometric analysis. the carotenoid content was calculated by the equation as follow:

$$\text{Carotenoids content} = \frac{25 \text{ x A x } 383}{100 \text{ x sample weight (g)}} \text{ x Dilution factor}$$

Note: A is the absorbance data and the value of 383 is calibration factor for β carotene

Color analyses of CPO: The color measurement (5) was conducted by Hunter's Lab Colorimetric Systems using Color Flex apparatus. The data obtained was expressed as L, a, b values and °Hue. Hunter's color rotation system is characterized by three values, namely L (Lightness), Description: a* (Redness) and b* (Yellowness). The L, a, and b values have scale intervals that indicate the color level of the material being tested. The notation L states the brightness parameter with a value range of 0-100, indicating dark to light. The notation a with a value range from (-80) - (+100) shows from green to red. The b notation with a value range from (-70) - (70) shows from blue to yellow. Apart from that, the Hue Degree (°Hue) was also calculated where the °Hue was used to see the direction of the color trend with the following reference °H = arctan(b/a) (if a is positive and b is positive), °H = 180 + arctan(b/a) (if a is negative and b is positive). °H = 180 + arctan (b/a) (if a is negative and b is negative), °H = 360 + arctan (b/a) (if a is positive and b is negative).

Measurement of β-ionone :β-ionone formed after the carotenoid oxidation process was determined by GCMS (22). The β-ionone dissolved in ethanol is then filtered using a 0.2 micro sieve. After that, put it into a vial. The sample is then injected into the GCMS system. For GCMS, several parameters are prepared, including injection conditions, separation conditions and detection conditions. For the injection process, the injection temperature was set at 180 °C and the mobile phase flow rate was 1 mL/min. The separation process was carried out on a semi-polar column with type MS-5. The separation temperature has been set from 60 °C to 200 °C with a constant temperature increase at a rate of 10 °C/min. The detection temperature was set at 250 °C. The detected peaks were synchronized with the Wiley spectral data base that was installed in the GCMS.

RESULT AND DISCUSSION

Oxygen concentration: In this research, it will found out the effect of oxygen concentration on the carotenoid degradation process in CPO, which was carried out under certain heat conditions (140 C) and a measured time (2 h). This condition was obtained according to the previous research on β-ionone formation from degradation of carotene based on temperature treatments(2). The oxygen that will entered the CPO sample comes from the air. The use of air was for application reasons. Air utilization will be easier and cheaper later if this process was carried out industrially because it required only compressor equipment. However, scientifically, to see the effectiveness of oxygen in assisting the degradation process, the oxygen concentration contained in the CPO sample during the degradation process needs to be calculated. For this reason, oxygen levels can be calculated using the following equation:

$$\textbf{Oxigen concentration}\ (\%) = \frac{\textbf{Air flow}\ (\mathbf{L.h^{-1}})\textbf{x oxigen concentration in air}\ (\%)\textbf{x 2 h}}{\textbf{vol sample}\ (\mathbf{0,005\ L})}$$

Where: air flow was the air flow treatments as 8 L/h, 12 L/h, 16 L/h and 20 L/h; Oxygen concentration in air (21 %); 2 h was the duration of degradation process and volume sample was 5 mL that was equal to 0,005 L). Therefore, the concentration of oxygen during degradation process of CPO in this study can be proposed as shown in Table (1).

Table 1. conversion of air flow to oxygen concentration

No	L/h	%	h	L	%	g/100 mL
1	8	21	2	0.005	67200	67
2	12	21	2	0.005	100800	101
3	16	21	2	0.005	134400	134
4	20	21	2	0.005	168000	168

Degradation process of carotenoid

Saponification process: The raw material used in this research was CPO obtained from PT Padang Raya Cakrawala at Teluk Bayur, West Sumatra. CPO contains many chemical compounds. A saponification process must be carried out to obtain carotenoid compounds in CPO since CPO includes a lot of fractions, such as oil or fat, instead of carotenoid. Before carrying out the saponification process, it must know the saponification number, where the saponification number was done to determine the number of milligrams of alkali base (KOH) needed to saponify one gram of fat. The greater the saponification number depends on the molecular mass of the oil; the more significant the oil or fat molecules, the greater the molecular mass, the lower the saponification number. According to the result, the average saponification number for CPO was 121.742 mg KOH/g. This saponification number value shows that the minimum amount of KOH needed in this research was 0.6 grams to saponify 5 g of CPO in the saponification process. The saponification process was carried out in this research to separate fatty acids from the unsaponified fraction found in CPO. Therefore, the unsaponified fraction referred to in this research can be considered as carotenoids. Finally, after saponification, a total carotenoid in CPO was found to be 168, 6 ± 20 µg/g. Compared to other publications, the content of carotenoid in this study was quite less. Most of study informed that the carotenoid level in the CPO approximately present in the range of 300- 500 µg/g (7,9). The optimization process of non-carotenoid component replacement need to be conducted in order to increase the amount of extracted carotenoid.

Color observation: Based on the treatment given during the carotenoid degradation process to produce β-ionone, it was known that degradation of carotenoid compounds has occurred from the minor oxygen concentration in the air treatment of 8 L/h. This phenomenon can be seen from the color change in the CPO sample given the heating airflow at a particular time. The color change has occurred from orange to yellow. Based on color observations, changes in CPO color during the degradation process can be seen in Table (2).

Table 2. Color analysis of degraded CPO

Oxygen concentration (g/100 mL)	L ± SD	a* ± SD	b* ± SD	º Hue ± SD	Colour appearance
67	34,14±5.38^{b}	-1,76±1.33^{a}	42,28±8.78^{b}	92,67±2.10^{a}	Yellow
101	34,07±2.36^{b}	-2,43±0.42^{ab}	39,39±2.45^{b}	93,43±0.64^{a}	Yellow
134	27,79±0.31^{ab}	-2,45±0.32^{ab}	22,70±2.91^{a}	96,28±1.53^{a}	Yellow
168	23,7 ±1.42^{a}	-4,47±0.09^{b}	19,51±3.20^{a}	103,11±2.05^{b}	Yellow

Note: Numbers in the same column followed by different lowercase letters are declared to be statistically significantly different at the 5% level

Based on the data in Table (2), differences in oxygen concentration greatly influence the color. Color is one of the parameters easily observed in a product. In Table 2, it can be seen that the measurement results show that the brightness value (L) is in the range of 34.14-23.7, which can be concluded that the difference in oxygen concentration affects the brightness value, where the resulting color becomes increasingly faded. The *a value in this study has a negative value, indicating that the color results will tend to be yellow or faded. It was found that with treatment, the effect of differences in air concentration decreased *a value, with an average of (-1.76) - (-4.47). The decreasing value of *a indicates that the degradation sample is yellow. The *b value has a positive value, indicating the sample is yellow; the *b value obtained decreases with an average of 42.28-19.51. The decreasing value of *b suggests that the degradation sample has a yellow color that is increasingly fading. This change in carotene color indicates damage to carotene due to heating. High temperatures cause damage to the chromophore group, which decreases the color intensity of the carotene (19). Heat and oxygen can change carotenoids from their original form, which is easily oxidized and decreases color and antioxidant activity. Apart from that, the º Hue value obtained ranges between 92.67 and 103.11, which can be concluded that the º Hue value is yellow. The º Hue obtained has yellow color criteria in all treatments. Surely, differences in oxygen concentration during the degradation process influence the color of carotene, which previously turned dark orange to yellow. The changes in the º Hue value are due to changes in the total carotenoid content due to thermal treatment. The amount of carotene decreases due to exposure to high temperatures and oxygen.

Carotenoid content: Carotenoid spectrum analysis before and after degradation was carried out using UV-Vis spectrophotometry at 200 – 800 nm wavelengths. The carotenoid spectrum can be seen in Figure (2). The figure above shows that the difference in oxygen concentration in the degradation process produces a carotenoid spectrum that is different from the carotenoid spectrum in CPO. Before the degradation process, a spectrum was visible in the 450 nm wavelength range; meanwhile, there was no visible absorption after the degradation process. This data applies to all treatments based on the amount of oxygen during degradation. Along with the loss of absorption in the 450 nm area, in samples that have undergone degradation, high-intensity absorption appears in the 200 -250 nm area. This trend has indicated the formation of substances containing short chromophores, most likely the products of carotene degradation. One of them might be as the β-ionone compound.

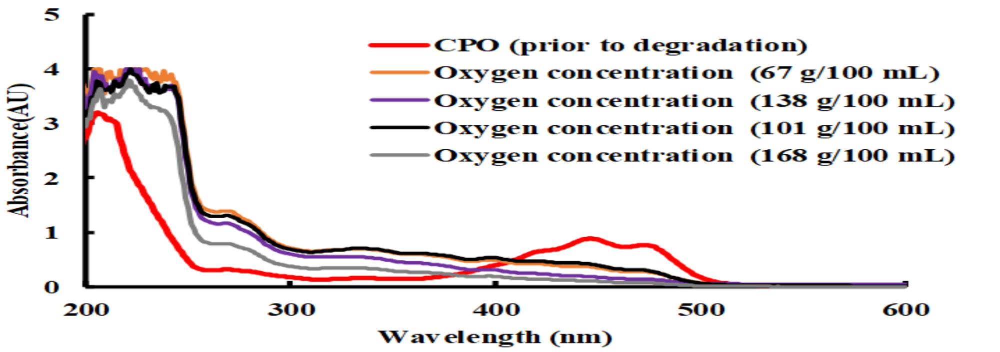


Fig. 2. UV-Vis Spectrum of carotenoid during degradation process with various oxygen concentration

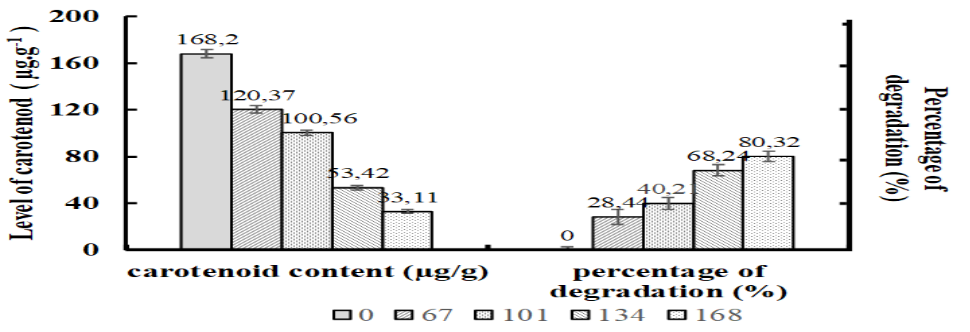


Fig. 3. Carotenoid content and its degradation relating oxygen concentration treatments

The percentage of carotenoid degradation can be calculated based on the photometric observations which shown in Figure 3. From Fig. (3), it can be seen that differences in oxygen concentration during degradation affected the carotene value. Based on the analysis of total carotenoids in this study, it was found that the total carotenoids were high, namely in treatment A (oxygen concentration 67 g/100 mL) of 120 µg/g, which experienced 29% degradation. In comparison, the low total carotenoids in treatment D (oxygen concentration 168 g/100 mL) experienced 80% degradation. These results show that the degradation process with an airflow rate of 20 L/h, which was produced 168 g/100 g of oxygen at a temperature of 140 °C for 2 hours, was the best treatment in the carotene degradation process. The higher the oxygen concentration, the more total carotenoids will be decreased. This finding was relate to the general hypothesis of oxidation reaction of carotenoid (23).

β-ionone formation: The formation of β-ionone compounds was detected using GCMS. Confirmation of the presence of ionone compounds is carried out by comparing the molecular ions of the compounds detected in the ethanol solution, which captures volatile compounds produced from the carotenoid degradation process of CPO. In addition, the retention time of the detected compounds was compared with the standard of β-ionone. Figure 4 shows the chromatogram of the solution that contains the volatile compounds resulting from the degradation of carotenoids from CPO.

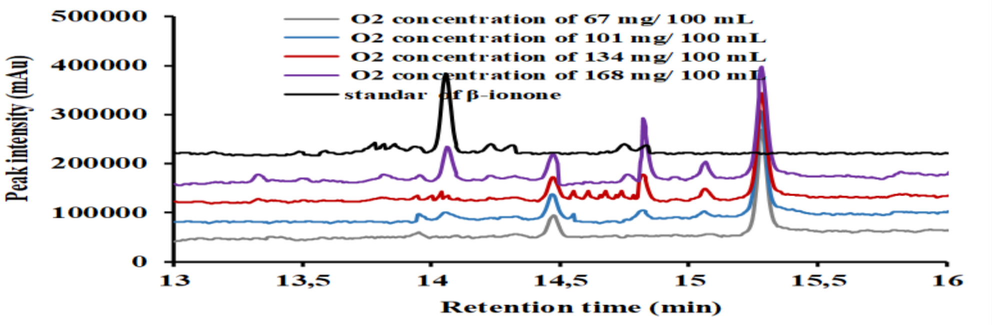
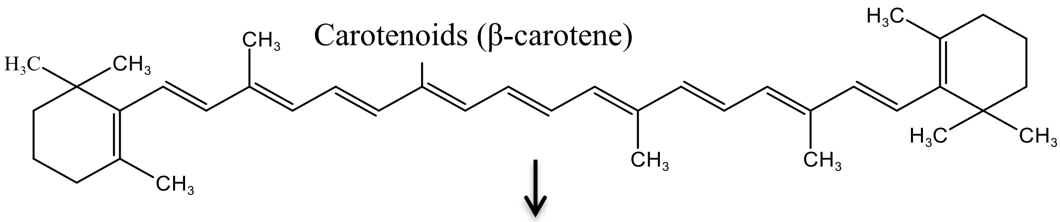


Fig. 4. Chromatogram of GCMS of β-ionone formation due to oxygen treatments

From Figure (4), several things can be observed. First, the formation of β-ionone can be detected in the chromatography system that has been carried out. β-ionone was detected at 14.08 minutes. Secondly, it was known that the treatment of the oxygen concentration during the degradation process has provided a different trend in the formation of β-ionone. As the oxygen concentrations up to 138 g/100 mL, the formation of β-ionone in the ethanol solution could not be observed. The formation of new β-ionone was start to be detected at an oxygen concentration of 168 g/mL. This 168 g/100 mL concentration was obtained by flowing air at a flow rate of 20 L/h for 2 hours. Based on this data, it can be concluded that oxygen concentration plays a vital role in forming β-ionone in the degradation process of carotenoid compounds. Furthermore, something interesting was also discovered in Figure (4). At 14.85 minutes, the formation of a compound was observed, and it increased as the oxygen concentration increased. Based on molecular ion information from the mass spectrometry system, it was known that the compound detected was Dihydroactinidiolide (dhA). The formation of dhA appears to occur gradually as the oxygen concentration increases. The detection of dhA formation makes the results of this research very interesting. dhA is a further compound that can be formed if β-ionone continues to be oxidized (1,17). The formation of dhA starts from the formation of β-ionone. This result may explain why, at 67 g/ 100 mL, 101 g/ 100 mL, and 134 g/ 100 mL, β-ionone did not seem to be detected at oxygen concentrations. This condition may be because the reaction has already progressed to the formation of dhA. The formation of β-ionone was not comparable to the formation of dhA since only a small amount of carotenoid was degraded. When the oxygen concentration reaches 168 g/100 mL, carotenoid degradation reaches 80%, so this condition induces the formation of more dhA as well as the formation of ionone. Even though β-ionone was detected, the amount was very little. The concentration of β-ionone formed was only 4,4 mg/L. This result was in line with previous research, which has stated that dhA will be formed along with the formation of β-ionone. Controlling the amount of oxygen was unable to restrain the formation of dhA from further oxidation of β-ionone. The formation of dhA naturally occurs due to heat, so β-ionone will produce 5,6-epoxy-β-ionone, which will then form the dhA compound[24]. This pathway can be seen in Figure 5. To fulfill the need for β-ionone on an industrial scale, this further reaction, which can form dhA, will be an obstacle. Therefore, optimization of reaction conditions that can only produce β-ionone from the degradation of carotenoid compounds but not produce dhA needs to be further developed.



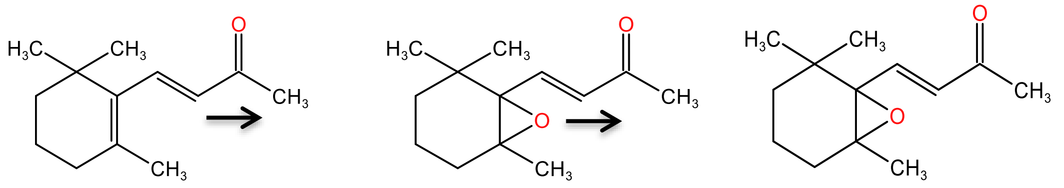


Fig. 5. pathway for the formation of dhA in the carotenoid degradation process

Conclusion: The degradation process of carotenoid compounds from CPO has been carried out using various oxygen concentrations. The amount of oxygen can affect the amount of β-ionone formed. The greater the oxygen concentration used, the more β-ionone will be produced. However, the amount of β-ionone formed is also influenced by the formation of Dihydroactinidiolide (dhA). This compound is a continuation result of the β-ionone degradation process. Perhaps the amount of oxygen can also trigger further degradation of β-ionone to dhA. Of course, this is detrimental because the amount of β-ionone formed is small. Further research to find optimum conditions for the formation of β-ionone from carotenoid compounds will be more challenging because it also has to consider how the degradation process only stops until the formation of β-ionone; the reaction does not continue until the formation of dhA.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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