



Effect of Degradation Time on Carotene Content in Crude Carotene Extract of Carrot for the Formation of its Derivative Aromatic Compounds

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ABSTRACT

Background: The difference in degradation time on the formation of carotene's derivative aromatic compounds [β -ionone and dihydroactinidiolide (dhA)] due to thermal degradation process of carotenes in crude carotenoid extract of carrot was studied.

Methods: β -ionone and dhA formation and that of carotenes degradation during thermal degradation ($T=140^{\circ}\text{C}$) was observed periodically for 4 h. The constant oxygen supply during degradation was maintained at approx. 7 L/h.

Result: The formation of β -ionone and dhA accumulated significantly with increasing duration of the degradation process. The maximum formation of β -ionone and dhA could be estimated with the pattern of carotenes degradation. After 4 h of degradation, more than 80% carotene has degraded. It can be concluded that the degradation process of crude carotene extract from carrots at temperature of 140°C will result in a maximum carotene degradation process when the degradation process is carried out for 5 h.

Key words: Carotene, Carrot, Derivative aromatic compounds, Fragrant.

INTRODUCTION

The fragrance industry's demand for raw materials is currently on the rise (Jugreet *et al.* 2021). Fragrance raw materials are usually produced synthetically and/or extracted from natural or agricultural sources. In comparison to synthetic raw materials, the need for natural raw materials would undoubtedly be more suitable. The distillation method is commonly used to remove fragrances from natural or crops (Burger *et al.* 2019). Since the yield of fragrances in natural ingredients is only 1-3 per cent, this process is known to require a large amount of raw materials (Stratakis and Kidis 2016).

To overcome this problem, a technology on process engineering has been developed to obtain fragrances without extraction from natural materials. The change of secondary metabolite compounds that have high yields in natural substances into aromatic compounds can be considered for this situation. As theoretically, the changing β -carotene compounds into raw materials for obtaining β -ionone and dhA compounds have been introduced (Zepka *et al.*, 2014). Carotenes including β -carotene are pigments that are mainly contained in several plants or crops such as oil palm, carrots, maize kernels and papaya (Pandey *et al.* 2003; Panghal *et al.* 2009; Pitambara and Nagarajan, 2016; Syukri and Chamel, 2021). This compound is present in large quantities and is not categorized as an aromatic compound. Meanwhile β -ionone and dhA are derivative compounds that can be produced by the process of oxidation carotene compounds. This compound is one of the aromatic compounds that are widely used as fragrances in processed products such as soap, shampoo and even food.

There have been many studies that have developed regarding the conversion of β -carotene to ionone and dhA.

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The conversion of β -carotene to β -ionone and dhA can be carried out chemically (oxidation) or enzymatically (Czajka *et al.*, 2018; Perveen *et al.*, 2019). To obtain β -carotene, it is necessary to carry out the process of isolating β -carotene from crude carotene extracts produced from agricultural materials. Carrying out the isolation process would require a considerable amount of effort and would be impractical in practice. The use of carotene crude extract without β -carotene isolation has also been done previously. The study concluded that the degradation process of crude carotene extract can also be used as a base material for obtaining β -ionone and dhA. The information obtained from this study is still limited. The research results only inform that the best condition for the degradation process is the thermal degradation process at a temperature of 140°C for 4 h (Hazrulrizawati *et al.* 2017). There is no information yet about

the degradation patterns of β -carotene and the patterns of β -ionone and dhA formation that occur. Therefore, in this study that matter will be clarified.

The process of β -ionone and dhA formation is carried out by thermal degradation from the crude carotene extract of carrot. The degradation process was carried out at a temperature of 140°C for 4 hours. The pattern of carotene degradation and ionone and dhA formation will be observed periodically. This data will be useful in developing this process on a pilot scale in the future.

MATERIALS AND METHODS

The study was arranged at the laboratory of Food and Agricultural Product's Phytonutrient, Department of Agricultural Product Technology and the laboratory of Animal Biotechnology, Department of Animal Production Andalas University, Indonesia on from February 2021 to May 2021.

Samples preparation

The dried carrots were made by soft heating of ground fresh carrots at a low temperature (60°C) until the moisture content was about 15%.

Carotene extraction

The extraction of carotene was conducted according Rifki *et al.* 2020 with modifications. A 200 mL hexane was applied to 100 g of dried sample. After that, the mixture was homogenized. Maceration was used to extract the carotenoid. The maceration process was supported by the use of an ultrasonic apparatus for 4 h and then proceeded without the use of the sonication method for another 8 h. The liquid phase was isolated from the residue after the mixture was filtered. The maceration process for the residue was repeated every 12 hours until the sample's color changed from orange to white, indicating that all of the carotene had been removed. The solvent was evaporated with a rotary evaporator until the viscous extract of carotene was obtained.

Thermal degradation of crude carotene extract

Degradation was carried out according to Hazrulrizawati *et al.* 2017 with some modification by using a Rancimat 743 apparatus (Herisau, Switzerland) with a temperature range of 80 to 220°C. A 0.5 g of Crude carotene extract was weighed and mixed with 4.5 g of glycerol in a 50 mL reaction vessel which was then placed in an electric heating block. The instrument was operated at 140°C with an air flow rate of 7 L/hr. The degradation process lasted 4 hours, with hourly sampling intervals. In a measuring vessel containing 10 mL of ethanol, effluent air containing volatile organic acids, including volatile organic compounds from crude carotene samples, was collected. Until injecting 2 mL of ethanol into the Gas Chromatography-Mass Spectrometer, it was filtered through a 0.2 M polytetrafluoroethylene membrane filter.

Analysis of total carotene

In a 25 mL amber volumetric flask, a viscous extract of 0.1 g was transferred. The volume was filled with hexane and

the sample was measured at 446 nm with a UV-VIS Spectrophotometer. The total carotenoid content was calculated using the following formula:

Carotenoids content:

$$\text{Carotene} \left(\frac{\mu\text{g}}{\text{g}} \right) = \frac{25 \times A \times 383}{100 \times \text{sample weight (g)}}$$

Where

A = absorbance at 446 nm, 383 = diffusion coefficient of caroten, V = value of hexane (mL) and W = weight of sample (g).

Detection of Aromatic volatile compounds

The detection of volatile compound was conducted according to Syukri *et al.* 2019 and Rini *et al.* 2021 with slight modification. A mass spectrometer (Shimadzu GCMS-QP2010 Ultra, Kyoto, Japan) was used to analyze the volatile compounds formed by crude carotene degradation 10,11. Helium served as the carrier gas. The DB-5 MS capillary column (30 m length, 0.25 mm inner diameter, 0.20 m film thickness, Agilent, USA) was used for matrix separation. The injector temperature was set to 220°C, the column oven temperature was raised from 60°C to 260°C and the detector interface temperature was set to 270°C. The separation was carried out using the following temperature program: the temperature was set to 60°C and held for 4,5 minutes. The temperature was then increased to 130°C at a 5°C min⁻¹ ramp rate and then to 260°C at a 10°C min⁻¹ ramp rate. For 2 minutes, the final column temperature was kept at 260°C. With split less injection mode, 1 mL of sample was injected for each sample. A mass spectral library search and a peak area normalization method to compare retention indexes were used to identify compounds. The results of the experiment were validated by repeating it at least three times.

Colour measurement

The carotenes sample were check for colour changes with a *hunterLab. colorFlex EZ*. The measurement referred to the method that described by Olgunoglu (2013). The measurement was conducted at least three times.

Analytical statistics

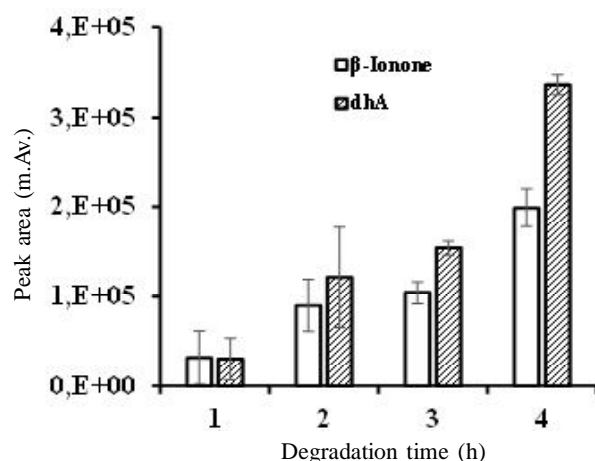
All determination's results are stated as the average of three replicates. Analysis of variance was used to assess statistical significance and the means were compared using Tukey's test at a 5% level of significance using R 3.3.2. (R Foundation for Statistical Computing).

RESULTS AND DISCUSSION

Fig 1 indicates the accumulation of β -ionone and dhA relating to carotenes degradation during thermal degradation at 140°C for 4 h. The accumulation of β -ionone and dhA increased significantly after 2 h degradation process. β -ionone increased four times after 4 h degradation while dhA increased six times. Previous reports indicated that the formation of dhA would be more than that of β -ionone

Table 1: Carotenes degradation during thermal degradation for 4 h.

Time (h)	Total Carotene content ($\mu\text{g}.\text{mg}^{-1}$)	Carotene degradation (%)
4	$1,34 \pm 0,21^a$	86,05
3	$3,49 \pm 0,41^b$	63,68
2	$3,72 \pm 0,81^b$	61,29
1	$6,59 \pm 1,07^c$	31,42
0	$9,61 \pm 1,01^d$	0

**Fig 1:** The accumulation pattern of β -ionone and dhA relating to carotenes degradation during thermal degradation at 140°C for 4 h.

(Kanasawut and Crouzet, 1990). The thermal degradation process of β -carotene isolated from palm oil has formed dhA fifteen times that of β -ionone. The degradation process of β -carotene will produce a higher amount of dhA than β -ionone (Wache *et al.* 2003; Shumbe *et al.* 2014; Ge *et al.* 2015; Hazrulrizawati *et al.* 2017). According to the proposed β -carotene degradation pathway, the formation of dhA occurs as the first oxidation product prior to the formation of β -ionone (Gloria *et al.*, 1993). Although there was another proposed β -carotene degradation pathways that suggest β -ionone formation occurs before dhA (Kanasawut and Crouzet, 1990; Sommerburg *et al.* 2003), the findings of this study suggested that the proposed pathway of Gloria *et al.* (1993) was more appropriate. Therefore, it can be concluded that the use of thermal reactions in the degradation process of carotene compounds produced β -ionone lower than dhA.

The degradation pattern of crude carotene during thermal degradations was observed using spectrophotometric analysis. The amount of carotenes that was degraded was influence by time. In this study, the sample used was a non-

polar extract of carrot. The extract contains many substances that solved in hexane. The maximum wavelength obtained was 446 nm, indicating the presence of carotene as the main compound of the carrot crude carotene extract (data not shown). Moreover, there was a significant degradation of carotenes during 4 h of degradation period (Table 1). There has been carotene degradation of 30% in the first hour of the degradation process and continues in the next hour. After 4 h of degradation period, more than 80% of the initial carotene was degraded. At initial, there was $9,61 \mu\text{g}.\text{mg}^{-1}$ of carotene in crude carotene extract of carrot, after 4 h of degradation only $1,34 \mu\text{g}.\text{mg}^{-1}$ of carotene was detected, therefore it can be estimated that the degradation process of crude carotene to form β -ionone and dhA might no more than 6 h. The result of this study indicated that carotenes including β -carotene degraded rapidly due to heat treatment. This result was in agreement with previous research conducted by Hazrulrizawati *et al.* (2017). However, in contrast with the condition of under light treatment, β -carotene degraded in low rate as mentioned by Limbo *et al.* (2007) and Torri *et al.* (2007). The presence of other non-polar matrices did not appear to have an effect on the degradation process of β -carotenes.

Table 2 indicates the colour observation of crude carotene extract of carrot during thermal degradation. The color observation of thermally degraded crude carotenes was observed on Hunter color Lab in the form of Hue, 'L', 'a' and 'b' values. There was an increasing of hue value of treated crude carotene extract thermal degradation from 32 to 39. In line with the carotene degradation pattern, the color change also occurred from orange to yellow which indicates the degradation of the carotene compound. On 'L', 'a' and 'b' values, there was no significant changes during first 3 h of degradation process however after 4 h degradation, there was a decreasing of 'L', 'a' and 'b' values. These data indicated, as degradation of carotenes occurred, other compounds shift, potentially darkening the color of the mixture. It can be suggested that the degradation of other non-polar compounds such as fatty acids might occurred at this time (at 4 h degradation). The browning reaction due to the maillard reaction and the formation of acrylamide might also occur due to the presence of non-polar vitamins, fats, proteins and carbohydrates (Reddy and Jialal, 2020; Moran and Johnson, 2017, Aprialis *et al.* 2021). Although present in a small amount, as a biochemical product, carrot extract also contains fat-soluble parts which will also be extracted during carotene extraction which will then react when the heat degradation process occurred.

Table 2: Colour of crude carotene extract of carrot during thermal degradation.

Time (h)	L*	a*	b*	C	°Hue
4	$6,28 \pm 0,03^a$	$1,52 \pm 0,03^a$	$1,24 \pm 0,04^a$	$1,96 \pm 0,05^a$	$39,26 \pm 2,75^b$
3	$9,50 \pm 0,06^b$	$5,98 \pm 0,03^c$	$4,65 \pm 0,03^d$	$7,58 \pm 0,04^d$	$37,85 \pm 0,21^b$
2	$9,11 \pm 0,05^c$	$5,59 \pm 0,05^b$	$4,26 \pm 0,21^c$	$7,02 \pm 0,15^c$	$37,29 \pm 1,23^b$
1	$8,34 \pm 0,06^d$	$5,52 \pm 0,04^b$	$3,51 \pm 0,01^b$	$6,54 \pm 0,02^b$	$32,44 \pm 0,24^a$

CONCLUSION

The formation of β -ionone and dhA was investigated through a thermal degradation of crude carotene extract of carrot. The formation of dhA was prominent compare to β -ionone. The process of degradation can be conducted optimally in about 5 hours where in this condition, all carotene has been completely degraded. The finding of this study can be useful in developing appropriate techniques in the utilization of non-aromatic substances from agricultural products to produce aromatic compounds that is useful as an alternative solution to the problem of procuring aromatic compounds which are constrained by the difficulty of raw materials.

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