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Short Communication

Production of Aromatic Compounds from Crude Carotene Extract of Carrots by Thermal Degradation: A Preliminary Study

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Abstract

Background and Objective: β -ionone and dihydroactinidiolide (dhA) are derived from carotenes and have a unique character, these compounds have been identified as flavour-producing additives. The formation of beneficial β -ionone and dihydroactinidiolide (dhA) by thermal degradation of β -carotene in crude carotene extract from carrot was studied. **Materials and Methods:** The crude extract of carotene was oxidized under the thermal condition with three different temperatures 120, 140 and 160°C with controlled air supply as much as 7 litres per hour for 1, 2, 3 and 4 hrs. The degradation process was conducted using a Rancimat 743[®] apparatus. The occurrence of degradation products of carotene was observed using gas chromatography-mass spectrometry. **Results:** Thermal degradation of crude carotene extract promoted the formation of β -ionone and dihydroactinidiolide influenced by the increase of temperature and oxidize time. The higher the degradation temperature and the longer the degradation process, the more degradation products formed. The formation of some others compounds such as alkenes, esters and fatty acid was also observed. Although crude carotene extract consists of a complex matrix, with the proper degradation conditions such as optimal temperature and time, valuable target compounds could still be produced. **Conclusion:** These findings provided wide opportunities in utilizing crude carotene extract as the source of the substrate in producing some beneficial aromatic compounds.

Key words: Aromatics, carotene, crude extract, degradation, oxidation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

β -ionone and dihydroactinidiolide (dhA) are aromatic compounds derived from carotenes that have a unique aroma that makes them highly usable in household products such as cosmetic, food and personal care¹. β -ionone is a prominent scent and aromatic molecule present in many flowers and fruits, such as blackberries, peaches and apricots, among others which are associated with violet scent and a woody odour character². Moreover, dhA is also recognized as an aromatic compound in plants such as tobacco and tea with ripe apricot red fruit woody aroma³. To date, these compounds have been identified as flavour-producing additives in non-alcoholic drinks, ice cream, candy, baked goods, gelatine and puddings, chewing gum and maraschino cherries, as well as cosmetic and personal care items like shampoo and soaps⁴.

Even though these products are extremely beneficial to the industry, extracting these aroma compounds directly from plant sources is costly and inefficient. Apart from its low concentration of plants, agricultural and environmental factors such as land scarcity and the difficulty of the extraction process are the main reason for this situation. A large number of chemical modifications of carotenes represents a very attractive alternative for the sustainable production of β -ionone and dhA recently^{5,6}.

Carotenes are one of the secondary metabolite compounds in plants which are a subgroup of isoprenoid compounds that contain more than 600 chemical structures and are distinguished by the presence of a conjugated tetraterpene matrix. They've been identified as precursors to a variety of other bioactive derivatives that are typically produced through oxidative degradation including β -ionone and dhA. Many reports have been published on the chemical and enzyme-assisted in the processing of β -ionone and dihydroactinidiolide, nonetheless, many studies have been done focusing only on the degradation of isolated β -carotene and no studies have been carried out on the degradation of crude carotene extract from plants^{7,8}.

Although the purity of β -carotene is essential for the production of β -ionone and dhA, isolation of β -carotene from crude carotene is difficult practically. It is apparent that many plants contain significant quantities of carotenes, which can be differentiated by their colour such as carrot. Carotene is classified as a non-polar compound that has similar properties to other lipids in plants such as fatty acids and membrane lipids⁹. Therefore, degradation of β -carotene in crude lipids or carotene extract might quite difficult compare to degradation of isolated β -carotene. It can be hypothesized that the use of

crude carotene extracts from carotene-rich plants can be developed into raw materials for the manufacture of β -ionone and dhA either by using the chemical or enzymatic technique. Therefore, in this study, the utilization of crude carotene extract from carrot had done as a substrate for the degradation process on the production of beneficial β -ionone and dhA. A very simple chemical degradation process using measurable heat and oxygen has been carried out in this study which can be the basis for the development of carotene biotransformation to produce β -ionone and dhA.

MATERIALS AND METHODS

Study area: The study was carried out during February-April, 2021. The study was conducted at the laboratory of crop processing engineering, Department of Crop Technology and Laboratory of Livestock Biotechnology, Faculty of Animal Science, Andalas University.

Samples preparation: Dried carrots were prepared by heating the grinded-fresh carrots at low temperature (60°C) until their moisture content was reached approx. 15%.

Carotene extraction: A hundred gram of dried sample was added to 200 mL of hexane (technical grade). The mixture was then homogenized. The extraction was conducted by maceration. The maceration process was assisted by the use of an ultrasonic apparatus for 4 hrs and then continued for another 8 hrs without the use of a sonication system. The mixture was then filtered where the liquid phase was separated from the solid phase. The maceration process for the solid phase was carried out continuously (every 12 hrs) until the colour of the sample, which was orange, turns white, which indicates that all the carotene has been extracted. The collected liquid phase was combined and 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 with a model A Metrohm Rancimat 743 (Herisau, Switzerland) with a temperature range of 80-220°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 120, 140 and 160°C with an airflow rate of 7 litres per hour. Degradation was carried out for 4 hrs with an hourly sampling time. Effluent air containing volatile organic acids including volatile organic compounds from the crude carotene samples were collected in a measuring vessel

containing 10 mL of ethanol. A 2 mL of ethanol was filtered through a 0.2 μ M polytetrafluoroethylene membrane filter before injected into Gas Chromatography-Mass Spectrometer.

Aromatic volatile compounds detection: The volatile compounds produced from crude carotene degradation was analysed by using mass spectrometer apparatus (Shimadzu GCMS-QP2010 Ultra, Kyoto, Japan)^{10,11}. The helium was used for carrier gas. The capillary column used for fatty acid separation was DB-5 MS (30 m length $\times$ 0.25 mm inner diameter $\times$ 0.20 μ m film thickness, Agilent, USA). The injector was set at 220°C, the column oven temperature was elevated from 60-260°C and the detector interface was set at 270°C. The separation was performed using a temperature program as follows; the temperature started at 60°C and hold for 4, 5 min. The temperature was then raised to 130°C at a ramp rate of 5°C min⁻¹ and then it further increased to 260°C at a ramp rate of 10°C min⁻¹. The final column temperature was maintained at 260°C for 2 min. A 1 mL of sample was injected for each sample with splitless injection mode. Compounds identification was performed using a mass spectral library search and a peak area normalization approach to compare retention indexes. The obtained data were confirmed by repeating the whole experiment at least three times.

RESULTS AND DISCUSSION

Figure 1 showed the GCMS chromatogram of the collecting solution (ethanol) that contain the products after degradation of crude carotene extract from carrot at 120, 140 and 160°C with periodic degradation time (1, 2, 3 and 4 hrs). Since glycerol was used as a medium for crude carotene extract on the degradation process, the accumulation of glycerol was observed during degradation at any temperature conditions. The longer the degradation process, the more intensity of glycerol accumulated in ethanol. In Fig. 1a, c and e, the accumulation of glycerol was pointed at the retention time of 7-9 min. The shift in glycerol retention time occurs due to its increasing intensity. The retention time on gas chromatography correlates with the polarity of separated compounds. Compounds resulting from the degradation of crude carotene appear after the peak of glycerol which indicates that these compounds have a low polarity than glycerol. Based on theory, the degraded compound from carotene is a carbonyl compound such as β -ionone and dhA which has a lower polarity than alcohol (glycerol).

The occurrence of degraded compounds has observed starting from the retention time of 14 min. In Fig. 1b, d and f, the presence of β -ionone and dhA was pointed at the

retention time of 14.03 and 14.8 min, respectively. The accumulation of β -ionone was not significant at degradation temperature of 120°C, on the other hand, the accumulation of dhA has been observed starting from the degradation temperature treatment at 120°C, the trend of increasing dhA intensity is getting clearer along with the length of the degradation process. Furthermore, in the first hour of each temperature conditions showed the same pattern with no significant accumulation of β -ionone and dhA in Fig. 1 b. The study on degradation of β -carotene has been carried out in the last few decades where the degradation temperature has proposed start at 120°C¹². The current result indicated that it would take time to degrade the carotenes from the crude extract of carrot when the degradation temperature was at 120°C. The complexity of crude carotene extract from carrot might delay the degradation process.

In this study, the crude carotene extract of carrot was obtained by the soxhlet extraction technique. This extraction technique could extract all non-polar substances. As mentioned by Hewavitharana *et al.*¹³, the non-polar crude extract of foods consist of oils and fat fractions include triacylglycerol and sterols that have high degradation temperatures that is more than 140°C¹⁴. Some literature had suggested that the degradation temperature of β -carotene to form its derivative compounds was achieved around 140°C¹². Furthermore, the accumulation of β -ionone and dhA was observed clearly from the product of degradation temperature of 140 and 160°C in Fig. 1d and f. These results were similar to previous research that proposed the degradation of β -carotene occurred at the temperature condition of more than 140°C¹². Although the degradation of β -carotene has started at 120°C, when it forms as the crude material, the degradation of β -carotene would become delay due to the presence of other non-polar substances.

Several compounds that formed in maximum intensity due to the degradation process of crude carotene extract from carrot based on GCMS database library searching were shown in Table 1. The degradation temperature of 120°C did not produce many compounds while the higher degradation temperatures produced more compounds. Although β -ionone and dhA had formed on the treatment of degradation temperature of 120°C, however, the intensity was very low. The significant accumulation of β -ionone and dhA was observed on the treatment of degradation temperature of 140 and 160°C from the early time of degradation process to the end of the degradation process. The formation of β -ionone and dhA appears to occur in the greatest amount under degradation conditions at 140 for 4 hrs. The higher the degradation temperature, the more degraded compounds

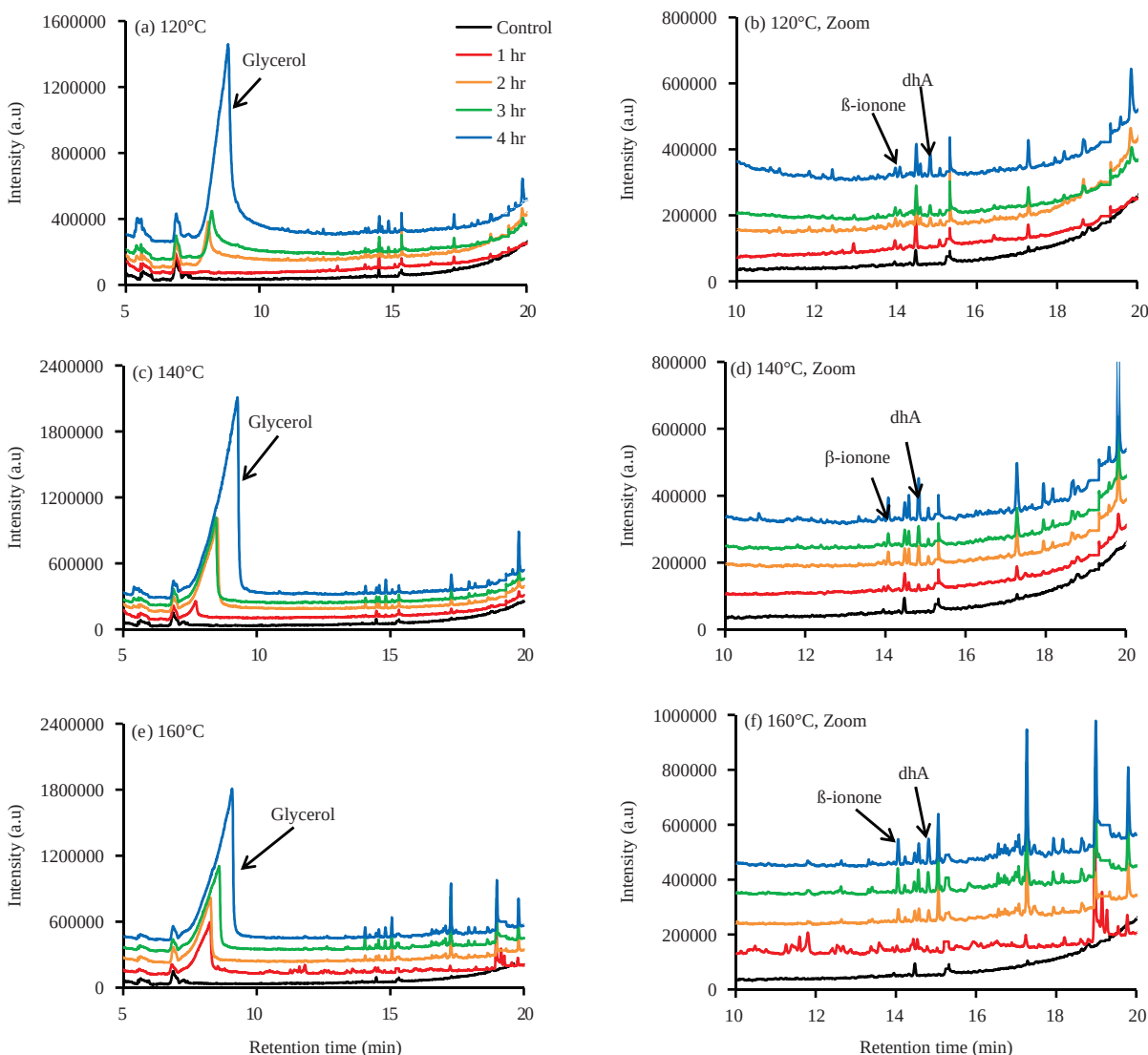


Fig. 1(a-f): Chromatogram GC/MS

(a) Chromatogram of the degraded product of crude carotene extract from carrot at 120°C, (b) Zoom of the chromatogram to eluate the degraded products of crude carotene extract from carrot at 120°C, (c) Chromatogram of the degraded product of crude carotene extract from carrot at 140°C, (d) Zoom of the chromatogram to eluate the degraded products of crude carotene extract from carrot at 140°C, (e) Chromatogram of the degraded product of crude carotene extract from carrot at 160°C and (f) Zoom of the chromatogram to eluate the degraded products of crude carotene extract from carrot at 160°C

produced. As the character of crude carotene extract of carrot that contains many non-polar substances, the degradation process might not only occur in carotenes. The compounds such as triacylglycerol including fats/oils would be also degraded. There were the accumulations of degraded hydrocarbon including aldehydes, ketone and carboxylic acids derivatives during the degradation process. At conditions of higher degradation temperatures, the formation of advanced oxidation compounds such as aldehydes and fatty acids is more dominant than the formation of β -ionone and dhA. At a degradation temperature of 140°C, the accumulation of furan

derivative compound at the retention time of 19.75 min has occurred in the highest intensity. The formation of furan due to thermal degradation of macromolecules, this kind of degradation have proposed by several studies due to the presence of non-polar amino acids substances^{15,16}. Moreover, at a degradation temperature of 160°C, the occurrence of alkenes (1-octadecene) and fatty acid (Heptadecanoic acid) was observed significantly. It seemed that the degradation of fat or oils had started on this condition. Several studies indicated decomposition of triglycerides results in the release of free fatty acids and alkenes^{14,17}.

Table 1: Thermal degradation products of crude carotene extract of carrot at various temperature for 4 hrs degradation process

Temperature (°C)	Duration (hr)	Produced compounds (relative intensity)	Retention time (min)	Relative intensity to all calculated peaks (%)
120	4	β -ionone	14.05	1.32
		Dihydroactinidiolide (dhA)	14.80	2.74
		1-Heptadecene	17.93	4.26
		1,2,4-Metheno-1H-cyclobuta[b]cyclopenta[d]furan, 2,2a,3a,4,6a,6b-hexahydro-3a-methyl-	19.8	6.15
140	4	β -ionone	14.05	2.68
		Dihydroactinidiolide	14.80	4.16
		1-Hexadecene	15.05	1.06
		1-Heptadecanol	17.27	6.68
		1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)-	18.10	1.13
		1,2,4-Metheno-1H-cyclobuta[b]cyclopenta[d]furan, 2,2a,3a,4,6a,6b-hexahydro-3a-methyl	19.75	13.81
160	4	β -ionone	14.05	3.01
		Dihydroactinidiolide	14.80	2.89
		1-Nonadecene	15.05	4.66
		1-Octadecene	17.27	13.37
		Levopimaric acid methyl ester	17.93	1.71
		Heptadecanoic acid	18.90	12.57
		Tricyclo[4.2.1.1(2,5)]deca-3,7-diene-9,10-diol, 9-methyl-, stereoisomer	19.75	8.12

The crude carotene extract of carrot consist of complex non-polar substances including carotenes and fats/oil compounds, the difference in the level of degradation temperature could make the pattern of formation of derivative compounds would be also different. From this study, it could be confirmed that the degradation temperature of carotenes is lower than oil compounds¹⁸⁻²⁰. The ratio of β -ionone and dhA was observed at the degradation condition of 140°C for 4 hrs in the value of 1:2, where this data was similar to the previous study that indicates the accumulation of dhA was higher than β -ionone¹².

CONCLUSION

β -ionone and dhA are the valuable aromatic compounds that could produce from the degradation of β -carotene. The utilization of crude carotene extracts from carrot as the raw material for the production of β -ionone and dhA was investigated. The temperature of carotene degradation is thought to be lower than that of other non-polar substances so that the ionone formation process is optimized without the formation of other derivative products. In this study, the condition at the temperature of 140°C with a 4 hrs degradation process was suggested as the proper degradation condition in the production of β -ionone and dhA from crude carotene extracts of carrot.

SIGNIFICANCE STATEMENT

This study discovered the potentiality of crude carotenoid extract of carrots for the development of some aromatic compounds such as β -ionone and dhA. This result can be beneficial for the fragrance industry to overcome the

limitation of raw materials. This study will help the researchers to further develop the technology for the production of aromatic compounds without the conventional distillation method.

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