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SEPARATION OF CAROTENOIDS IN TOMATO

(PEMISAHAN KAROTENOID DALAM TOMAT)

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ABSTRAK

Telah dilakukan penelitian mengenai pemisahan karotenoid dalam zat warna (pigmen) tomat. Pemisahan zat warna atas golongan karoten, monol, diol dan poliol dila-kukan dengan kromatografi kolom, sedangkan pemisahan komponen dari tiap-tiap golongan dilakukan dengan kromo-tografi lapisan tipis.

Identifikasi dilakukan dengan pengukuran penyerapan maksimum sinar tampak, Rf dan pengujian gugus epoksi dan dibandingkan dengan literatur. Sebelas senyawa secara tentatif telah berhasil diidentifikasi, yakni 6 dari golongan karoten, 1 dari golongan monol, 3 dari golongan diol dan 1 dari golongan poliol.

INTRODUCTION

Carotenoids are an important group of naturally pigment and distributed in green leafy vegetables and in orange or red coloured fruits. Carotenoids are also found in insects, birds and other animals. It was estimated more than 100 milion tons of carotenoid produced in nature a year (Weedon, 1971).

Carotenoids play 2 important roles in foods, especialy in fruits and vegetables. Firstly, due the presence of many conjugated double bonds in the molecule, they are brightly coloured and, therefore, enhance, the aesthetic value of foods. Secondly, many carotenoids have nutritional value due to their ability to be metabolised in the body to retinol (vitamin A). which is essential to maintain proper functioning of occular systems.

Recenty, epidemioligical investigations have indicated that the level of dietary or blood carotenoids may be ralated to the incidence of human cancer, A range of carotenes and xanthophylls, with or without vitamin A activity, have been found in laboratory studies with animals to retard the development of some types of cancer (Hirayama, 1979).

Tomato is a type of fruit which contains carotenoids and a number of reports has been published concerning carotenoid composition of tamato (Curl, 1961; Zakaria *et al*. 1979 and Hirota *et al*, 1982). However, there is no report about the composition of Indonesian tomato and the method used for separating the components. This paper reports the method used for separating individual components of Indonesian tamato as well as tentatively identifying the components.

MATERIAL AND METHOD

All chemicals used were of pro-analysis grade. Potassium hydroxide, sodium hydroxide, sodium chloride, sodium sulphate, phenolphtalein, anhydride acetate, chloride acid, hyflo-super-cel, dimethylamine, butyl hydroxy toluene, silica gel, alumina and magnesium oxide were from Merck. All sovents used for column cromatography, thin layer cromatography and spectroscopy were of pro-analysis grade. Chloroform was washed with double distilled water to remove trace acids prior to use. Tomato fruits for experiments were obtained from local retail market in Padang, West Sumatra.

Extraction of Carotenoids

Tomato pigment were extracted according to the method of Zakaria *et al*. (1979) with a slighdy modification, by replacing petroleum ether with dietyl ether. Tomato fruits were cut off into pieces and homogenized with blender. The homogenates were extracted with aceton and filtered through Wahtman paper. Residues were re-extracted with acetone until they became colourless.

Acetone extracts were added diethyl ether with te same volume and sufficient double distilled water was then added. After shaking the mixture was allowed to settle until separation between aqueous layer and organic layer was obtained. Aqueous layer was then re-extracted with diethly ether. Organic extracts were combined and washed with double distilled water to remove acetone and dried over anhydrous sodium sulphate. During the process all conta-iners were protected from sulight by covering with carbon paper. The extracts were purged with nitrogen gas for oxygen exclusion.

Saponification

For removing unwanted materials, such as chlorophylls, fats as well as hydrolysing ester carotenoids, pigment extracts were saponified. For this saponification the method of Curl (1953) was routinely used during the experiments. The saponification was carried out by adding 20% potassium hydroxide-methanol solution into solution of carotenoid in diethyl ether and leaving the mixture overnight in the dark. Unreacted alkali was washed wih water until aqueous layer became colourless with phenophtalein. Organic layer was dried over anhydrous sodium sulphate. After filtering, solvent was removed in vacuo and residue was re-dissolved with chloroform for further experiment.

Separation into Carotene, Monol, Diol and Polyol Groups

Separation of carotenoid into carotene, monol, diol and polyol groups was adopted from the method of Gross *et al.* (1971). Pigment solution in chloroform was subjected on the top of column with adsorbent of magnesium oxide-hyflo-super-cel (1:1). Carotene fraction was eluted with 5% acetone in petroleum ether, monol with 10% acetone in petroleum ether, and diol-polyol mixture with petroleum ether-ethanol (1:1). The mixture of diol-polyol was then separated with 10% acetone in petroleumether-ethanol (97:3).

Separation of Individual Components of Carotene Fraction

Carotene fraction was separated into individual compenents with column chromatography using the same adsorbent and eluted with petroleum ether to give 3 sub-fractions. The first two sub-fractions were colourless and after collection each fraction gave absorption maxima at 277,285,298 nm and 298,330,365 nm respectively.

The third sub-fraction was pale yellow broad band separated with thin layer chromatography using calcium hydroxide-silica gel G (6:1) and eluted with petroleum ether-benzena (9:1) to give 4 sub-sub-factions. Each sub-sub-fraction absorbed at 423,450,477 nm; 376,400,425 nm; 430,460,488 nm and 445,472,504 nm in petroleum ether respectively. Chromatogarphic behavour showed that each sub-sub-fraction gave Rf at 0.85,0.60,0.40 and 0.20 on calcium hydroxide-silica gel G(6:1) when eluted with petrolum ether-benzene (49:1).

Separation of Individual Components of Monol Fraktion

Solution of monol in choloform was separated with preparative thin layer chromatography of alumina. Eluting with 5% acetone in petroleum ether gave 2 sub-fractions. Sub-fraction 1 gave sharp band with dark red colour, while sub-fraction 2 was yellow broad band. Both sub-fractions were scraped off, exctracted with chloroform and filtered to remove the adsorbent. sub-fraction 1 did not give any colour change when treated with 0.1 N hydrochloric acid in ethanol. Sub-fration 1 absorbed at 426,448,477 nm in petroleum ether and gave Rf of 0.72 on adsorbent of silica gel with eluent of methylene cholride-ethylacetate (4:1). Sub-fraction 2 was a mixture and was unsuccesfull to separate either by increasing or decreasing the polarity of eluent and it was not examined for further identification.

Separation of Diol Fraction

Solution diol fraction in chloroform was subjected on thin layer cromatography of silica gel G and eluted with petrolum ether-ethyl acetate-isopropanol (95:10:5) to give 5 sub-fraction. Sub fraction 1, 2 and 4 were red sharp band, while sub-fractions 3 and 5 were red broad band.

All sub-fractions were scraped off, extracted with chloroform, filtered to remove the adsorbent and evaporated the solvent in vacuo. Sub-fractions 1, 2 and 4 gave absorption maxima at 420,446,473 nm; 478,450,422 nm and 467,439,417 nm

in ethanol respectively. When treated with 0.1 N hydro chloric acid in ethanol, sub-fractions 1 and 2 did not show any colour change, but sub-fraction 4 gave dark blue colour, Chromatographic behaviur showed that sub-fraction 1,2 and 4 had Rfs of 0.36, 0.25 and 0.20 respectively on on thin layer chromatography of silica gel G with eluent of methylene chloride-ethyl acetate (4:1). Sub-fration 3 and 5 were mixtures, as they showed broad spots on thin layer chromatography when developed with various eluents. Both sub-fractions were unsuccessful to separate due to small amount of material and decomposed during saparation to give green to blue colour.

Separation of Polyol Fraction

Solution of polyol fraction in chloform was applied on preparative thin layer chromatography of silica gel G and developed with petroleum ether-ethyl acetate-isopropanol (95:10:1) to give 3 sub-fractions.Sub-fractions 1 and 3 were yellow broad bands, while sub-fraction 2 was dark red sharp band.

All sub-fraction were scraped off, extracted with chloroform, filtered to remove the adsorbent and evapora-ted the sovent in vacuo. Sub-fraction 1 and 3 did not give good separation when developed with various solvents on thin layer chromatography and were not examined for further analysis.

Sub-farction 2 gave absorption maxima at 468,442,420 nm in ethanol. When treated with 0.1 N hydrochloric acid in ethanol the green colour was produced. This sub-fraction was then developed on thin layer chromatography of silica gel G with eluent of n-hexane-ethyl acetate (19:1) and showed Rf of 0.70.

RESULT AND DISCUSSION

Separation of Carotenoids into Caratene, Monol, Diol and Polyol Groups

Adsorbent of magnesium oxide-hyflo-super-cel (1:1) gave good result for separating cartenoids into carotene, monol, diol and polyol groups. The constrain to work with this adsorbent was difficult to pack it and sometimes it gives poor separation. The advantage of this adsorbent was stability of carotenoids during the separation, as there was no any colour change during the process. From the colour intensity on column during separation, it was assumed that more carotenes than xanthophylls were available in tomato.

Separation of Carotene Fraction

Separation with column and thin layer chromato- graphies showed that carotene group consisted of 6 components.

Component 1 (sub-fraction 1) gave absorption maxima at 298,285,277 nm in n-hexane and had Rf of 0.20 on thin layer chromatography of silica gel when developed with petroleum ether. By comparing with reported in literature component 1 is assumend to be phytoene. According to Davies *et al.* (1961)

phytoene (2) has Rf of 0.21 on silica gel G developed with petroleum ether and shows absortion maxima at 276,286,298 nm. Gross *et al.* (1971) reported that phytoene was obtained as fraction 1 when eluted from column of magnesium oxide-hyflo-super-cel with petroleum ether. This component was also fraction 1 when eluted with the same eluent and adsorbent.

Component 2 (sub-fraction) gave adsorption maxima at 365,346,330 nm in petroleum ether and had Rf 0.1 on thin layer chromatography of silica gel G developed with petroleum ether Compared with a number of reports in literature, component 2 is suggested to be phytofluene (3). Curl (1961) reported that phytofluene (3) gave adsorption maxima at 366,348,331 nm. In other report Foppen wrote that phytofluene had Rf of 0.12 on thin layer chromatography of silica G, eluted with petroleum ether. Gross *et al.* (1971) also found that phytofluene was the second component when chromatographed on the same column and eluent.

Sub-fraction 3 gave broad band on column chromatography, was then separated on thin layer chromatography of calcium hydroxide-silica gel (6:1) to give 4 components (sub-sub-fractions 1,2,3 and 4) component 3 (sub-sub-fraction 1) absorbed at 477,450,423 nm in petroleum ether with Rf of 0.85 on the above adsorbent eluted with petroleum ether-benzene (49:1). By comparing with report in literature it is proposed that component 3 to be beta-carotene, since according to Foppen (1971) beta-carotene gave absorption maxima at 478, 451, 421 nm and Gross *et al.* (1971) reported that beta-carotene absorbed at 475,450,425 nm in petroleum ether.

Component 4 (sub-sub-fraction 2) absorbed at 425,400,376 nm in petroleum ether and had Rf of 0.60 on thin layer chromatography of calcium hydroxide-silica gel G (6:1) with eluent of petroleum ether-benzene (49:1). These data correspond to those of zeta-caroten which were reported by Gross *et al.* (1971) and Foppen (1971) who found that zeta-carotene gave absorption maxima of 425,400,378 nm and 418,398,376 nm, respectively in petroleum ether. In further report mentioned that zeta-carotene had Rf of 0.61 on thin layer chromatography of silica gel G with the same eluent as described above. As absorption maxima and Rf of the component was identical with those of zeta caroten it is assumed that this component to be zeta-carotone.

Component 5 (sub-sub-fraction 3) is suggested to be gamma-carotene. Since absorption maxima and Rf of this component correspond to those of gamma-carotene as reported in literature. This component gave absortion maxima at 430,460,488 nm in petroleum ether and chromatographic behavour showed that Rf of this component was 0.40 on thin layer chromatography of calcium hydroxide-silica gel G (6:1). In literature reported that gamma-carotene showed absorption maxima : 485,456,428 nm (Gross, *et al.*,1971); 490, 461,431 nm (Curl, 1961); 494,461,437 nm (Foppen. 1971) and Rf = 0.45 (Foppen, 1971), in which the condition was the same with this experiment.

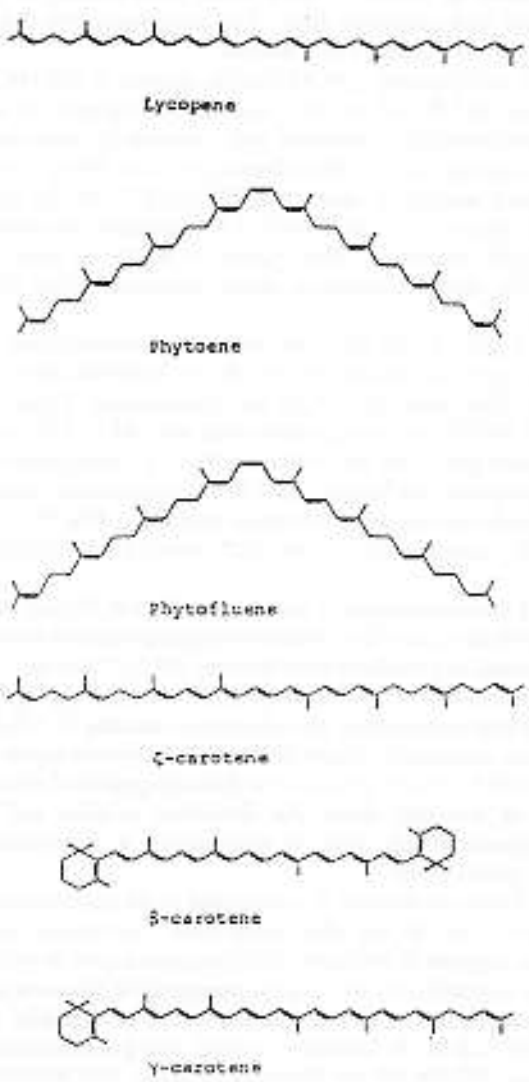


Figure 1. A number of carotenes isolated from tomato

Component 6 (Sub-sub-fraction 4) had absorption maxima at 504, 472,445 nm in petroleum ether and Rf 0.20 on thin layer chromatography of calcium hydroxide-silica gel G with eluent of petroleum ether-benzen (49:1). Based on these parameters it is assumed that this component was lycopene. This assumption by by comparing a number of reports in literature. Curl (1961) and Foppen (1971) reported that lycopene gave absorption maxima at 519, 485, 457 nm and 505, 472, 446 nm, respectively. More over Curl (1961) also reported that lycopene gave Rf of 0.15, as the condition was the same with this experiment.

Separation of Monol Fraction

Monol fraction only gave 2 sub-fractions when separated with thin layer chromatography. Sub-fraction showed sharp band on preparative thin layer chromatography and gave absorption maxima at 477, 448, 426 nm in petroleum ether. When compared with reports in literature, this component is assumed to be cryptoxanthin as this compound absorbed at 475, 450, 425 nm. This assumption was supported by chromatographic behaviour, since this component gave Rf of 0.72 and cryptoxanthin was reported to have Rf. of 0.75 (Foppen, 1971) on the same condition with this experiment, namely adsorbent of silica gel G and eluent of methylene chloride-ethyl acetate (4:1).

Separation of Diol Fraction

It can be seen from experiment that diol fraction gave 5 sub-fractions when separated on thin layer chromatography of silica gel G with eluent of petroleum ether-ethyl acetate-isopropanol (95:10:5). Only sub-fractions 1,2 and 4 gave sharp bands on thin layer chromatography, while other 2 sub-fractions i.e. sub-fraction 3 and 5 were broad bands. Sub-fractions 3 and 5 always showed broad spots on various conditions of thin layer chromatography and no separation can be made. When treated with 0.1 hydrochloric acid in ethanol these sub-fractions gave colour change from green to blue. This indicated that these sub-fractions were mixtures of mono and diepoxides.

Sub-fraction 1, 2 and 4 always showed single sharp spot on thin layer chromatography in various conditions. This suggested that these sub-fractions were single compound. When treated with 0.1 N hydrochloric acid in ethanol sub-fractions 1 and 2 did not show any colour change. On the other hand sub-fraction 4 produced blue colour. This indicated that sub-fractions 1 and 2 were not epoxides and sub-fraction 4 was a diepoxide.

Sub-fraction 1 showed absorption maxima at 420, 446, 473 nm in ethanol and gave Rf of 0.36 on silica gel G and eluent of methylene chloride-ethyl acetate (4:1). When compared with reports in literature this sub-fraction is assumed to be lutein, since this compound showed absorption maxima at 420, 445, 475 in petroleum ether (Chapman, 1966) and had Rf of 0.35 on silica gel G with eluent of methylene chloride-ethyl acetate (4:1), (Foppen, 1971). More over by comparing with literature sub-fraction 2 is predicted to be zeaxanthin, since this component showed absorption maxima of 478, 450, 422 nm in ethanol and Rf of 0.25 on silica

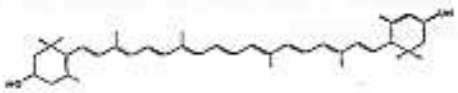
gel G with eluent of methylene chloride-ethyl acetate (4:1). In literature it was reported that zeaxanthin showed absorption maxima at 424, 448, 475 nm in ethanol and Rf of 0.24 on silica gel G with eluent of methylene chloride-ethyl acetate (4:1).

Sub-fraction 4 showed absorption maxima at 467, 439, 417 nm in petroleum ether and Rf of 0.20 on silica gel G with eluent of methylene chloride-ethyl acetate (4:1). When compared with reports in literature this component is suggested to be violaxanthin, since violaxanthin gave absorption maxima at 467, 439,416 nm in ethanol anf Rf of 0.19 on silica gel G with eluent of methylene chloride-ethyl acetate (4:1) (Davies, 1965 and Stobart, 1967). This suggestion was also supported by epoxide test. This component was a diepoxide, since it produced blue colour with 0.1 hydrochloric acid in ethanol which correspond to violaxanthin.

Separation of Polyol Fraction

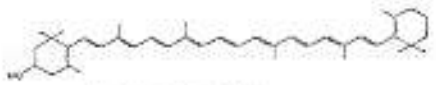
When separated on thin layer chromatography of silica gel G with eluent of petroleum ether-ethyl acetate-isopropanol (95:10:5) this fraction gave 3 sub-fractions. Two of them, namely sub-fraction 1 and 3 were broad bands and only subfraction 2 was sharp band. This component always showed sharp single spot when developed on thin layer chromatography in various conditions. Solution of this component in ethanol gave absorption maxima at 468.442.420 nm. When treated with 0.1 N hydrochloric acid in ethanol produced green colour, indicating that this component was a monoepoxide. If compared with report in literature, this component was assumed to be neoxanthin. Gross *et all.* (1971) reported that neoxanthin gave absorption maxima at 464.437.416 nm and showed green colour on treating with 0.1 N hydrochloric acid in ethanol. In other experiment Foppen (1971) found that neoxanthin had Rf of 0.69 on thin layer chromatography of silica gel G with eluent of n-hexane-ethyla acetate (19:1). In this experiment sub-fraction 2 was found to have Rf of 0.70. Subfractions 1 and 3 always showed broad spots on thin layer chromatography on various conditions and were not examined for further analysis.

From all experiments above can be seen that a number of sub-fractions which were mixtures, indicated by broad spots on thin layer chromatography. There is not assumption or suggestion can be made for this components. There fore, the components tentatively identified were much less than reported in literature. According to Curl (1961) tomato pigments contained 9 components of carotenes and 22 components carotenes of xanthophylls. In this experiment 6 carotenes and 5 xanthopylls were tentatively identified. This showed that the method used still need development.

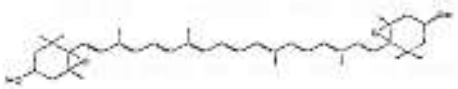


Lutein

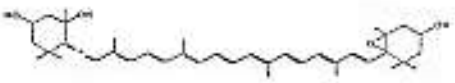
Zeaxanthin



β-cryptoxanthin



Violaxanthin



Neoxanthin

Figure 2. A number of xanthophylls isolated from tomato

CONCLUSION

This experiment showed that tomato pigments contained carotene, monol, diol dan polyol groups. A number of components were still mixtures and no identifications can be assumed. Only six carotenes and 5 xanthophylls were tentatively identied and were much less than reported by Curl (1961) in literature. Identification was carried out only by comparing absorption and Rf of the compounds with those reported in literature since the compounds isolated were in small amount and not enough for finding all spectral data required for structural elucidation.

Based on the experiment it is suggested to continue this work until all the components separated and identified.

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