DETERMINATION OF CAROTENOIDS BY THIN LAYER CHROMATOGRAPHY

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Abstract

The carotenoids of yellow, red and tangerine tomato fruits were separated and identified by thin-layer chromatography (TLC). β-carotene is extracted with a mixture content hexan and acetone. The various systems of adsorbents and solvents for TLC of carote and tomato carotenes were examined, and it was revealed that the most suitable TLC system for separation and identification was silica gel plates F_{254} and the solvent system of mobile phase acetone-water 9:1. In the tomato, β-carotene was identified and was caltulate R_f of β-carotene.

Key words: (maximum 6): Carotenoids, β-caroten, TLC, cromatographic system

INTRODUCTION

Carotenoids are widely-spread natural pigments yellow or orange in colour. They are found in many flowers, fruits, seeds or roots. (Hura, 2004)

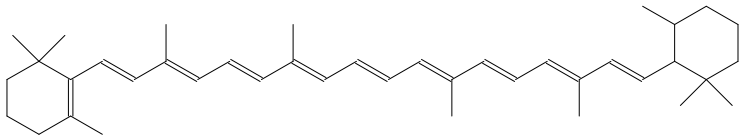
Around 400 known carotenoids colored orange, yellow or red are known (Oranescu, 2008). The most common is β-carotene, the orange pigment in carrots. Equally popular are alpha- and gamma-carotene, lycopene, zeaxanthin, cryptoxanthin, lutein. Some carotenoids are cleaved in the intestinal tissue to vitamin A, which is then transported via the lymph and blood, and stored in the liver. Improperly functioning digestive enzymes along with a protein-deficient diet, can limit the production of vitamin A.

Carotenoids contain long chains, formed of 22 or more carbon atoms, where simple covalent bonds between carbon atoms alternate with double bonds. This gives them properties of extensive electron systems, able to absorb and inactivate reactive oxygen species and other free radicals.

In the plant kingdom they are found in fruits, vegetables, mushrooms, flowers, algae and in the animal kingdom in eggs, liver, butter etc. So, **β-carotene** is an orange-yellow dye (E 160a II) found in oranges, carrots, green plants, algae. **Lycopene** (E 160d), a red-orange dye is present in tomatoes. **Lutein** (E 161b) is found in green plants and egg yolk. **Zeaxanthin** is found in corn, **cryptoxanthin** in papaya, **violaxanthine** in the yellow pansy and the red-orange **cantoxanthin** is found in fungi, crustaceans and green plants. **Capsanthin** and **capsorubin** (E 160c) are found in paprika, **oleoresins** (E 160c) coloured red, are present in red

peppers, **β-apo-8'carotenal** (E 160e), red-orange, found in the orange peel and **crocetin** is a bright yellow dye found in saffron. **Annatto, bixin, norbixin** (E 160b) coloured peachy-orange are present in the seeds of a tropical plant Bixa Orellana L. (Oranescu, 2008; Britton, 2008)

Of the total carotenoids, carrots contain 70% β-Carotene and melons contain 85% β-carotene. Tomatoes contain a large amount of lycopene, 85% of total carotenoids and watermelons 81%. Also all herbs and green vegetables are rich in carotenoids and lutein. A lot of lutein is found in the leaves of cabbage, spinach (10 mg%), pepper (7 mg%) and parsley (up to 10 mg%). [5]



1,3,3-Trimethyl-2-[3,7,12,16-tetramethyl-18-(2,6,6-trimethylciclohex-1-en-1-il)octadeca-1,3,5,7,9,11,13,15,17-nonaen-1-il]ciclohex-1-ene

Carotenoids are insoluble in water but soluble in organic solvents. They present specific absorption aspects that help in their identification. In contact with air they self-oxidate quickly and degrade. (Britton, 2008)

The physical properties of carotenoids are dictated by conjugated polyene system and the nature of the terminal groups. C_{40} carotenoids contain at least one polyene system with 8-9 conjugated bonds, thus giving the color and the specific absorption in the UV-VIS. Conjugated double bonds have little influence on the color and absorption in the UV and VIS. (Waksmundzka, et all, 2008; Leyton, et all, 2013; Pereira, et all, 2011)

One of the methods of quantitative and qualitative analysis for carotenoids are chromatographic methods. Column chromatography is a method of separation of the carotenoids in various extracts. Alumina and silicic acid are used as the stationary phase to separate the extract into fractions containing groups of carotenoids with approximately equal polarity. (Rodriguez, et all, 2001; Jeyanthi, et all, 2014; Pereira, et all, 2011)

The structural properties of carotenoids, the long chromophore of the conjugated double bond is a major factor that determines a number of specific practical aspects of CSS of carotenoids. Among them: (Abdel-Kader, 1991; Bureau, et all, 1986; Fan, et all, 1993)

- light absorption properties and, therefore, the color, which enables easy detection
- susceptibility to oxidative degradation
- affinity for some basic inorganic adsorbents.
- folded and linear structure of trans and cis isomers

Carotenoids have higher molar absorption coefficients and therefore are strongly coloured and easily detected with the naked eye in under microgram amounts on the white background of the TLC plates.

MATERIAL AND METHOD

Chemicals and Reagents

Reagents that was used ware analytical grade: hexane, acetone, toluene, methanol, butanol, methyl acetate, petroleum ether, chloroform, acetic acid, distilled water, Berzelius and Erlenmeyer glass, calibrated capillaries, pipette, mortar and pestle, boards, sheets with sicagel F_{254} submitted on plastic sheets with alumina foil deposited on aluminum (Merck). Was used vegetable products: carrots, tomatoes.

Sample preparation

A mixture of hexane is prepared: acetone 1: 1; 20 g of vegetables are cut into small pieces, placed in a mortar and triturated with 10 ml solvent mixture (hexane + acetone). When the separation is complete, the suspension obtained is filtered and the filter material is washed with 5 ml of the mixed solvents. The filtrate containing β-carotene is extracted and analyzed trough thin layer chromatography.

Procedure

In a first stage of the study, it has been determined the optimal composition of the mobile phase used for the proper separation of carotenoids from the carrot extract. The mobile phase compositions shown in Table 1 were analised for this study.

In the chromatographic chamber, the covered beaker, a piece of filter paper was introduced on one side and it was immersed in eluent to provide an atmosphere saturated in developant. In these glasses the eluent mixture is introduced to a height of 0.5 cm, so that the samples deposited on chromatographic plates will not be dipped.

RESULTS AND DISCUSSION

Samples in the form of uniform and small spots are deposited on chromatographic plates, using calibrated capillaries. The cromatographic plates are dried and inserted in the developer camera. The development is watched, it occurs until the solvent front reaches approximately 1 cm from the top of the plate. The plate is removed from the beaker and dried.

Thw followin solvent systems (Table 1) were used for chromatography.

Table 1

Eluents and ratio of components

No.	Eluent composition	The ratio of combination of the components
1	Hexane-Acetone	1:1
2	Butanol-Water-Acetic acid	20:12:5
3	Hexane-Acetone	9:1
4	Toluene-Methanol	9:1
5	Ethyl ether-Toluene	1.1
6	Toluene-acetic acid -diethyl ether-methanol	120:30:30:2
7	Petroleum ether-methanol-chloroform	10:10:10
8	Acetate-methanol-water-acetic acid	20:3:1:2
9	Petroleum ether-Acetone	3:2
10	Petroleum ether-Acetone	2:3
11	Petroleum ether-Acetone	4:1
12	Acetone:water	9:1

The compound is colored in yellow and was not required further processing for viewing spots.

The mixture of acetone-water 9:1 was the mobile phase that achieved a good separation of the β-carotene from the carrot, obtaining the separation factor R_f of 0,56 when using the silica gel plates, respectively 0,53 in the case of alumina.

Not all solvent systems used to develop, evaluate gave satisfactory results in the separation and identification of carotenoids from carrots.

An efficient separation was obtained with the use of developer systems shown in Table 2.

Table 2

Results of TLC analysis

Eluent composition	The ratio of components	TLC Plate	
		Silicagel F_{254}	Alumine
Hexane-Acetone	1:1	Necor.	Necor.
Butanol-Water-Acetic acid	20:12:5	$R_f = 0,89$	$R_f = 0,88$
Hexane-Acetone	9:1	$R_f = 0,92$	$R_f = 0,95$
Toluene-Methanol	9:1	Necor.	Necor.
Ethyl ether-Toluene	1.1	Necor	Necor
Toluene-acetic acid -diethyl ether-methanol	120:30:30:2	Necor	Necor.
Petroleum ether-methanol-chloroform	10:10:10	$R_f = 0,51$	$R_f = 0,64$
Acetate-methanol-water-acetic acid	20:3:1:2	$R_f = 0,43$	$R_f = 0,66$
Petroleum ether-Acetone	3:2	Necor	Necor
Petroleum ether-Acetone	2:3	$R_f = 0,93$	Pic cu coadă
Petroleum ether-Acetone	4:1	Necor	Necor
Acetone:water	**9:1**	**$R_f = 0,63$**	**$R_f = 0,53$**

The mobile phase that was achieved good separation of β-carotene in carrots was the mixture of acetone - water 9: 1, to give the separation factors Rf of 0.56 when using respectively 0.53 silica gel plates in the case of alumina.

Then, we determined the quality of β-carotene in the tomato extract. The sample was prepared according to the method described above. We used silica gel plates. Data in the literature indicate better stability of the β-carotene on silica gel plates in comparison with the alumina. The mobile phase used was acetone-water mixture 9: 1.(Table 3), shows the experimental results and figure 1 a plate shows the separation of β-carotene from carrot and tomato extract.

Table 3

Experimental results

Vegetable product	R_f
Carrot	0,63
Tomato	0,63

Fig. 1. Resulting chromatographic plate resulting when separating β-carotene from carrot and tomato.

CONCLUSIONS

The experimental results prove the presence of β-carotene in tomatoes, the chromatographic system, mobile phase acetone-water and silica gel plates F_{254} allowing good separation of these compounds.

The proposed method allows the qualitative detection of β-carotene in plant extracts and food products.

REFERENCES

1. Hura, C., 2004, Aditivi alimentari. Ed. Cermi, Iaşi
2. Orănescu, E., 2008, Aditivii alimentari, necesitate şi risc. Ed. AGIR, Bucureşti

3. Britton, G., Liaaen-Jensen, S. and Pfander H., 2008, Carotenoids. Natural Functions, Vol.4, Ed. BirkhäuserVerlag, Basel
4. Safta, M., 2002, Antioxidanţii naturali în alimentaţie şi medicină. Ed. Sudura, Timişoara
5. Britton, G., Pfander, H., 2008, Carotenoides, Birkhauser Verlag. Berlin, 19-37
6. Waksmundzka-Hajnos, M., Sherma, J., Kowalska, T., 2008, Thin Layer Chromatography in Phytochemistry. Chromatographic Science Series; Vol. 99
7. Leyton-Forero, G., Abdo, R., Prieto, L., Poveda-Pisco, J.C., Ceron-Lasso, M.S., 2013, Carotenoids Extracted from Promising Clones of CriollanPotato (Solanum tuberosum Group Phureja) for Food Industry, Revista Latinoamericana De La Papa; Vol. 17 (2), 104-114
8. Pereira F., J., Egle Siqueira, M. A., Fontes Vieira, R., Tania Da Silveira Agostini-Costa., 2011, Fruits of butia capitata (mart.) becc as good sources of β -carotene and provitamina. Rev. Bras. Frutic., Jaboticabal – SP; Vol. Especial, E. 612-617
9. Rodriguez-Amaya, D. B., Ph., D., 2001, A Guide To Carotenoid Analysis In Foods OMNI Research ILSI Human Nutrition Institute.
10. Jeyanthi, L.R., Sharmila, S., Merina Paul Das and Seshiah, C., 2014, Extraction and purification of carotenoids from vegetables. Journal of Chemical and Pharmaceutical Research; 6(4), 594-598
11. Fereshteh F., Gholamreza J., Masoud Y., Bahman B. G., Mehraban S., Shayan H., 2013, Evaluation of Food Color Consumption and Determining Color Type by Thin Layer Chromatography. American Journal of Applied Sciences; Vol. 10 (2), 172-178
12. Abdel-Kader, Z. M., 1991, Determination of carotenoids in foods by high-performance liquid chromatography. Nahrung. 35: 689-693
13. Arya, S. S., Natesan, V., Parihar D. B. and Vijayaraghavan P. K.., 1979, Stability of carotenoids in dehydrated carrots. J. Food Technol. 14:579-586
14. Bureau, J. L. and Bushway, R. J.,1986,. HPLC determination of carotenoids in fruits and vegetables in the United States. J. Food Sci. 51:128-130
15. Fan, J. J., Lee, H. C. and Yen, G. C.,1993, Quantification and identification of carotenoids and chlorophylls in raw and pickled mustard, (in Chinese). J. Chinese Agric. Chem. Soc. 31:765-775
16. ***http://www.clickcaster.com/users/modulbiochimie/assets/pigmen_i_-_oral.pdf