

THE ANALYSIS OF CAROTENOIDS FROM MINT EXTRACTS

Mihaela Ciurea¹, I. Jianu²

¹Aurel Vlaicu University of Arad, Faculty of Food Engineering, Tourism and Environmental Protection, str. Elena Drăgoi nr. 2, Arad

²Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, Calea Aradului nr. 119, Timișoara, RO-300645

Abstract

This paperwork proposed to establish the efficiency of the carotenoids extracts with aqueous ethanol in processing and analytical scope. Aqueous ethanolic phase was processed and used in HPLC analysis. Extracts, which have in their composition carotenoids pigments, are being put under saponification for releasing carotenoids and discharging chlorophylls and fats that can be saponificated. In the etheric phase carotenoids pigments have been extracted, and then the value of total carotenoids has been read for spectrophotometric evaluation.

Keywords: *carotenoids pigments, extraction, saponification, HPLC, spectrophotometric VIS.*

Introduction

There are many known varieties of mint. The mint oils, obtained by extraction from these numerous varieties have multiple utility functions in farmaceutical industry, cosmetics, food and chemical industry. These oils composition depends on the plants variety and climateric conditions of the place they are grown at. Thus, the major components of these oils can differ very much quantitively and qualitively.

Knowing the composition is very important as much in the control phase of raw oils as in checking of the process of separation, purification or of chemical transformation of some components. Let's not forget the utility of making the analysis for cosmetic products, farmaceutical products and food flavor.

Experimental

Extraction: The carotenoids from alcoholic extract diluted in 10% NaCl, were repeatedly and completely extracted with ethyl ether. The

reunited etheric phases were dried on anhydrous sodium sulphate. The total carotenoids evaluation was made spectrophotometrically at 450 nm (table 1). For further treatment the extract in ethyl ether was split in two parts.

The total unsaponificated extract (TUE) was obtained by under pressure distillation of ether, and this was kept at - 20°C under an inert gas pillow (nitrogen). The other part was put under saponification as it is written below.

Saponification: The extracts that contain carotenoids pigments are usually submitted to saponification for the scope of rejecting the carotenoids that are under ester form and to release the fats that can be saponificated and chlorophylls (in case of green tissue extracts – for example mint extract).

To the ethyl ether extract a volume equal to KOH 30% in methanol was added. The saponification happened in a closed flask in darkness and under continuous stirring for 8 hours at 22°C. The obtained extract was washed with 1% NaCl at neutral pH. The etheric phase containing the carotenoidic pigments was evaporated and kept at -20°C, representing the total saponificated extract (TSE). The obtained total solids were introduced into a known volume of chromatographic ethyl acetate, filtrated through Millipore filter and used in HPLC analysis.

The quantity of carotenoids pigments from the samples was calculated with Britton (1995) formula. The registration of absorption specter and the reading of absorption value were done at 450 nm with a Spectrophotometer UV-VIS M 40.

The separation of the carotenoids pigments through HPLC happened with a system which includes a Kontron 322 pump system, a chromatographic column in reversed phase Discovery C18, with a length of 250 mm and 4.6 mm, in diameter, and the particles diameter of 5 µm. The detection was done with a photodiode array detector Waters 990.

The following system was used:

Solvent A: Acetonitrile : Water (9 : 1, v/v) + 0.5 % EPA (ethyl izopropilamine)

Solvent B: Acetic ether + 0.5 % EPA

The used programs were:

System A (the short program): at $t = 0'$: 5% B in A; $t = 16'$: 60% B in A; $t = 25'$: 60% B in A; $t = 27'$: 5% B in A.

System B (the long program): at $t = 0'$: 5% B in A; $t = 16'$: 60% B in A; $t = 25'$: 60% B in A; $t = 30'$: 75% B in A; $t = 50'$: 75% B in A; $t = 55'$: 5% B in A.

The rate of flow of the mobile phase was 1 ml/min.

The retention times of the carotenoids were compared to the retention time of separated available standards in the same condition (figure 1). The other compounds were identified on the base of the chromatographic compartment correlation and the absorption spectrum in UV-VIS, carotenoids characteristic (figure 2 and 3) (Britton, 2004).

Results and Discussions

The evaluation of total carotenoids from alcoholic extract spectrophotometrically made at 450 nm is presented in table 1.

Table 1. Total carotenoidic dosage.

Sample/pigments	mg of total carotenoids/ peak area %
Mint	0.02
Lutheine	62.4
Zeaxanthine	10.8
β -criptoxanthine	2.1
β -carothine	15.3
α -carothine	signs
Neoxanthine	3.2
Violaxanthine	3.6

It must be mentioned that these quantities refer to the whole quantity of alcoholic extract available (400 ml). In the literature, (Goodwin, 1980) the quantity of carotenoids changes a lot depending on the analyzed hybrid (variety), cultivation conditions etc. Above all following the analysis we have found that the extraction with aqueous ethanol is not efficient in the case of carotenoids, comparatively with the extraction which uses unpolarized solvents: petroleum ether, hexane, ethyl ether, acetone, acetic ether etc. The most efficient extractants are halogenated derivations that present the disadvantage of developing peroxides.

Carotenoids pigments can be by nature hydrocarbonated or oxidized derivations (xantophyll). Xantophylls with hydroxyl groups or with carboxyl groups can be found under esther form with fat acids of esther – glycosides or glycosides (Britton, 1995).

That's the reason why two programs, identical in the first part, were used. The difference between these two programs is in the first place the delaying of program A until the 55th minute, because carotenoids esther have much longer retention time. Solvent B's percentage has been modified too, for the scope of reducing the retention time.

It has been worked on the saponificated sample for mint extract, because of it's high quantity of chlorophyll and their absorption in 400-500 nm domain can distort the results. These results are presented in table 2.

The peaks with retention times under 5 minutes are not carotenoids pigments, but compounds of other nature that couldn't be identified (sterols, flavonoids, degradation products). On the chromatogram relatively small amounts of other carotenoids compounds are present, probably deteriorated and those that couldn't be identified on the base of absorption spectrum.

Table 2. Identified and dosed pigments in mint extract using HPLC

Pigments	Pigment nr. on chromatogram	Standard retention time	TSE retention time	TUE retention time
Lutheine	1	19.10	18.90	20.00
Zeaxanthine	2	20.05	20.00	20.10
β-cryptoxanthine	3	22.11	22.11	22.80
β-carothine	4	25.46	-	24.90
α-carothine	5	25.02	25.02	-
Neoxanthine	7	5.07	5.00	5.45
Violaxanthine	8	6.72	6.10	6.30
Clorophyll A*	ca	21.49	-	21.50
Clorophyll B*	cb	21.74	-	21.80

*- only in unsaponificated extract

** - in petroleum ether

The analyzed extract does not contain carotenoids under esterificated shape. All the chromatograms were recorded in the long program too (figures are not presented) and in none of them have been identified carotenoids compounds with retention time longer than 30 minutes. Because of this the recorded chromatograms were processed with the short program.

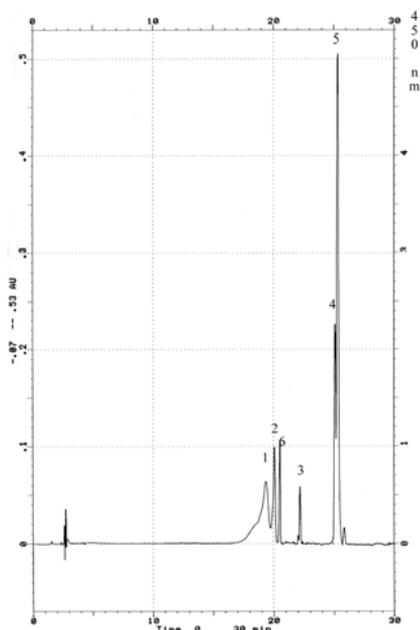


Fig. 1. The HPLC chromatogram of a standard mixture. 1- Lutheine, 2 - Zeaxanthine, 3 - β -criptoxanthine, 4 - α -carothine, 5 - β -carothine, 6 - Cantaxanthine.

The carotenoids pigments are extremely sensitive to oxidation in the presence of the oxygen in the air, and because of this it is recommended to do the extraction under an inert gas pillow in the presence of the antioxidants. On the other side in light presence they suffer isomerisation thing that makes it necessary to take extreme measures like working in darken etc.

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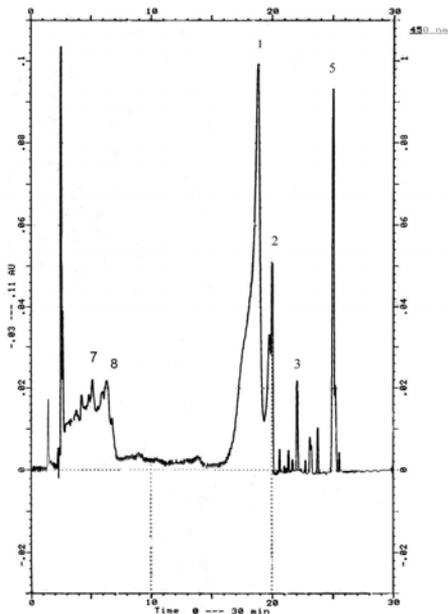


Fig. 2. The HPLC chromatogram of saponified mint extract

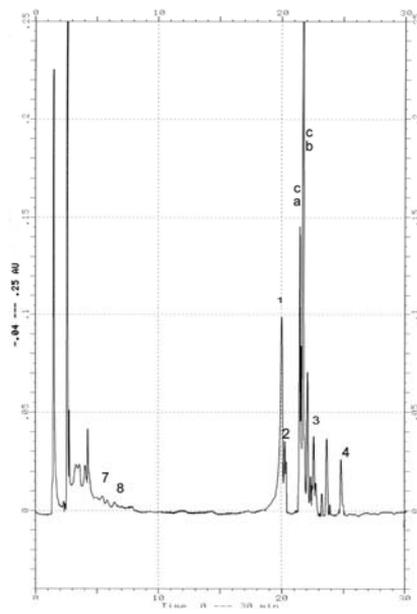


Fig. 3. The HPLC chromatogram of unsaponified mint extract.

Conclusions

The extraction with aqueous ethanol is not efficient in the case of carotenoids. The carotenoids can be efficiently extracted with unpolarized solvents: petroleum ether, hexane, ethyl ether, acetone, acetic ether etc. The most efficient extractants are halogenated derivations, which are not used because they develop peroxides.

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