ANALYSIS OF CAROTENOIDS, VITAMINS AND FOLATES IN TRADITIONAL FOODS FROM BLACK SEA AREA

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INTRODUCTION

Nowadays, consumers are much more aware of nutritional composition and show especially interest in compounds with putative health benefits. Therefore, in the last few years, great attention has been devoted to the study of bioactive compounds in order to promote the consumption of traditional foods.

The European project BaSeFood (Sustainable Exploitation of Bioactive Components Black Sea Area Traditional Foods) aims to study traditional foods from Black Sea Area countries (BSAC), namely their nutritional and bioactive composition [1]. In the frame of this project, the present work has analysed carotenoids, vitamins and folates in 33 traditional foods.

MATERIALS AND METHODS

	Carotenoids, retinol and α-tocopherol	L-ascorbic acid	Riboflavin	Total folates
Sample extraction	Homogenized samples + $MgCO_3$ + Hexane/Ethanol 4:3 (v/v) ⇓ 5 min in an Ultra Turrax® and centrifugation (5 min at 1500 rpm, 4 °C) (Figure 1 A) ⇓ Re-extraction ⇓ Pool organic phases and evaporate to dryness at 25 °C* ⇓ Filtration (0.22 µm PTFE filter)	Homogenized samples + Stabilization solution (10% (v/v) perchloric acid + 1% (w/v) metaphosphoric acid in ultrapure water) ⇓ Vortex and dilution with mobile phase ⇓ Filtration (filter paper and 0.45 µm PVDF filter)	Homogenized samples + HCl 0.1 M for 30 min in an autoclave at 121 ºC ⇓ pH was adjusted to 4.5 with sodium acetate 2.5 M ⇓ Overnight enzymatic treatment at 37 ºC with takadiastase and β-amylase (Figure 2A) ⇓ After cooling at room temperature, dilution with ultrapure water and filtration (filter paper and 0.45 µm PET filter)	The determination of the total folate content in Traditional foods was carried out by a microbiological assay (EN 14131:2003) with turbidimetric detection of the growth of the microorganism Lactobacillus casei, subspecies rhamnosus (ATCC 7469). The analyses were performed in an accredited laboratory according to ISO/IEC/17025.
Chromatographic analysis	**Ultra-high Pressure Liquid Chromatography- Photodiode Array Detector (PDA) (Figure 1 B)** ➢ **Guard-Column :** ACQUITY UPLC® BEH, C18 guard column (2.1 x 5 mm I.D., 1.7 µm particle size) ➢ **Column:** ACQUITY UPLC® BEH C18 reverse phase column (2.1 x 50 mm I.D., 1.7 µm particle size) ➢ **Column temperature:** 20 ºC ➢ **Injection volume:** 10 µL ➢ **Flow rate:** 0.5 mL/min ➢ **Detection:** λ =295 nm (α-tocopherol); λ = 325 nm (retinol); λ = 450 nm (carotenoids) ➢ **Gradient mobile phase:** (A) ultrapure water; (B) acetonitrile/methanol (with 0.05 M ammonium acetate)/dichloromethane (75:20:5)	**HPLC- Photodiode Array Detector (PDA)** ➢ **Guard-Column :** SecurityGuard Cartridge AQ C18 (40 2.0 mm I.D., 5 µm particle size) ➢ **Column:** Phenomenex, Synergi™Hydro-RP (150 4.6 mm I.D., 4.0 µm particle size) ➢ **Column temperature:** 30 ºC ➢ **Injection volume:** 20 µL ➢ **Flow rate:** 0.6 mL/min ➢ **Detection:** λ = 454 nm ➢ **Mobile phase:** 20 mM ammonium dihydrogen phosphate, pH 3.5 (adjusted with orthophosphoric acid 85%), containing 0.015% (w/v) of metaphosphoric acid	**HPLC-Fluorescence Detector (Figure 2 B)** ➢ **Column:** Phenomenex Luna C18 (250 x 4.6 mm I.D., 5 µm particle size) ➢ **Column temperature:** 37 ºC ➢ **Injection volume:** 50 µL ➢ **Flow rate:** 1 mL/min ➢ **Detection:** λ_{ex} = 422 nm and λ_{em} = 522 nm ➢ **Mobile phase:** 0.05 M acetate buffer + methanol (70:30, v/v)	

*** Samples were also saponified with 10 % (w/v) methanolic KOH during 4h at room temperature.**

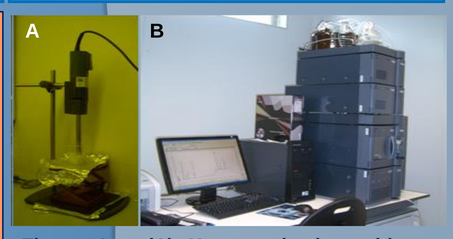

Figure 1 – (A) Homogenization with an Ultra Turrax®. (B) Ultra Performance Liquid Chromatograph.

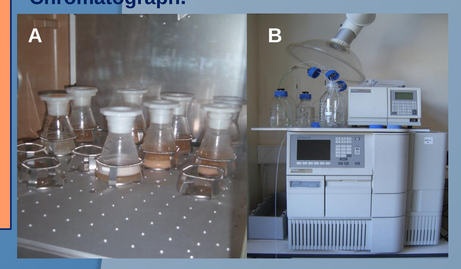

Figure 2 – (A) Overnight incubation. (B) HPLC equipment with fluorescence detector.

RESULTS

Carotenoids, retinol and α-tocopherol

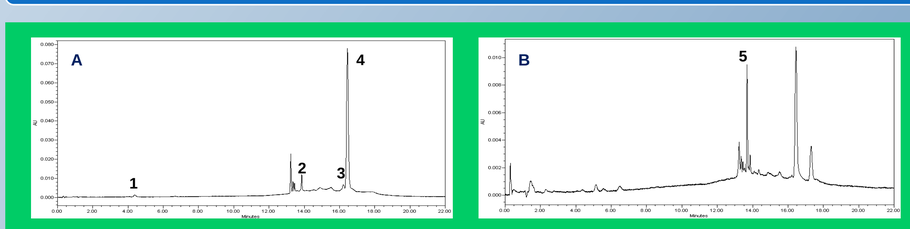

Figure 3 - Chromatogram of plums jam from Romania. (A) λ = 450 nm, extract with saponification; (B) λ = 295 nm, extract without saponification (1 - Lutein; 2 - β-cryptoxanthin; 3 - α-carotene; 4 - β-carotene; 5 - α-tocopherol.

Depending on the sample and on the studied compound, saponification might be needed. Therefore, both extraction procedures (with and without saponification) where carried out for all matrices. Most of the samples contain α-tocopherol (Figure 3) and do not present retinol. Great variability was found in carotenoids content of BSAC samples.

L-ascorbic acid

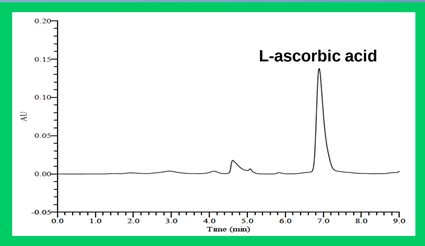

Figure 4 - Chromatogram of L-ascorbic acid content for fruit of the evergreen cherry laurel (29.7 mg/100 g of edible portion) from Turkey.

One of the samples with highest L-ascorbic acid content was the fruit of the evergreen cherry laurel (Figure 4). However, most of the samples did not contain L-ascorbic acid (limit of quantification <0.04 mg/100 g of edible portion).

Riboflavin/Total folates

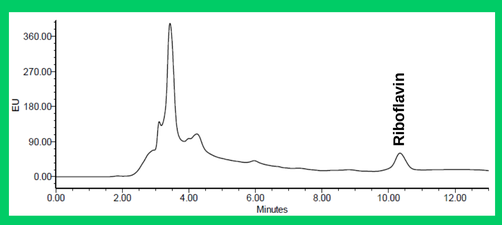

Figure 5 – Chromatogram of riboflavin content for Churchkhela, (0.064 µg/mL) from Georgia.

Roasted sunflower seeds presented the highest riboflavin (0.19 mg/100 g of edible portion) and total folates (113 µg/100 g of edible portion) content. About 58% of the foods analysed had contents below the quantification limit (<0.02 mg/100 g of edible portion). Figure 5 shows a chromatogram of riboflavin content found in Churchkhela (0.064 µg/mL).

REFERENCES

[1] D'Antuono L.F., Soares Costa H., Sanches-Silva A. (2010). BaSeFood: Sustainable exploitation of bioactive components from the Black Sea Area traditional foods. Nutrition Bulletin, 35, 272-278.

ACKNOWLEDGEMENTS

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CONCLUSIONS

The analysed traditional foods from BSAC can be considered good sources of bioactive compounds, although it was found a great variability on the content of carotenoids, vitamins and total folates. Due to the putative health benefits of these compounds, the consumption of those with higher content of bioactive compounds should be encouraged/promoted.