BIOACTIVE PHYTOSTEROLS AND FATTY ACIDS PROFILE OF TRADITIONAL FOODS FROM BLACK SEA AREA COUNTRIES

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on behalf of the BaSeFood Black Sea area partners*

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INTRODUCTION/AIM

Phytosterols (PS) are abundant in foods of plant origin and vegetable oils. These compounds have received particular attention due to their capability to lower serum cholesterol levels, resulting in significant reduction of the risk of heart disease [1]. Also, the consumption of fatty acids (FA) is important because it can be associated with both negative and beneficial health effects, depending on the FA. This work was performed within the collaborative research program Sustainable exploitation of bioactive components from the Black Sea Area traditional foods (BaSeFood), funded by the European Commission [2]. Traditional foods from Black sea region are presently being studied for their potential positive effects on human health, especially focusing on its bioactives compounds. The aim of this study was to analyse the bioactive PS, total fat and FA profile of 33 Traditional Foods from six Black Sea Area countries (BSAC) (Figure 1).

BULGARIA

(A) Baked layers of pastry stuffed with pumpkin; (B) Halva; (C) Millet ale; (D). Mursal tea; (E) Rodopian dried beans; (F) Rose jam

GEORGIA

(A) Nettles with walnut; (B) Tsiteli doli bread; (C) Churchkhela; (D) Flax oil; (E) Wild plum sauce.

ROMANIA

(A) Cornmeal mush; (B) Nettle sour soup; (C) Herbal dish; (D) Elderberry soft drink; (E) Plums jam.

TURKEY

(A) Black tea; (B) Bulgur pilaf; (C) Fruit of the evergreen cherry laurel; (D) Sautéed pickled green beans; (E) Kale soup.

RUSSIAN FEDERATION

(A) Buckwheat porridge crumby; (B) Kvass southern; (C) Watermelon juice; (D) Mustard oil; (E) Vegetable okroshka.

UKRAINE

(A) Sauerkraut; (B) UKrainian borsch; (C) Transcarpathian green borsch; (D) Roasted sunflower seeds; (E) Uzvar; (F) Sour rye bread; (G) Cottage cheese with green and garlic.

Figure 1. Selected Traditional Foods from Black Sea Area countries.

METHODS AND RESULTS

TOTAL FAT

- 0.2 – 10 g of sample
- 75 mL ultra-pure water + 45 mL HCl (37%)
- Boiled for 20 min
- Filtered with a filter paper (Whatman G40, 150 mm Ø) (Figure 2 A)
- Extracted using a Soxhlet, with petroleum ether (Figure 2 B)
- Residue was dried for 1 h 30 min at 101 °C ± 2 °C, until constant weight

(1) Baked layers of pastry stuffed with pumpkin; (2) Tsiteli Doli Bread; (3) Cornmeal mush; (4) Buckwheat porridge crumby; (5) Bulgur pilaf; (6) Sour rye bread; (7) Rodopian dried beans; (8) Nettles with walnut sauce; (9) Nettle sour soup; (10) Kale soup; (11) Transcarpathian green borsch; (12) Ukrainian borsch; (13) Churchkhela; (14) Plums jam; (15) Uzvar; (16) Halva; (17) Flax oil; (18) Mustard oil; (19) Roasted sunflower seeds; (20) Herbal dish; (21) Pomazanka; (22) Millet ale; (23) Sautéed pickled green beans

Figure 3. Total fat (g/100 g of edible portion) content of traditional foods from BSAC.

- ✓ Mustard oil and flax oil had the highest content of fat (99.9 g/100 g of edible portion) (Figure 3)
- ✓ Uzvar had a low content of fat (0.138 g/100 g of edible portion) (Figure 3)
- ✓ 30% of the analysed traditional foods had fat contents lower than the limit of quantification (<0.1 g/100 g of edible portion)

FATTY ACIDS

- 0.2 – 0.5 g of sample
- 2 mL of toluene + 3 mL of methanolic HCl (5:95, v/v) (Figure 2 C)
- Water bath (70 °C for 2 h)
- 5 mL of K_2CO_3 (6%) + 1 mL of toluene
- Centrifugation at 1100 rpm (5 min)
- Organic phase was dried with Na_2SO_4
- Filtration with a 0.45 µm PTFE syringe filter

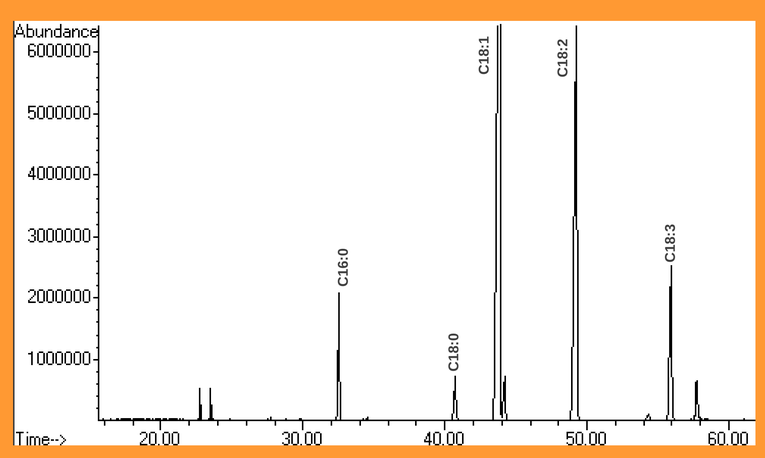
Chromatographic conditions

Equipment: HP 6890 N (Figure 2 D)
Column: HP-88 column (100 m x 0.25 mm i.d., 0.25 µm)
Detector: MS
Injection volume: 1.0 µL
Carrier gas: Helium

Ramp	°C/min	Next °C	Hold
Initial		50	11.0
Ramp 1	25.0	77	0.00
Ramp 2	17.0	168	32.0
Ramp 3	1.50	195	0.00
Ramp 4	0.50	199	0.00
Ramp 5	1.00	220	3.00
Ramp 6	25.0	235	0.00

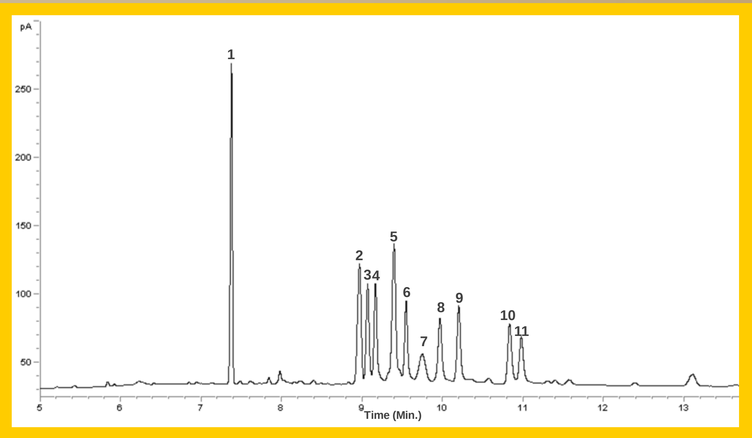
- ✓ The applied method for fatty acids determination in the 33 selected traditional foods, allowed the identification of 51 different fatty acids, including 11 *trans* fatty acids isomers
- ✓ Mustard oil has the highest content of oleic acid (C18:1) (Figure 4)
- ✓ Roasted sunflower seeds have the highest content of γ-linolenic acid (C18:3, n-3)
- ✓ Flax oil has the highest content of linolenic acid (C18:3, n-6)

Figure 2. (A) Filtration. (B) Fatty acids extraction. (C) Soxhlet apparatus. (D) Gas chromatograph.



Palmitic acid (C16:0); Stearic acid (C18:0) ; Oleic acid (C18:1); Linoleic acid (C18:2); γ-Linolenic acid (C18:3)

Figure 4. Chromatogram of mustard oil.



(1) 5 α-Cholestane; (2) α-Tocopherol; (3) Cholesterol; (4) Dihydrocholesterol; (5) Desmosterol + Brassicasterol; (6) Lathosterol; (7) Ergosterol; (8) Campesterol; (9) Stigmasterol; (10) β-Sitosterol; (11) Stigmastanol

Figure 5. Chromatogram of a standards mix of 11 phytosterols and vitamin E (α-Tocopherol).

PHYTOSTEROLS

- 0.2 g of sample
- 100 µL of internal standard (50 µg/mL) + 2 mL KOH (1 M, in 90% EtOH)
- Water bath (85 °C, 30 min)
- After cooling, 1 mL of n-hexane was added
- Centrifugation at 1200 rpm (5 min)
- Upper layer was separated and evaporated to dryness under nitrogen
- Residue was derivatized with 200 µL of BSTFA:TMCS (99:1, v/v) at 60 °C, 15 min

Chromatographic conditions

Equipment: HP 6890 N
Column: J&W DB-5 MS column (30 m × 0.25 mm i.d., 0.25 µm)
Detector: FID
Injection volume: 2.0 µL
Injector temperature: 290 °C
Detector temperature: 300 °C
Flow: 1.0 mL/min
Carrier gas: Helium
Ramp: The column initial temperature was set at 250 °C, during 1 min, followed by an increase of 10 °C/min to 290 °C, which was maintained for 20 min

Method optimization

- Extraction
  - Sample amount (0.1 to 0.5 g)
- Saponification
  - KOH (1 M, 2 M and 3 M) in 90% EtOH
  - Water bath at 85 °C, for 30 and 60 min
- Derivatization
  - BSTFA:TMCS volume (50, 100 and 200 µL)
  - Derivatization at 60 °C, for 15 and 30 min at 1200 rpm

The optimal conditions for method application were:
- ✓0.2 g of sample
- ✓ KOH (1 M) in 90% EtOH
- ✓ Water bath at 85 °C, for 30 min
- ✓ BSTFA:TMCS volume 200 µL
- ✓ 60 °C, for 15 min at 1200 rpm

CONCLUSIONS

Our results show that some of the traditional foods from BSAC are a good source of polyunsaturated fatty acids to the diet, especially γ-linolenic n-3 and linolenic n-6 fatty acids which are related to health benefits, namely regarding cardiovascular diseases. With respect to total fat content, a great variability was found and the highest content was found in the oilseeds group. The method developed for phytosterols analysis, is rapid, easy to handle and allows the determination of 11 sterols and α-tocopherol, simultaneously (Figure 5).

REFERENCES

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