Stabilitätspakt für Südosteuropa
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The impact of chemical composition on the antioxidant, antibacterial and antifungal activity of commercial Macedonian cold-pressed oils

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Cold pressed edible oils

• the most important foodstuff - polyunsaturated fatty acids and tocopherols (Vitamin-*E*-active compounds)
• reduced risk of coronary hearth diseases, the level of LDL, degenerative diseases and cancer
• minor grope of phenolic components as powerful antioxidants responsible for human health benefits.

Cold pressed walnut oil

- The highest level of γ-tocopherol
- Improves blood circulation
- Lowers heart disease risk
- Prevents eczema
- Maintains hormone balance

Cold pressed almond oil

- the highest level of α-tocopherol
- retains moisture in the skin
- provides a protective barrier that resists infections in premature infants

Poppy seed oil

- prevents of diabetes
- prevents of inflammations
- reduces blood pressure
- prevents Asthma and Rheumatoid Arthritis

Wheat germ oil

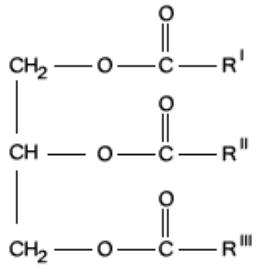
- aids in cellular metabolism
- booths immune system
- reduces blood pressure
- helps to improve stamina and performance

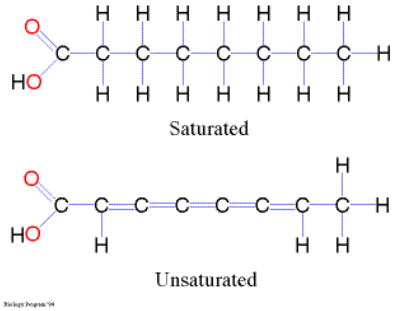
Process of cold pressing

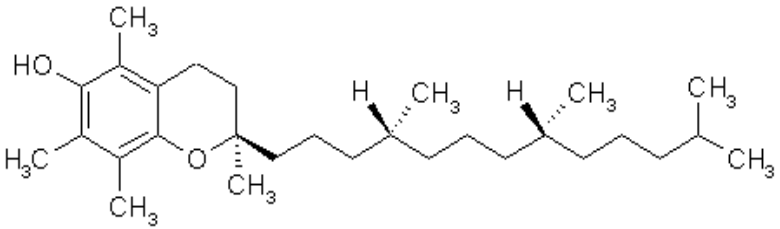
1. Pressing of the seeds under high pressure (the temperature did not increase 40°C)
2. Sedimentation of waxes and other impurities
3. Decantation after sedimentation of pure virgin oil
4. Filtration with high porous filter
5. Filtration with very fine filter

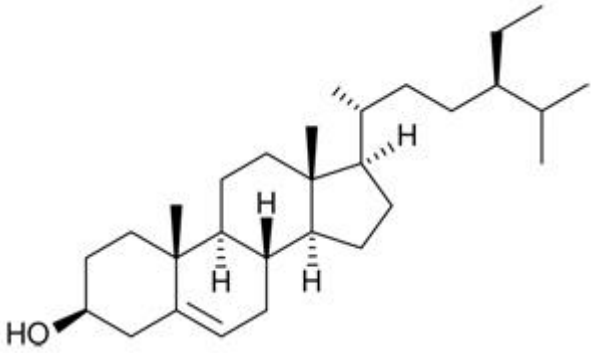
Composition of cold pressed oils

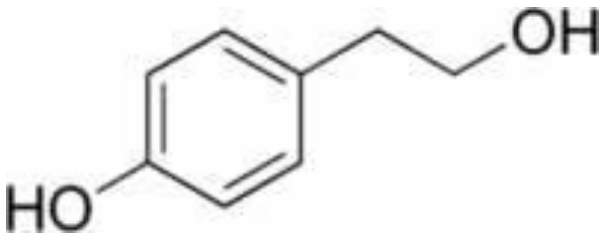
Complex mixture











Determination of fatty acid profile by GC-FID

• **Preparation of fatty acid methyl esters using trimethyl sulfonium hydroxide (TMSH)**

The sample was dissolved in *tert*-butyl methyl ether (TBME) and mixed with a methanolic solution of trimethylsulfonium hydroxide (TMS-OH). Glycerides are base-catalysed transesterified and fatty acid methyl esters are formed.

• **Determination of fatty acid methyl esters by GC-FID**

The column - HP88 (100 m x 250 μm x 0.2 μm)
Temperature program 175°C for 5 min and 5°C/min to 250°C
Column flow rate -1mL/min
Split ratio 100:1

Fatty acids in oils (%)

Oil type	saturated	ω-7	ω-9	ω-9	ω-9	ω-6	ω-3
	Palmitic acid	**Palmitoleic acid**	***cis*-Oleic acid**	***trans*-Oleic acid**	**Gondoic acid**	**Linoleic acid**	**γ-Linolenic acid (ALA)**
Poppy seed oil	8.51±0.03	0.13±0.01	**14.35±0.02**	1.06±0.01	0.08±0.01	**72.28±0.06**	0.89±0.01
Walnut oil	5.93±0.02	0.07±0.00	**17.89±0.01**	0.78±0.00	0.20±0.00	**60.73±0.01**	**11.74±0.01**
Almond oil	6.38±0.01	0.42±0.01	**67.57±0.02**	1.04±0.00	0.07±0.00	**20.96±0.01**	0.39±0.00
Wheat germ oil	9.29±0.03	0.08±0.00	**38.14±0.04**	0.97±0.00	0.82±0.00	**37.71±0.01**	2.23±0.00

Determination of tocopherols and tocotrienols in oils by RP-HPLC-DAD

- oils were dissolved in *n*-hepane
- Column: Kinetex 50 × 4.6 mm
- UV dectector on 292 nm
- The mobile phase (methanol:water-96:4) and the eluation was performed at a flow rate of 2 mL/min.
- identification by retantion times and quantification by calibration curves obtained from pure standards from tocopherols and tocotrienols

Tocopherols and tocotrienols (Vitamin E) in oils

(mg/kg of oil)

Oil type	α-t	α-T3	β-t	γ-t	Plast 8	γ-T3	δ-t	Total
Poppy seed oil	1.91±0.00^{a}	ND^{a}	0.03±0.00^{a}	**15.72±0.01^{b}**	0.17±0.00^{a}	0.14±0.00^{a}	0.22±0.00^{a}	18.19±0.00
Walnut oil	1.03±0.01^{a}	ND^{a}	0.12±0.00^{a}	**21.89±0.01^{c}**	ND^{a}	0.06±0.00^{a}	**2.38±0.01^{b}**	25.48±0.03
Almond oil	**23.77±0.01^{c}**	0.31±0.00^{a}	0.23±0.00^{a}	1.58±0.00^{a}	0.37±0.05^{a}	0.16±0.00^{a}	0.04±0.01^{a}	26.46±0.07
Wheat germ oil	5.80±0.06^{b}	ND^{a}	0.49±0.02^{a}	**19.68±0.04^{c}**	0.58±0.06^{a}	0.30±0.02^{a}	5.62±0.04^{b}	**32.47±0.24**

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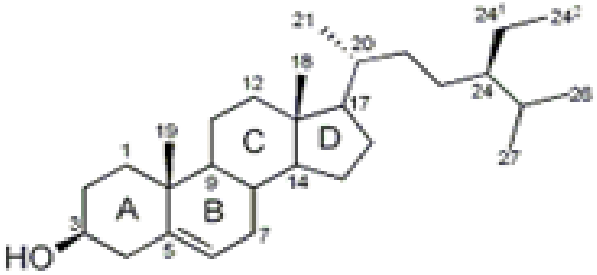
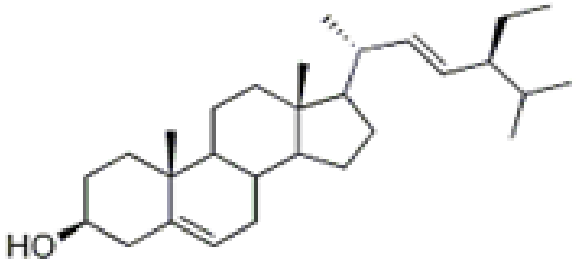
Phytosterols

Steroid compounds in plants with similar structure as cholesterol and differ only in carbon side chain and presence or absence of double bonds.

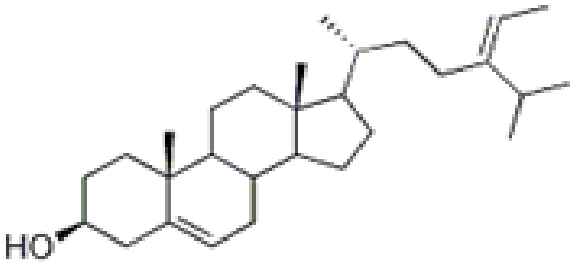
The main role of phytosterols – lower cholesterol in blood



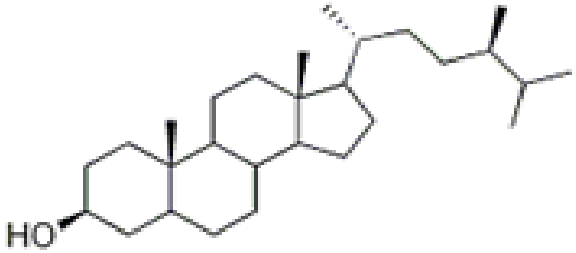
β–Sitosterol



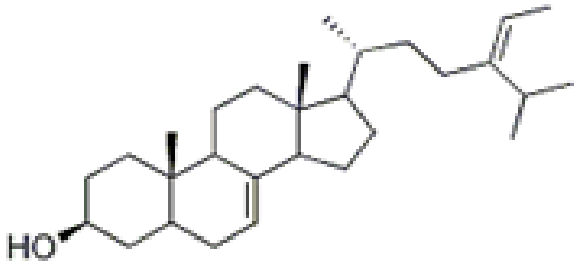
Stigmasterol



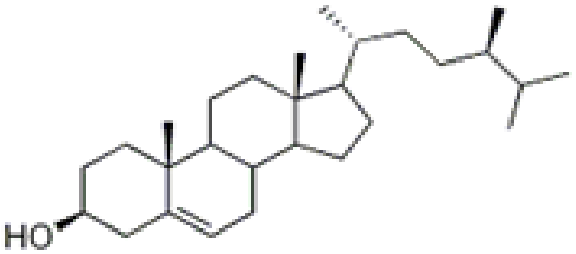
Δ5–Avenasterol



Campestanol



Δ7-Avenasterol



Campesterol

Determination of phytosterols by TLC and GC-FID

- the sample is hydrolyzed with hydrochloric acid (~3.5 M) with reflux at 100ºC
- saponification with 2.5 M methanolic KOH is added directly to the oil sample
- reaction is heated 1 h on 80°C
- isolation of main classes of phytosterols on TLC with reagent for development (hexan:dietlyether)
- derivatisation by *N*-methyltrimethylsilyltrifluoroacetamide (MSTFA)
- GC-FID analyses

Phytosterols in oils (mg/kg)

Phytosterols	**Walnut oil**	**Poppy seed oil**	**Almond oil**	**Wheat germ oil**
Cholesterol	7.17±0.39^{a}	1.91±0.32^{a}	35.27±1.44^{b}	**12.21±7.18^{c}**
Brassicasterol	ND^{a}	9.19±1.88^{b}	ND^{a}	ND^{a}
24-Metylencholesterol	2.02±2.02^{a}	**92.67±4.55^{c}**	11.75±1.47^{b}	83.67±2.14^{d}
Campesterol	**80.68±2.33^{a}**	**587.86±5.33^{c}**	**129.99±7.44^{b}**	**1039.10±15.98^{d}**
Campestanol	ND^{a}	1.91±0.23^{a}	ND^{a}	72.65±1.49^{b}
Stigmasterol	6.75±0.38^{a}	**986.16±8.52^{d}**	32.66±3.14^{b}	**822.46±7.99^{c}**
Δ7-Campesterol	ND^{a}	ND^{a}	ND^{a}	75.89±4.96^{b}
5,23-Stigmastadienol	16.13±0.35^{a}	27.57±1.55^{ab}	48.66±2.14^{b}	**162.15±9.22^{c}**
Chlerosterol	38.80±0.42^{a}	46.72±1.28^{b}	54.87±0.69a^{b}	81.73±11.48^{c}
β-Sitosterol	**1476.47±13.50^{a}**	**1739.08±12.57^{b}**	**2396.35±13.59^{c}**	**3148.44±49.33^{d}**
Sitostanol	14.02±0.32^{b}	6.51±0.11^{a}	54.87±0.71c	**129.07±28.12^{d}**
Δ5-Avenasterol	**118.75±1.75^{b}**	**273.83±4.29^{c}**	**365.15±3.27^{d}**	70.70±4.67a
5,24-Stigmastadienol	28.37±1.39^{a}	32.55±2.07^{a}	60.42±1.51^{b}	**240.64±19.54^{c}**
Δ7-Stigmastenol	**309.85±3.48^{c}**	10.72±0.98^{a}	57.16±2.78^{b}	**345.71±29.14^{c}**
Δ7-Avenasterol	10.22±0.61^{a}	13.40±1.12^{ab}	19.27±1.17^{b}	**101.18±5.47^{c}**
Total	**2109.23±26.94**	**3750.08±44.77**	**3266.42±39.05**	**6485.6±196.71**

TPC and antioxidant assays

Samples	DPPH assay for (mg of α-tocopherol/L oil)	TPC assay (mg/L GAE)	DPPH assay for methanol extracts (mg Trolox/L oil)	TEAC assay for methanol extracts (mg of Trolox/L oil)
Almond oil	1379.19 ± 46.57^{b}	**558.82 ± 10.335^{c}**	**160.30±7.10^{c}**	**124.23 ± 1.17^{c}**
Walnut oil	1704.92 ± 27.17^{c}	524.78 ± 18.246^{c}	66.69±1.03^{b}	98.00 ± 1.65^{b}
Poppy seed oil	1160.17± 5.55^{a}	368.23 ± 17.717^{b}	56.47±3.43^{b}	88.78 ± 3.68^{b}
Wheat germ oil	**2015.67 ± 21.86^{d}**	61.57 ± 3.816^{a}	27.89±13.61^{a}	59.13 ± 15.71^{a}

Antimicrobial tests

• Antibacterial activity against two *gram-positive bacterial strains*: ***Listeria monocytogenes* (ATCC 13076)**, and ***Staphylococcus aureus* (ATCC 49444)**, and against two *gram-negative bacterial strains*: ***Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (ATCC 25922)**, and against antifungal activity using: ***Candida albicans* (ATCC 10231)**

• Each microorganism was suspended in Mueller Hinton (MH) broth and diluted approximately to 10E6 colony forming unit (cfu)/mL.

• The plates were incubated at 37 °C and the diameters of the growth inhibition zones were measured after 24 h. Gentamicin (10 μg/well) was used as positive control. The negative control was performed with only sterile broth cultured 24 h with 10 μL of 70% ethanol.

Antimicrobial tests

Antimicrobial activity

Samples	Inhibition zone in diameter (mm)				
	Staphylococcus aureus	**Listeria monocytogenes**	**Salmonella enteritidis**	**Escherichia coli**	**Candida albicans**
Almond oil	8.0±0.0	8.0±0.0	8.0±1.0	8.0±0.5	**14.0±1.0**
Walnut oil	8.0±0.0	8.0±1.0	8.0±1.0	8.0±1.0	**14.0±0.5**
Poppy seed oil	8.0±1.0	**10.0±0.5**	8.0±2.0	8.0±0.5	**16.0±0.5**
Wheat germ oil	8.0±0.5	8.0±0.5	8.0±1.0	8.0±1.5	8.0±0.5

Future prospective studies:

- in vivo analyses
- tocopherols

α>β>γ>δ

antioxidant activity ≠ biological activity

Aknowledgements

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