A multi-methodological approach for the hemp seed oils characterization
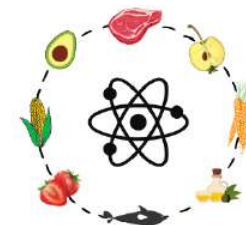
Giacomo Di Matteo

PhD student
Sapienza University of Rome,
Laboratory of Food Chemistry

SISSG Congress 2022:

Edible oils and fats: Innovation and sustainability
in production and control

Perugia, 15 June 2022

Cannabis

Cannabis

This article is about the plant genus. For therapeutic use, see Medical cannabis. For the psychoactive drug, see Cannabis (drug). For other uses, see Cannabis (disambiguation).

Cannabis (/ˈkænəbɪs/)[2] is a genus of flowering plants in the family Cannabaceae. The number of species within the genus is disputed. Three species may be recognized: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*; C. *ruderalis* may be included within *C. sativa*; all three may be treated as subspecies of a single species, *C. sativa*;[1][3][4][5] or *C. sativa* may be accepted as a single undivided species.[6] The genus is widely accepted as being indigenous to and originating from Asia.[7][8][9]

Cannabis

Temporal range: Early Miocene - Present 19.6–0 Ma
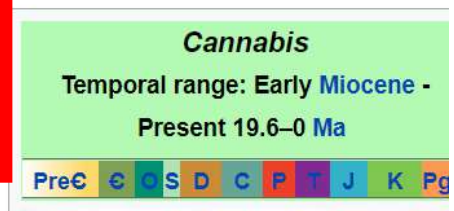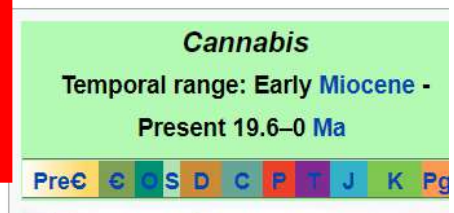
| PreЄ | Є | O | S | D | C | P | T | J | K | Pg |

Cannabis

Cannabis

From Wikipedia, the free encyclopedia

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Cannabis ?

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Cannabis
Temporal range: Early Miocene - Present 19.6–0 Ma

PreЄ Є O S D C P T J K Pg

Cannabis :   Sativa   Indica   Ruderalis

Cannabis

Cannabis

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Cannabis

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| PreꞒ | Ꞓ | O | S | D | C | P | T | J | K | Pg |

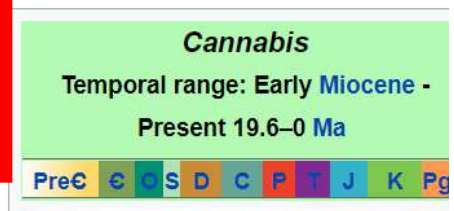**Cannabis :   Sativa   Indica   Ruderalis**

Cannabis

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Cannabis
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Cannabis : Sativa Indica Ruderalis

Cannabis

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PreꞒ Ꞓ O S D C P T J K Pg

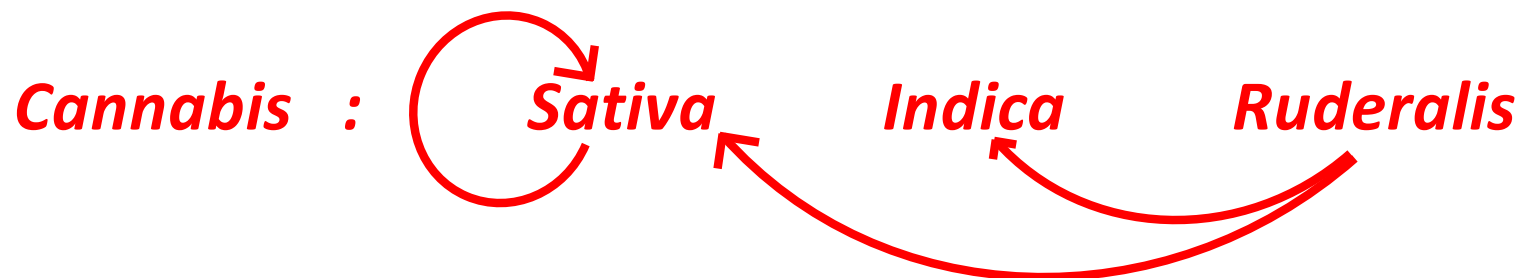**Cannabis : Sativa**

Cannabis sativa L.

Taxonomy	
Family	Cannabaceae
Genus	*Cannabis*
Species	*C. Sativa*

Classification		
Chemotype I	THC/CBD > 1	drug type
Chemotype II	THC/CBD ~ 1	
Chemotype III	THC/CBD < 1	*fiber type*

Cannabis sativa L.

- Annual plant, good soil and climate adaptability

- Dioecious or monoecious

- Rich chemical profile: more than 100 compounds typical only of this plant

Cannabinoids: THC, CBD, CBG, CBN, CBDV, ..

Brenneisen R., Chemistry and Analysis of Phytocannabinoids and Other Cannabis Constituents, 2007

Cannabis sativa L. – Legislation

1937
USA
Marihuana Tax Act
defined *C. sativa* as narcotic drug and introduced it in the schedule I of controlled substances.

1938
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1971
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Convention on Psychotropic Substances
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1975
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1998
Canada
The state of Canada legalized the cultivation of *C. sativa* varieties with a THC content < 0.3% of the dry weight of leaves and flowering parts of the plant, after the release of license from the Office of Controlled Substances of Health Canada.

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Cannabis sativa L. – Legislation

75TH CONGRESS, 1ST SESSION—CHS. 552, 553—AUGUST 2, 1937

transfer to a common carrier for the purpose of transporting marihuana.

SEC. 2. (a) Every person who imports, manufactures, produces, compounds, sells, deals in, dispenses, prescribes, administers, or gives *Special occupational tax levied.*

1937

USA Marihuana Tax Act defined *C. sativa* as narcotic drug and introduced it in the schedule I of controlled substances.

552   75TH CONGRESS, 1ST SESSION—CH. 553—AUGUST 2, 1937

away marihuana shall (1) within fifteen days after the effective date of this Act, or (2) before engaging after the expiration of such fifteen-day period in any of the above-mentioned activities, and (3) thereafter, on or before July 1 of each year, pay the following special taxes respectively:
(1) Importers, manufacturers, and compounders of marihuana, $24 per year.

Act and the application of such provision to other persons or circumstances shall not be affected thereby.

Effective date. SEC. 17. This Act shall take effect on the first day of the second month after the month during which it is enacted.
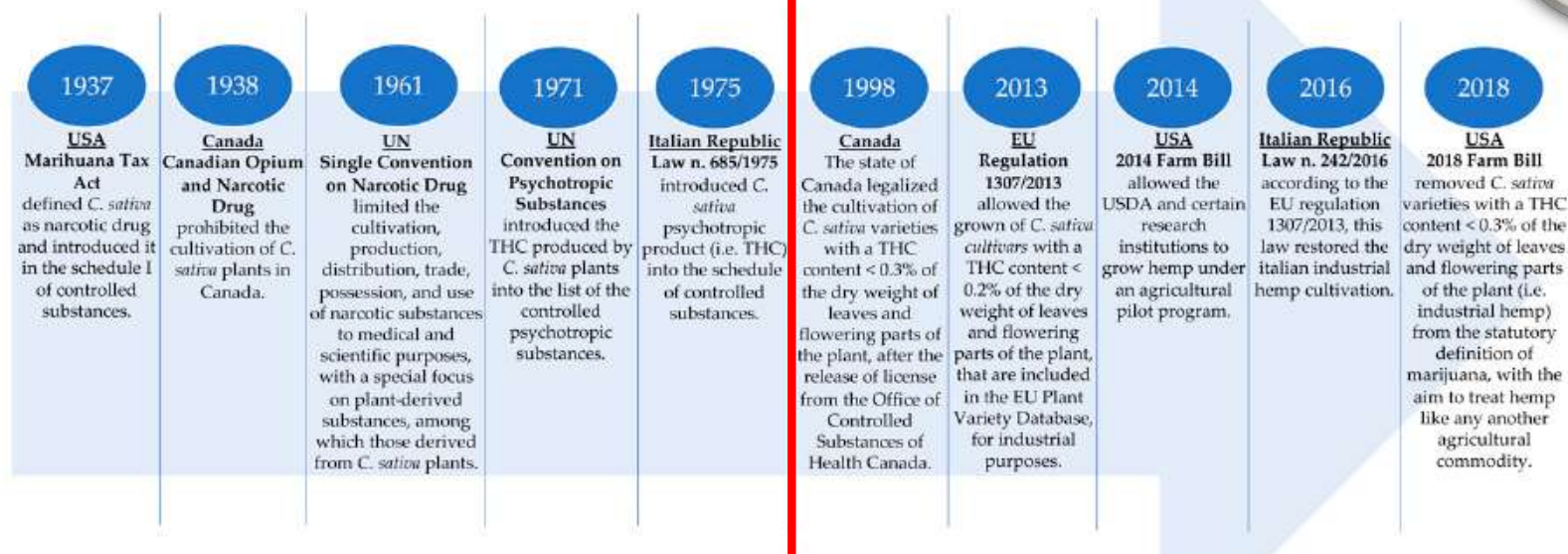
Short title. SEC. 18. This Act may be cited as the 'Marihuana Tax Act of 1937."

Approved, August 2, 1937.

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Farinon et al., 2020, doi:10.3390/nu12071935

Cannabis sativa L. – Legislation

The New York Times

THE 1996 ELECTIONS: THE NATION -- THE STATES

In California, Voters Bar Preferences Based on Race
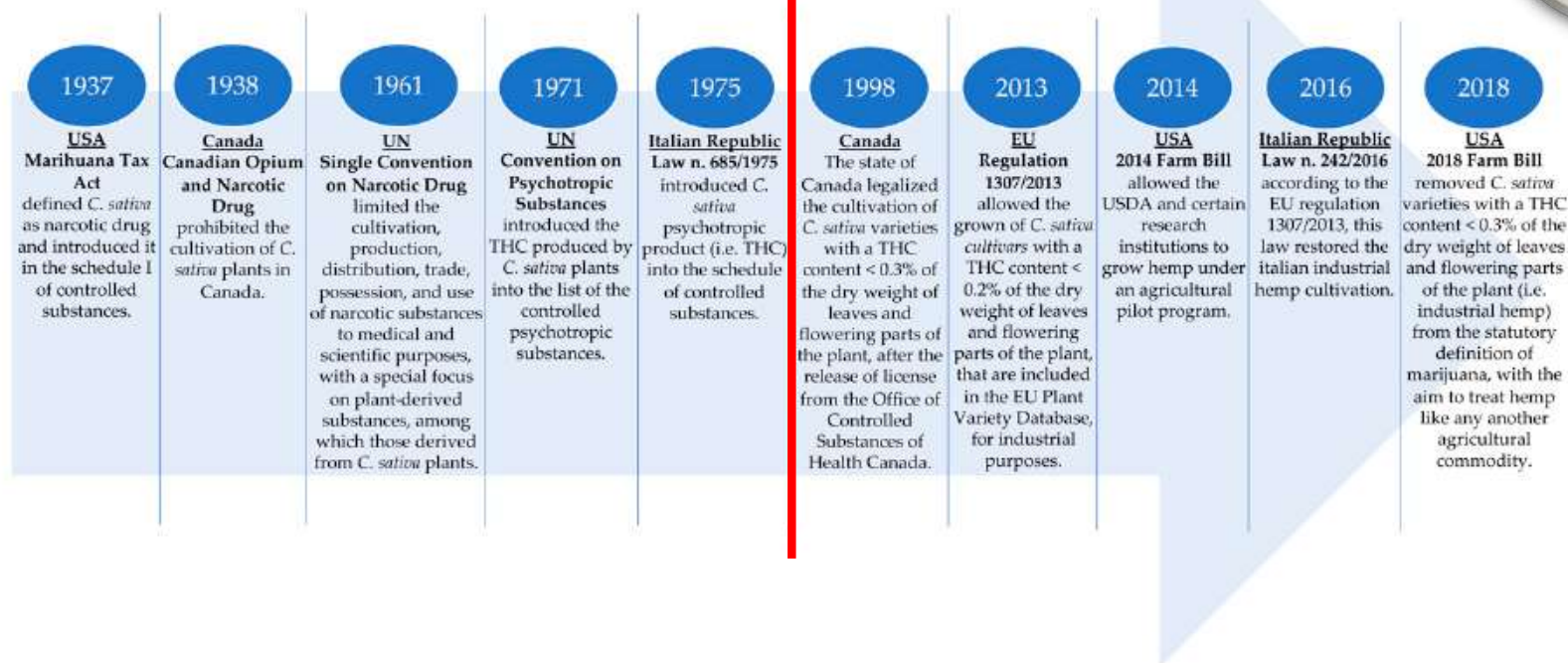
By Robert Pear

Nov. 6, 1996

California voters also approved legalizing the cultivation, possession and use of marijuana for medical purposes. Arizona approved a measure allowing doctors to prescribe marijuana to relieve the pain and suffering of patients who are seriously ill.

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Cannabis sativa L. – The case of Italy

50' years

- Italy is the second largest producer of Cannabis (100000 ha)

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1975

- Law n. 685/1975 – Prohibition of hemp cultivation

- Law n. 685/1975 – Psychotropic compounds from hemp were included in the list "Tabelle delle sostanze soggette a controllo"

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Cannabis sativa L. – The case of Italy

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- Law n. 242/2016 – Restore of the industrial hemp cultivation

Cannabis sativa L. – EU authorized cultivars

EU Plant variety database

Plant variety database ∨   Plant reproductive material ∨

European Commission

Agricultural species - Varieties

SEARCH CRITERIA | **SHOW CURRENT** YES | **SPECIES** A - 85 - HEMP - CANNABIS SATIVA ...

Adzelvieši	Dacia Secuieni	Finola	Lipko	Santhica 70
Armanca	Delta-405	Futura 75	Lovrin 110	Secuieni Jubileu
Asso	Delta-llosa	Futura 83	Marcello	Silvana
Austa SK	Dioica 88	Glecia	Marina	Sofia
Balaton	Earlina 8 FC	Gliana	Markant	Succesiv
Beniko	Eletta Campana	Glyana	Matrix	Teodora
"Bialobrzeskie	Epsilon 68	Helena	MGC 1013	Tiborszallasi
- Białobrzeskie"	Fedora 17	Henola	Mietko	Tisza
Białobrzeskie	Felina 32	Ivory	Monoica	Tygra
Cannakomp	"Férimon	KC Bonusz	Olivia	Uniko B
Carma	- Ferimon"	KC Dora	Orion 33	Uso-31
Carmagnola	Ferimon	KC Virtus	Pūriņi	Villanova
Carmaleonte	Fibranova	KC Zuzana	Rajan	Wielkopolskie
Chamaeleon	Fibrante	KCA Borana	Ratza	Wojko
Codimono	Fibrol	Kompolti hibrid TC	Santhica 23	Zenit
CS	Fibror 79	Kompolti	Santhica 27	

Allegato XII art. 7bis par. 1 del Regolamento europeo CE 2860/2000

Cannabis sativa L. – EU authorized cultivars

EU Plant variety database

Plant variety database ⌄   Plant reproductive material ⌄

European Commission

THC < 0.2 % (p/p)

Agricultural species - Varieties

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CS	Fibror 79	Kompolti	Santhica 27	

Allegato XII art. 7bis par. 1 del Regolamento europeo CE 2860/2000

Cannabis sativa L. – a chimeric plant
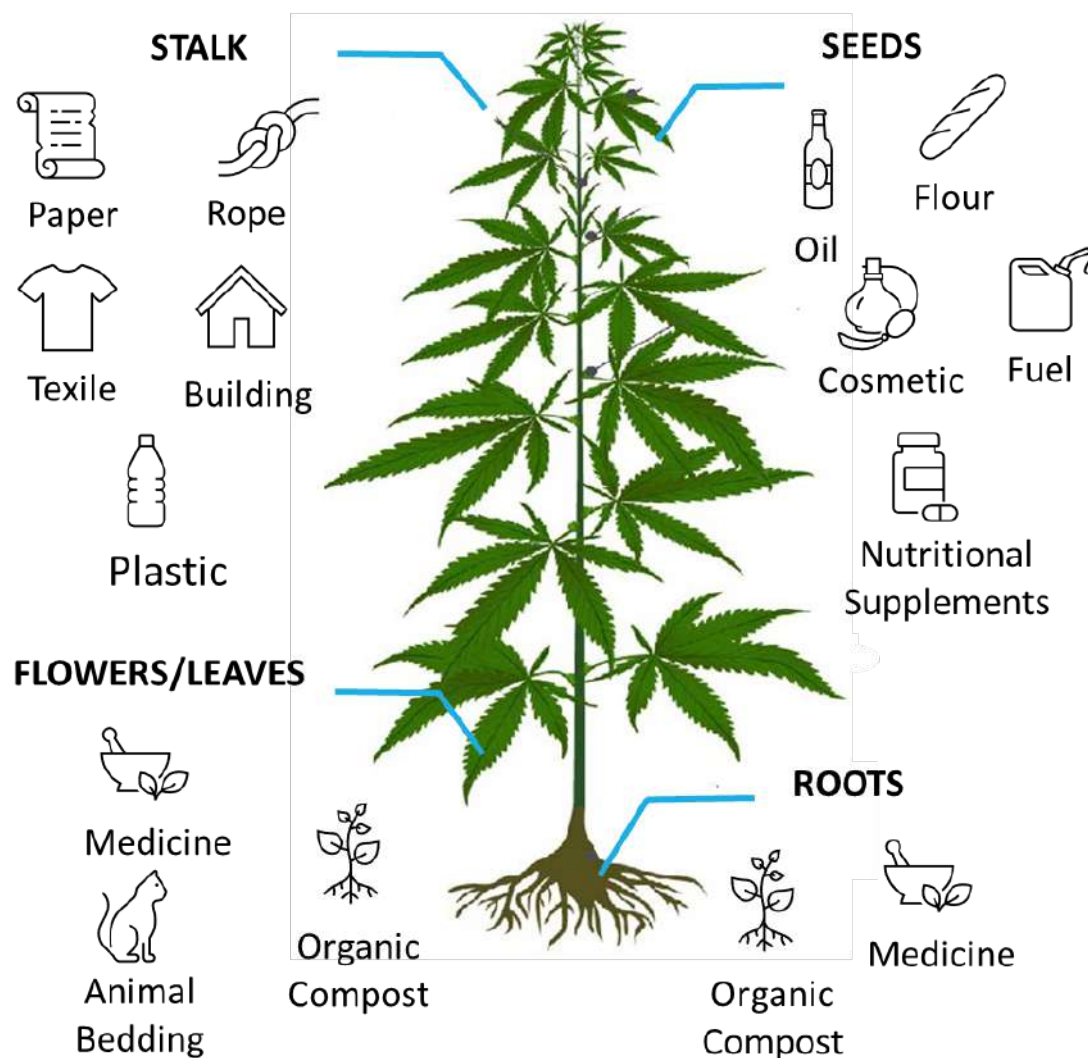
Cannabis sativa L. – various foodstuffs

Decree of the Ministry of Health of 4 Novembre 2019

- Hemp seeds

- Hemp flour

- Hemp seed oil

Cannabis sativa L. – various foodstuffs

Decree of the Ministry of Health of 4 Novembre 2019

- Hemp seeds

- Hemp flour

- Hemp seed oil

✔ Good source of nutritive compounds (sterols, essential fatty acids ω-3 and ω-6, ..)

🚫 An official definition of quality parameters is still needed for the matrix

Sampling

ERBOLOGY
20 €/L

FIORI DI LOTO
24 €/L

CRUDOLIO
25 €/L

BAULE VOLANTE
26 €/L

GERMINAL BIO
30 €/L

HEMP FARM
44 €/L

HANFOL NU3
52 €/L

PIPKIN HEMP OIL
64 €/L

SABO
80 €/L

Industrial Hemp: Development and valorization of a new eco-sustainable agro-food chain
June 2018 – October 2020

The multi-methodological approach

Oil analysis

- Conventional analyses used for extra virgin olive oils (Regulation EU 2015/1830)

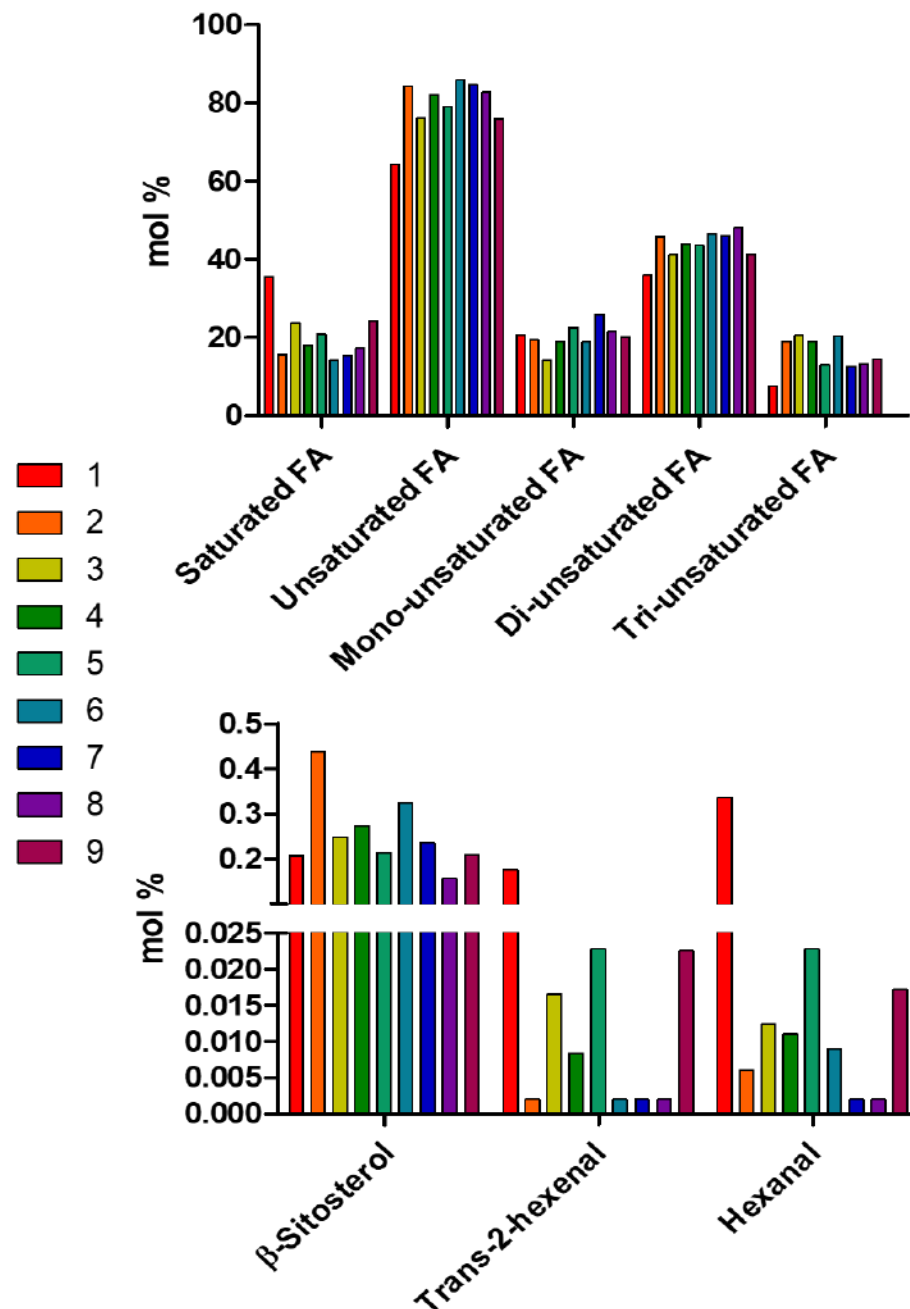- Nuclear Magnetic Resonance (NMR) methodology

Spano M, Di Matteo G, Rapa M., et al. Commercial hemp seed oils: A multimethodological characterization. Appl Sci. 2020;10(19):1–15.

The multi-methodological approach

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Conventional analysis

- Free acidity
- Peroxides number
- Anisidine number
- Spectrophotometric parameters (K_{232}, K_{270}, ΔK)

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NMR – Pros and cons

- ✓ Non-destructive
- ✓ Reproducibility
- ✓ Resolution of complex mixtures with a single experiment
- ✗ Low sensibility
- ✗ High costs
- ✗ Qualified personnel
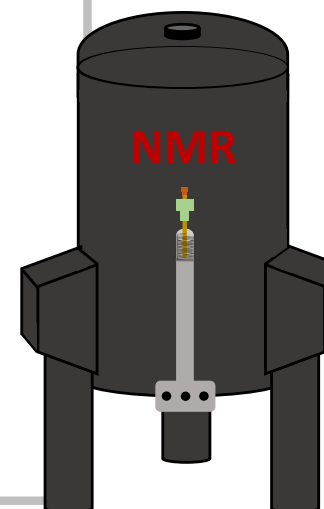
NMR

Spano M, Di Matteo G, Rapa M., et al. Commercial hemp seed oils: A multimethodological characterization. Appl Sci. 2020;10(19):1–15.

NMR analytical protocol

Sample preparation

20 µL oil
20 µL DMSO-d$_6$
700 µL CDCl$_3$

Spectra acquisition

The ^{1}H NMR spectra were acquired at 301K on a Bruker 600 MHz using the following experimental conditions:

- Number of scans 1024

- 90° pulse

- TD 64K data points

- Relaxation delay 0.5 sec

- Acquisition time 2.96 sec

- Spectral width 18 ppm
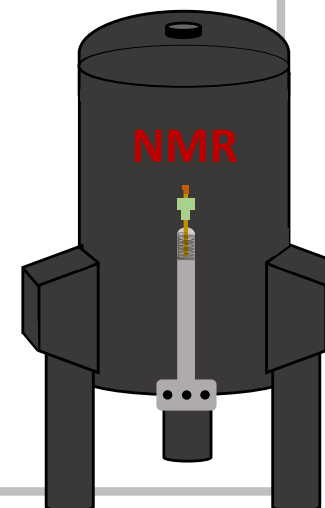
Spectra processing

- Calibration: CDCl$_3$ signal set at 7.26 ppm

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NMR spectrum
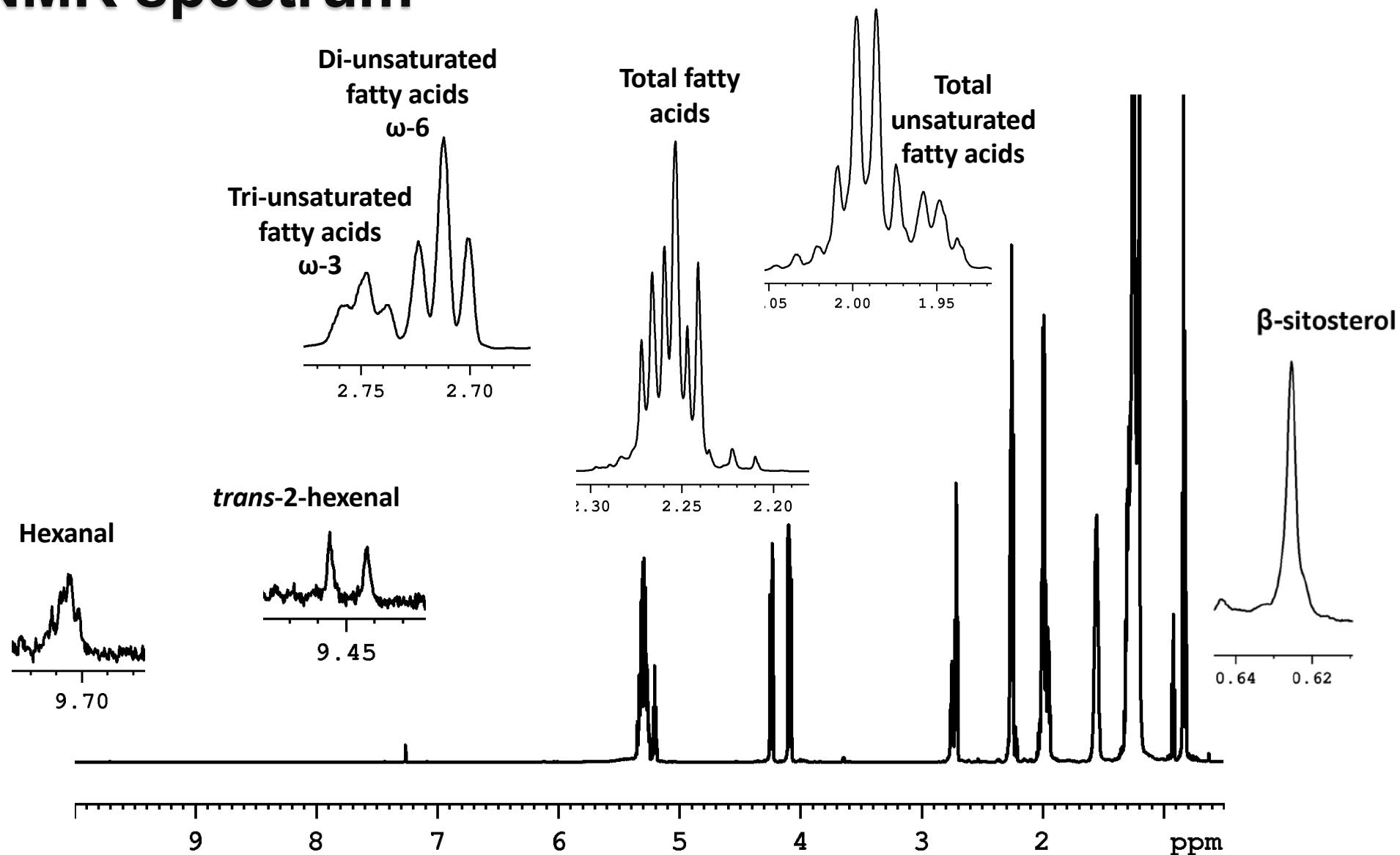
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NMR results

- Tri-unsaturated fatty chains (8-20%)
  < Mono-unsaturated (15-28%)
  < Di-unsaturated (35-48%)

- The content of aldehydes are very low in all the samples

- Sample 1 have the lowest level of tri-unsaturated fatty chain

- Sample 1 have the highest content of hexanal (0.34%) and trans-2-hexenal (0.17%)

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Conventional analysis results

Sample	A%
1	17.24 ± 0.63
2	1.86 ± 0.07
3	0.40 ± 0.01
4	4.15 ± 0.12
5	1.47 ± 0.08
6	2.22 ± 0.02
7	0.76 ± 0.03
8	0.43 ± 0.02
9	1.50 ± 0.06

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Conventional analysis results

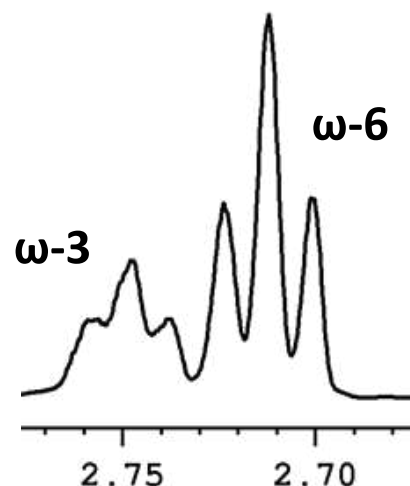| Sample	A%	P.N. (meqO$_2$/Kg)	A.N.
1	17.24 ± 0.63	4.31 ± 0.37	3.58 ± 0.17
2	1.86 ± 0.07	16.68 ± 1.28	1.14 ± 0.04
3	0.40 ± 0.01	16.42± 1.39	2.84 ± 0.11
4	4.15 ± 0.12	8.36 ± 0.75	1.60 ± 0.07
5	1.47 ± 0.08	10.69 ± 0.96	1.17 ± 0.03
6	2.22 ± 0.02	22.14± 1.88	3.30 ± 0.28
7	0.76 ± 0.03	10.50 ± 1.26	0.11 ± 0.02
8	0.43 ± 0.02	17.13 ± 0.89	0.12 ± 0.01
9	1.50 ± 0.06	17.03 ± 1.17	2.80 ± 0.02

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Conventional analysis results

Sample	A%	P.N. (meqO$_2$/Kg)	A.N.	K$_{232}$	K$_{270}$	ΔK
1	17.24 ± 0.63	4.31 ± 0.37	3.58 ± 0.17	2.65 ± 0.09	0.69 ± 0.01	0.03
2	1.86 ± 0.07	16.68 ± 1.28	1.14 ± 0.04	2.04 ± 0.02	0.41 ± 0.01	0.01
3	0.40 ± 0.01	16.42± 1.39	2.84 ± 0.11	2.35 ± 0.04	0.57 ± 0.01	0.02
4	4.15 ± 0.12	8.36 ± 0.75	1.60 ± 0.07	2.12 ±0.03	0.47 ± 0.01	0.01
5	1.47 ± 0.08	10.69 ± 0.96	1.17 ± 0.03	2.01 ± 0.17	0.39 ± 0.01	0.01
6	2.22 ± 0.02	22.14± 1.88	3.30 ± 0.28	2.26 ± 0.05	0.49 ± 0.01	0.01
7	0.76 ± 0.03	10.50 ± 1.26	0.11 ± 0.02	1.85 ± 0.02	0.26 ± 0.01	0.01
8	0.43 ± 0.02	17.13 ± 0.89	0.12 ± 0.01	1.72 ± 0.01	0.19 ± 0.01	0.01
9	1.50 ± 0.06	17.03 ± 1.17	2.80 ± 0.02	2.21 ± 0.02	0.46 ± 0.01	0.02

Spano M, Di Matteo G, Rapa M., et al. Commercial hemp seed oils: A multimethodological characterization. Appl Sci. 2020;10(19):1–15.

A multi-methodological approach for the hemp seed oils characterization

The interesting part

ω-6:ω-3 value of 3:1

⬇

Ideal for the human consumption

ω-3

ω-6

2.75 2.70
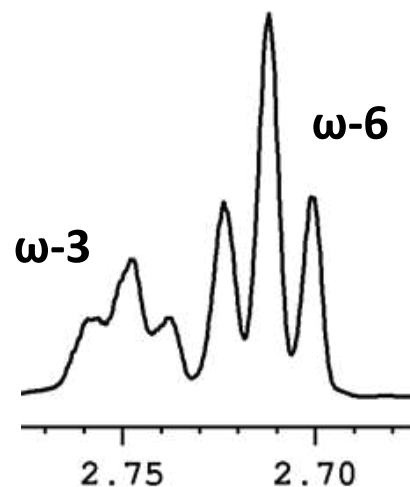
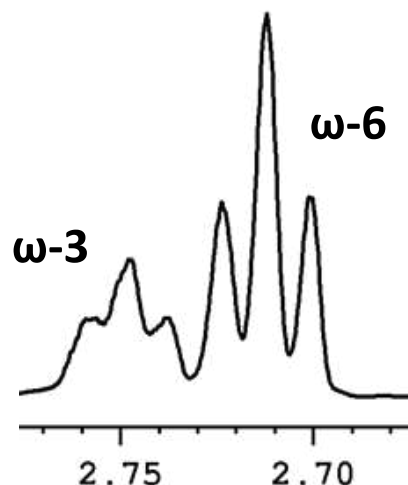NMR represents a rapid and powerfull tool for its determination

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The interesting part

ω-6:ω-3 value of 3:1

↓

Ideal for the human consumption

ω-3

ω-6

2.75   2.70

NMR represents a rapid and powerfull tool for its determination

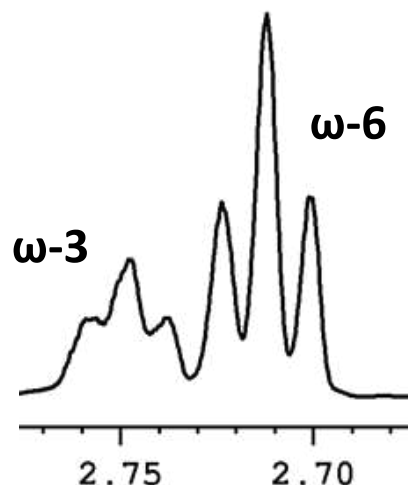| Sample	1	2	3	4	5	6	7	8	9
Origin	Italy	EU	EU	NON-EU	NON-EU	Italy	NON-EU	NON-EU	Italy
Price (€/L)	44	20	24	25	26	30	52	64	80
ω-6/ω-3 label	4.20	2.80	3.37	3.17	3.21	2.39	3.11	3.37	2.57
ω-6/ω-3 NMR	4.71	2.40	1.99	2.30	3.36	2.27	3.64	3.61	2.86

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- In some cases, the effective ω-6/ω-3 value was very different from those indicated in the label
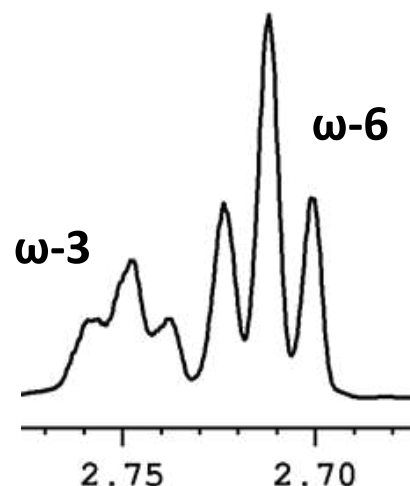- In some cases, the ω-6/ω-3 value wasn't correlated with the price

Spano M, Di Matteo G, Rapa M., et al. Commercial hemp seed oils: A multimethodological characterization. Appl Sci. 2020;10(19):1–15.

The interesting part

ω-6:ω-3 value of 3:1

↓

Ideal for the human consumption

ω-3   ω-6
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Conclusion

- Potential application of the developed method as an official tool for hemp seed oil quality determination

- NMR ω-6/ω-3 value in hemp seed oil can be used as a benchmark for quality determination

- Need of an official protocol and harmonized guidelines for hemp seed oil quality determination

- Monitor the fatty acid variations during storage
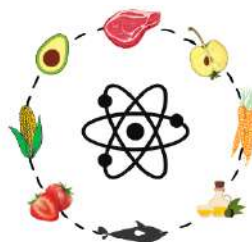
Thanks for your kind attention

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