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By G. Lotti and V. Averna

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Seed Lipids of the Water Plants (*)

By

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1. For a long time we have been studying the problems relating to the chemistry of fatty substances in vegetable substances, their distribution in the various parts of the plants, their analytical characteristics in regard to the species and variety and the variations in the seeds and oleic fruits during the process of ripening. We refer particularly to our previous researches on the lipidic composition of almonds (1), of olives (2), and of pistachios cultivated in Sicily and those on the

(*) Work undertaken with help from the National Research Council of Italy.

characteristics of fats present in the seeds and hypogeous organs of the same plants, root-stocks (4), bulbs (5), and tubers (6) and those relating to the analytical characteristics of oils from seeds of plants belonging to the same genus (7,8,9).

With a view to adding to our knowledge of the acidic composition and the analytical characteristics of fats extracted from seeds, so as to look for new oils and fats suitable for food consumption or for pharmaceutical or industrial uses, and to identify the particular fatty acids which are of particular interest in the field of vegetable bio-chemistry, we have examined in this present research the fats extracted from a group of water plants.

The study of the lipidic composition of seeds of water plants is of particular importance from the point of view of vegetable chemistry insofar as these plants show in relation to terrestrial plants, some biological peculiarities in their composition which condition the development of their general metabolism, with possible consequences that are both qualitative and quantitative on the chemical composition of their products of synthesis — in our case, the lipids.

The water plants, infact, show among others, notable modifications in the organs that are normally located under water, with an increase of plasticity and the presence of a particular tissue of aereation ('air chemistry') which enables the submerged tissues to breathe. The development of the roots also is greatly modified, so much so that in some plants these are entirely absent. Moreover, many water plants have heterophyllia. From a metabolic point of view, in the water plants deprived of roots, the function of mineral nutrition is largely undertaken by the submerged leaves which also take in Co_2 dissolved in water, in this form or as bicarbonate of calcium, transforming it into carbonate and thus giving origin to calcerous crusts (ex. Potamogeton). The mechanism of transpiration is also altered insofar as the water plants tend to limit the absorption of water on the part of submerged organs (absence of stomae) and increase the rhythm of transpiration.

It is well-known that the so-called water plants may be divided into those that live completely submerged in water and plants with hypogeous organs (roots, root-stocks or tubers fixed to the bottom and with leaves spread out on the water surface (Hydrophytae). Moreover, those hydrophytae that are to be found in damp places (marshes, submerged fields, etc.) belong to the family of water plants, having their roots in the land usually under water and the leaves, instead, above water as in the case of non-aquatic plants (Helophytae) (10).

Research on the lipidic composition of water plants is rather scarce while the fats of algae (11, 12, 13, 14) and of marine animals appear to have been more commonly studied and in these large quantities of polyunsaturated, in part conjugated acids have been found. It would therefore be interesting to evaluate if and to what extent this is valid for water plants. J.A.Lovern (12) found in the fats of Anacharis alsinatrum considerable quantities of polyunsaturated acids, while H.P.Kaufmann (15) showed the presence of conjugated, polyunsaturated acids in water plants and marsh plants such as some species of Nymphea and Iris, asserting that most probably in nature the said conjugated acids are much more common than is supposed to be the case.

2. In this present research, we have examined the oils extracted from the seeds of 21 species of vegetable plants, furnished partly by the Botanical Gardens of the University of Palermo and collected partly in the region along the coastline of Tuscany, belonging to the families of the Nymphaeaceae, Polygonaceae, Alismataceae, Cyperaceae, Oenoteraceae, Iridaceae, Labiatae, Borraginaceae, Typhaceae. The seeds of Cyperus Papyrus and Cyperus flavescens came from the edge of the River Anapo (Syracuse) where these plants have grown easily right from the time they were introduced by the Saracens (Arabs - Trans).

Of the species examined by us, only those belonging to the genus Polygonum, Nelumbuim and Nymphaea are true water plants (Hydrophytae). This last genus, from the point of view of vegetable geographic association belongs to the order of Nupharetum. All the other species considered belong to the Helophytae. In particular, with regard to the plants, the geographic association Scirpeto-Phragmitetum comprises the genuses Scirpus, Typha and Cladium, while the genuses Rumex, Iris and Alisma belong to the Glycerietum aquaticae and the Cyperus to Papyretum. The genus Carex forms part of plants that live close to the small reeds (association Magnocaricetum) while the genuses Lycopus, Epilobium, Symphytum and Myosotis are part of amphibious plants that exist on the banks of streams or along the ditches of fields.

With regard to the composition and the analytical characteristics of the fats extracted from the seeds being examined by us, the literature available refers only to the data already noted by us on the seeds of Nymphea and of Iris (15), those by H.A.Schuette and L.Gagneron (16) and by J.R.Clopton and R.W.Von Korff (17) on seeds of Typha latifolia and those of R.Kleiman, F.R.Earle et al. (18) on fats of seeds of Symphytum officinale and Myosotis sylvatica which showed the presence non-conjugated tetraenoic acids.

The oil was extracted from seeds which were previously dessicated at 70°C and finely ground, using petroleum ether (fraction 40-60°C) on Soxhlet. The etheric extracts appeared on the whole oily and of a color ranging from bright yellow to brown. Some of these and especially those dervived from the seeds of <u>Carex dioica</u> and <u>C. flava</u>, from <u>Cyperus</u>, <u>Polygonum</u>, <u>Rumex</u>, <u>Nymphaea</u> and <u>Typha</u> showed a wax-like consistency and generally a light brown color.

In table I is reproduced the analysis on the seeds and the fundamental analytical characteristics of the oils extracted, together with values of specific extinction in the U.V. field at 232, 262, 268, 274 and 308 mμ and of the value of Δ K measured on pure fatty acids (19). From the data in the table it appears that the seeds being examined contain small quantities of total nitrogen (varying from 1.11 to 3.77%); the ash content is somewhat variable and reaches a maximum of 17.7% in the seeds of <u>Carex dioica</u>, while the extract of ether is somewhat modest, being 1.2% in the seeds of <u>Nimphaea coerulea</u> and going up to 9.9% in those of <u>Iris pseudacorus</u>. Only in a very few cases and specifically in the seeds of <u>Epilobium hirsutum</u>, <u>Lycopus europaeus</u> and <u>Myosotis palustris</u>, the ether extract reaches somewhat high values, being respectively 21.6, 23.2 and 33.8%.

The content in non-saponifiable fatty substances, determined according to the official methods of analysis is, with very few exceptions, very high and reaches 41.0% in the extract of Nymphaea coerulea and the values of the pure fatty acids vary correspondingly. As we have already noted in the case of other seeds (4,5,6), in some particular case we may find a considerable difference between the sum of the values of the non-saponifiables and of the total fatty acids, and the content of the ether extract may refer to the presence in the extract of volatile or soluble substances which are removed by the non-saponifiables.

The refraction index at 25°C in cases where it was possible to effect it, varies from 1.4703 to 1.4883 while the number of iodine of the total fatty acids shows naturally considerable variations owing to the total unsaturation of the different fats. The saponification number, instead, has only limited variations, remaining between 183.2 and 196.9.

The values of specific extinction in the U.V. field measured of the fatty acids dissolved in cyclohexane show in all the fats, except those from the seeds of Cladium Mariscus and Rumex aquaticus the presence of more or less high quantities of conjugated doubles, which calculated by the noted formulae of Brice and Swain reach a maximum value of around 10% in the oil of seeds of Carex pendula. In some cases further, there are found also small quantities of conjugated, triples shown up by values of ΔK; this was so in the case of fats from the seeds of Polygonum amphibium, Alisma Plantago, Lycopus europaeus and Rumex aqaticus, but these never exceeded 0.25% while we were unable to find conjugated triples in Nymphaea and in Iris pseudacorus (15).

3. The fatty acids separated from the etheric extracts, methylated in a phial with methylic alcohol and zinc chloride, according to the official Methods were later analyzed by means of gas-chromatography using a "Fractovap model C" apparatus with thermo-conductivity detector and columns of 2m. filled with "Weas", utilizing helium as the carrier gas.

The acids were identified in the chromatograms by means of the number of carbons and determined quantitatively through internal normalization.

The results obtained are reproduced in Table 2, together with the values of the ratio of oleic acid/linoleic acid and unsaturated acids/saturated acids. In Fig. 1, in order to demonstrate this, we have reproduced the chromatogram of the total fatty acids present in the seed of Symphytum officinale.

An appraisal of the data in the table shows very marked differences in the acidic composition of the oils of the different water plants; these differences are both qualitative and quantitative depending upon the species and the family to which the plants belong. The range of the fatty acids present appears considerable even if from the point of view of quantity, the most important ones were always palmitic acid, stearic acid, oleic acid, linoleic acid and sometimes linolenic acid.

Table I - Analytical characteristics and U.V. spectrophotometry of oils extracted from seeds of water plants

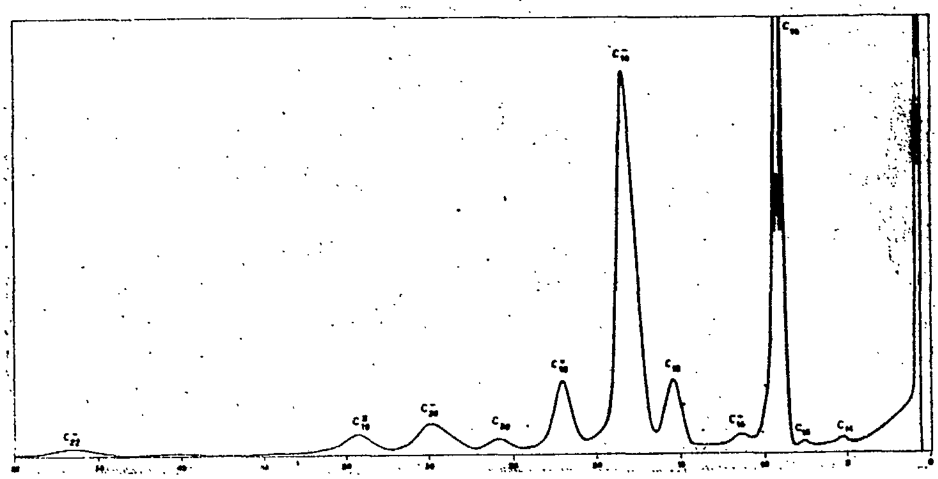
N°	Species & botanical family	Analysis of the seeds (Values on s.s)			Analysis of the Oils										
		Azoto totale %	Ceneri %	Estratto etereo %	Insaponificabile %	Acidi grassi puri %	Indice rifrazione a 25°C	N° di iodio Wijs	N° di saponificazione	K_{232}	K_{262}	K_{268}	K_{274}	K_{308}	ΔK
	Hydrophytae														
1	Nelumbium speciosum L. (Nymphaeaceae)	2,20	—	6,2	16,8	78,2	1,4735	70,2	186,0	21,09	8,20	7,96	7,42	3,12	+ 0,15
2	Nymphaea coerulea Sav. (Nymphaeaceae)	2,18	3,1	1,2	41,0	57,3	—	86,1	191,1	29,85	6,80	6,53	6,42	12,50	— 0,08
3	Nymphaea stellata Casp. (Nymphaeaceae)	2,25	4,4	1,7	35,2	45,5	—	89,4	191,7	12,92	4,27	3,94	3,77	2,51	— 0,08
4	Polygonum amphibium L. (Polygonaceae)	1,83	3,7	2,9	28,6	58,1	—	113,7	192,3	22,86	9,54	11,67	9,80	8,55	+ 2,00
	Helophytae														
5	Alisma Plantago L. (Alismataceae)	1,76	4,2	5,6	9,5	79,4	1,4883	184,5	183,2	15,72	2,62	3,42	2,21	1,20	+ 1,01
6	Carex dioica L. (Cyperaceae)	1,66	17,7	5,0	11,2	76,0	—	132,5	191,5	17,14	5,35	5,00	4,64	2,14	0
7	Carex flava L. (Cyperaceae)	1,11	6,0	2,5	34,8	61,9	1,4791	103,2	191,2	20,68	3,24	2,93	2,77	1,39	— 0,08
8	Carex pendula Huds. (Cyperaceae)	1,92	4,9	8,0	2,5	89,7	1,4789	146,2	191,1	98,33	19,16	18,80	18,30	6,66	+ 0,07
9	Cladium Mariscus R. Br. (Cyperaceae)	1,25	3,8	5,0	4,4	90,8	1,4703	97,7	190,7	6,37	2,46	2,38	2,29	1,10	+ 0,01
10	Cyperus flavescens L. (Cyperaceae)	2,67	3,4	8,1	17,3	49,3	—	57,6	196,9	36,31	14,66	15,20	14,52	10,47	+ 0,61
11	Cyperus Papyrus L. (Cyperaceae)	3,07	7,9	3,6	18,1	61,7	—	98,3	187,1	40,00	14,72	14,54	14,20	20,63	+ 0,08
12	Epilobium hirsutum L. (Oenoteraceae)	3,77	7,5	21,6	5,3	83,2	1,4748	124,4	193,1	67,62	19,87	18,23	17,00	9,22	— 0,20
13	Iris pseudacorus L. (Iridaceae)	2,48	4,2	9,9	33,8	57,2	1,4827	116,1	191,0	24,59	11,76	11,76	11,76	7,10	0
14	Lycopus europaeus L. (Labiatae)	2,26	6,4	23,2	12,6	78,1	1,4832	184,5	183,3	18,57	4,28	5,38	4,28	2,02	+ 1,10
15	Myosotis palustris Hill. (Borraginaceae)	2,65	11,5	33,8	2,1	63,7	1,4765	65,3	191,5	29,20	13,78	13,31	12,61	8,41	+ 0,12
16	Rumex aquaticus L. (Polygonaceae)	2,22	2,3	2,8	22,0	75,0	1,4754	100,5	191,1	8,8	9,96	10,90	9,25	2,54	+ 1,30
17	Rumex sanguineus L. (Polygonaceae)	1,98	3,9	1,4	24,0	66,2	—	98,8	191,6	19,12	11,47	11,47	11,47	9,83	0
18	Scirpus holoschoenus L. (Cyperaceae)	1,35	5,2	3,5	16,9	69,8	—	109,6	190,3	52,08	4,46	4,46	4,46	2,32	0
19	Scirpus lacuster L. (Cyperaceae)	1,12	2,9	3,4	13,4	82,7	—	144,0	190,9	20,91	4,31	3,97	3,75	0,91	— 0,06
20	Symphytum officinale L. (Borraginac.)	2,29	12,9	5,7	4,1	87,2	1,4725	60,7	194,0	19,30	9,17	9,01	8,70	3,16	+ 0,08
21	Typha latifolia L. (Typhaceae)	1,91	10,8	1,7	18,8	58,1	—	95,2	191,4	33,45	8,90	8,00	7,45	2,76	— 0,17
		Total Nitrogen	Ash	Ether Extract	Non-Saponifiable	Pure Fatty Acids	Refraction index at 25°C	Iodine No.	Saponification No.						

TABLE II

Acidic composition, percentage wise of oils extracted from the seeds of water plants

N	Botanical Species	C_{12}	C_{14}	C_{14}^{-}	C_{15}	C_{15}^{-}	C_{16}	C_{16}^{-}	C_{17}	C_{17}^{-}	C_{18}	C_{18}^{-}	C_{18}^{--}	C_{18}^{---}	C_{20}	C_{20}^{-}	C_{22}	C_{22}^{-}	C_{22}^{--}	C_{24}	oleic lin. oleic	unsatu- rated saturated
	Hydrophytae																					
1	Nelumbium speciosum L.	tr.	tr.				19,2	tr.	1,9	tr.	2,8	70,1	6,0	tr.							11,6	3,1
2	Nymphaea coerulea Sav.		tr.				19,6	tr.			9,9	26,5	35,4	0,6	4,7	0,6	1,5			1,2	0,7	1,7
3	Nymphaea stellata Casp.		0,3	0,2	0,2	0,1	16,2	0,5			19,1	16,9	30,1	6,6	2,1	6,2	1,3				0,5	1,5
4	Polygonum amphibium L.	0,3	1,0	0,4		0,1	12,7	4,8		0,3	2,7	29,4	40,0	5,2	0,9	1,3	0,9				0,7	4,0
	Helophytae																					
5	Alisma Plantago L.		0,5		0,2		13,1				1,9	12,2	18,7	52,4		0,2	0,8				0,6	5,0
6	Carex dioica L.		0,1	0,1			8,0		2,4	0,2	3,6	16,8	67,2	0,6	0,2	0,1	0,7				0,2	5,6
7	Carex flava L.		0,4	tr.	0,1		19,6	0,2	0,2		3,7	24,8	41,7	1,9	1,9	2,6	2,0			0,9	0,6	2,4
8	Carex pendula Huds.		0,3				5,8	tr.	0,1		0,6	15,3	76,1	0,4	0,4	0,7	0,3				0,2	12,2
9	Cladium Mariscus R.Br.						7,5				0,7	70,4	20,2	0,7	tr.	0,5	tr.				3,5	11,1
10	Cyperus flavescens L.		0,3		0,6		41,1	1,5	0,5		5,0	34,3	10,0	3,6	3,1		tr.				3,4	1,0
11	Cyperus Papyrus L.	tr.	tr.		1,0		10,0	0,8	0,5		2,4	24,1	43,4	0,2	4,5	1,7	9,4			2,0	0,5	3,0
12	Epilobium hirsutum L.		0,3		0,2		17,0	0,5			4,0	9,7	66,0	0,6	1,7						0,1	3,3
13	Iris pseudacorus L.		0,1				7,3	0,3			1,1	46,2	44,1	tr.	0,9	tr.					1,0	9,5
14	Lycopus europaeus L.		tr.				7,0		0,3	0,2	2,1	12,3	33,9	43,8		0,4					0,3	9,6
15	Myosotis palustris Hill.		1,0		0,4		27,6	0,2			6,3	41,0	8,1	3,7	3,2	2,0		3,1		3,4	5,0	1,3
16	Rumex aquaticus L.		tr.				13,6	tr.			2,1	53,0	25,1	2,4	0,6	1,0				2,2	2,1	4,4
17	Rumex sanguineus L.						14,6				1,7	64,2	15,4	1,6	0,2	1,5	0,3		0,5		4,2	4,9
18	Scirpus holoschoenus L.		0,2		0,1		14,9	tr.			2,9	22,9	50,8		2,8		2,6		1,3	1,5	0,4	3,0
19	Scirpus lacuster L.		tr.				5,0	0,4		0,1	0,3	21,0	72,0			1,2	tr.				0,3	17,8
20	Symphytum officinale L.		0,1		0,1		33,3	0,2			7,5	41,2	7,1	3,1	1,4	4,3		1,7			5,7	1,3
21	Typha latifolia L.		0,4		0,2		14,0	tr.	1,0	1,0	4,0	27,8	42,9	0,4	1,3	0,6	3,5		0,9	2,0	0,6	2,8

These are met with also in many cases myristic acid, palmitoleic acid, arachidic acid, eicosenoic and behenoic acids while more rarely do we find also lauric acid myristoleic acid pentadecanoic acid, pentadecenoic acid, heptadecanoic acid, heptadecenoic acid, erucic acid, lignoceric acid and in the fats from seeds of <u>Rumex sanguineus</u>, <u>Scirpus holoschoenus</u>, and <u>Typha latifolia</u>, an acid that may be related on the basis of the time of retention, to a C_{22} with two double bonds.



<u>FIG.I</u> Gaschromatograph of the total fatty acids present in the seeds of <u>Symphytum officinale</u>, made using a "Fractovap Mod. C." apparatus, with thermo-conductivity detector. Quantity injected; 1 μl. Column of 2m, Ø 6x5 filled with "Weas", carrier gas used, helium; temperature of evaporator 300°C; temperature of the column 210°C.

From a quantitative point of view the acids mainly represented were, in almost all the species examined, oleic and linoleic acids, the former reaching the value of 70.4% in the oil of <u>Cladium Mariscus</u> and the latter 76.1% in the oil of <u>Carex pendula</u>. Only in the fats of <u>Alisma Plantago</u> and <u>Lycopus europaeus</u> the acid present in a higher quantity proved to be linolenic acid, which reached the values respectively of 52.4 and 43.8%, and in that of <u>Cyperus flavescens</u> the palmitic acid content was 41.1%. All the other acids were present in considerably smaller quantities. Stearic acid had a maximum value of 19.1% in the fat of the seeds of <u>Nymphaea stellata</u>, arachidic of 4.7% in that of <u>Nymphaea coerulea</u>, eicosenic of 6.2% in the lipids of <u>Nymphaea stellata</u> and behenic of 9.4% in those of <u>Cyperus Papyrus</u>. Among the fatty acids present eratically, we noted 4.8% of palmitoleic acid in the lipids of <u>Polygonum amphibium</u>, 3.1% of erucic acid and 3.4% of lignoceric acid in <u>Myosotis palustris</u>.

Considering now the different species, it is interesting to note that the <u>Hydrophytae</u> examined had all of them somewhat low quantities of linolenic acid and rather high values of saturated acids as we can see from the ratio of unsaturated / saturated acids which never exceeded 4.

In the <u>Helophytae</u>, instead, the unsaturated acids were present often in rather high quantities (value of the ratio of unsaturated / saturated acids reached 17.8

in the case of Scirpus Lacuster). The Cyperaceae especially had all of them more or less high values of behenic acid, while the quantities related to oleic and linoleic acids did not appear with any regularity.

In comparing the results obtained by us with those in the literature on the subject, we noted marked differences especially in the case of Borraginaceae (Symphytum and Myosotis) probably as a result of the differing conditions of the ambient.

In the oils from the seeds of the aforesaid species, we were unable to find any trace of tetraenoic acids, which had instead been recorded by the authors cited above (18).
On fats extracted from seeds free in pure carbonic sulphur for purposes of spectroscopy, infra-red spectrums were also made from 1 to 16 μ, using the "Beekman 1. R5A" apparatus.

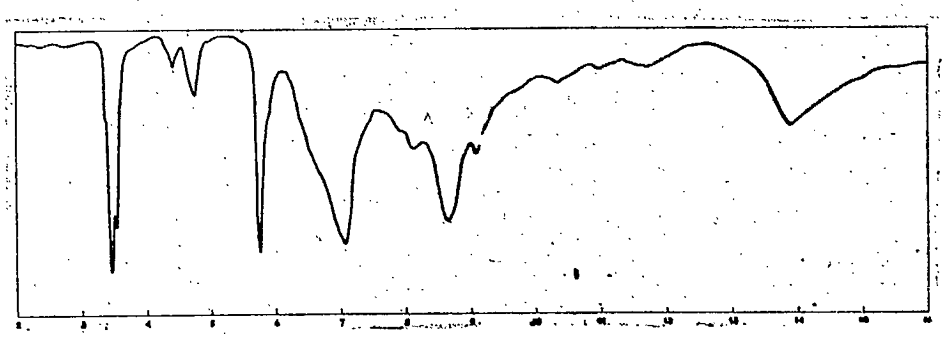


Fig.2 Infra-red spectrum of oil from seeds of Carex dioica. Solution at 1% in CS_2 . Thickness of the flask 0.5mm. Speed of recording 1 μ/min.

In Fig. 2 is reproduced the I.R. spectrum of the oil from the seeds of Carex dioica. The greater part of the oils examined showed a rather simple spectrum, so characteristic of vegetable oils in general. It must be pointed out, however, that the oils from the seeds of Symphytum officinale, Alisma Plantago, Scirpus holoschoenus, Epitobium hirsutum, Myosotis palustris, showed a peak at 2.70 μ, typical of the presence of free hydroxyls; those of Carex flava, Carex pendula, Nymphaea stellata, Lycopus europaeus, Alisma Plantago showed tops at 11.0 and 11.6μ, characteristic of epoxidic forms, the lipids of the seeds of Scirpus holoschoenus, Alisma Plantago, Symphytum officinale, Nymphaea stellata, Cladium Mariscus showed peaks at 10.20 and 10.6 μ. This may be the index of the double conjugated cis-trans found also by L.J.Morris and M.O.Marshall (20) in the oils of Dimorphoteca sinnata e Crepis rubra. The oils of Lycopus europaeus, Epilobium hirsutum, Myosotis palustris e Cyperus flavescens also showed a peak at 10.36 μ related to the isolated trans double bond.

In conclusion, the analysis made on the total lipids of a group of water plants has enabled us to draw the following conclusions:

a) For the very first time it was possible to determine the analytical characteristics and the acidic composition of the oils derived from the seeds of 16 species of water plants belonging to 6 different families.

b) An examination of the U.V. showed the presence in the greater part of the fats, of conjugated doubles and sometimes of conjugated trebles while the infra-red spectrums sometimes showed peaks related to the presence of hydroxyls, epoxidic forms and trans fatty acids.

c) The acidic composition appears very different in the various species and families. Water plants showed generally they had high quantities of polyunsaturated acids even if the analysis made by us did not show the presence of tetraenoic acids.

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