SYMPOSIUM: CRUCIFEROUS OILSEEDS

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L.-A. APPELQVIST, Program Chairman

Composition of Seeds of Cruciferous Oil Crops[1]

L.-Å. APPELQVIST, Division of Physiological Chemistry, Chemical Center, University of Lund, Lund, Sweden

ABSTRACT

Several species of the Cruciferae family are presently used as oilseed crops, viz., *Brassica campestris* (turnip rape and sarson), *B. juncea* (brown or yellow mustard), *B. napus* (rape), *Crambe abyssinica* (crambe), and *Sinapis alba* (white or yellow mustard). Seed oils of these species are characterized by variable but generally large amounts of erucic acid (22:1) in the triacylglycerols, which make up 95-98% of the total lipids of high quality, viable seeds. In addition to erucic acid, the major fatty acids are oleic (typically 10-25%), linoleic (10-20%), linolenic (7-11%) and eicosenoic (5-10%). However cultivars of rapeseed lacking erucic acid and having about 55-60% oleic, 20-25% linoleic and ca. 10% linolenic acid have been developed. The eicosenoic and erucic acids are located exclusively at the 1 and 3 positions of the triacylglycerol. As a consequence, major triacylglycerol types have carbon numbers 54, 56, 58, 60 and 62. The phospholipids of rapeseed are essentially devoid of erucic acid and have palmitic, oleic and linoleic acids as major fatty acids. Sterols generally amount to about 0.5% of the oil with β-sitosterol, campesterol and brassicasterol as major constituents (about 55%, 25% and 15%, respectively, of the total sterols). A few per cent of the total sterol fractions is cholesterol. The tocopherol content of rapeseed oil is about 800 ppm with α- and γ-tocopherol as major components. Cruciferous seeds contain a fairly large number of storage proteins. Thus approximately 50 components have been detected in alkaline extracts of *Brassica napus*, a major portion of which are in the molecular weight range 120-150,000. The protein spectrum of *B. napus* (rape) is more complex than that of *B. campestris* (turnip rape) since the former species is an allotetraploid with *B. oleracea* (kale, cabbage, etc.) and *B. campestris* as parents. Approximately 5% of the fat free seed meal is composed of glucosinolates, which are split upon enzymatic hydrolysis to antinutritional factors: isothiocyanates, oxazolidinethiones and nitriles. The different crucifers discussed have both qualitative and quantitative differences with respect to glucosinolate content.

[1]One of nine papers presented at the Symposium, "Cruciferous Oilseeds," ISF-AOCS World Congress, Chicago, September 1970.

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INTRODUCTION

Several species of the family Cruciferae are utilized as oil seed crops. Thus the terms rapeseed and rapeseed oil on the world market can mean seeds and oils of *Brassica napus* (rape) or *Brassica campestris* (turnip rape or yellow sarson) or a mixture of both. Furthermore there are both summer annual and winter annual types of *Brassica napus* and *Brassica campestris* with slight differences in chemical composition. Commercial seed and oil named "mustard" can be extracted from *Brassica juncea* (brown mustard, leaf mustard or sometimes yellow mustard) or from *Sinapis alba* (white mustard or yellow mustard). Also the nomenclature rules of the Codex Alimentarius Commission of FAO/WHO allow the inclusion of seeds from *Brassica tournefortii,* a noxious weed in Australia, in the term "rapeseed." The existence of several crop types with the group names "rapeseed" and "mustard seed" is one reason for a greater compositional variation in commercial samples of these seeds than in those of soybeans, sunflowers, groundnuts, etc., where only one species is behind each commodity name. Less known oil seed crops in this group are *Crambe abyssinica* and *Camelina sativa,* the last one probably not grown at all at the present time.

When "typical data" for rapeseed and mustard seed are presented in this paper and elsewhere it must be remembered that there is very often a large range of variation in composition with genotype. This variation is discussed by another author in this symposium (1), and consequently this paper will not dwell on the details of the interspecific variation. Rather it will review the chemical composition of "typical samples," if such exist, of the Cruciferous oil seeds. Quite naturally most of the data will be on the two dominating species on the world market, namely *Brassica*

TABLE I

Typical Ranges of Variation in Content of Common Fatty Acids in the Oils From Some Cultivars or Breeding Lines of Some Cruciferous Seeds

Species and type	Ranges in percentage content						References
	Palmitic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic acid	
Brassica campestris							
Winter turnip rape	2-3	14-16	13-17	8-12	8-10	42-46	9,10,11,12,13[a]
Summer turnip rape, classical cultivars	2-3	17-34	14-18	9-11	10-12	24-40	9,12,14,15[a]
Summer turnip rape, low erucic acid lines[b]	4-7	48-55	27-31	10-14	0-1	0	5,15
Sarson and toria	2-3	9-16	11-16	6-9	3-8	46-61	10,15[a]
B. juncea	2-4	7-22	12-24	10-15	6-14	18-49	10,11[a]
B. napus							
Winter rape, classical cultivars	3-4	8-14	11-15	6-11	6-10	45-54	9,10,11,13,16,17[a]
Winter rape, low erucic acid cultivars or lines[b]	4-5	40-48	15-25	10-15	3-19	3-11	17,18
Summer rape, classical cultivars[c]	3-4	12-23	12-16	5-10	9-14	41-47	9,10,14,15,19[a]
Summer rape, low erucic acid cultivars or lines[b]	5	52-55	24-31	10-13	0-2	0-1	5,15,18
B. Tournefortii	2-4	6-12	11-16	10-16	6-8	46-52	25,26
Crambe abyssinica	2-3	13-22	7-12	4-9	2-3	51-62	3,11,20,22,23,24[a]
Sinapis alba	2-3	16-28	7-10	9-12	6-11	33-51	9,10,12,20,23,25, 27,28,29

[a]References 15 and 21 contain data for variation in erucic acid content among many breeding samples of *Brassica campestris, B. juncea* and *B. napus;* References 3, 21 and 24 also for *Crambe abyssinica;* and Reference 21 for *Sinapis alba* (named *Brassica hirta* in Reference 1). These data are not included in the ranges given above.

[b]Results from only a few samples available. The range of variation in fatty acid compositions is expected to increase as new cultivars are being released.

[c]Except for the Polish cultivar Bronowski, which is not grown to any significant extent. This cultivar has ca. 10% erucic acid (See Reference 10).

campestris (turnip rape and sarson) and *Brassica napus* (rapeseed).

GROSS COMPOSITION OF SEED PARTS

The seeds of rape and mustard contain a seed coat or hull, 15-20% of the total seed weight, two cotyledons and another embryonic structure, mainly hypocotyl. There is also a very small endosperm, generally only a few cells in thickness. Whereas rape and mustard seeds occur in pods carrying about 10-40 seeds each, *Crambe abyssinica* has one, or occasionally two, seeds in each pod and a very small seed coat or hull.

The oil and protein are not equally distributed between the different seed parts. For example the seed coat or hull is low in oil and protein and high in polysaccharides. In *Brassica campestris* and *B. napus* the hulls or seed coats typically comprise 15-20% of the total seed weight with about 15% oil, 15% protein and almost 30% "crude fiber" (cellulose, hemicellulose and lignin), whereas the remainder of the seed contains 45-47% oil and 28-30% protein but only about 3% crude fiber (2). In *Crambe abyssinica* the seed coat tissue forms a smaller portion of the total seed weight, namely about 8%, with typically 17% oil and 23% protein compared to 38% and 34% in the hypocotyl, and 55% and 23% in the cotyledons (3). The differences in oil content that have been recorded for the morphological sub-units of *Sinapis alba* are of a similar order except that the oil content of the seed coats was much lower, 5-10% (4). Other interesting compositional differences between the parts of Cruciferous seeds are recorded in the literature (3,5).

As a consequence of the high oil content of rapeseed, the natural moisture level is generally between 6% and 8% when the seed is in equilibrium with the environmental moisture. Data reported in the literature on an "as is" basis can therefore be assumed to refer to about 7% moisture.

The predominant part of the literature data on the chemical constituents of the seeds is obtained with entire seeds and not on the seed parts. However data on the seed parts are of interest for two reasons: (A) The seed parts are biologically different. The seed coat contains maternal and endospermic tissue, whereas the cotyledons are embryonic tissue; this difference is important in studies on the control of the content of a certain component; (B) In the course of industrial processing it is possible to remove the seed coat, if desired, for example in the preparation of rapeseed flour or protein concentrates, as discussed in this symposium (6).

FATTY ACID COMPOSITION

The oil from Cruciferous seeds is composed of about 95-98% triacylglycerols (or triglycerides). The total content of free fatty acids is low, about 0.3-0.5% in fully mature rape and mustard seed when adequately handled after harvest (7). Improper handling causes elevated free fatty acid levels, as is well known (8). The amount of mono- and diacylglycerols is generally very small in a high quality seed.

Until recently the triacylglycerols have mainly been characterized by their total fatty acid compositions. Actually, because of the predominance of the triacylglycerols, the fatty acid data reported very often refer to the total lipids extracted. A very large amount of such data on the fatty acid composition of crude lipids of *Brassica* seeds has been published. Critical reviews have been made earlier, to which the reader is referred for details on this matter (9,10).

In this paper, ranges of variation for important fatty acids of some Cruciferous oilseeds have been compiled from only a few recent papers, in which gas chromatographic data were reported. From Table I the following seems to be noteworthy: The range of variation in linolenic acid content of European and Canadian cultivars of both rape and turnip rape is rather low, and much lower than that

TABLE II

Fatty Acid Composition of Cotyledons, Hypocotyl and Seed Coat of *Brassica napus*[a]

Seed part	Total amounts of fatty acids obtained from 100 seed parts, mg	Fatty acid composition, wt %											Isomer proportions (*n*-9)/(*n*-7)		
		16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1	22:2	24:0	24:1	18:1	20:1	22:1
Cotyledons	61.5	3.3	0.3	1.2	12.4	15.2	9.0	0.8	9.5	0.7	0.5	44.6	1.0	0.4	1.1	12/1	8/1	25/1
Hypocotyl	11.0	6.6	0.6	1.6	15.3	21.3	9.8	0.8	10.3	0.8	0.5	29.9	0.6	0.4	1.5	8/1	8/1	16/1
Seed coat	4.8	5.2	2.0[b]	1.2	18.7	17.0	6.1	0.6	14.4	0.6	0.7	30.0	0.7	0.6	1.1	0.9/1	0.3/1	3/1

[a]Cultivar Regina II. Percentage composition and total amounts of fatty acids determined by gas liquid chromatography analysis on a packed column; monoene isomer proportions estimated from analysis on a capillary column (5).

[b]Also 1.1 per cent of 16:3.

given in several of the classical textbooks. Previous very low figures were probably a consequence of inappropriate methods in determinations of the linolenic acid content. Also the range of variation in erucic acid content for the presently predominant rapeseed crop in Northern Europe, winter rape, is rather small; the level is always close to 50%. The two next important oil crops for Europe are summer rape and winter turnip rape. Typical samples of these contain about 45-50% erucic acid except for the Polish summer rape, Bronowski, with ca. 10%. Small variations exist in linoleic and linolenic acid contents. In contrast to the European crop types, the predominant type in Canada, summer turnip rape, is generally lower in erucic acid. Sarson, grown in India and Pakistan, has often 50-60% erucic acid. White mustard, *Sinapis alba,* is characterized by a rather low linoleic acid content so that the linoleic to linolenic proportion is distinctly different in most cases in *Sinapis alba* compared to that of the predominant *Brassica* crops. *Crambe abyssinica* is very high in erucic acid, about 60%, which is the basis for the great interest paid to this species as a raw material for the synthesis of erucic acid derivatives (30).

Brassica juncea shows very large variations in erucic acid content and also in oleic, linoleic and eicosenoic acid contents. The *B. juncea* samples investigated in our own studies could be pooled into two distinct groups, one with about 20% erucic and the other with about 45% erucic acid. This might be of interest since mustard oil of Indian or Pakistanian origin is generally extracted from *B. juncea.* All the Indian samples had high erucic acid content.

Finally a look at *Brassica tournefortii* reveals that all samples so far reported in the modern literature appear to be very similar in fatty acid composition, with about 50% erucic acid. This again might be of interest for the industrial oil chemists, in view of the possibility that the Australian shipments of so-called rapeseed oil could come from *B. tournefortii.*

The renewed interest in the zero or low erucic acid cultivars of rapeseed, which is a consequence of the pathological effects reported from feeding experimental animals with large doses of "classical" rapeseed oil (31), has hastened the marketing of such cultivars (1). At present it is impossible to predict accurately the time for phasing over from classical rapeseed cultivars to the new ones with little or no erucic acid. However most probably the data in Table I for Canadian and European rapeseed will have only historical interest in the mid 70's.

FATTY ACID ISOMERS

The use of enrichment techniques such as urea fractionation or argentation chromatography has resulted in the detection of a large amount of minor fatty acids in rapeseed oil and white mustard seed oil. Thus 51 different fatty acids were reported to occur in white mustard seed obtained from Denmark (28), many of them in very small quantities (<0.01%). Whether all of these are true constituents of pure white mustard seed, or some of them are contaminants either of the seed material or the laboratory equipment remains to be established (cf., 32 and *loc. cit.*).

The presence of positional isomers of monoene fatty acids in rapeseed, mainly isomers of oleic, eicosenoic and erucic acids have been reported (33,34). These analyses were performed on samples of commercial rapeseed oil. In our laboratory we investigated the monoene proportions and the gross fatty acid composition of the various morphological parts of rapeseed (5). The erucic acid content is considerably lower in the hypocotyl and seed coat compared to the major lipid storage parts, the cotyledons (Table II). Most interestingly, the seed coat fatty acids were very rich in the less common isomers, the so-called ω-7 or (n-7)-acids, which can be considered

elongation products of palmitoleic acid, an (*n*-7)-acid. Noteworthy is the apparent absence of the (*n*-7)-isomer for the C-24 monoene, both in a published chromatogram (34) and in our chromatograms. This is taken to mean that rapeseed contains acids elongated in three successive steps in each monoene series. This observation might be of interest if a new industrial process for oil and protein manufacture is developed in which the seed coat is removed from the remainder of the seed, and thus could be processed for oil separately to yield uncommon fatty acid isomers. For instance the ω-7 isomer of erucic acid would, upon ozonolysis, yield a C-15 dicarboxylic acid which might have specific industrial uses.

Most of the fatty acids of Cruciferous seeds so far discussed are shown in Figure 1, which also indicates their proposed or established metabolic relationships. Recent studies on German rapeseed oil established the (*n*-9)-structure for the C-20 and C-22 di- and tri-enoic acids of rapeseed oil (35). The presence of dienes and trienes in rapeseed oil other than linoleic and linolenic acids could be of significance for the specific off-flavors noted for rapeseed oil.

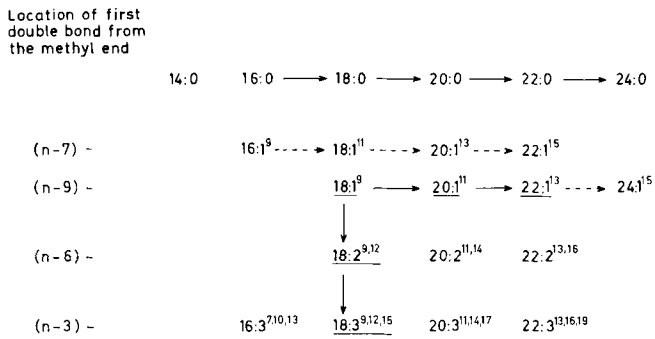

FIG. 1. Structures of and possible metabolic relationships among fatty acids of Cruciferous seeds. Major components of oils from classical cultivars are underlined. Metabolic relationships indicated by full-lined arrows refer to published data on higher plants, those indicated by a dashed arrow to analogous precursor-product relations suggested by the present author.

TRIACYLGLYCEROL STRUCTURE

The long chain fatty acids, eicosenoic and erucic, together with minor amounts of saturated acids, are exclusively or almost exclusively esterified at the outer position of the glycerol molecule, as reported by several authors (20,23,27) and demonstrated in Table III. Furthermore direct gas chromatography of triacylglycerols from Cruciferous oils has shown major peaks for the carbon numbers 54, 58 and 62, as reported in the literature (36) and demonstrated in Table IV. The C-62 peak contains the monooleo-, monolinoleo- and monolinoleno-dierucyl glycerols. On the other hand only a very minor peak is observed for C-64 and none at all for C-66, which should contain trierucylglycerol if present.

The absence of erucic acid in the 2 position or its presence in trace amounts seems to be under strong genetic control, and indicates that the enzymes synthesizing the triacylglycerols of brassicas can not introduce erucic acid into the central position. Recently thousands of samples were analyzed for fatty acid composition in a breeding program for increased erucic acid content. However no single inbred plant or offspring from crossing "high erucic x high erucic" yielded an oil with more than 67 mole % of long chain fatty acids plus saturated acid (24). This finding is disappointing for the USDA projects aiming at the highest possible erucic content in the Cruciferous oil seeds (cf., 3 and 30). Since there is at least one species that can synthesize trierucyl glycerol, namely *Tropaeolum majus* (36), studies on the biosynthesis of triacylglycerols in *Brassica napus* compared to that in *Tropaeolum majus* have been initiated at our laboratory.

Some minor yet not unimportant differences between *Brassica napus* and *Sinapis alba* with respect to fatty acid positioning were observed in our studies (23). The pancreatic lipase data indicated a less strict positioning in *Sinapis alba* than in a *Brassica napus* sample with very similar erucic acid content (see Table III). This difference has been verified by others using gas liquid chromatography (GLC) analysis of the intact triacylglycerols and other techniques (Table IV and Reference 37).

Furthermore we have found that the positioning of linoleic and linolenic acid is slightly different in zero erucic rapeseed oil from that in soybean oil. Thus the linolenic in soybean oil seems to be randomly distributed among the inner and outer positions, whereas the linolenic acid seems to be preferentially but not exclusively located in the central position in "zero erucic" rapeseed oil (23). This could perhaps be of some industrial significance for the oxidative stability of the oil, since it has been reported that the inner position is more protected (38).

NONSAPONIFIABLES

The total content of so-called nonsaponifiable lipids is reported to be from about 0.5-0.1% of the oil (9,39). The major component among the nonsaponifiable lipids consists of the sterols. A typical value for sterols is slightly over 60% of the total nonsaponifiables; hydrocarbons comprised 9%, squalene 4%, aliphatic alcohols 7% and triterpenoid alcohols about 9% in a sample of rapeseed oil (40).

It should be pointed out that the nonsaponifiables comprise a mixture of native constituents, e.g., hydrocarbons, and compounds which have appeared as a result of the saponification, e.g., alcohols and acids being formed from esters. Hence it is highly desirable that more information on the native nonglyceride components be accumulated. However at present most data on these compounds have been obtained after a primary saponification of the total lipids, although methods have been developed for the isolation of the nonglyceride components in native form (40).

Analysis of the hydrocarbon fraction of a sample of rapeseed oil of unreported origin showed it to contain 36 components, which were normal hydrocarbons from C-11 to C-31 iso and/or ante-iso hydrocarbons from C-11 to C-17, C-19 to C-21. The major component, about 8-10% of the total hydrocarbons, was the saturated C-29 (41).

The triterpenoid alcohol fraction of seed oils has a complex qualitative composition. Among a large number of seed oils studied (40), none had exactly the same GLC profile and that of rapeseed was the most complex of all. At least 13 components were present in the rapeseed extract, major components being β-amyrin, cycloartenol and 4-methylcycloartenol. If the triterpenoid alcohol fractions of classical and zero erucic rapeseed are similar—and there is no biochemical reason to assume the reverse—the GLC profile of the triterpenoid alcohols could be an excellent "fingerprint" of *Brassica* oils regardless of their fatty acid composition in the same manner as yellow seed coat has been suggested as a "marker" for the zero erucic seeds (1).

The major part of the sterols occur as sterol esters, but few data are available on the proportions of the various sterol fractions. The qualitative and quantitative composition of the total sterols have been studied in detail by two different laboratories (42,43) using gas chromatography-mass spectrometry (see Table V). Obviously β-sitosterol predominates, followed by campesterol and brassicasterol. It should be noticed that cholesterol has been identified as a constituent of rapeseed lipids in two independent laboratories using GLC/mass spectrometry. The data presented so far do not indicate any major differences in sterol

TABLE III

The Fatty Acid Composition of Triacylglycerols (1, 2, 3 Position Acids) and of Monoacylglycerols Derived by Pancreatic Lipase Action (2 Position Acids) From Some Cruciferous Seeds (23)

Species and cultivar	Fatty acid composition, mole %										
	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0+ 22:1	22:2
Brassica campestris, Yellow Sarson											
Triacylglycerol	2.1	0.5	1.1	12.6	14.8	8.9	1.2	4.1	0.8	53.6	0.4
2 position	2.2	0.8	1.0	31.6	38.5	22.1	---	1.5	---	2.3	---
Proportion[a]	35	53	30	84	87	83		12		1	
B. napus, cv. Victor											
Triacylglycerol	3.3	0.3	0.7	11.4	15.4	10.7	0.7	8.1	0.5	48.1	0.9
2 position	2.0	0.6	0.9	28.0	40.6	25.1	---	0.6	---	2.1	---
Proportion[a]	20	67	43	82	88	78		3		2	
B. napus, cv. Regina											
Triacylglycerol	4.0	0.4	1.1	13.9	17.5	10.2	1.5	10.7	0.7	39.6	0.4
2 position	0.6	0.5	0.1	27.9	43.0	25.8	---	0.9	---	1.2	---
Proportion[a]	5	42	3	67	82	84		3		1	
B. napus, zero erucic acid line											
Triacylglycerol	5.0	0.3	0.9	52.7	24.9	12.8	1.0	2.1	---	0.3	---
2 position	1.4	0.4	0.2	40.6	37.6	19.7	---	0.2	---	---	---
Proportion[a]	9	44	7	26	49	51		32			
Crambe abyssinica											
Triacylglycerol	2.4	0.4	0.7	17.1	10.1	9.0	0.9	2.9	---	56.3	0.2
2 position	3.0	1.0	1.4	44.4	25.3	19.5	---	1.2	---	4.2	---
Proportion[a]	42	83	67	87	84	72		14		3	
Sinapis alba, cv. Seco											
Triacylglycerol	2.4	0.3	0.8	26.3	10.4	12.1	1.3	9.1	0.6	35.6	1.1
2 position	1.2	0.6	0.1	45.7	21.2	26.7	---	1.3	---	3.1	---
Proportion[a]	17	67	4	58	68	74		5		5	

[a](2 position/tracylglycerol x 3) x 100 = Proportion, i.e., percentage of fatty acid type esterified with the 2 position.

TABLE IV

Triacylglycerol Patterns by Carbon Number of Seed Oils From Rape, Turnip Rape and White Mustard (Persmark, pers. comm.)[a]

Species and cultivar	Triacylglycerol pattern, wt % Carbon number								
	50	52	54	56	58	60	62	64	66
Brassica campestris, winter type, cv. Duro	Trace	1.2	2.1	3.3	17.5	22.8	50.0	3.0	Trace
B. campestris, summer type, cv. Bele	Trace	2.3	7.8	13.5	38.4	20.1	16.2	1.7	---
B. napus, winter type, cv. Victor	Trace	0.6	1.7	6.0	7.8	17.6	60.8	5.3	Trace
B. napus, summer type, cv. Regina	0.2	1.2	2.8	8.4	15.4	20.9	47.1	4.0	Trace
Sinapis alba, cv. Seco	0.3	1.8	3.3	8.0	23.3	18.5	39.4	5.4	Trace

[a]See Table III for the fatty acid patterns of cv. Victor, Regina and Seco; see Reference 9 for those of cv. Duro and Bele with approximately 44% and 28% erucic acid respectively.

pattern between related species or cultivars.

The tocopherol content of rapeseed is in recent investigations generally reported to be about 800 ppm. The relative proportions of the individual tocopherols are about 35% α- and about 65% γ- with traces of δ-tocopherol (44 and U. Persmark, unpublished).

PIGMENTS

Mature rapeseed contains rather small amounts of chlorophylls and related pigments (5-10 ppm in the oil), whereas elevated levels of these compounds can occur in certain areas and crop years as a consequence of unfavorable harvesting conditions. It has been reported that German and Polish samples of rapeseed oil contained no chlorophyll but only the magnesium-less compounds, the pheophytins (45,46). Studies on oils extracted carefully in our laboratory from high quality seeds revealed mainly chlorophyll and very little, if any, pheophytins. We found, however, that seeds which in various ways were demaged could contain pheophytin even in oil carefully extracted in the laboratory (L.-Å. Appelqvist and S.-Å. Johansson, unpublished data).

The total content of carotenoid pigments is about 25-50 ppm. Very little β-carotene is present, and the major components are neo-lutein A and neo-lutein B (45,46).

POLAR LIPIDS

Whereas the triacylglycerols and most of the nonsaponifiable materials are easily extracted with nonpolar solvents and thus occur in commercial rapeseed oil, this is not the case with all of the polar lipids, the phospholipids and the galactolipids, some of which are hexane soluble after seed "cooking." They form the basis of the production of rapeseed lecithin, the composition of which has been reported recently (47,48). The following appears noteworthy: (A) Phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol are the major phospholipids in rapeseed "lecithin." Compared to soybean lecithin, the most obvious difference is in the higher proportion of phosphatidyl ethanolamine; (B) The phospholipid fatty acid composition is very different from that of the triacylglycerols of the same seeds, with large amounts of palmitic and linoleic acids, little of eicosenoic and only a trace of erucic acid in the phospholipids, cf., Table I. Thus the fatty acid composition of the rapeseed phospholipids is rather similar to that of soybean phospholipids and most phospholipids of higher plants.

It should be noted that the polar lipid fraction of mature rapeseed also contains some galactolipids (49). The monogalactosyl diglyceride is characterized by considerable amounts of 16:3, hexadecatrienoic acid. Both the monogalactosyl and the digalactosyl diglyceride are rich in linolenic acid and contain little or no eicosenoic and erucic acid (L.-Å. Appelqvist, unpublished). Recently monogalactosyl and digalactosyl diglycerides have been isolated from commercial rapeseed lecithin in our laboratory.

PROTEINS

The predominant mass of the seed proteins, about 25% of the dry matter in the Cruciferous species discussed in this paper, can probably be considered as storage elements with no catalytic function. On the other hand a quantitatively small fraction is assumed to have specific functions as structural elements in different organelles, e.g., plastids or mitochondria. Finally certain proteins are associated with catalytic activity, e.g., myrosinase (correctly named thioglucoside glucohydrolase), lipase and lipoxygenase.

This section of the present paper will be a review of some work on rapeseed proteins from this more metabolic point of view. The gross amino acid composition of all the proteins of the Cruciferous seed meals and the solubility characteristics are of more direct concern to the food technologist and animal nutritionist, and are consequently discussed in detail in two other papers in this symposium (6,50).

The proteins of *Brassica campestris, B. napus, B. juncea, B. oleracea, B. nigra, B. carinata* and *Sinapis alba* were recently compared in studies using electrophoretic and serological techniques (51-53). These studies were designed to provide a better understanding of the genetic relationships among these species. Besides applying serological techniques in the development of the electrophoretic strips, the proteins separated were assayed for β-galactosidase, β-glucosidase, esterase and myrosinase. From these studies many results of taxonomic significance have emerged, e.g., the generic status of *Sinapis alba* (53).

It is emphasized that theproteins detected in seed extracts of the allotetraploid species are generally the sum of those detectable in the parental diploid species (see discussion in Reference 51). However the proportions can vary within wide ranges, thereby masking part of this additive pattern. The greater complexity of the seed protein pattern from the allotetraploid species rapeseed *Brassica napus* (containing the sum of the chromosomes of *B. oleracea* and *B. campestris*) and leaf mustard or brown

TABLE V

The Quantitative Composition of Total Sterols (Free and Esterified) in Seeds of *Brassica campestris, B. napus* and *Sinapis alba*

Sample	Sterol composition, %				
	Brassicasterol	Campesterol	Cholesterol	β-Sitosterol	Reference
Brassica campestris, cv. Golden Ball[a,b]	13.4	22.4	2.7	61.5	43
B. campestris, cv. Wallace[a,b]	19.6	25.2	0.3	53.4	43
B. napus, cv. Matador	13.0	31.9	1.7	53.4	42
B. napus, cv. Regina II	10.1	25.6	3.8	60.6	42
B. napus, summer rape[b]	16.1	27.8	Trace	52.3	43
Sinapis alba[c]	5.2	34.6	3.2	43.8	43

[a]In the original paper named *Brassica rapa*, a designation now considered less appropriate for the subspecies *Brassica campestris* var. *rapifera*.

[b]Minor amounts of other sterols also detected.

[c]Also 13.2% of 24-metylidenecholesterol.

mustard, *B. juncea* (addition of chromosomes from *B. nigra* and *B. campestris*) makes it potentially more complicated to isolate the proteins of these species for industrial utilization than would be the case with the diploid *B. campestris*, turnip rape or sarson; cf., another paper of this symposium (6).

The phosphate buffer and alkaline soluble proteins of *Brassica napus*, winter type cultivar Panter, are being studied in great detail at the Institute of Biochemistry at Uppsala, Sweden, but so far nothing of this work has been published. It has been reported, however, that about 50 bands are detected after isoelectric focusing in polyacrylamide gel of such extracts. There are 4-6 basic proteins, about 20 weakly acidic, and about 20 neutral proteins. The basic proteins were all in the molecular weight range 15,000-20,000 and accounted for ca. 20% of the soluble proteins. The major portion of the soluble proteins had molecular weights from ca. 120,000-150,000, whereas the residual 5% were in the molecular weight range of 50-75,000 (Jansson, J.C., S.-Å. Liden, B. Lonnerdal and J. Porath, unpublished).

Recent Canadian studies have demonstrated dramatic differences in the proportions of two selected proteins or protein fractions as a function of genotype and cultivation conditions. One of the proteins studied is basic with a molecular weight of about 14,000, and the other is a large, globular protein which easily dissociates into sub-units (54). The great effect of mineral nutrition on the amounts of selected proteins of rapeseed, (c.f., Nugget 1 and Nugget 2 in Table VI) is indeed noteworthy and should be compared to the generally rather small nutritional effect on rapeseed fatty acid patterns (19). As seen from Table VI, there are large variations in the content of specific proteins between cultivars of each species, e.g., Bronowski vs. Target, Echo vs. Yellow Sarson, but only minor ones between representative samples of well-nourished seeds of a single cultivar, Target 1 vs. Target 2. Whereas relatively large differences in amino acid composition of the two selected proteins were recorded between cultivars, a very small one was noted as a result of the mineral nutrition, although the amount of the basic protein (denoted A-IV-S) varied by a factor of 3.4 (see Table VI). This is expected from what is known about the strong genetic control of any "template-governed" synthesis of a macromolecule. The large differences recorded in protein patterns among cultivars and species obviously underlines the importance of working with seeds of well-defined biological status in all seed protein studies.

Studies on the proteins of *Crambe abyssinica* have demonstrated a "N-solubility vs. pH" profile entirely different from that of soybeans. A considerably higher pH (about 11) was necessary to dissolve 95% of the protein, and two solubility minima were obtained at ca. pH 4 and 8, at which about 40% of the protein was dissolved in contrast to a single minimum at pH ca. 4, where only ca. 10% is soluble in the case of soybean meal (55). The solubility profile of winter rape cultivar Panter is reported to be rather similar to that of crambe (Jansson, J.C., unpublished). These problems are further discussed in another paper given at this symposium (see Table VII in Reference 6). No systematic studies on the proteins of crambe seem to have been reported to date, but a polypeptide with prolamine-like solubility and a molecular weight of about 5,000 has been isolated from crambe seeds (56). The name "crambin" has been proposed for the compound, which has been crystallized from aqueous acetone extracts in yields of approximately 1% of the seed protein.

Catabolic enzymes of significance for the oil and meal quality are lipase, lipoxygenase, myrosinase and possibly others. Extracts with lipase and lipoxygenase activity have been detected in Cruciferous seeds but so far no detailed studies on pure lipase or lipoxygenase preparations from such seeds have been reported (57-60).

On the other hand, myrosinase has been purified from *Brassica juncea* (61) and *B. napus* (Lönnerdal, B., unpublished), as well as from *Sinapis alba* (62 and Björkman, R., unpublished). It appears that there is more than one protein in some of these seeds associated with myrosinase activity (63,64 and Lönnerdal, B., unpublished) and that the myrosinase of *Brassica napus* is different from that of *Sinapis alba* in some respects (Björkman, R., unpublished and Lönnerdal, B., unpublished). The myrosinase activity in autolysing meals and possible activity of other glucosinolate-metabolite-attacking enzymes or factors of *Crambe abyssinica* and *Brassica napus* have been studied in some detail, but so far nothing has been reported about purified myrosinase preparations from crambe (65,66).

CARBOHYDRATES

The low molecular weight carbohydrates of *Brassica napus* have been reported to be fructose, glucose, sucrose,

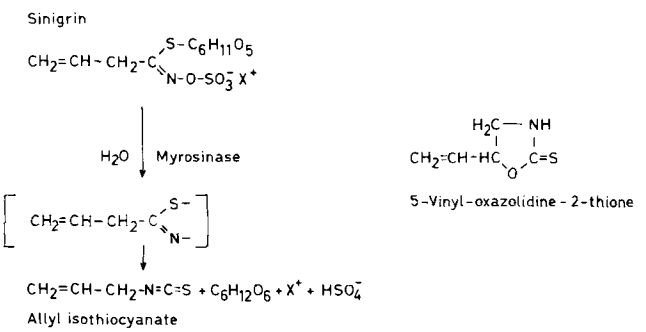

FIG. 2. The structures of allylglucosinolate or sinigrin and the cleavage products formed by myrosinase action at neutral pH, in the absence of seed meal or in the presence of specially heat-treated meals (65,70,72). The structure of 1-5-vinyl-oxazolidinethione, a major cleavage product of rape and crambe glucosinolates under the aforementioned conditions, is shown to the right.

raffinose and stachyose in levels from about 0.2% to about 2% in fat free meal (67). Only sucrose and stachyose were mentioned as constituents of the similar fraction of *Sinapis alba* (68), but this difference must not be related to interspecific variation unless verified in direct comparisons.

Very little seems to be known about the polysaccarhides of Cruciferous seeds. It has, however, been reported that the polysaccharides of white mustard are quite complex and that both qualitative and quantitative differences are obvious between the seed coat and the remainder of the seed (68). A pure homopolysaccharide, an araban, has been isolated from *Sinapis alba* extracts. No evidence was found for the presence of starch or fructans by the same authors. Among the more easily soluble materials, arabinose, galactose and galacturonic acid predominated as monomers, whereas xylose and glucose were the major constituents of the less soluble cellulosic and "hemicellulosic" fractions (68). From current Canadian studies on the polysaccharides of *Brassica campestris*, a paper reporting the isolation in small amounts of a homogeneous, highly branched, polysaccharide, so-called amyloid, appeared while this manuscript was in preparation (69).

GLUCOSINOLATES

Since this class of compounds is of great importance for the utilization of the rapeseed meal, qualitative and quantitative data on the glucosinolates are presented in three other papers in this symposium (1,6,50). In this context their general formula and a few supplementary viewpoints will be mentioned. The name glucosinolate was coined about ten years ago by a pioneering group in this field, which established the correct structure of sinigrin or allylglucosinolate (see Figure 2), a compound that had been known since 1840 but until 1956 had been associated with an erroneous structural formula (70). Alternative names in the past were "thioglucosides" and "mustard oil glucosides," of which at least the former is still being used.

The glucosinolates are digested by endogenous seed enzymes, called myrosinase or thioglucoside glucohydrolase (EC 3.2.3.1) to isothiocyanates glucose and sulfate (see Figure 2). Isothiocyanates carrying a β-OH-group cyclize spontaneously to oxazolidinethiones. This simple pattern of digestion holds true at pH 6 and above in pure "substrate-enzyme" solutions and in specially heat-treated dilute slurries of seed meals of *Brassica napus* and *Crambe abyssinica* (65,71,72). Under autolytic conditions in seed meal slurries a variety of products arises, e.g., (R)-1-cyano-2-hydroxy-3-butene and (2R)-1-cyano-2-hydroxy-3,4-epithio-butanes from *Brassica napus* (see 65 and *loc. cit.*).

It should be pointed out that these antinutritional factors occupy a significant portion of the fat free substance in these seeds: typically about 3% in *Brassica*

TABLE VI

Amounts of Protein Fractions BI and AIVS Isolated From Seeds of Different Cultivars of Rape and Turnip Rape (54)[a]

Species and cultivar	Proteins			
	BI, large globulin		AIVS, small basic protein	
	Weight, mg	% N	weight, mg	% N
Brassica campestris				
Echo	221.0	17.5	172.0	17.4
S-7165	350.0	16.5	43.6	14.0
Yellow Sarson	46.0	16.4	2.0-3.0	—
B. napus				
Target 1[b]	322.0	16.8	108.0	16.1
Target 2[b]	275.0	17.0	121.0	16.2
Bronowski	480.0	16.5	60.5	16.2
Nugget 1[c]	120.0	17.4	100.0	16.8
Nugget 2[d]	144.0	17.0	340.0	15.0

[a]Weights based on 10.0 g whole seed.

[b]Target 1 and Target 2 were sampled from experimental plots with adequate amounts of moisture and mineral fertilizer.

[c]Grown on nutrient deficient soil and supplied with N-fertilizer.

[d]Grown on nutrient deficient soil and supplied with N-, P-, K- and S-fertilizers.

campestris (73); 4-5% in the summer types of *B. napus* and slightly more, 6-7% in the winter types (73); about 5-7% in *B. juncea* (74 and E. Josefsson, unpublished); about 8-10% in *Crambe abyssinica* (72); and 9% in *Sinapis alba* (75).

MISCELLANEOUS COMPOUNDS

Under this heading two different groups of compounds will be discussed; sinapine and its derivatives as well as flavonoid polymers or tannins. The former group of substances is of interest because one of the longest known glucosinolates, sinalbin, was isolated as its sinapine salt. The levels in defatted meals are reported as about 1% in *Brassica campestris* and *B. napus* (76), 0.3-0.6% in *Crambe abyssinica* (76), and about 1.5% in *Sinapis alba* (77). It appears that at least some of the sinapine of *Crambe abyssinica* is present as glucoside; see (76) for further discussion on this group of compounds. No adverse physiological effects seem to be attributed to the ingestion of sinapine, but it has a bitter taste.

Recently studies on the tannins or condensed polyphenols of *Brassica campestris* have been published (78). Besides this report and an earlier note on color reactions for polyphenols in extracts from *B. napus* and *B. nigra* (79), little seems to have been reported on these compounds from Crucifers, although it has been suggested that the tannins may adversely effect the value of rapeseed meal or isolated protein.

DISCUSSION

It appears highly likely that the great interest presently paid to rapeseed will result in extensive compositional studies of several Crucifers, and consequently several compounds hitherto unknown as constituents of Cruciferous seeds may be reported in the near future. There is no doubt that the approximately 60% nonlipid material of Cruciferous seeds represents a greatly "under-researched" area in view of its potential value.

In conclusion, it is tempting to metaphorically apply to research on Cruciferous oil seeds what the great scientist Carl Linnaeus stated more than 200 years ago about the advantages of rapeseed cropping: "... those who become interested in this crop have no reason to regret their toil, when they in this manner can derive rich remuneration from a well cultivated soil." (80).

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