Investigation of the Phase Behavior of *Cruciferae* Seed Oils by Temperature Programmed X-Ray Diffraction

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Abstract

The phase behavior of the seed oils from different strains of *Brassica napus, Brassica campestris, Sinapis alba, Crambe abyssinica* and *Erysimum orientale* is reported. The rapeseed oils and white mustardseed oils include products from plant breeding work in Canada and Sweden. The experimental technique includes the continuous recording of X-ray diffraction patterns vs. temperatures between −50 C and complete melting of the most stable solid phases with a new DPT (Diffraction-Pattern-Temperature) camera. The crystallization and melting of oils with an erucic acid content above 8% can be described in terms of the polymorphic phases α, β_2 and β_1 (in the order of increasing stability). The designation β_2 is tentative as the phase cannot be classified unambiguously by X-ray diffraction data alone. The phase transition temperatures for a certain species form continuous functions of the erucic acid content, thus providing a basis for the prediction of some of the properties that are relevant for the utilization of the oils in the food industry.

Introduction

Species of *Cruciferae,* e.g., *B. napus* and *B. campestris,* are the subject of considerable plant breeding efforts with the purpose of reducing the content of longer fatty acids in the glycerides in order to increase the nutritional value of the seed oils and to achieve technological advantages for their utilization in the food industry (1). A knowledge of how the properties of the oils depend on the composition of fatty acids and glycerides is useful for the prediction of the range of utilization and consequently also for selection studies in plant breeding. The behavior of the oils upon crystallization and melting has a certain significance, as it is characteristic for the glyceride composition of the oils and gives direct information about the utilization possibilities at refrigeration temperatures where solid phases often occur in these oils. As a part of a continuing investigation on the phase behavior of fats and oils (2), the melting and crystallization of some *Cruciferae* seed oils with varying composition will be reported.

No investigations with X-ray diffraction methods, which enable the identification of solid phases on the basis of their crystal structure, seem to have been reported previously for oils. However, Hannewijk and Haighton (3) clearly demonstrated polymorphism in rapeseed oils and other oils with Differential Thermal Analysis (DTA). In addition the heats of fusion of two polymorphic phases of rapeseed oil have been estimated by Differential Scanning Calorimetry (4).

Nomenclature

The crystallization of triglycerides and fats in different polymorphic forms has been known for a long time but lack of agreement has prevailed about the classification of the different polymorphic forms (5,6). In the present paper the nomenclature proposed by Larsson (7), which is based on an analysis of the chain packings in terms of the arrangement of the carbon atom planes, will be used to designate different polymorphic phases: (a) a form which gives only one strong short spacing line near 4.15 A is termed α; (b) a form showing two strong short spacing lines near 4.20 and 3.80 A or three strong lines near 4.27, 3.97 and 3.71 A and which also exhibits a doublet in the 720 cm^{-1} region of the infrared absorption spectrum, is called β'; (c) a form which does not satisfy criteria a or b is called β. When two or more crystal forms of a compound receive the same name they should be distinguished by subscripts, e.g., β_1, β_2, and it is recommended that they are numbered in the order of decreasing melting points.

This nomenclature is an extension of that introduced by Lutton (8) and makes it possible to unambiguously denote new forms of at least compounds or mixtures with significant content of saturated fatty acids. As will be indicated in this paper it seems not to be as easily applicable to the description of the polymorphism of all triglycerides and liquid oils containing predominantly unsaturated fatty acids in the *cis* configuration, but it still seems to be the best nomenclature available at present time.

The expression polymorphic phase, used analogously to the expression polymorphic form in the text, indicates solid phases with homogeneous chain packing.

Experimental Procedures

The X-ray diffraction pattern as a function of temperature was determined with a so-called DPT (Diffraction-Pattern-Temperature) camera that was constructed by Abrahamsson (Abrahamsson, personal communication), and is a new construction of the DPT camera described by Stenhagen (9). A Philips X-ray generator, PW 1009, and a Philips 1 kW X-ray tube type 25623/62 with a Cu-anode were used. The radiation was filtered through a Ni foil. The film was moving in the film holder at a constant speed in front of variable slits and the sample temperature was controlled by a linear temperature programmer which can be run between −100 C and +100 C at different heating and cooling rates.

DTA curves were recorded with low temperature DTA equipment from Linseis KG, Selb, West Germany, operating between −200 C and 500 C.

The samples were obtained in the form of seeds from the Swedish Seed Association, Svalöf, Sweden, except for the sample of *B. napus,* "Canbra," which was obtained as bleached oil through the courtesy of the Department of Industry, Ottawa, Canada (Table I).

The oils were extracted from the seeds by batchwise hydraulic pressing at 100 C in a doublespindle press. The crude oils obtained were purified in laboratory equipment according to the following procedure. Desludging was carried out at 80 C by agitation with 4% water for 20 min followed by centrifugation at 8000 rpm. After drying, treatment with concentrated phosphoric acid, 0.1 ml/100 g of oil, was performed

TABLE I

Fatty Acid Composition of Oils Investigated

Samples	IV[a]	GLC, weight percentage Me esters							
		16:0	18:0	18:1	18:2	18:3	20:1	22:1	others
Brassica napus									
Summer type									
Sv. Regina II	105	3.4	1.3	13.2	17.1	9.0	10.2	45.3	0.5
64–101	105	3.2	1.1	14.2	16.2	9.2	10.4	45.3	0.4
64–124	108	4.9	1.8	27.2	16.0	10.6	16.3	22.6	0.6
64–110	112	4.1	1.8	36.3	18.6	10.4	13.8	14.7	0.3
66–3001	114	4.6	1.6	32.5	20.7	11.7	12.4	13.7	2.8
66–3002	123	4.9	1.7	32.4	25.8	14.4	8.0	9.7	3.1
64–111	113	3.8	1.9	45.5	17.9	10.6	10.6	9.7	0.0
67–154	117	4.4	1.7	47.3	21.8	11.4	5.2	6.3	1.9
67–209	119	4.1	1.4	56.6	20.5	12.2	2.8	1.1	1.3
64–131	121	4.4	1.4	55.6	23.5	11.3	2.4	1.1	0.3
64–136	121	4.2	1.6	55.3	23.9	11.2	2.6	1.0	0.2
64–127	121	4.2	1.6	55.9	24.0	11.2	2.6	.....	0.5
64–135	122	4.2	1.5	55.8	23.7	11.7	2.8	.....	0.3
Canbra	112	4.6	2.1	58.0	21.2	9.3	1.8	1.4	1.6
Brassica napus									
Winter type									
Sv. Victor	102	3.0	0.6	9.6	14.3	9.5	7.0	53.1	2.9
67–4087	111	3.7	1.6	37.6	17.4	11.4	11.2	14.4	2.7
67–4086	117	4.5	1.1	54.6	20.6	11.5	2.9	3.0	1.8
Brassica campestris									
Summer type									
Sv. Bele	110	2.2	0.7	24.1	17.8	10.6	11.8	29.7	3.1
Brassica campestris									
Winter									
Sv. Duro	102	1.8	0.7	11.8	13.7	10.2	8.0	49.0	4.8
Sinapis alba									
Sv. 0405	101	2.6	0.8	22.9	10.2	8.9	9.5	44.9	0.2
Sv. Seco	104	2.6	0.8	24.9	9.6	10.9	9.4	41.7	0.1
64–350	102	2.4	0.1	28.6	9.6	9.5	9.9	39.6	0.3
64–351	100	2.7	1.0	35.1	9.5	8.0	10.5	32.8	0.4
64–348	106	3.0	1.0	39.1	10.2	10.8	12.7	22.9	0.3
Crambe abyssinica	88	2.0	0.5	13.3	10.5	4.6	1.3	63.1	4.7
Erysimum orientale	93	2.1	0.4	6.3	24.5	2.3	21.5	31.4	11.5

[a] Calculated from the GLC results.

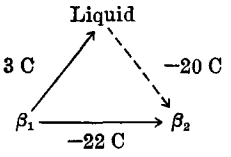
at 90 C for 5 min. The phosphoric acid and the free fatty acids were neutralized with 4 M sodium hydroxide solution at 60 C. The oils were then washed 15 times with small portions of hot water, dried and treated with 1.5% bleaching earth (Tonsil LFF80) at 90 C and 5 mm Hg for 30 min. After filtration, steam deodorization was performed at 3 mm Hg and 220 C for 2 hr. Finally 0.006% citric acid was added as a 50% solution in ethanol under vacuum. Fatty acid compositions and iodine values for the purified samples are recorded in Table I. The content of free fatty acids in all samples was below 0.1%.

Results and Discussion

DPT diagrams were recorded for the oils between room temperature and −50 C. Routinely the phase changes during slow cooling from the melted state to −50 C were followed, and then the melted samples were subjected to rapid cooling to this temperature after which the phase changes during slow heating to room temperature were followed. One such DPT heating diagram for a rapeseed oil (*B. napus*) with 53% erucic acid is reproduced in Figure 1.

It may be seen from the Figure that the diffraction lines for the solid phase formed do not undergo visible changes until the temperature reaches ca. −22 C, where a transformation takes place. The new diffraction pattern vanishes at 3 C and is replaced by the diffuse liquid scattering. The short spacings of the low melting phase are 5.20 w, 4.80 w, 4.48 s, 4.10 m, 3.80 m and 3.62 w A where the letters w, s and m designate the relative intensities of the spacings: w, weak; s, strong; and m, medium. According to the nomenclature this phase is called β_2. The high melting phase has the short spacings for the usual triclinic chain packing (5,7) with parallel chain planes with the strongest short spacing at 4.6 A and additional spacings between 4.1 and 3.7 A, and is thus designated β_1. The thermal effects that accompany the sharp phase change can be visualized in the DTA curve that corresponds to the course of Figure 1, and is shown in Figure 2. It is evident that the transformation at ca. −20 C is considerably exothermic.

On slow cooling (0.5 C/min) of this rapeseed oil, the β_2 form was formed from the melt at ca. −20 C and was not changed by further cooling. The polymorphism of this oil can be summarized as follows.



The dashed arrow designates a transformation during cooling and the lined arrows designate transformations during heating.

This polymorphism has not been found previously among pure triglycerides (5,10) and fats (6). Usually these crystallize in α forms or phases from the melt and transform later monotropically to more stable forms, often, but not always, in the order α to β' to β. It could therefore be questioned whether the β_2 phase in the above scheme is e.g., a mixture of α and β' phases or has in fact a homogeneous chain packing with parallel chain planes as presumed for β forms in the Larsson nomenclature (7). A mixed chain packing can be ruled out for several reasons. The phase lacks properties of such mixtures established in other fats and oils (2), e.g., changes in widths and intensities of spacings with temperature. Conclusive evidence against the involvement of α packing in the phase named β_2 seems to be that some samples first exhibited a weak but sharp spacing at

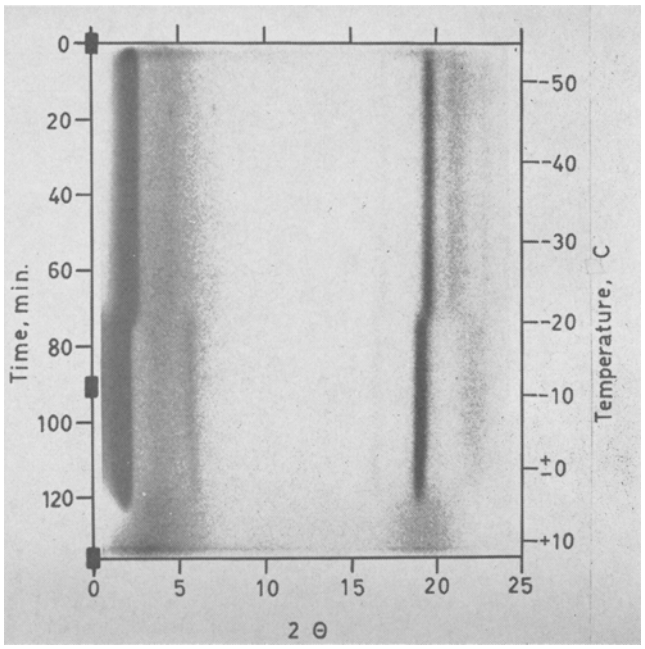


FIG. 1. DPT diagram for heating rapidly cooled rapeseed oil at 0.5 C/min.

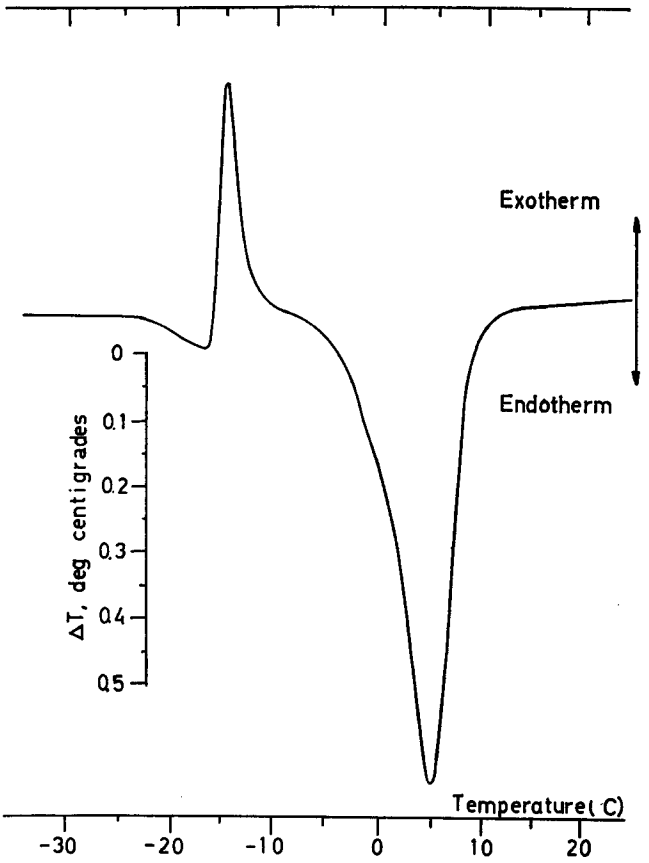
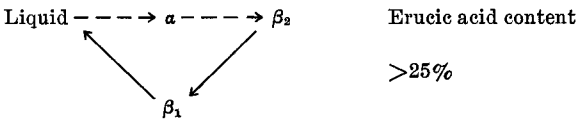


FIG. 2. DTA curve for heating rapidly cooled rapeseed oil at 2 C/min.

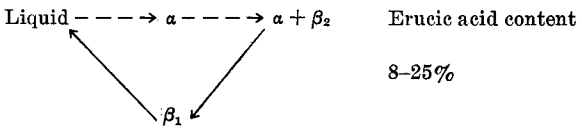
4.15 A together with a very long spacing at ca. 75 A on slow cooling of the melts, indicating the formation of α phase from the melt before β_2. It may be seen from Table II that the spacings for α are not involved in the spacings for β_2. On the other hand, mixtures of α and β_2 were identified in samples with lower erucic acid content, as will be shown below. It is, however, more uncertain that the requirement of parallel chain planes is fulfilled in the β_2 phase of *Cruciferae* oils. The doubt arises when a comparison with the very few reported data on pure *cis*-unsaturated triglycerides is made. Melting points and X-ray diffraction patterns for three polymorphic forms, α, β' and β, of triolein were reported by Ferguson and Lutton (11) and the chain packings were further analyzed by Chapman (12) with IR spectroscopy. The β' form of triolein was shown to have the stronger short spacings at 5.22 w+, 4.35 s, 4.09 w+, and 3.87 m A and two peaks at 729 cm^{-1} and 722 cm^{-1} in the IR spectrum. Although the short spacings do not fit exactly into the definition of β' forms the double peak undoubtedly indicates a chain packing with perpendicular chain planes (5). Therefore the designation β' should be correct. The β' form of trierucin was characterized by Carter and Malkin (13) by the short spacings 4.53 m, 4.28 m, 4.07 and 3.88 m. In the present work practically the same spacings for this form were determined with a sample greater than 99% pure but the relative intensities were found to be as follows: 4.50 w, 4.30 s, 4.05 m and 3.87 m. In analogy with triolein this is probably also correctly designated as β'. Comparing these data with the short spacings of the β_2 phase of the *Cruciferae* oils investigated here (Table II), it does not seem possible to decide whether the chain planes are parallel or perpendicular in the β_2 form on the basis of short spacing data alone. The question of the chain packing is left open until further evidence is available, and the designation β_2 should be regarded as tentative.

In samples with erucic acid contents above ca. 25% α phases could be detected only incidentally. It still seems probable that the crystal nuclei formed in the melt are of the α type in all samples (5) but that the phase itself cannot be observed with the methods used. Therefore the following scheme of phase transitions in *Cruciferae* oils with erucic acid content above 25% is considered more general than the one previously given.

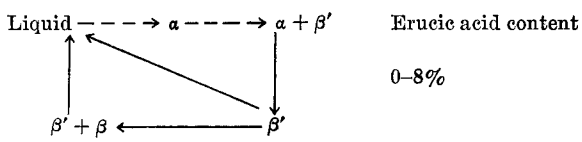


Scheme 1

Analogously, the phase transitions on cooling and heating oils with lower erucic acid contents are described in Schemes 2 and 3.



Scheme 2



Scheme 3

It appears that α phases can be identified more frequently in samples with low erucic acid content and that mixtures of α and β_2 phases were obtained when oils with 8% to 25% erucic acid were slowly cooled. The oils with low erucic acid content (Scheme 3) showed, instead of the previously discussed β_2

TABLE II

X-ray Powder Data for Polymorphic Phases of *Cruciferae* Oils

Polymorphic phase	Long spacings,[a] A	Short spacings,[a] A
α	75 w	4.15 w
β'	45 s 15 w	4.38 m 3.90 m
β_2	37 s 18.5 w 12.0 w	5.20 w 4.80 w 4.48 s 4.10 m 3.80 m 3.62 w
β_1	45 s 15 m	4.6 s 4.1–3.7 m (d)

[a] Abbreviations: s, strong; m, medium; w, weak; (d), diffused.

phase, phases with the strongest short spacings near 4.35 A and 3.90 A, the structure of which should be isomorphous with the β' form of triolein (5,11). This phase appeared in mixtures with α and β phases. The β' phases are also frequently found in other oils of commercial interest (2). It should, however, be emphasized that *Cruciferae* oils exhibit, compared to other oils, a characteristic and specific polymorphism, i.e., the three schemes described have not been found in the following oils: corn, cottonseed, peanut, safflower, soybean and sunflower (2). The explanation is found in the specific content of C_{20} and C_{22} fatty acids combined with the very high preference for the positioning of eicosenoic and erucic acids in the 1- and 3-positions of the glycerides (14). Consequently the polymorphic phases of interesterified rapeseed oil with a high content of erucic acid were found to be different from the original oil and analogous to those of trierucin. In randomized samples the content of trierucin was estimated to be 10–15%, while only traces of C_{66} triglycerides could be detected with GLC in the original oils. The X-ray data on *Cruciferae* oils are summarized in Table II. The spacings depend on the temperature of observation and should be regarded as representative averages at temperatures where the phases are stable.

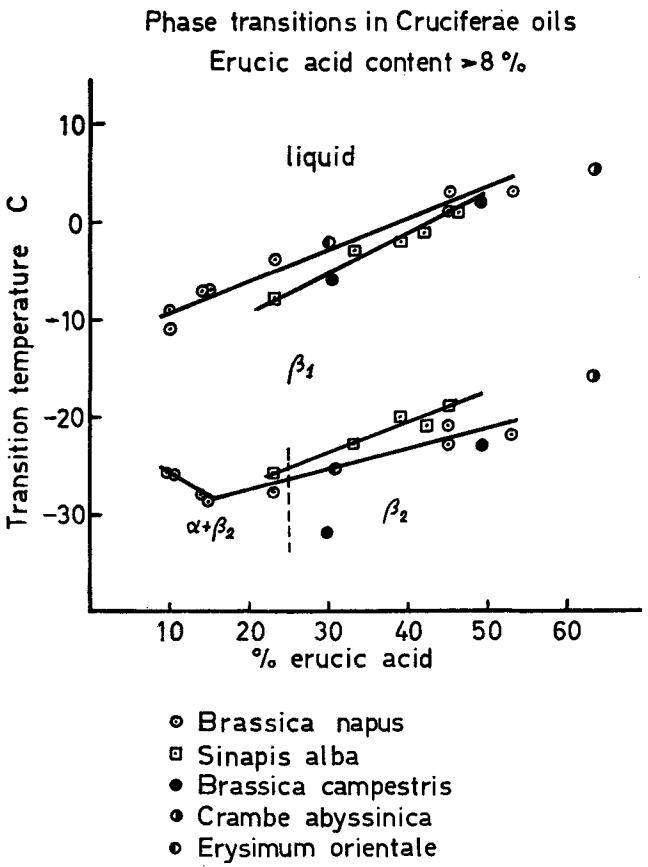


FIG. 3. Phase transitions in *Cruciferae* oils. Erucic acid content > 8%.

TABLE III

Cold Tests for Some Cultivated *Cruciferae* Oils

Samples	$C_{22:1}$ %	DTA minimum point, C	DPT β_1 melting point, C	Cold test 7 days at 0 C
Brassica napus				
Sv. Regina II	45	4,5	3	Opaque
Sv. Victor	53	6,0	3	Opaque
Brassica campestris				
Sv. Bele	30	−1,8	−6	Clear
Sv. Duro	49	5,6	2	Opaque
Sinapis alba				
Sv. Seco	42	1,5	−1	Clear

The transition temperatures between different phases were fairly independent of the rate of cooling and heating for oils with $C_{22:1}$ (erucic acid) > 8%, i.e., those that follow Schemes 1 and 2. For these the transition temperatures recorded on the DPT films for β_2 to β_1, $\alpha + \beta_2$ to β_1 and β_1 to liquid are shown in Figure 3 as a function of the erucic acid percentage. The points for rapeseed oils and white mustardseed oils have been linked together.

The deviation of the observations from the lines can be explained by some irregularities in the composition of fatty acids other than $C_{22:1}$, mainly in the relative amounts of oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids (Table I). The difference between the transition temperatures of *B. napus* and *S. alba* is, however, significant, which was also shown by the DTA work. The fact that the curve for the melting of the β_1 phases is lower for *S. alba* than for *B. napus* can however not be explained by the composition of the remainder of the fatty acids composing the glycerides. At constant $C_{22:1}$ content the *S. alba* oils contain more $C_{18:1}$ and less $C_{18:2}$ than the oils from *B. napus* (Table I), which should give higher melting temperatures for the former oils. The explanation must instead be sought in the fatty acid positioning in the triglycerides, and in fact a greater preference of $C_{18:2}$ and $C_{18:3}$ for the 2-position in rapeseed oil than in mustardseed oil has been reported (15). Consequently the triglyceride distribution should be more homogeneous in the rapeseed oils, which can provide an explanation for their higher melting temperatures at the same erucic acid content. The fact that the points for the other species fit in fairly well on the lines indicates that greater differences in fatty acid positioning are not to be expected between the different oils examined. For the oils with lower than 8% contents of erucic acid, showing the phase behavior of Scheme 3, the transition temperatures are less definite. The final melting temperature is in all observed cases equal to or less than −10 C.

The connection between these results and the practical evaluation of the precipitation of crystalline material under refrigerated storage of oils is shown in Table III for some seed oils that are cultivated in Sweden. In this Table the erucic acid content is given together with the temperature for the minimum point in the DTA curve (Fig. 2) that is caused by the melting of the β_1 phase. Column 3 of Table III gives the β_1 melting point, determined from the DPT films. The summer form of *B. campestris*, Sv. Bele, and the *S. alba*, Sv. Seco, remain clear at 0 C for 7 days or longer while the others crystallize to a marked extent. The β_1 melting points determined with the DPT technique are in good agreement with the cold test results while the DTA minimum points depend more on experimental conditions. From the dependence of the β_1 melting point on the erucic acid content

(Fig. 3) it can be concluded that the requirement for a clear oil at 0 C is, $C_{22:1} \leq 39\%$ for *B. napus* and $C_{22:1} \leq 43\%$ for *S. alba*.

The selection of species for cultivation as well as the evaluation of oils for different uses, including hydrogenation, can thus to a large extent be made on the basis of the erucic acid content alone as far as the phase behavior is concerned.

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