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Variation in the fatty acid composition of developing seeds of rapeseed

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Summary. A study was made of the variation in the fatty acid composition of the oil in the developing seed of the 'Rafal' cultivar of the *Brassica napus* L. cultivated in central Italy. The oil content reaches its maximum level 60 days after the petals fall. The increase in the percentage of oleic acid is negatively correlated with the palmitic, stearic and linoleic acid content.

The seed of the rapeseed provides a quantity of oil ranging from 40 to 53% of dry matter, depending on the cultivar, and on the region, year and soil in which it is grown[2].

The characteristics of this oil are that it contains eicosenoic and erucic acids which are typical fatty acids of the Cruciferae family to which the rapeseed belongs. This seed is also rich in other fatty acids, namely oleic, linoleic and linolenic acids. In the past, use of the rapeseed in various oleiferous food products was limited because of its high erucic acid content, which sometimes accounts for up to 53% of the total acids[3].

Over the last few years, by means of plant breeding, it has been possible to develop rapeseed cultivars with an extremely low level of eicosenoic acid and which are free from, or contain only a very small amount of erucic acid with, at the same time, a higher oleic acid content[4,5]. Currently there are many varieties of rapeseed with a low erucic acid content which have been grown in very different environments from that of Italy (Canada, Sweden, Germany, France, etc.). Many problems have still to be solved in relation to the varieties, cultivation areas and choice of agronomic techniques.

The scope of this paper is to investigate variations in the fatty acid content, from fertilization to maturity, of the seeds of a cultivar with a zero erucic acid content, cultivated in central Italy. Cultivar Rafal was preferred as it is considered to be well suited to this area[6].

Plant material. Seeds of the 'Rafal' cultivar of the *Brassica napus* L. ssp. *oleifera* DC., winter rape, were sown on October 5 in an open field at S. Apollinare, Perugia (lat. 43 °08′ N; 300 m above sea level). Average temperatures in the coldest month, on flowering and at maturity were 4.4, 8.5 and 18 °C respectively.

Flowering commenced 180 days (April 5) after sowing. Petal fall was taken as a sign that fertilization had taken place. The first samples of developing seeds were taken 10 days after petal fall (APF). Further samples were taken at intervals of 10 days up to the 70th day APF.

Determination of fatty acid. The developing seeds were removed from the plants and extracted with ethyl ether in a Soxhlet extractor and subsequent preparation of fatty acid methyl esters was carried out according to Appelqvist[7]. These samples were analyzed in a Perkin-Elmer 3920 B chromatograph fitted with a hydrogen flame ionization detector and equipped with a 200-cm column packed with 15% w/w diethylene glycol succinate (DEGS) coated on Chromosorb W/HP. The nitrogen flow rate was 20 ml/min and the injector, column and detector temperatures were 250, 200 and 220 °C respectively.

Quantitative estimation of proportions of individual fatty acids was carried out by the integrator Sigma 10 Chromatography Data Station. Determination of absolute amounts of fatty acid was achieved by the use of methyl heptadecanoate as internal standard. The identification of fatty acids was based on the comparison of retention times with those of known reference mixtures.

Samples of other seeds at a similar stage of maturity were taken, for determination of dry weight by oven-drying at 105 °C.

Results. The variation in the percentages of dry weight and oil content during seed development is shown in the figure. The dry weight drops during the first 30 days APF and then

Table 1. Variation in the fatty acid composition of developing seeds of the Rafal cultivar of *Brassica napus* L.

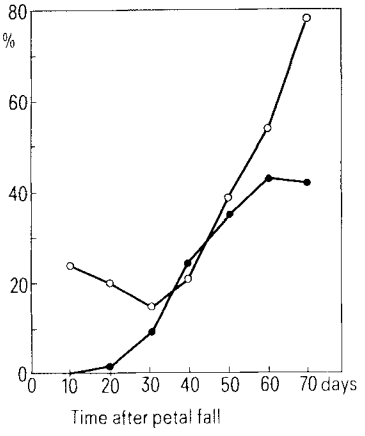
Time after petal fall, days	Fatty acid composition, % 16:0	16:1	18:0	18:1	18:2	18:3	20:1	20:2	22:0	22:1	Other acids
20	11.7	0.6	3.7	40.6	26.2	9.1	0.1	5.0	0.8	0.2	2.0
30	12.7	0.8	3.2	45.6	21.2	11.5	0.2	2.2	0.2		2.4
40	9.1	0.8	3.1	58.3	18.4	7.4	1.2	0.5	0.4		0.8
50	6.1	0.5	1.8	61.5	19.5	8.5	1.2	0.1	0.4		0.4
60	5.5	0.5	1.5	62.0	19.5	8.7	1.2	0.2	0.3		0.6
70	5.7	0.4	1.6	64.0	17.9	8.3	1.2		0.3		0.6

increases linearly up to 70 days APF, reaching 78% dry matter.

Oil was not detected in the first samples taken 10 days APF. At 20 days APF the oil content accounted for approximately 1.5% of the total weight of the seed; subsequently the oil content rose rapidly owing to the synthesis of the triglycerides, which reaches its maximum level 60 days APF and shows a tendency to decrease once the seed has fully developed.

The fatty acid composition of the Rafal cultivar of the developing rapeseed is illustrated in table 1. The percentage of oleic and eicosenoic acids increases during seed development. The oleic acid varies from 40.6% 20 days APF to 64.0% 70 days APF, whereas the eicosenoic acid increases from 0.1% to 1.2%. The palmitic, palmitoleic, stearic, linoleic and linolenic acid content decreases progressively during development. Traces of erucic acid are only found 20 days APF; the 5% of eicosadienoic acid found 20 days APF decreases linearly, and was no long detected in mature seeds.

Table 2 gives the values of the correlation coefficients[8] of the fatty acids determined while the seed was maturing. It is interesting to note the negative correlation, significant at the 99% level, of the oleic acid content with the palmitic, stearic and linoleic acids. The correlation between the eicosenoic and palmitic acids was equally negative and highly significant, whereas the correlation between the eicosenoic and stearic acids and between the linoleic and eicosenoic acids was significant at the 95% level. The coefficient correlation is positive and highly significant between the oleic and eicosenoic acids and between the palmitic and stearic acids; positive but significant at the 95% level between the stearic and palmitoleic acids and between the linoleic and behenic acids. No correlation of any significance was noted for the other acids.



Variation in the percentage of dry weight (○) and the percentage of the oil content (●) in relation to the dry matter during the development of the seed of the rapeseed cultivar Rafal.

Table 2. Correlation coefficients between fatty acid

Oleic/palmitic	− 0.932**
Oleic/stearic	− 0.886**
Oleic/linoleic	− 0.891**
Oleic/linolenic	− 0.615 n.s.
Oleic/palmitoleic	− 0.534 n.s.
Oleic/eicosenoic	0.977**
Oleic/behenic	− 0.534 n.s.
Palmitic/stearic	0.938**
Palmitic/linoleic	0.668 n.s.
Palmitic/linolenic	0.629 n.s.
Palmitic/palmitoleic	0.585 n.s.
Palmitic/eicosenoic	− 0.899**
Palmitic/behenic	− 0.036 n.s.
Stearic/linoleic	0.694 n.s.
Stearic/linolenic	0.613 n.s.
Stearic/palmitoleic	0.761*
Stearic/eicosenoic	− 0.790*
Stearic/behenic	0.157 n.s.
Linoleic/linolenic	0.393 n.s.
Linoleic/palmitoleic	0.173 n.s.
Linoleic/eicosenoic	− 0.857*
Linoleic/behenic	0.785*
Linolenic/palmitoleic	0.345 n.s.
Linolenic/eicosenoic	− 0.740 n.s.
Linolenic/behenic	− 0.363 n.s.
Palmitoleic/eicosenoic	− 0.440 n.s.
Palmitoleic/behenic	− 0.400 n.s.
Eicosenoic/behenic	0.123 n.s.

** Significant correlation at the 1% level; * significant at the 5% level; n.s., no significant correlation.

Discussion. The data collected in relation to the accumulation of oil during the development of the seed of a cultivar of the rapeseed free from erucic acid and cultivated in central Italy, are consistent with the findings of other researchers in Canada[9] and in Europe[10,11]. The total quantity of the oil level, 42% in relation to dry matter, is equal to that reported by Morice and Chone[11], which confirms the potential interest of using the Rafal cultivar in the area under examination[6]. The oil content is at its maximum level 60 days after fertilization, when the moisture level reaches approximately 50%, as noted by Rollier[12]. During the last 10 days there was a slight drop in the oil content which is characteristic of the winter cultivars[2].

The composition of the fatty acids which the plant has accumulated during maturation is in agreement with the data reported by Downey et al.[13] and by Morice and Chone[11]. The total absence of erucic acid and the low eicosenoic acid content gives rise to a high content of oleic acid (64.0%) and linoleic acid (17.9%) which is responsible for making this oil of a sufficiently good quality suitable for nutritional purposes[14]. In particular the oleic acid was the main constituent affecting the composition of the oil. The oil content increased substantially during maturation, as opposed to the palmitic, stearic and linoleic acids, as already observed by Woyke[15].

The Rafal cultivar, because of its high oil content and suitable fatty acid composition is considered to be the most suitable for breeding programs to combine the absence of erucic acid with the desirable agronomic characteristics of the rapeseed. This cultivar would be suitable as a cultivated crop for dry land in central Italy.

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