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# Characterization of the morphological changes and fatty acid profile of developing *Camelina sativa* seeds

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ABSTRACT

Camelina is an annual crop that produces seeds containing up to 40% oil. Although Camelina oil is a natural source of ω-3 and ω-6 fatty acids (found mainly as linoleic and linolenic acids), representing about 50% of their total fatty acid content, it is mostly used for non-food applications. To better understand *Camelina* oil synthesis, we analyzed the fatty acid content and composition of developing *Camelina* seeds. In the early stages of seed development (up to 18 days after flowering; DAF) the fatty acid profile differed to that of mature seeds, with high levels of palmitic, stearic and oleic acids. During the oil accumulation period (18–24 DAF) the fatty acid composition progressively changed to resemble that of the final developmental stages (from 24 DAF onwards), at which the oil content of the seeds increased due to desiccation. Analysis of the oil from 40 different accessions of *Camelina sativa* seeds grown in Córdoba (Spain) revealed an average content of: 7.0% palmitic acid, 2.5% stearic acid, 6.9% oleic acid, 14.5% linoleic acid, 41.0% linolenic acid, 10.9% gondoic acid, and 3.5% erucic acid. From these accessions we identified that with the most relevant characteristics for industrial applications, those with: the lowest PUFA/MUFA index (1.40, CAS-CS16); the lowest C20-C24/C16-C18 ratio (0.25, CAS-CS13); and the highest oil content (41.7% of CAS-CS38).



## 1. Introduction

In recent years several lines of research have sought to further our knowledge of lipid biochemistry in oilseed crops. This involves understanding the processes that regulate the amount and composition of stored oil, and determining the specific potential of genetically engineered oil crops (Battey et al., 1989). *Camelina sativa* is a member of the *Brassicaceae* family (Al-Shehbaz, 1987), also known as gold-of-pleasure or false flax, that may play an important role in oilseed biotechnology in the coming years. This oil crop accumulates high levels of oil in its seeds, it can be grown in marginal lands and it is easily transformed by floral dipping with *Agrobacterium tumefaciens*. *Camelina* has been cultivated in Europe since the Bronze Age (Bouby, 1998) and it was extensively grown in Europe in the nineteenth century, although it fell into disfavor when more productive crops were developed, such as wheat and rapeseed, with its production gradually declining and almost vanishing by the mid 20th century.

*C. sativa* is a small bushy plant that begins to grow as a rosette of leaves, from which a stem with numerous leaves and branches subsequently emerges. The florescence is a cluster of largely autogamous, cruciferous, pale yellow flowers of about 5–7 mm in diameter (Zubr, 1997). The small (4–5 mm) pear-shaped capsule has a tip that is 2–3 mm long and that contains about 15 oval-shaped yellow seeds that turn dark-brown during maturation. The development of *Brassicaceae* seeds can be divided into three distinct phases (Gurr, 1980): the intial "growth phase" that immediately follow fecundation and that involves rapid cell division but little deposition of storage material; a second "accumulation phase" in which storage oil and protein are synthesized rapidly; and the final "desiccation phase" that is characterized by dehydration and little synthetic activity (Appelqvist, 1975).

Many studies of *Camelina* have highlighted its potential as an promising oilseed crop for sustainable agriculture systems (Putnam et al., 1993). *Camelina* is a low-input oilseed crop with very high nutrient efficiency that can grow with limited nitrogen fertilization. *Camelina* produces antimicrobial phytoalexins that confer resistance to plant pathogens and insect pests, and it is an allelopathic crop, producing and releasing into the environment secondary metabolites that inhibit the development of neighboring plants

*Abbreviations:* ROA, rate of oil accumulation; DAF, days after flowering; LCSFA, long saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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(Lovett and Jackson, 1980). This crop is particularly competitive in semi-arid regions and in relatively infertile or saline soils, and it is extremely resistant to adverse environmental conditions (*e.g.*, drought), probably due to the polyploid nature of its genome. Moreover, *Camelina* has a rather short vegetative period of about 4 months, and thus, it can be incorporated into double cropping systems during cool periods of growth. Together, these agronomic features makes *Camelina* an ideal crop for the industrial production of biodiesel and indeed, the methylation proccess required for biodiesel production from *Camelina* oil is identical to that used for rapeseed oil (Fröhlich and Rice, 2005).

In the present study, we performed a morphological and chemical analyses of developing *Camelina* seeds from the CAS-CS0 variant, characterizing the oil content and fatty acid variation of seeds and vegetative tissues. Moreover, a population of 40 accessions of *Camelina* grown at the same location was studied to identify new varieties with potential applications in the oleochemical industry. Based on these findings, we discuss the potential of *Camelina* oil as a raw material for industrial applications.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

*C. sativa* of the CAS-CS0 cultivar used in the characterization study was cultivated in growth chambers at 25/15 °C (day/night) with a 16-h photoperiod and a light intensity of 250 $\mu E\, m^{-2}\, s^{-1}$. Samples of cotyledon, hypocotyl, stem, root, and leaves were taken up to 10 days after sowing (DAS). Seeds for analysis were harvested from 6 to 50 DAF, kept at −80 °C and processed. The 40 accessions of *C. sativa* studied for phenotype selection were grown in the field in Cordoba, Spain. For each experiment vegetative tissues and seed samples from at least three different plants were analyzed.

### 2.2. Determination of oil and water content

Lipids were extracted from *Camelina* seeds and vegetative tissues using a modified version of the method described by Hara and Radin (1978). Approximately, 30–60 mg of seeds at different phases of development or vegetative tissues were triturated in 3.0 ml of hexane/2-propanol 3:2 (v/v) using a glass homogenizer. A volume of 1.5 ml of 6.7% (w/w) $Na_2SO_4$ was then added to the homogenate and the phases were separated by centrifugation at 2000 rpm for 5 min. The upper organic phase was transferred to a fresh tared tube, while the resulting aqueous phase was extracted with a volume of 1.5 ml hexane/2-propanol 7:2 (v/v) and the phases were again separated by centrifugation under the same conditions. The organic phases were combined, the solvent was removed and the extract was dried under a flow of $N_2$ gas. The lipid residue obtained was weighed and the water content of the *Camelina* seeds was determined as the weight difference of approximately 30 *Camelina* seeds before and after desiccation at 80 °C for 48 h.

### 2.3. Protein determination

Protein content was determined by elemental analysis of 5–10 dry *Camelina* seeds using a LECO CHNS-932 analyzer (St. Joseph, MI). The total protein content was calculated as the percentage of nitrogen multiplied by a factor of 6.25 (Bulletin of the International Dairy Federation, 2006).

### 2.4. Fatty acid determination

Lipids from developing seeds and vegetative tissues were quantified by converting their fatty acids to the corresponding fatty acid methyl esters (FAMEs), which were subsequently analyzed by gas chromatography. For the transmethylation reaction, total lipid extracts from *Camelina* seeds were evaporated under $N_2$ after the addition of heptadecanoic acid (17:0) as an internal standard, and the resulting residues were incubated at 80 °C for 1 h after dissolving them in 1 ml of 1.25 M HCl in methanol (Sigma). Once cooled, the FAMEs were extracted with 1 ml of heptane and analyzed on a Hewlett-Packard 5890A gas Chromatography apparatus (Palo Alto, CA) with a fused silica (Bellefonte, PA) Supelco SP2380 capillary column (30 m length; 0.32 mm i.d.; 0.20 mm film thickness) with hydrogen flame ionization detection (FID). Hydrogen was used as carrier gas at a linear gas rate of 28 cm/s. The injector and detector temperatures were 220 °C, the oven temperature 170 °C, and the injection split ratio was 1:50. Fatty acids were identified by comparison of their retention times with those of known standards.

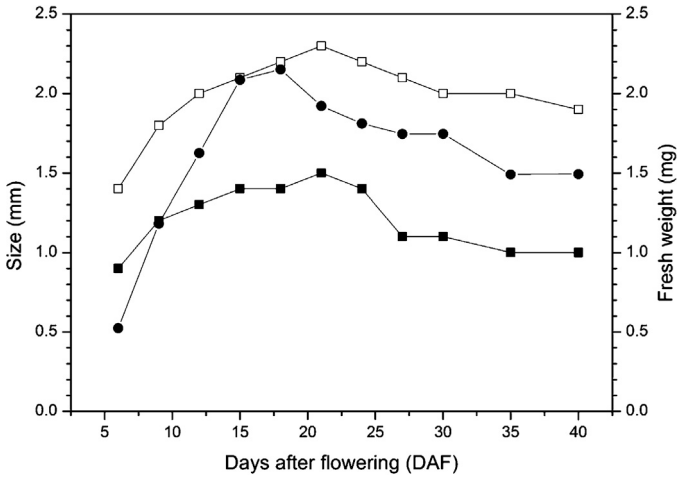


**Fig. 1.** Size and fresh weight of *Camelina sativa* seeds during development: length (□), width (■) and fresh weight (●).

## 3. Results and discussion

### 3.1. Morphological changes during C. sativa seed development

The CAS-CS0 *C. sativa* line was studied in all experiments, a line that is used commercially in Spain and is part of the oilseed collection at the Instituto de la Grasa in Seville, Spain. *Camelina* seeds varied in size from 1.4 mm × 0.9 mm to 1.9 mm × 1.0 mm (length × width) during development (Fig. 1). This variation was not linear as most seeds grew mainly during the first developmental stage (up to 12 DAF), after which seed size stabilized. A similar pattern was also observed for fresh weight, which ranged from 0.52 to 1.49 mg/seed and that peaked at about 20 DAF. From fertilization to 12 DAF, the growth phase of seed development, the maternal tissue surrounding the ovary progressively increased in size (Fig. 2, panel 2, A–C).

The morphological changes that take place in the *Camelina* siliques, seeds and embryos were studied (Fig. 2). The siliques of *Camelina* remained green during the first half of seed development, from fecundation to 24 DAF (Fig. 2, panel 1), rapidly turning brown upon reaching their maximum size. At 6 DAF, the nucleate endosperm was enclosed in a thick inner and a thin transparent outer integument, which subsequently turned green (9 DAF) due to the development of the photosynthetic machinery in this organ. The embryo also became visible at this point, displaying a short radicle and two small cotyledons (Fig. 2, panel 3), the size of these cotyledons increasing rapidly between 12 and 18 DAF (Fig. 2, panel 2). At 12 DAF, the fresh weight and volume of the developing seed was comparable to that of mature seed, although these values varied slightly during later stages of development.