Seed Protein in *Camelina sativa* (L.) Crantz var. Calena

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Authors' contributions

This work was carried out in collaboration between both authors. The authors of this manuscript worked together to design, conduct, analyze and interpret the findings of this experiment. Both authors read and approved the final manuscript.

ABSTRACT

Camelina sativa (L.) Crantz is an oilseed crop used for biofuel production. By-products from oil extraction are high in protein (about 35%) and can be used for animal feed. The aim of this study was to characterize the protein fraction of camelina meal. The protein fraction of camelina meal is composed by 60% of globulins. The amino acid profile showed an interesting content of sulfur amino acids, but it was rather deficient in lysine. Seed storage proteins were mainly accumulated between 14 and 42 days after pollination, indicating that, at maturity, the accumulation of protein is already finished. SDS-Page separation of meal protein during the development of the seed showed that the 12S globulin is the principal storage protein.

1. INTRODUCTION

Camelina sativa (L.) Crantz (known also as Gold of pleasure) has been grown in Europe for centuries and, in the Iron and Bronze ages, was an important agricultural crop. Camelina is an oilseed crop which has several agrotechnical benefits: cultivation of the crop is simple and environmentally friendly, application of pesticides/herbicides is not needed, a short

growing season (85–100 days), the plant is adaptable to marginal soils and needs few water [1-4]. Camelina oil is currently very much appreciated for the production of jetfuels (bio-kerosin) [5]. In Europe, the ITAKA project (Initiative Towards sustAinable Kerosene for Aviation) is expected to support the development of aviation biofuels in an economically, socially, and environmentally sustainable manner [6].

The exploitation of by-products after oil extraction is a key factor for the production of jetfuel from camelina and this could reduce costs and promote environmental sustainability. The evaluation of camelina meal as a potential ingredient in livestock rations is a critical factor to further increase the economic value of the plant. The meal (by-product of oil production) is a good source of protein (30-40% depending on the variety) for animal feed [7]. In Europe, the total protein crop production currently occupies only 3% of the european arable land [8]. Therefore, growing camelina in Europe could be a new source of protein for the feed industry which could reduce the import of other protein sources (mainly soybean from South America) [9]. The aim of the present study was to give information on camelina seed protein and the kinetics of their accumulation. This latter aspect may be important for deciding the period of seed collection.

2. MATERIALS AND METHODS

2.1 Meal Preparation, Protein Extraction and Assay

Seeds of *Camelina sativa* (L.) Crantz var. Calena were used. Plants were grown near Casazza (45°45′N - 9°54′E; 450 m AMSL; Lombardia, Italy) in spring 2010. Immature seeds were collected at different stages of development (every 7 days) from 7 days after pollination (DAP) until maturity (49 DAP). The seeds were collected from plants with a stage of development similar. Three different samples were performed.

Camelina seeds or immature seeds were ground in a mortar and mixed with hexane (1:10, w/v). The solution was vigorously shaken for 30 minutes. After centrifugation, the upper-liquid was collected and the extraction procedure repeated. The dried flour was used for protein extraction. Protein was extracted using a Plant Total Protein Extraction Kit (Sigma-Aldrich, St. Louis, USA). Protein content was determined via a Quantum Protein Kit (Euroclone, Milan, Italy), using Bovine Serum Albumin (BSA) as standard.

2.2 Amino Acid Analysis

50 µL of protein extract (approximately 2.5 mg of protein) was subjected to acid hydrolysis (3 different hydrolysis) in 3 mol L^{-1} mercaptoethanesulfonic acid containing 2 g kg^{-1} Na-azide at 110°C for 16 h. Hydrolyzed samples were then diluted 10 times with water before amino acid analysis.

Amino acid composition was determined by HPLC analysis of o-phthaldialdehyde (OPA) derivatives according to Reggiani et al. [10]. The separation of OPA derivatives was performed at a flow rate of 0.8 mL min^{-1} on a 150 × 4.6 mm Water Spherisorb ODS-2 3 µm reverse-phase column (Grace Davison Discovery Sciences, Sedriano, Italy). Two mobile phases were used: (A) 100 mmol L^{-1} Na-acetate (pH 7.13)–tetrahydrofuran (99.5:0.5, v/v); (B) methanol. Phase B was maintained at 5% (v/v) for 2 min, increased linearly to 50% (v/v) over 37 min, increased linearly to 100% (v/v) over 3 min, and then returned to 5% (v/v) for 2 min to regenerate the system. Proline was assayed in hydrolyzed samples by an acid ninhydrin method [11].

2.3 Differential Protein Extraction and SDS-Page

Differential protein extraction was according to Osborne and Mendel [12] and further modified by Moureaux and Landry [13]. Separation of polypeptides from 3 independent extractions by 12% SDS-Page was according to Laemmli [14]. As molecular weight marker was used Full Range Rainbow Recombinant Protein Molecular Weight Marker (GE Healthcare UK Limited, UK).

3. RESULTS AND DISCUSSION

3.1 Osborne Protein Fractionation and Amino Acid Profile

The distribution of protein fractions in mature seeds according to the Osborne method modified by Moureaux and Landry [13] is shown in Fig. 1. The globulin fraction is the most abundant representing 60% of total protein. The rest was made up of albumin (30.3%) and, in small part, by glutelin (9.7%). The content of globulins in camelina results higher than that observed in "canola meals (37-44%) [15]".
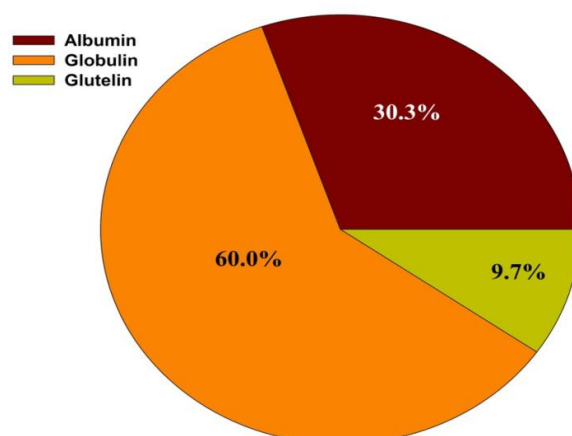
Fig. 1. Protein fractionation according to osborne protocol modified by moureaux and landry [1968]

Protein amino acid composition of meal is shown in Table 1. The amino acid profile of camelina meal, when compared with soybean meal (main ingredient of feed in Europe), had a higher content of sulfur amino acids. Camelina protein contained 4.39% of sulfur amino acids compared to just 2.76% in soybean [16]. Feedstuffs rich in methionine are in demand for those industries that require methionine additive analogues to ensure animal growth. In fact, methionine is clearly recognized as first limiting amino acid in poultry, high-yielding cows and third limiting amino acid in pigs [17]. Instead, camelina meal is deficient in lysine. Therefore, for the use as animal feed, camelina meal has to be mixed with meal rich in this amino acid (6.67% in soy) or free lysine should be added to the diet.

3.2 Accumulation of Storage Proteins during Seed Development

Seed storage proteins are biological reserves of amino acids of the plants and are progressively accumulated during the development of the seed. In Fig. 2 is reported the protein content of camelina meal obtained at different stages after pollination. As can be seen, the concentration of protein gradually increased from 7 to 42 DAP and after 6 weeks stopped. The rate of deposition of protein resulted quite linear between 21 and 42 DAP (Fig. 2).

In Fig. 3 is shown the SDS-Page profile of proteins at different stages of seed development. As evident from the gel, storage proteins became evident especially from 14 DAP forward. Since there is a close relationship between camelina and *Arabidopsis thaliana* [18], the protein profile

by SDS-Page resulted very similar (especially for subunits of 12S globulin) [18,19]. Coomassie staining indicated that the predominant polypeptides were in 29-33 and 19-21 kDa ranges (Fig. 3). In *Arabidopsis thaliana*, polypeptides of 29.2 and 34.7 kDa correspond to α subunits while those of 20.9 and 21.2 to β subunits of cruciferin (globulin of *Arabidopsis*) [20]. Other polypeptides of 14 and 70 kDa increased with the development of the seed. The polypeptide lighter could match the 2S albumin [19]. The SDS-Page profile confirms that the globulin fraction is predominant as also observed in Fig. 1.

Table 1. Amino acid profile of protein from meal of *Camelina sativa* var. Calena

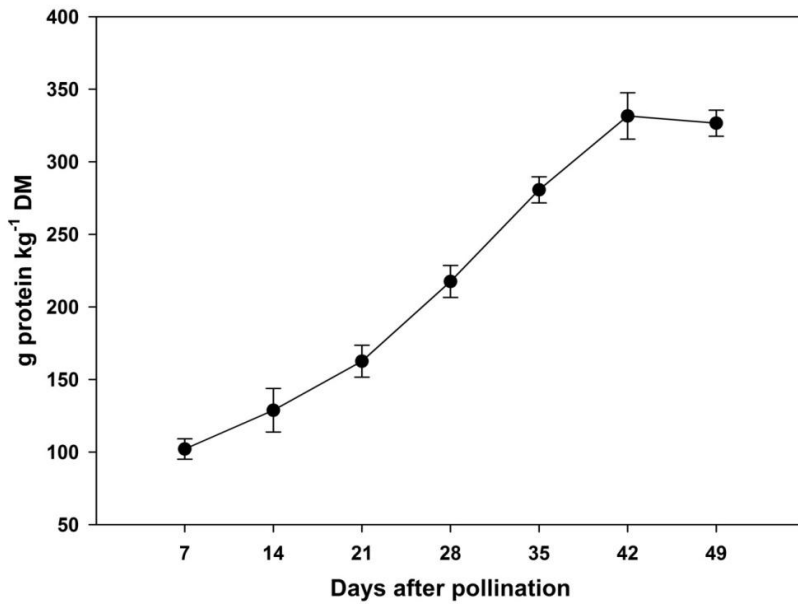| Amino acid	g 100 kg^{-1} protein ± SEM
Glutamate	14.98±0.87
Aspartate	9.03±0.47
Arginine	8.27±0.47
Leucine	6.91±0.28
Glycine	6.07±0.68
Valine	6.04±0.45
Proline	6.02±0.54
Alanine	5.98±0.49
Serine	5.96±0.52
Phenylalanine	5.21±0.38
Isoleucine	4.64±0.24
Lysine	4.49±0.47
Histidine	4.21±0.26
Tyrosine	3.64±0.33
Threonine	2.88±0.16
Methionine	2.48±0.21
Cystine	1.91±0.24
Tryptophan	1.28±0.09

Fig. 2. Protein accumulation in meal during the development of *Camelina sativa* seed var. Calena. Data are expressed on a dry matter (DM) basis
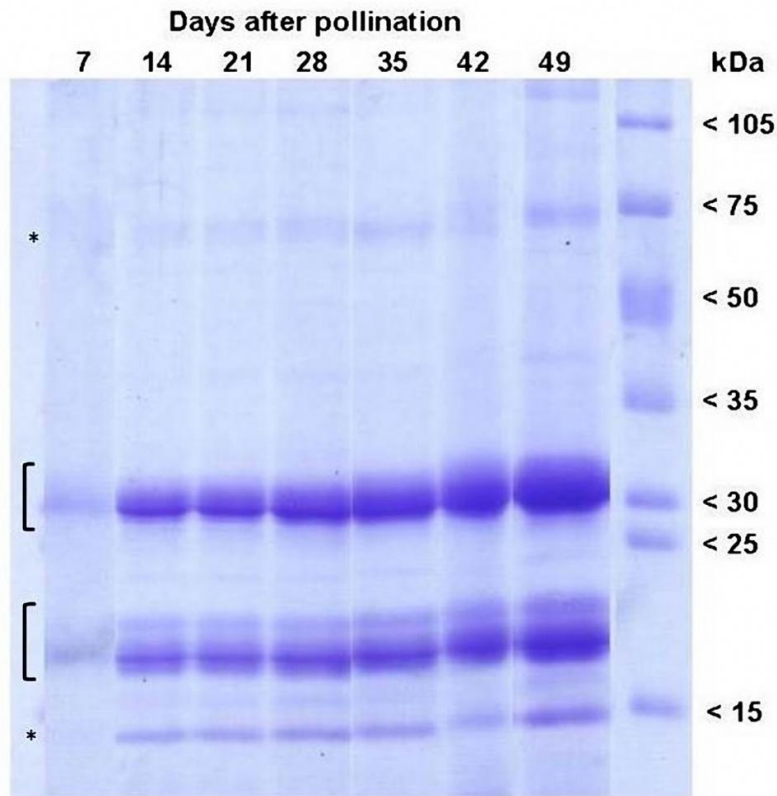

Fig. 3. SDS-page protein profile at different stages of development of *Camelina sativa* meal var. Calena

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4. CONCLUSION

Camelina meal has potential for use in animal rations both as a high-protein source and for the short cycle of the plant. The accumulation of proteins was complete at 42 DAP, then the collection of the seed can be carried out after 6 weeks of flowering, thus avoiding dehiscence observed in camelina. CS protein may represent an important ingredient to complement the content of sulfur amino acids in feed instead of legumes. However, implementation of breeding programs for increasing lysine content is recommend. Otherwise, it might be searched seed polypeptides (other than 12S) richer in lysine.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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