Camelina [Camelina sativa (L.) Crantz] seeds as a multi-purpose feedstock for bio-based applications

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Barbara Alberghini[a], Federica Zanetti[a]*, Massimiliano Corso[b], Stéphanie Boutet[b], Loïc Lepiniec[b], Angela Vecchi[a], Andrea Monti[a]

[a] Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum – Università di Bologna, Bologna, Italy

[b] Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000, Versailles, France.

*Corresponding author: federica.zanetti5@unibo.it

Abstract

Camelina [*Camelina sativa* (L.) Crantz] is an oilseed crop belonging to the *Brassicaceae* family that has attracted worldwide attention because of its agronomic and qualitative characteristics. This crop can adapt well to different environments and produce oil suitable for multiple bio-based uses. The most commonly measured and reported components of camelina seeds are fatty acids, proteins, and vitamins. However, they also contain specialized metabolites (SMs, formerly known as "secondary metabolites") retained in the meal, which have not been fully characterized. This work presents a long-term study conducted from 2015 to 2019 at the experimental farm of the University of Bologna (Italy), aimed at comparing six camelina cultivars (Cypress, Midas, 789-02, Pearl, Omega, and WUR) for their agronomic and oil-compositional parameters and the SM content and composition of their seeds. Cypress was the best genotype in terms of agronomic characteristics, i.e., stable and high seed yields and increased 1000-seed weight (TKW). Pearl and 789-02 were identified as the most suitable for specific bio-based applications because of the increased n-3:n-6 ratio of the oil. Among the SM classes, PAs, and flavonols were influenced by the growing conditions and genotype. Pearl was the cultivar in which specialized metabolites were affected most by variation in meteorological

conditions. Therefore, this variety may represent a starting point for future research targeting the increase/decrease of specific SM classes and the desired content of specific fatty acids by selecting the growing environment. The content and composition of camelina SMs confirm its nature as a multi-use crop, corroborating its key role in the circular economy.

Abbreviations

FA, fatty acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, α-linolenic acid; n-3/n-6 = omega-3/omega-6; TKW, thousand kernel weight; GDD, growing degree days; SMs, specialized metabolites; PAs, proanthocyanidins.

Keywords: Seed yield; fatty acid composition; specialized metabolites; bioactive compounds; oleic acid; linolenic acid; glucosinolates

1. Introduction

One of the main aims of the European Green Deal, launched by the European Union at the end of 2020, is to achieve a fully circular economy by 2050. The principal goal of a circular economy is to minimize the loss of resources by highly evaluating products and materials in the production process. In this context, camelina [*Camelina sativa* (L.) Crantz] can play a major role as it is a low-input, multi-purpose crop (Berti et al., 2016; Righini et al., 2016; Mondor and Hernández-Álvarez, 2021; Zanetti et al., 2021). Camelina is an oilseed crop belonging to the *Brassicaceae* family and is becoming well-known. Certain remarkable traits are the driving forces behind the research conducted on camelina. From an agronomic point of view, camelina can adapt to various environments because of its resistance to biotic and abiotic stresses (Berti et al., 2016). Camelina oil has a unique composition in that it has high contents of polyunsaturated fatty acids (PUFA), in particular, linoleic acid (C18:2) and α-linolenic acid (C18:3), which have different applications in the bio-based industry

(Kim et al., 2015), animal feed, and medicinal use (Waraich et al., 2013; Righini et al., 2016). Moreover, the oil is characterized by the presence of vitamin E, particularly tocopherols, which enhance its stability, with γ-tocopherol as the predominant isoform (Zubr and Matthäus, 2002). Many studies have been carried out to investigate these characteristics, demonstrating that oil content, fatty acid, and tocopherol composition are highly dependent on environmental conditions during the crop cycle (Zubr and Matthäus, 2002; Kirkhus et al., 2013; Zanetti et al., 2017, Righini et al., 2019). Nevertheless, camelina has other compositional features that have not been extensively studied, such as the presence of specialized metabolites.

Specialized metabolites (SMs), also known as "secondary metabolites," are bioactive compounds with multiple roles in plants. SMs are synthesized in different plant tissues and organs, particularly seeds, where large amounts of various SM classes with an important impact on physiological functions accumulate (Corso et al., 2020; 2021). Although SMs are not directly involved in the physiological processes of plant growth and development (Edreva et al., 2008; Mazid et al., 2011), they are essential for plant survival under stressful conditions and are largely influenced by the environment (Boutet et al., 2022). It has been demonstrated that biotic and abiotic stresses lead plants to increase the production of SMs under challenging conditions (Bartwal et al., 2013; Balestrini et al., 2021). However, these compounds are not only involved in the plant defense system but are also responsible for their fragrances and pigmentations, thus enabling the plant to better interact with the surrounding environment (i.e., pollinators, predators, and neighboring plants) (Cowan, 1999; Pietta, 2000; Bartwal et al., 2013; Balestrini et al., 2021; Corso et al., 2020). A wide range of SMs is known and can be traced back to three main classes: phenolics (or phenylpropanoids), terpenes, and nitrogen-containing SMs, including glucosinolates and alkaloids (Bartwal et al., 2013). While most studies have focused on SM in vegetative tissues, the ability of seeds to produce these compounds has largely been neglected. The possibility of using seed SMs for nutraceutical, cosmetic, and medicinal applications has recently attracted increased attention towards these compounds (Edreva et al., 2008; Bartwal et al., 2013; Corso et al., 2021).

In camelina, SMs accumulate throughout the plant, from vegetative tissues (Onyilagha et al., 2003; Karamać et al., 2020) to seeds (Terpnic et al., 2012; Quéro et al., 2016; Boutet et al., 2022). During seed pressing, most of the bioactive compounds are retained in the seed meal (Terpnic et al., 2012; Kurasiak-Popowska et al., 2019), and as a result, about 10% of the camelina meal consists of phytochemicals such as phenolics, glucosinolates, and terpenoids (Berhow et al., 2014; Kurasiak-Popowska et al., 2019), while flavonols are mainly concentrated in the oil (Kurasiak-Popowska et al., 2019). These compounds have protective roles against a wide range of human diseases, including cancer, senile dementia, Alzheimer's disease, and diabetes (Berhow et al., 2013; Niciforović and Abramović, 2014; Martin and Li, 2017; Bondonno et al., 2019), as natural preservatives (Terpnic et al., 2012; Kumar et al., 2015; Kumar and Pathak, 2016), and in pharmaceutical applications (Kumar et al., 2015). Once it is understood whether the adopted agronomic management and genetic background of camelina influence their presence, these traits might further increase the value of camelina seeds and co-products. However, there is a great need to fully characterize SMs in camelina seeds and improve our knowledge of variations in response to different growing conditions. This contrasts with all the knowledge acquired on the more investigated characteristics of camelina (i.e., oil and protein content and fatty acid composition), particularly in response to variety choice, growing conditions, and agronomic management. Previous work by Boutet et al. (2022) detailed camelina seed SM diversity and plasticity by means of metabolomic analyses. However, the present study aimed to characterize the content, variation, and interaction of the main specialized metabolic families of six camelina genotypes (with different genetic backgrounds grown in open field conditions for five consecutive years in northern Italy), with environmental or agronomical parameters. We presumed that the differences would be strictly related to meteorological conditions occurring during the camelina growing cycle since the experimental site stayed the same.

2. Materials and Methods

2.1 Agronomic trial set-up

Six spring camelina cultivars from different breeding programs and characterized by different agronomic and quality traits [i.e., seed size, oil quality, earliness, seed yield potential (Zanetti et al., 2017)], were grown for five consecutive years (2015–2019) at the experimental farm of the University of Bologna at Cadriano (44° 33' N, 11° 23' E, 32 m a.s.l.), which is characterized by a homogenous soil type. The site has silty-clay-loam soil (27% sand, 29% clay, 44% silt, soil organic matter =1.25%, pH = 7), a long-term mean annual temperature of 13.2 °C, and cumulative precipitation of 613 mm. The cultivars included in the trial were Midas (Agriculture and Agri-Food Canada, Saskatoon, Canada), Cypress, 789-02, and Pearl (Smart Earth Camelina, Saskatoon, Canada), Omega (Poznan University of Life Sciences, Poznan, Poland), and WUR (Wageningen University and Research, Wageningen, The Netherlands). Camelina was sown from mid-March to the beginning of April during each growing season (Table 1). The experimental design was a completely randomized block, with three replicates for 2015–2017 and four replicates for 2018–2019. The same plot dimension (10.5 m^2), row distance (0.13 m), and seeding rate (500 seeds m^{-2}) were maintained during all years of the trial. N fertilization was manually supplied at 50 kg/ha as urea, assumed to be a non-limiting amount for camelina under the conditions. Since the preceding crop was winter wheat that is always fertilized with P and S, these nutrients were not considered as limiting and, therefore, not applied. All the trials were rainfed. At full maturity, the central portion of each plot (6.5 m^2) was manually mowed and threshed using a mechanical thresher. Representative sub-seed samples from each plot were collected, cleaned, and stored for analysis.

2.2. Crop cycle and meteorological data

The key dates and main meteorological data (GDD, air temperatures, i.e., T_{max} and T_{min}, and cumulative precipitation) of the five years of the trial are reported in Table 1. Meteorological data were collected from a weather station located at the trial site. GDD were calculated using the following formula: $GDD = \Sigma [(T_{max} + T_{min})/2 - T_{base}]$, where 4°C was used as the base for the calculation (Gesch and Cermak, 2011). Phenological stages were surveyed during the camelina cycle

following the BBCH scale proposed by Martinelli and Galasso (2011), assuming BBCH 605 as the 50% flowering stage. Meteorological data were considered for the entire crop cycle, from sowing to harvest (identified as period "A"), and for the period from 50% flowering to harvest (identified as period "C"), as the latter was formerly identified as a "critical period" for final oil composition of camelina by Righini et al. (2019). When the crop reached full maturity (i.e. residual seed moisture <10%), it was harvested as described above. Biomass, straw, and seed residual moisture were determined after oven-drying the subsamples from each plot at 105°C, and weighed when a constant weight was reached. All yield values were reported on a dry matter (DM) basis.

2.3 Seed quality analysis

Thousand Kernel Weight (TKW) was determined using the Seed Counter S-25 machine by Data Technologies (DATA Detection Technologies Ltd., IL) at the Seed Research and Testing Laboratory (LaRAS) of the University of Bologna. Seeds were analyzed by Agriculture and Agri-Food Canada (Saskatoon, Canada) to determine seed oil and protein content and fatty acid composition, following the procedures described by Zanetti et al. (2017). Seed oil and protein contents were evaluated in representative seed samples by near-infrared spectroscopy (NIRS), while fatty acids were determined by gas chromatography after seed sample methylation.

2.4 Specialized metabolites determination

Untargeted metabolomic analyses of the same genotypes characterized in this study have been described by Boutet et al. (2022). Metabolite extraction and analyses were performed on dry seeds of six Camelina genotypes harvested during five consecutive growing seasons. Briefly, polar and non-polar metabolites were extracted from 60 mg of dry seeds using a MeOH:methyl-tert-butyl:H_2O (1:3:1) buffer. Polar and semipolar metabolites were separated from the oil and protein fractions using a MeOH:H_2O (1:3) buffer. Polar and semipolar metabolite fractions were used for metabolomic analyses. Untargeted metabolomic data were acquired using a UHPLC system (Ultimate 3000

156 Thermo) coupled with a quadrupole time-of-flight mass spectrometer (Q-Tof Impact II Bruker
157 Daltonics, Bremen, Germany). A Nucleoshell RP 18 and a reversed-phase column were used for
158 chromatographic separation. Samples were injected in positive and negative ionization modes (ESI+
159 and ESI-).
160 ESI+ and ESI- were processed using MZmine 2.52 software (http://mzmine.github.io/).
161 Metabolite annotation was performed in four steps:
162 i) LC-MS/MS data were compared using a homemade library (IJPB metabolomic platform)
163 containing more than 150 standards or common experimental features.
164 ii) LC-MS/MS data were searched against available MS^2 spectral libraries (Massbank NA, GNPS
165 Public Spectral Library, NIST14 Tandem, NIH Natural Product, and MS-Dial).
166 iii) Molecular network analysis was used to assign non-annotated metabolites to a chemical
167 family (Boutet et al., 2022).
168 iv) The Sirius software (Bio. informatik. uni-jena.de/software/sirius/) was used to assign putative
169 annotations to metabolic features that were not annotated during the previous steps.
170 Metabolite abundance was expressed in relative units (r.u.) Corso et al., 2018), which
171 corresponds to the peak area of each metabolite normalized to the internal standard (apigenin, 200
172 ng/sample) and weight of seeds used for the extraction. In contrast to Boutet et al. (2022), the
173 accumulation of metabolic classes (e.g., flavonols and glucosinolates) was calculated by totaling the
174 intensities of metabolites belonging to each category. These data were used for further statistical
175 analyses. LC-MS/MS untargeted metabolomic data and metadata were deposited in the INRAE data
176 repository portal (data INRAE) with the identifier https://doi.org/10.15454/ATTENN.
177
178 *2.5 Statistical analysis*
179 Prior to ANOVA, homoscedasticity of the data was tested using Bartlett's test for $P \leq 0.05$.
180 Two-way ANOVA was performed, considering year and cultivar as the main factors. When ANOVA
181 was significant ($P \leq 0.05$), Fisher's test was applied to separate the different means ($P \leq 0.05$). A

correlation study was conducted to evaluate the relationships between seed and oil characteristics (oil content, protein content, and FAs) and SMs for all cultivars combined and individually. Another correlation study was conducted to evaluate the relationships among the main SM classes and meteorological data for individual cultivars, considering either the whole growing cycle or the 50% flowering to harvest period. When the correlations were found to be significant at $P \leq 0.05$, Pearson's correlation coefficient (r) was reported. All statistical analyses were performed using the Statgraphics Centurion 18 software (ver. 18.1.13, Statgraphics Technologies Inc., Virginia, USA).

3. Results

3.1 Weather conditions

Camelina completed its cycle accumulating 1147–1388 GDD (Table 1) from sowing to harvest. Meteorological conditions during the entire growth cycle varied across the study years. The hottest year was 2018, with an average temperature of 18.8°C, while the coldest year was 2019 when a mean temperature of 16.2°C was registered. The lowest mean minimum temperature was recorded in 2017 (9.8°C), and the highest mean maximum temperature in 2018 (24.8°C). The wettest year was 2019, with cumulative precipitation of 229 mm, and the driest was 2017 with only 95.2 mm. Regarding the period from 50% flowering to harvest, the highest mean temperature was recorded in 2019 (23.0°C), and the lowest in 2016 (19.9°C). The lowest mean minimum temperature was recorded in 2017 (13.5°C), and the highest mean maximum temperature in 2018 (29.3°C). In 2016, the highest precipitation was observed (126.6 mm), whereas the driest year was 2017, with a 52.2 mm cumulative precipitation from 50% flowering to harvest (Table 1).

3.2 Camelina productive performance and compositional characteristics

For all the surveyed agronomic (seed yield, TKW) and compositional (oil content, protein content, oleic acid content, linoleic acid content, linolenic acid content, n-3:n-6 ratio) traits, the main factors (i.e., "year" and "cultivar") and their interaction were found significant ($P \leq 0.05$), except for

oil and protein content for which only the main factors were significant (Table 2). Mean seed yield (Fig. 1) varied significantly among cultivars and across years. In general, 2017 (the coolest and driest year) had the greatest seed production (2.13 Mg DM ha^{-1}), while the lowest value (0.78 Mg DM ha^{-1}) was observed in 2019 (the wettest year and the hottest from flowering to harvest). The highest coefficients of variation (CV) for seed yield values were reported for cultivars 789-02 (CV = 0.41), Cypress (CV = 0.36), and WUR (CV = 0.39). In particular, 789-02 and Cypress halved their seed yields when comparing the most and least productive years, which corresponded to 2015 (greatest) and 2016 (smallest) for 789-02, and 2017 (greatest) and 2018 (smallest) for Cypress (Fig. 1). Nevertheless, Cypress was characterized by a significantly greater seed yield than that of 789-02 (grand mean: 1.73 vs. 1.51 Mg DM ha^{-1}, Cypress vs. 789-02, respectively $P \leq 0.05$). In contrast, WUR was characterized by the lowest yield potential (grand mean: 1.29 Mg ha^{-1} DM), but in the most productive year (2017) it reached close to 2 Mg ha^{-1} DM. It is worth noting that both Cypress and WUR reached the greatest seed yield in the driest and coolest year (2017), while 789-02 reported comparable and greater seed yields in 2015 and 2017 compared with all the other growing seasons. All the tested genotypes had minimum production in 2019, which was characterized by uneven precipitation distribution and the highest temperatures in the period from sowing to harvest.

Camelina 1000-seed weight (TKW) was influenced by the main factors “year” and “cultivar” and their interaction (Table 2). Among the cultivars, Cypress had the greatest TKW (1.49 g), and Omega had the smallest (1.00 g). TKW was negatively affected in 2019, reporting the minimum value (0.94 g), while 2018 produced the heaviest seeds (1.37 g). The cultivars’ CV for TKW was less variable than that of seed yield, ranging from 0.11 for 789-02 to 0.23 for WUR. The interaction “year x cultivar” on TKW is reported in Fig. 2. The lowest TKW for all cultivars were observed in 2017 and 2019 (grand mean values: 1.05 g and 0.94 g, in 2017 and 2019, respectively), while a consistent increase in seed weight was registered for all tested genotypes in 2018 (grand mean: 1.37 g).

Seed oil and protein contents were significantly affected by the “year” and “cultivar” (Table 2). In 2016, which was characterized by a longer period from 50% flowering to harvest, i.e., > GDD,

234 the greatest seed oil content was observed (39.3% DM), while the lowest value was in 2019 (37.6%,
235 Fig. 3A), characterized by very high temperatures from 50% flowering to harvest (Table 1). Seed
236 protein reached the maximum value in 2017 (32.6%) and the lowest in 2015 (27.9%, Fig. 3A). Despite
237 producing the heaviest seeds, Cypress was characterized by the highest seed oil content (39.4%), but
238 not different from 789-02 (Fig. 3B). Cypress had the lowest seed protein content (29.2%, Fig. 3B),
239 while 789-02, Pearl, and WUR had the greatest seed protein concentration (Fig. 3B). The CVs for
240 seed oil and protein were more stable than those for seed yield and TKW. The cultivar with the highest
241 seed oil content was Pearl (CV = 0.03), whereas WUR had the lowest (CV = 0.03). For seed protein,
242 Midas had the lowest CV (0.06), and Cypress had the highest CV (0.08).
243 All the main FAs characterizing camelina oil (i.e., oleic (C18:1), linoleic (C18:2), and
244 linolenic (C18:3) acids, and the n-3:n-6 ratio) were significantly influenced by cultivar, year, and
245 their interaction (Table 2, Fig. 4). Oleic acid content reached its highest level in 2019 (20.5% DM),
246 while 2016 and 2018 were the lowest amounts (16% DM). Regarding cultivar choice, Pearl had the
247 highest C18:1 content (22.9% DM), and Cypress had the lowest (14.2% DM). The CV for C18:1
248 content was the lowest in Cypress (0.09) and the highest in Omega (0.14). Concerning the "year x
249 cultivar" interaction (Table 3) C18:1 accumulation was promoted in all the cultivars with the
250 exception of Midas in 2019. This genotype was the only one presenting increased C18:1 content in
251 2017 (15.9%) and 2018 (20.7%) and an intermediate value in 2019 (17.4%). Pearl was the cultivar
252 with the greatest increase from 2018 (18.1%) to 2019 (27.6%). Linoleic acid (C18:2) accumulation
253 reached 21.2% DM in 2019 and 16.5% DM in 2016 (grand mean: year, $P \leq 0.05$). Among cultivars,
254 Pearl reported the lowest mean value for C18:2 (15.4% DM), while Cypress and WUR had the highest
255 with 21.3 and 21.1% DM, respectively. The CVs for C18:2 ranged from 0.9 to 0.13 among all
256 cultivars. The growing seasons of 2018 and 2019 had the highest values of C18:2 for all cultivars,
257 apart from Midas and 789-02, which accumulated higher amounts in 2017 (Table 3). Linolenic acid
258 (C18:3) was the FA representing the highest share in all camelina cultivars (Table 3), in particular,
259 789-02 had the highest mean value (31.5% DM), and WUR, Cypress, and Midas reported the lowest

share with an average of 29.7% DM. The cultivar CVs for C18:3 were very limited compared with the other main FAs and ranged from 0.10 to 0.11 among all genotypes. Analyzing the effect of year, 2019 was the hottest from 50% flowering to harvest and had the lowest value (25.5% DM). In contrast, 2016 and 2018 reported the highest mean with 34.0 and 33.7% DM of C18:3, respectively. Concerning the "year x cultivar" interaction, Omega had a different behavior than the other genotypes, presenting higher amounts in 2017, 2018, and 2019, and the lowest in 2015 and 2016, while 789-02 reached the highest value (Table 3).

The n-3:n-6 ratio is a very important parameter for vegetable oils, influencing possible end uses, and it was also significantly influenced by year, cultivar, and their interaction (Table 2). The lowest value was in 2019, and the highest was in 2016, reporting 1.19 and 2.02, respectively ($P \leq 0.05$). Pearl was characterized by the highest n-3:n-6 ratio (1.97), whereas WUR and Cypress had the lowest (1.36, both). The CVs for n-3:n-6 were much higher than the ones for the main FAs, ranging from 0.19 in Omega to 0.24 in Midas. The interaction "year x cultivar" was also significant (Tables 2 and 3), again, Omega reported a different behavior than the other genotypes, with the highest ratio in 2018 and one of the lowest in 2017, while it was the opposite for all other cultivars (Table 3).

3.3. Specialized metabolite accumulation in camelina seeds

Twelve classes of SMs were identified, namely alkaloids, amino acids and derivatives, benzoic acids, carboxylic acids, cinnamic acids, flavan-3-ols and PAs (proanthocyanidins), flavanones, flavones, flavonols, glucosinolates, isoflavones, and terpenes. Among them, the most representative classes are amino acids and derivatives, cinnamic acids, flavan-3-ols and PAs, flavonols, and glucosinolates (Boutet et al., 2022). Therefore, the authors decided to focus only on those SMs. The ANOVA results are reported in Table 4, and different from what was surveyed for the agronomic and compositional traits, only year was found significant for all the SMs. Cultivar had a significant effect only on flavan-3-ols and PAs, and flavonols, and the interaction "year x cultivar" was never significant. The SMs classes presenting the highest variation were amino acids and

286 derivatives (CV = 0.74) and glucosinolates (CV = 0.63), with ranges of 63.3–214.1 relative units
287 (r.u.), and 38.6–106.6 r.u., respectively. Amino acids and derivatives significantly changed across
288 years (Fig. 5A), and in 2016 particularly, the amino acid content was approximately three times that
289 observed in 2018 and 2019. The variations in cinnamic acids, flavan-3-ols and PAs, and flavonols
290 across years (Fig. 5B, C, D) were significant ($P \leq 0.05$) but less remarkable. In particular, 2015 was
291 the growing season in which the lowest amounts were determined compared with all the others, apart
292 from cinnamic acids, for which 2019 had significantly lower production. Finally, the variation in
293 glucosinolate content across years was highly significant ($P \leq 0.05$), with 2016 and 2017 reporting
294 the highest amounts and 2018 and 2019 being characterized by lower amounts (Fig. 5E). Concerning
295 the cultivar choice, only flavan-3-ols and PAs, and flavonols showed different accumulation patterns
296 in the studied genotypes. For flavan-3-ols and PAs (Fig. 6A), Cypress accumulated the highest
297 amount, which was not significantly different from that of Midas, while 789-02 and Pearl had the
298 lowest amounts. For flavonols, Cypress was one of the cultivars with the highest amount, but this
299 time it was not different from that of Midas and Omega, while 789-02 was confirmed as the genotype
300 with the lowest accumulation in the seeds.

301 To reveal possible relationships between the agronomic and qualitative traits and SM
302 accumulation and diversity, a correlation study was performed considering the mean values for all
303 the cultivars and breaking down the study for each camelina cultivar to understand if certain
304 genotypes had peculiar compositional traits. Only linolenic acid (C18:2) and amino acid and
305 derivative contents showed a significant ($P \leq 0.05$) but negative correlation when considering all
306 cultivars with r=-0.91 (Table 5). The same trend was evident when considering single genotypes,
307 such as Cypress and 789-02, for which r increased to -0.96 and -0.97, respectively. Since year
308 significantly affected all identified SM classes in camelina seeds, a second correlation study was
309 carried out to determine if there were any significant relationships between meteorological conditions
310 experienced, either during the entire growth cycle or just in the final part (i.e., after 50% flowering
311 stage), and the accumulated SMs. Again, this second study was carried out either by including all

312 camelina cultivars or a break down by cultivar. When considering all cultivars, amino acids and
313 derivatives were negatively correlated with the mean and the maximum temperature after 50%
314 flowering (r = -0.92 and r = -0.92, for mean T_{mean} and T_{max}, $P \leq 0.05$, respectively) (Table 5). When
315 investigating the relationships within a single cultivar, Pearl was the only one in which many SMs
316 were related to the surveyed meteorological variables (Table 5), demonstrating a greater susceptibility
317 to environmental conditions than the others. In particular, a negative correlation between minimum
318 temperature after 50% flowering and cinnamic acids (r = -0.95) and between total GDD after 50%
319 flowering and flavonols (r = -0.95) were surveyed. Lastly, negative correlations were also identified
320 between glucosinolates and mean temperature from full flowering to harvest (r = -0.92), and between
321 glucosinolates and maximum temperature from full flowering to harvest (r = -0.91) in Pearl.
322
323 4. Discussion
324 The present results on camelina seed yield and quality confirmed existing knowledge. By
325 setting up the ANOVA considering both "year" and "cultivar" as factors and running the trials at the
326 same experimental site, characterized by homogenous soil characteristics, it was possible to
327 determine which seed traits were affected by growing conditions (i.e., the different meteorological
328 pattern of each study year), and which ones were mainly under genetic control. The seed yield values
329 were in line with those reported in the literature (Zanetti et al., 2021), although the mean (1.59 Mg
330 DM ha^{-1}) was slightly lower than that observed by Zanetti et al. (2017) in the same location as the
331 present work. Seed yield was higher in growing seasons characterized by milder temperature, in
332 accordance with Krzyżaniak et al. (2019). Moreover, Cypress (former line 787-08) proved to be the
333 best performing cultivar both in terms of seed yield and seed weight, as was stated by Zanetti et al.
334 (2017). The fact that Cypress showed the highest TKW in four out of five years while maintaining a
335 satisfactory seed yield and above-average seed oil content further demonstrated that this cultivar has
336 outstanding agronomic potential, these dimensions being some of the main constraints limiting the
337 adoption of camelina by farmers. Both oil and protein content were influenced by environmental

conditions. Oil content was fostered by lower maximum temperatures after full flowering (2016) and reduced in the wettest year (2019), confirming the results reported by other authors (Krzyżaniak et al., 2019; Righini et al., 2019). Conversely, the protein content was promoted by low cumulative precipitation, reaching the highest content in the driest year (2017). This season was also characterized by the highest seed yield and smallest seed size; thus, a compensation effect might have occurred between seed production and oil accumulation in the seeds. As expected, the FA composition was also affected by meteorological conditions, particularly by temperature. Higher temperatures caused an increase in oleic and linoleic acid content while reducing α-linolenic acid, whereas cooler temperatures boosted α-linolenic acid accumulation, as reported by Zanetti et al. (2017) and Righini et al. (2019). The present study also identified two cultivars, Pearl (former line 887) and 789-02, as genetically characterized by increased oleic and α-linolenic acid contents, confirming the results of Zanetti et al. (2017). These two cultivars showed intermediate seed yields compared to the others, but 789-02 was characterized by reduced variation coefficients across study years for all the surveyed compositional traits, apart from seed yield. These characteristics might be of interest because they could represent a benchmark cultivar for the bio-based industry, which is always looking for feedstock with a stable composition to fit specific transformation processes (John et al., 2019).

The actual scientific knowledge of camelina mainly focuses on its seed oil and protein content and quality and how it could be affected by genetic and environmental factors. Nevertheless, the full valorization of this emerging oilseed crop encompasses the use of all its seed components with a biorefinery approach. To the best of our knowledge, this is one of the first studies, together with Boutet et al. (2022), in which the two seed components have been studied. Additionally, the scope included understanding whether some relationships that could be exploited exist. Among the surveyed SM classes characterizing camelina seeds, some are considered noxious compounds; thus, a clear understanding of whether some genetic or environmental drivers exist in their accumulation may open the route to future new crop development. The most relevant are cinnamic acids, including sinapine,

364 which is considered an undesired component in animal feed, causing a fishy taint in eggs when
365 included in egg-laying hen diets (Matthäus and Zubr, 2000; Matthäus and Angelini, 2005; Berhow et
366 al., 2014), and glucosinolates, which decrease the palatability of animal feed by causing increased
367 astringency (Matthäus and Angelini, 2005; Corso et al., 2021), and are recognized as antinutritional
368 factors, reducing animal growth and being toxic, particularly in monogastrics (Matthäus and Angelini,
369 2005; Berhow et al., 2014; Corso et al., 2021). Similarly, PAs can also reduce the tastiness of forage
370 and lower its nutritive value (Dixon et al., 2004). However, these SMs, together with flavonols, are
371 considered bioactive compounds with antioxidant, antiviral, antimicrobial, and anticarcinogenic
372 properties (Cowan et al., 1999; Cushnie and Lamb; 2005; Salminen et al., 2006; Terpnic et al., 2012;
373 Berhow et al., 2013; Berhow et al., 2014; Corso et al., 2020). These characteristics suggest the use of
374 SMs obtained from camelina seed meal for medicinal and nutraceutical applications (Edreva et al.,
375 2008; Bartwal et al., 2013; Berhow et al., 2013; Niciforović and Abramović, 2014), as well as their
376 use as natural preservatives in the food industry (Terpnic et al., 2012; Kumar et al., 2015; Kumar and
377 Pathak, 2016). The yearly variability reported for SMs patterns highlighted that environmental
378 conditions during crop development are key factors in defining the final camelina seed composition,
379 as reported in other studies (Del Carmen Martinez-Ballesta et al., 2013; Berhow et al., 2014;
380 Balestrini et al., 2021; Boutet et al., 2022). Amino acids and derivatives seemed to be promoted by
381 high cumulative precipitation and lower maximum temperature after full flowering (i.e., 2016), while
382 their content was reduced in growing seasons with higher temperatures during the seed-filling stage
383 (i.e., 2018-19). Cinnamic acids, flavan-3-ols and PAs, flavonols, and glucosinolates showed similar
384 accumulation patterns across years and were elevated in growing seasons characterized by different
385 meteorological conditions (i.e., 2016–2018). SMs, such as cinnamic acids and flavonoids, are well-
386 known antioxidants that can protect seed embryos and coats from several abiotic stresses, such as
387 drought and high temperature, and strongly contribute to seed adaptation to adverse environmental
388 conditions (Chen et al., 2015; Corso et al., 2021; Boutet et al., 2022). These compounds are also part
389 of the defense mechanisms of plants against biotic stresses (e.g., herbivores, predators, pathogens)

(Dixon et al., 2004; Russo and Reggiani, 2012; Paauw et al., 2019; Corso et al., 2020; Corso et al., 2021). Hence, it can be presumed that in addition to abiotic stress (i.e., related to meteorological conditions), such biotic stresses were also responsible for SM variations across the study years. The high flavan-3-ols, PAs, and flavonol contents displayed by Cypress, Midas, and Omega make these genotypes interesting for further studies (Boutet et al., 2022) to deploy their potential as a source of these SMs, which may have relevant bio-based applications. Additionally, the significant relationships between meteorological conditions after full flowering and many SMs in Pearl may represent a further starting point for the widespread production of camelina because a specific seed quality may be achieved by carefully choosing the growing environment, not only for producing oil with a specific FA composition (Righini et al., 2019) but also for meals characterized by different SMs for particular bio-based applications.

5. Conclusions

Camelina is a relatively new oilseed crop. A full understanding of its seed compositional qualities and its physiology, and the consequent tailoring of specific agronomic management represent the main goals for its future deployment. In the present long-term study, by considering year and cultivar as factors in statistical analysis, it was possible to identify environmental and genetic features influencing camelina seed productive performance and qualitative characteristics of seed oil and meal. The results indicated that it might be feasible to choose specific genotypes or growing conditions (i.e., specific environments characterized by milder temperatures or higher lower precipitation) that can maximize the crop's profit in terms of end-uses. The productive performance and oil quality of the tested genotypes showed remarkable variation among years and genotypes. Some of them (i.e., Pearl and 789-02) seemed more appropriate for specific bio-based applications (i.e., those requiring an increased n-3:n-6 ratio), and others (i.e., Cypress) were suitable for achieving high and stable yields. The results on seed SMs confirmed that these compounds are more under environmental than genetic control. Nevertheless, the identification of a cultivar highly responsive to

growing conditions for SMs accumulation, such as Pearl, and characterized by a peculiar FA composition, might open interesting routes for the future development of camelina as a biorefinery oilseed crop.

CRediT authorship contribution statement

Barbara Alberghini: Data curation, Formal analysis, Writing – original draft, Writing – review and editing; **Federica Zanetti**: Conceptualization, Data curation, Methodology, Formal analysis, Writing – original draft, Writing – review and editing; **Massimiliano Corso**: Investigation; Methodology, Data curation, Writing – original draft, Writing – review and editing; **Stéphanie Boutet**: Methodology, Data curation, Writing – review and editing; **Loïc Lepiniec**: Methodology, Project administration, Writing – review and editing; **Angela Vecchi**: Investigation, Data curation; **Andrea Monti**: Project administration, Supervision, Validation, Resources, Writing – review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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Figure 1. Mean seed yield (Mg DM ha^{-1}) in response to the interaction "year x cultivar" in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).

Figure 2. Mean 1000-seed weight (TKW, g) in response to the interaction "year x cultivar" in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).

Figure 3. Mean seed oil content and protein content (% DM) in response to year (A) and cultivar (B) in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars: standard error. Different letters within each parameter: significantly different means ($P \leq 0.05$, LSD Fisher's test).

Figure 4. Mean seed oleic acid content (C18:1, % DM) in response to the interaction "year x cultivar" in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).

Figure 5. Mean values of amino acids and derivatives (A), cinnamic acids (B), flavan-3-ols and PAs (C), flavonols (D), and glucosinolates (E) in camelina seeds, in response to year in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).

Figure 6. Mean values of flavan-3-ols and PAs (A) and flavonols (B) in camelina seeds, response to cultivar in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).

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Table 1. Sowing and harvest dates, and main meteorological data (GDD, minimum and maximum temperatures - T_{min} and T_{max}, and cumulative precipitation - Prec) surveyed during the 5-year trial on spring camelina carried out in Bologna (Italy) from 2015 to 2019[1].

Year	Sowing date	Harvest date	*Sowing to harvest*				*50% flowering to harvest*			
			GDD[1]	T_{min}	T_{max}	Prec	GDD[2]	T_{min}	T_{max}	Prec
				°C		mm		°C		mm
2015	1 April	26 June	1203	11.7	24.1	190.4	696	14.4	26.6	96.2
2016	17 March	29 June	1331	11.1	22.5	190.8	795	14.1	25.6	126.6
2017	15 March	14 June	1147	9.8	23.2	95.2	656	13.5	26.6	52.2
2018	27 March	28 June	1375	12.8	24.8	114.8	710	16.2	28.2	70.4
2019	11 March	2 July	1388	9.9	22.4	229.4	785	16.6	29.3	66.4

[1] See also Boutet et al. (2022) for additional information.

[2]Base temperature for GDD calculation = 4°C (Gesch and Cermak, 2014).

607 Table 2. ANOVA table with *F* values and statistical significance for agronomic and oil composition
608 traits of camelina in the multi-year study.

Source of variation	Seed yield	TKW	Oil content	Protein content	C 18:1	C 18:2	C 18:3	n-3:n-6
Y	94.6 **	335.5 **	14.0 **	114.2 **	250.5 **	257.4 **	467.6 **	362.5 **
C	6.48 **	315.5 **	19.4 **	3.55 **	629.6 **	451.9 **	15.9 **	223.1 **
Y x C	1.72 *	19.5 **	1.55 ns	0.69 ns	31.0 **	27.4 **	2.08 *	16.7 **

609 *, ** Significant at the 0.05, 0.01 probability levels, respectively (LSD Fishers' test); ns = not
610 significant.
611 Considered factors were year (Y) and cultivar (C).
612 C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = linolenic acid, n-3:n-6 = ratio of omega-3 to
613 omega-6 FAs.

Table 3. Main fatty acid content (% DM) (C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; n-3:n-6, ratio of omega-3 to omega-6 FAs) of the six camelina genotypes during the 5-year trial carried out in Bologna (Italy) from 2015 to 2019 in all years of the trial.

	C 18:1	C 18:2	C 18:3	n-3:n-6
2015	*19.2 ± 0.70 b*	*19.3 ± 0.60 b*	*28.9 ± 0.23 b*	*1.49 ± 0.06 c*
Pearl	24.2 ± 0.25 b	14.7 ± 0.17 k	29.7 ± 0.46 ghi	1.97 ± 0.05 cd
Cypress	14.9 ± 0.31 n	21.6 ± 0.16 def	28.8 ± 0.36 ijk	1.28 ± 0.02 hi
789-02	23.2 ± 0.37 c	16.4 ± 0.16 j	30.6 ± 0.24 g	1.81 ± 0.02 e
Midas	17.1 ± 0.15 kl	22.7 ± 0.19 bc	27.7 ± 0.21 lm	1.18 ± 0.01 ij
Omega	18.0 ± 0.15 hij	19.5 ± 0.07 h	28.3 ± 0.16 jkl	1.40 ± 0.01 g
WUR	17.7 ± 0.20 ijk	21.1 ± 0.22 fg	28.2 ± 0.37 kl	1.29 ± 0.03 h
2016	*16.0 ± 0.60 d*	*16.5 ± 0.49 c*	*34.0 ± 0.23 a*	*2.02 ± 0.08 a*
Pearl	20.7 ± 0.14 e	12.7 ± 0.18 l	34.7 ± 0.28 ab	2.63 ± 0.06 a
Cypress	13.1 ± 0.44 q	17.9 ± 0.10 i	34.0 ± 0.18 bcd	1.79 ± 0.02 b
789-02	19.1 ± 0.30 fg	14.2 ± 0.11 k	35.6 ± 0.28 a	2.40 ± 0.04 e
Midas	13.9 ± 0.13 op	19.5 ± 0.16 h	32.5 ± 0.32 f	1.58 ± 0.03 f
Omega	15.1 ± 0.10 n	16.5 ± 0.15 j	34.0 ± 0.22 bcd	1.95 ± 0.03 cd
WUR	14.3 ± 0.33 no	17.9 ± 0.15 i	33.3 ± 0.28 def	1.76 ± 0.03 e
2017	*18.4 ± 0.70 c*	*19.3 ± 0.58 b*	*28.7 ± 0.24 b*	*1.49 ± 0.06 c*
Pearl	23.9 ± 0.10 b	14.6 ± 0.22 k	29.3 ± 0.26 hij	1.96 ± 0.04 cd
Cypress	14.5 ± 0.28 no	21.3 ± 0.19 efg	28.0 ± 0.49 kl	1.27 ± 0.03 hi
789-02	21.7 ± 0.24 d	16.6 ± 0.26 j	30.0 ± 0.68 gh	1.76 ± 0.06 e
Midas	15.9 ± 0.28 m	21.5 ± 0.37 def	28.7 ± 0.61 ijkl	1.29 ± 0.05 h
Omega	17.8 ± 0.32 ijk	19.6 ± 0.36 h	28.6 ± 0.61 jkl	1.43 ± 0.05 g
WUR	16.4 ± 0.14 lm	21.9 ± 0.33 de	27.7 ± 0.24 lm	1.23 ± 0.03 hi
2018	*16.0 ± 0.79 d*	*19.5 ± 0.53 b*	*33.7 ± 0.19 a*	*1.70 ± 0.05 b*
Pearl	18.1 ± 0.41 hij	18.3 ± 0.15 i	33.4 ± 0.17 cdef	1.78 ± 0.03 e
Cypress	12.6 ± 0.37 q	22.2 ± 0.31 cd	32.8 ± 0.53 ef	1.43 ± 0.04 g
789-02	18.6 ± 0.09 gh	18.0 ± 0.50 i	34.5 ± 0.45 abc	1.86 ± 0.08 de
Midas	20.7 ± 0.36 e	16.3 ± 0.17 j	34.0 ± 0.13 bcde	2.02 ± 0.02 c

Omega	12.9 ± 0.28 q	20.8 ± 0.28 g	34.1 ± 0.43 bcd	1.59 ± 0.04 f
WUR	13.2 ± 0.15 pq	21.6 ± 0.64 def	33.6 ± 0.54 bcdef	1.51 ± 0.07 fg
2019	*20.5 ± 0.99 a*	*21.2 ± 0.68 a*	*25.5 ± 0.23 c*	*1.19 ± 0.05 d*
Pearl	27.6 ± 0.66 a	16.5 ± 0.09 j	25.2 ± 0.67 op	1.50 ± 0.04 fg
Cypress	16.0 ± 0.07 m	23.8 ± 0.20 a	25.3 ± 0.31 op	1.03 ± 0.02 k
789-02	24.0 ± 0.65 b	18.5 ± 0.56 i	26.6 ± 0.25 mn	1.41 ± 0.04 g
Midas	17.4 ± 0.19 jk	23.5 ± 0.45 a	26.1 ± 1.70 no	1.08 ± 0.05 jk
Omega	19.6 ± 0.47 f	21.8 ± 0.23 def	25.0 ± 0.25 op	1.11 ± 0.02 jk
WUR	18.4 ± 0.30 ghi	23.2 ± 0.22 ab	24.6 ± 0.28 p	1.03 ± 0.02 k

617 Mean values ± standard error.

618 Different letters mean significantly different values ($P \leq 0.05$, LSD Fisher's test) for the interaction

619 "year x cultivar" and for the main factor "year" (in Italics).

620 Table 4. ANOVA table with *F* values and statistical significance for specialized metabolites surveyed
621 during the 5-year trial on spring camelina carried out in Bologna (Italy) from 2015 to 2019.
622 Considered factors: year (Y) and cultivar (C).

Source of variation	Amino acids	Cinnamic acids	Flavan-3-ols and PAs	Flavonols	Glucosinolates
Y	13.39 **	3.92 **	4.49 **	4.09 **	11.34 **
C	2.30 ns	0.83 ns	8.04 **	4.04 **	2.08 ns
Y x C	1.29 ns	0.85 ns	1.11 ns	1.13 ns	1.29 ns

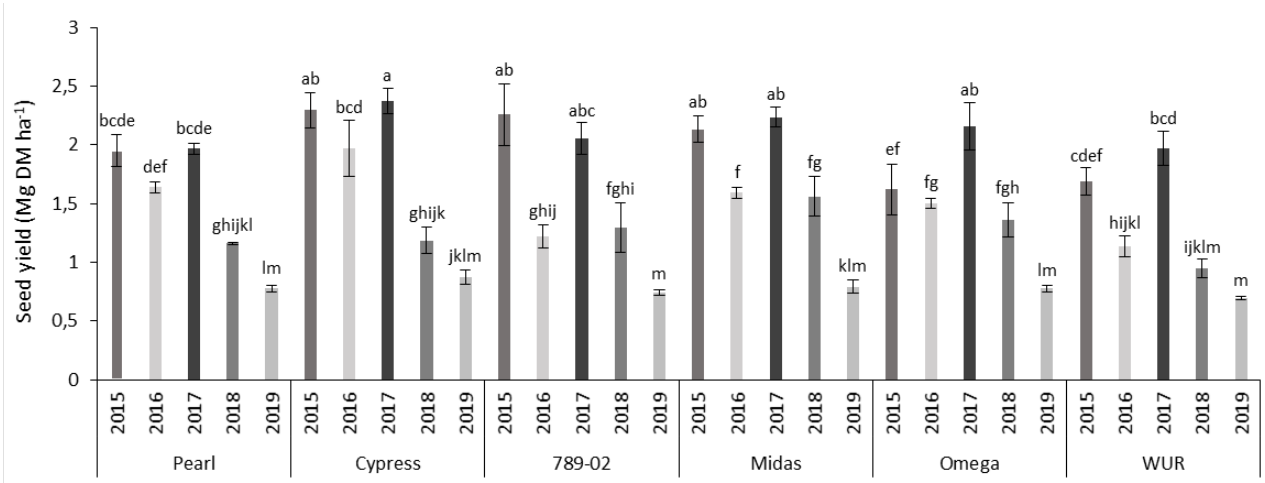
623 *, ** Significant at the 0.05, 0.01 probability levels, respectively (LSD Fishers' test); ns = not
624 significant.

Table 5. Pearson's coefficient (*r*) and *P*-values (in parenthesis) for the significant linear regressions between amino acids and meteorological variables calculated for the 50% flowering to harvest period, and main fatty acids (C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid) considering the mean values for all the cultivars (grey column); and for cinnamic acids, flavonols, glucosinolates and meteorological variables calculated for the 50% flowering to harvest period, only for the cultivar Pearl.

Variables	Amino acids	Cinnamic acids	Flavonols	Glucosinolates
Tmin[1]	–	-0.95 *(0.01)*	–	–
Tmean[1]	-0.92 *(0.03)*		–	-0.92 *(0.02)*
Tmax [1]	-0.92 *(0.03)*		–	-0.91 *(0.03)*
GDD[1, 2]	–		-0.95 *(0.02)*	–
C18:1	–			
C18:2	-0.91 *(0.03)*			
C18:3	–			

[1]Meteorological variables: Tmin mean = average minimum temperature from 50% flowering to harvest; Tmean mean = average mean temperature from 50% flowering to harvest; Tmax mean = average maximum temperature from 50% flowering to harvest; GDD = Growing Degree Days from 50% flowering to harvest.

[2]Base temperature for GDD calculation = 4°C (Gesch and Cermak, 2014).



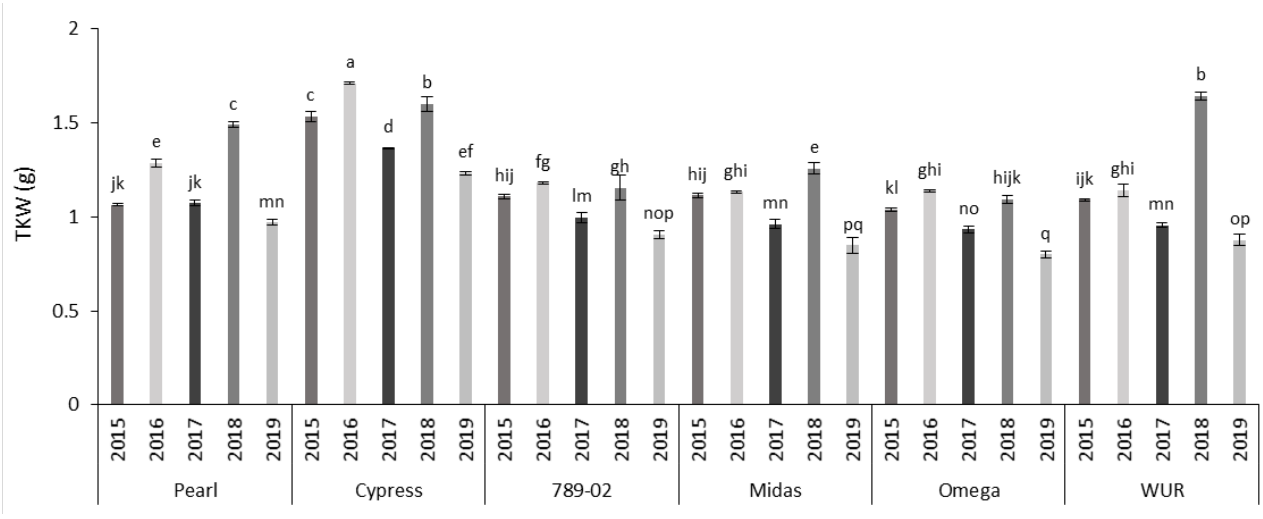
637

638 Figure 1. Mean seed yield (Mg DM ha^{-1}) in response to the interaction "year x cultivar" in the multi-

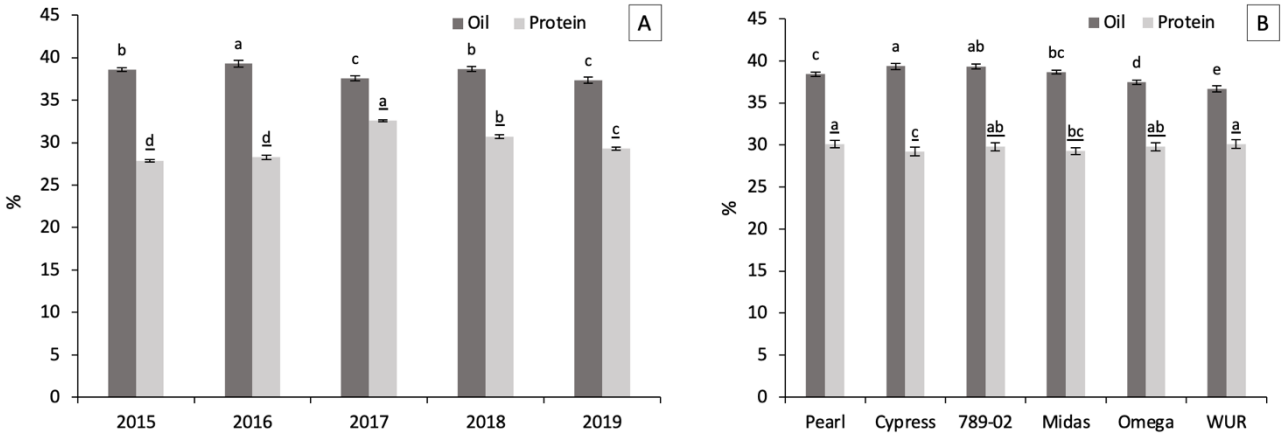
639 year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars:

640 standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).

641



642 Figure 2. Mean 1000-seed weight (TKW, g) in response to the interaction "year x cultivar" in the
643 multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars:
644 standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).



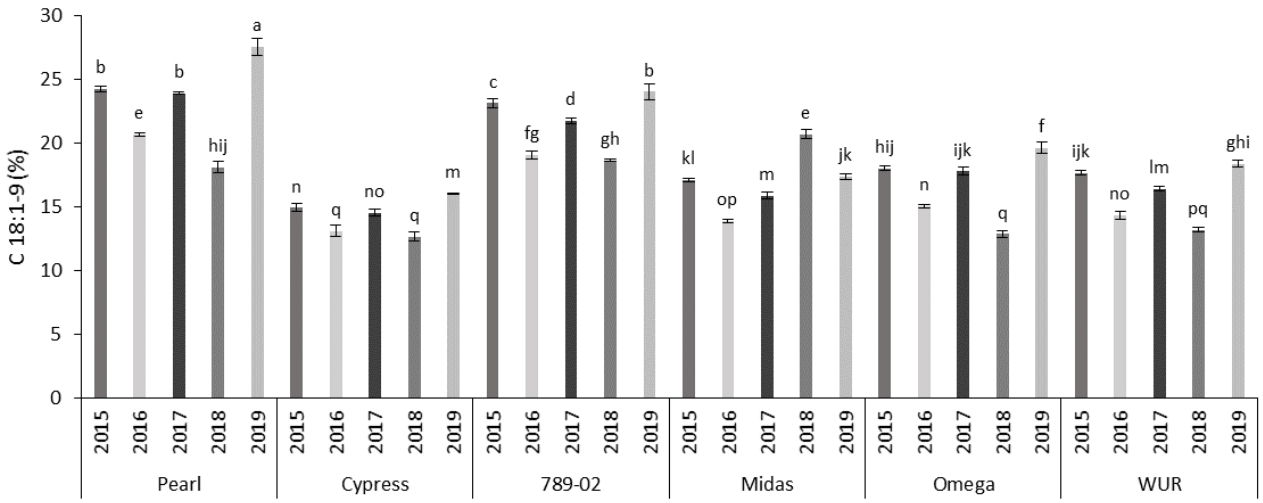
645

646 Figure 3. Mean seed oil content and protein content (% DM) in response to year (A) and to cultivar

647 (B) in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019.

648 Vertical bars: standard error. Different letters within each parameter: significantly different means

649 ($P \leq 0.05$, LSD Fisher's test).



650

651 Figure 4. Mean seed oleic acid content (C18:1, % DM) in response to the interaction "year x cultivar"

652 in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical

653 bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).

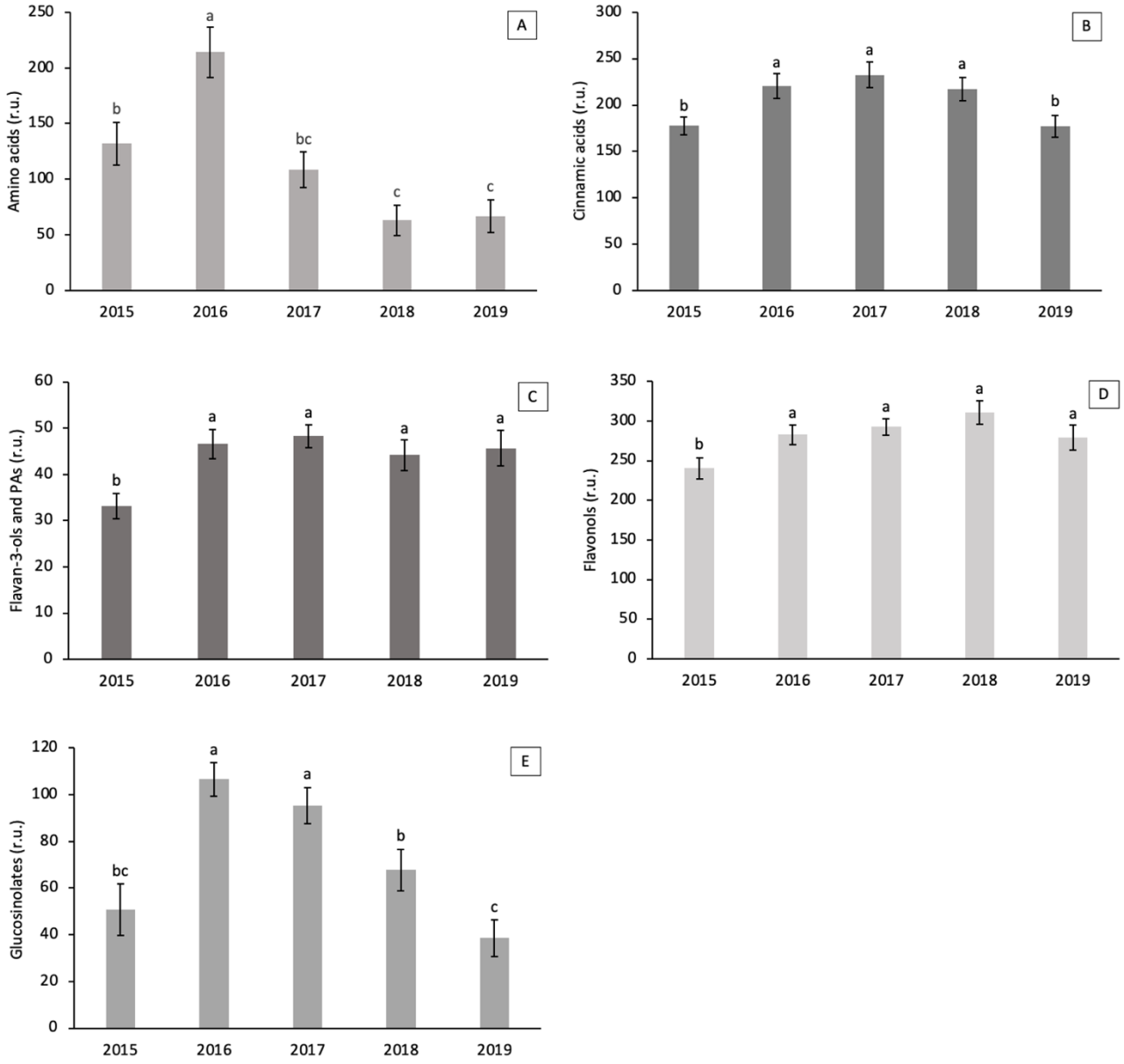
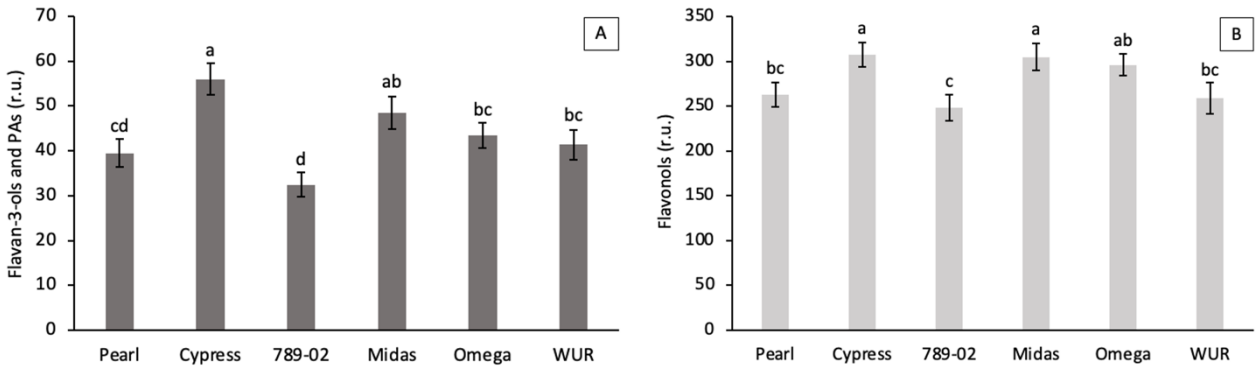


Figure 5. Mean values (r.u.) of amino acids and derivatives (A), cinnamic acids (B), flavan-3-ols and PAs (C), flavonols (D), and glucosinolates (E) in camelina seeds, in response to year in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to 2019. Vertical bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD Fisher's test).



659

660 Figure 6. Mean values (r.u.) of flavan-3-ols and PAs (A) and flavonols (B) in camelina seeds, response
661 to cultivar in the multi-year study on spring camelina carried out in Bologna (Italy) from 2015 to
662 2019. Vertical bars: standard error. Different letters: significantly different means ($P \leq 0.05$, LSD
663 Fisher's test).