Exploring the *Camelina sativa* value chain: a new opportunity for bio-based products and overall crop sustainability

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Camelina sativa L. Crantz (Camelina) is an oilseed crop, useful for both industrial and healthy food applications. It belongs to the Brassicaceae family and, nowadays, its popularity is growing. The chemical features of Camelina feedstock (seeds, defatted seed meals, and straws) were discussed in terms of yields of bioactive molecules per-hectare in open field trials laid out in Northern and Central Italy. Chemical and phytochemical features, such as oil fatty acid profile, defatted seed meal polyphenol, flavonol, glucosinolate and protein content and antioxidant activities, were examined pointing out the Camelina products potential applications in food, as well as in the pharma sector. At the same time, the valorisation of residual lignocellulosic biomass was discussed. Detailed glucosinolate and fatty acid profiles evidenced slight differences in the two trials, as well as total phenol and flavonoid contents. Fibre chemical compositions indicate that, in Central Italy, Camelina straws were characterised by a higher content of cellulose and hemicellulose and a lower amount of lignin in comparison to Northern Italy, while no differences were found in the ash contents. All together, these data open new perspectives for the valorisation of Camelina in a sustainable green chemistry approach based on the real yields of bioactive molecules and the exploitation of whole biomass.

Keywords: *Camelina sativa* L. Crantz; feedstock; phytochemicals; antioxidant activity; biomass exploitation.

1. INTRODUCTION

Camelina (*Camelina sativa* L. Crantz) is an ancient oilseed crop, belonging to the Brassicaceae family, also known as false flax (UK) and gold of pleasure (USA). It can adapt to sandy and degraded soils and it has lower input and water requirements than other traditional oilseed crops [1]. Winter varieties were rarely cultivated in the world except in North-eastern Europe. In Mediterranean regions, they usually give higher yields in comparison to spring varieties that are used mostly in Western Europe for the organic production of oil and press cake for feed, mixing it with legumes, to enhance the nitrogen supply and avoid weeds [1,2]. In Upper Midwest, where corn and soybean are the dominant crops, winter Camelina recently proved to be useful in a double-cropping system. This approach speeded up the Camelina harvest two weeks behind, whilst oil yields and quality were not affected. At the same time, it potentially allowed the earlier seeding of a second more profitable crop, thus improving overall production and biodiversity [3]. Camelina ultimately gained an emerging economic potential for biofuel and vegetable oil production, especially in temperate regions [4]. In order to generate a higher economic value in the oilseed and protein crops sector, defatted seed meals - as co-products of oil extraction - and crop residues - as lignocellulosic biomass - need to have proper qualities for the exploitation in a wide range of applications. Camelina can be used both for industrial and healthy food applications, thanks to the wide range

of products that can be derived from its cultivation. Camelina seeds and seed meals can be ingredients in porridge as in bread preparation, while its oil can find application as dietary oil for human consumption, with acknowledged beneficial effects in folk medicine [5]. To enlarge the number of these new exploitations, the characterisation of the chemical properties of crops and, at the same time, the bioactive molecule productivities per hectare are two crucial starting points for the conception of new extraction processes and the design of innovative bio-based products. It is undeniable that the renewed interest for Camelina has been linked to its typical fatty acid (FA) profile, characterised by a very high content of polyunsaturated fatty acids, with linoleic (LA) and alpha linolenic acid (ALA) accounting for more than 50% [6]. Furthermore, camelina oil is naturally poor in erucic acid, while, at the same time, it is a plant-based source of the essential ALA, whose content may be interesting for covering, at least partially, the recommended daily intake of 2 g/day according to Regulation EU n° 432/2012 [7]. Alpha-linolenic acid is considered a precursor in the desaturation and elongation pathway for the production of the very-long chain metabolites eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are associated with health benefits in humans, in the prevention of coronary heart disease, arrhythmias and thrombosis [6, 8]. However, the efficiency of ALA and its rate of conversion are still questionable [9]. A recent study on 79 human subjects with impaired glucose metabolism showed how Camelina oil could effectively improve serum lipid profile just after 12 weeks of treatment compared to diets enriched in lean or fatty fish designed to provide approximately 1 g (EPA) + (DHA) per day, confirming, the ability of being metabolised in long chain fatty acids for ALA derived from Camelina oil [10]. These traits, joined to the fibres from straws and bioactive molecules in the defatted seed meal, account for the potential health benefits of Camelina that to date cannot be ignored. Among all these compounds, Camelina seeds contain three methylsulfinyl-alkyl glucosinolates (GSLs): glucoarabin (9-(methylsulfinyl) nonylglucosinolate; GSL9), glucocamelinin (10-(methylsulfinyl) decylglucosinolate; GSL10), and (11(methylsulfinyl) undecylglucosinolate; GSL11). Glucosinolates are claimed to be active components responsible for many beneficial effects, which were assayed *in vitro*, *in vivo*, in human and epidemiological studies [11]. Alkyl sulfinyl GLSs are good inducers of detoxifying phase 2 enzymes regardless the length of their alkyl chain [12-15]. Glucoraphanin, the GSL present at high concentration in Tuscan black kale (*Brassica oleracea* L. *var. acephala sabellica*) sprouts and seeds [16, 17], is definitely the best known and extensively studied GSL with reference to the beneficial properties of its corresponding isothiocyanate, the sulforaphane [18, 19] and to its no adverse effects in healthy adults up to 400 µmol a day for two weeks [20]. Camelina GSLs and derived isothiocyanates appear as a "natural extension" of the alkyl sulfinyl side chain of glucoraphanin and sulforaphane. They have not yet been studied for their effects in humans, even if their activities were compared to those of sulforaphane in Hepa-1c1c7 cells. GSL9, GSL10 and Camelina seed extract have proved to be monofunctional inducers of the phase II detoxification enzyme quinone reductase (NQO1), in this cellular model, whereas they have no effect on phase I cytochrome P450 (CYP) 1A1 activity [15]. Interestingly, both Camelina seed extracts and Broccoli contain the bioactive flavonoid quercetin in several glycosidic forms and both GSL9 and GSL10 hydrolysed by myrosinase and sulforaphane exhibit a synergistic upregulation of NQO1 with quercetin, suggesting that isothiocyanates taken through their whole biomass natural source may be more effective than purified isothiocyanates [15]. Camelina beneficial effects on environment and health were also suggested by the antibacterial and antifungal effect of seed extracts, which were able to inhibit the growth of several laboratory bacterial strains or fungal pathogens in a way comparable with the large spectrum antibiotic kanamycin or clotrimazole, a standard drug for fungal infections [21, 22]. In this context, the aim of this study was to evaluate the biochemical features of seeds, defatted seed meals and residual lignocellulosic biomasses of Camelina after the cultivation in two environments of Central and Northern Italy, with different agronomic managements. Yields and crop characteristics have been assessed through a two-year open field experiments adopting an intensive crop rotation with cereals and exploring the possibility to maintain a quite uniformed yield in term of biomasses and potential ingredients for food and pharma industries both in spring and autumn sowing. In particular:

i) seeds were analysed for oil, fatty acid composition, protein and GSL content;

ii) defatted seed meals were characterised for total phenols and flavonoid content, and for the total antioxidant capacity;

iii) crop residues were evaluated through their composition in cellulose, hemicellulose and lignin.

The main components and the active compounds, listed above, have been expressed in terms of yield per hectare to better frame their potential economic value in a biorefinery approach for valorisation of the biomass as a whole.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL AND GROWTH CONDITIONS

Camelina cv Italia seeds were available in the seed collection of Brassicaceae of CREA-CI (Bologna) [23].

A two-year experiment (2014-2015) was laid out at open field trial with a single plot design and a plot size of 500 m^2, in two different environments, representative of Northern and Central Italy pedo-climatic conditions. The cultivation site in Northern Italy was located at the CREA experimental farm at Budrio (Bologna) in the Po Valley area (44°32'00" N; 11°29'33" E, 28 m above sea level). The cultivation site in Central Italy was located at San Piero a Grado (Pisa), in the coastal plain (43°40'N latitude, 10°19'E longitude, 1 m above sea level) at the Centre for Agro-Environmental Research "Enrico Avanzi" (CiRAA) of the University of Pisa. Both areas were characterised by flat land with alluvial deep loam soils with medium level of total nitrogen (1.4 g kg^{-1}) and organic matter (2%) content. In Bologna, Camelina was sown on 16th October 2013, and 20th October 2014, while harvest times were accomplished on 29th May 2014 and 4th of June 2015. In Pisa, Camelina was sown on 17th March and 12th March in 2014 and 2015 respectively, while harvest times were accomplished on 25nd June and 24th June in 2014 and 2015, respectively. The cropping techniques and mechanization methods were defined in relation to the specific characteristics of each area, and an intensive crop rotation with cereals was adopted, with the aim of performing the experiments under low input management in emphasized stress conditions. In both experimental sites, low fertilization rates were defined according to the soil chemical characteristics, evaluated in soil samples collected at the beginning of the trials. In Pisa, mineral nitrogen fertilization was applied after seeding, at the rate of 80 kg N ha^{-1} split in two applications (as ammonium sulphate and ammonium nitrate, in the 1st and 2nd application, respectively). In Bologna fertilization was provided through pelletized cattle and horse manure-based amendment (organic C 30%, organic N 2%, moisture 18%) at sowing time at the rate of 600 kg ha^{-1} (equal to 12 kg N ha^{-1}). At full seed maturity, in both trials, three sampling areas of 1 m^2 were randomly collected within each experimental plot to assess: thousand seed weight (TSW), evaluated according to ISTA rules (2005) and the residual above ground biomass. After sampling, the entire surface was mechanically harvested by a plot combine, to assess the grain yield. The harvest index (HI) was calculated as: [grain yield / (residual above ground biomass + grain yield)] × 100.

2.2 SEED CHARACTERIZATION AND ANALYSIS

After harvesting, seeds were cleaned, partially dried, ground to 0.5 mm size and analysed for their main qualitative characteristics. Seed moisture was determined by oven-drying the seeds at 40°C until constant weight for dry weight determination and the moisture content was calculated as the difference between the seed weight before and after the treatment. Seed oil content was measured by NMR (Nuclear Magnetic Resonance) technique by an MQC benchtop NMR analyser (Oxford Instruments) [24]. The quantitative determination was based on a specific calibration for Camelina seeds defined by the Soxhlet official method [25]. Fatty acid composition of Camelina oil was analysed after oil extraction from ground seeds by hexane, followed by trans-methylation in 2N KOH methanol solution. Fatty acid methyl esters (FAMEs) were evaluated by gas chromatography and the internal normalization method [26] was used for determining the fatty acid composition. Total content of (C-H-N) was determined by Elemental analyser LECO CHN TruSpec according to the American Society for Testing Materials [27]. The crude protein content was expressed as a percentage on dry matter and calculated from nitrogen using the conventional factor of 6.25. Total GSL content and profile were determined by HPLC analysis of desulfo-GSLs following the ISO 9167-1 method [28], with some minor modifications [29]. The desulfo-GSLs were detected monitoring their absorbance at 229 nm and identified with respect to their retention time [30]. Their amounts were estimated using sinigrin as internal standard; the response factor 1 was arbitrarily adopted.

2.3 DEFATTED SEED MEAL PHYTOCHEMICAL CONTENT AND ANTIRADICAL CAPACITY EVALUATION

Seed meals were obtained by grinding 10-15 g of seeds, subsequently sieved at 0.75 mm and defatted by hexane. For total phenolic content (TPC), total flavonoid content (TFC), and antiradical capacity determination, the meals were extracted with 80% methanol with a 1:20 (w/v) ratio by using ultrasound-assisted extraction (25 min). The extracts were centrifuged (30,500 g, 15 min, 4°C) and supernatants were filtered. Final extracts were kept on ice for the subsequent analysis that were performed at the same day or stored at -20°C. For each defatted seed meal sample three extracts were prepared, and the results were expressed on a dry matter basis (DM).

2.3.1 Total phenols

Total phenolic content was determined by the Folin-Ciocalteu method according to Dewanto *et al*. [31]. Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent (GAE) g^{-1} of meal.

2.3.2 Total flavonoids

Total flavonoid content was determined spectrophotometrically according to Jing *et al*. [32]. Catechin was used as external standard for calibration curve and the results were expressed as mg of catechin equivalents (CE) g^{-1} of meal.

2.3.3 Antiradical capacity

Antiradical capacity was assessed by measuring the ability of the extracts to scavenge synthetic radicals [i.e. 2, 2-diphenyl-1-picryl-hydrazyl-hydrate, DPPH• and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), ABTS•+]. Extracts serial dilutions in 80% methanol were tested and both DPPH• and ABTS•+ quenching capacities were estimated spectrophotometrically by solution discoloration at 515 nm and 734 nm respectively. Radical quenching was calculated by using the formula:

$$1- ((A_{sample} \times A_{blank})/(A_{control} - A_{blank})), \text{ where } A_{sample}$$

was the absorbance of the extract in presence of the radical solution; A_{blank} was the absorbance of the extracts plus solvent without radical; $A_{control}$ was the absorbance of the extraction solvent in presence of the radical solution. The concentration required to obtain a 50% antioxidant effect (EC_{50}) was determined as the concentration of extract (mg of defatted seed meal × mL^{-1} of extraction solvent) causing the 50% inhibition of the initial colour production. Spectrophotometric assays were performed by using an Infinite M200 PRO microplate reader, with 20 µL and 180 µL of extracts and radical solution (or solvent) respectively. Measurements were replicated two times for each pool.

2.4 FIBRE CHEMICAL COMPOSITION

Chemical fibre composition of residual lignocellulosic biomass (cellulose, hemicellulose and lignin) and total proteins were evaluated according to ASTM D5373 and Van Soest *et al.* [27, 33].
The tests were performed by using an ANKOM Fibre Analyser model A 200. Ash content was determined by heating the residues in an electric muffle furnace at 550°C for four hours.

2.5 STATISTICAL ANALYSIS

Described analysis were carried out in triplicate and statistical analysis of data, expressed as mean ± standard deviation, including EC_{50} estimation, were performed by using the R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), unless differently specified. Data were then analysed by a one-way ANOVA and LSD test to assess significant differences between samples ($P < 0.05$).

3. RESULTS AND DISCUSSION

The average grain yields obtained in 2014-2015 were 0.46 and 0.77 Mg ha^{-1} in Bologna and Pisa respectively, and those values were used to calculate the seed protein, oil and GSL yields reported in Table I. Although a same TSW obtained in both cultivation sites, lower grain yield and harvest index were observed in Bologna in comparison to Pisa (Table I). The mean oil content was lower in the Pisa trial (33%), in comparison to Bologna trials where a mean oil content of 39% was reached, even if the higher combined grain yield value determined a highest overall oil yield per hectare in Pisa. The protein content was similar in both cultivation sites, attesting around the levels reported in the literature [34]. Regarding the GSL seed content, total GSL yield per hectare in Bologna trial was lower than that achieved in Pisa, due to the lower seed yield obtained (see also Table II for a detailed analysis on GSL seed content and composition). Seeds from both environments showed a similar GSL profile (Table II), and the micromoles per gram of dried seed were slightly higher in Bologna than in Pisa for GSL10 and GSL11. The fatty acid profiles of the oils obtained in the two environments are reported in Table III. The oil extracted from Bologna seeds was characterised by a higher content of ALA (+13%) and a lower content of oleic and linoleic acids (about -7% and -15%, respectively), if compared to the oil produced in Pisa. Similarly, the detected content of polyunsaturated fatty acid (PUFA) resulted higher in Bologna, contrariwise monounsaturated fatty acid (MUFA) resulted higher in Pisa, whilst saturated fatty acids (SFA) contents were similar in both environments. The ratio between Ω 6 and Ω 3 ranged from 0.56 in Bologna to 0.7 in Pisa, accordingly to new recommendations for human health, which establish that a reduction in the dietary intake of LA/ARA ratios and ARA-derived metabolites could increase Ω3 circulating long chain-PUFAs in most individuals [35]. Phenolic and flavonoid contents of Camelina defatted seed meals were reported in Table IV. In the two environments, no significant differences were found in phenolic and flavonoid contents as well as for total antioxidant capacities of defatted seed meals (Table IV). Furthermore, the lowest EC_{50} values for DPPH• assay suggested a higher antiradical efficacy deriving from small molecules or from low steric hindrance molecules. The residual above ground biomass was quite similar in both sites, with 2.73 and 2.63 Mg ha^{-1} in Bologna and Pisa, respectively. The total proteins from the two environments accounted for 5.8% ± 0.4% and 4% ± 1%, for Bologna and Pisa respectively. The straw chemical composition (Table V) indicated that holocellulose accounted for more than 60% of the dry crude biomass by weight, in both locations. In Pisa, Camelina straws were characterised by a higher content of cellulose and hemicellulose and a lower amount of lignin in comparison to Bologna, while no significant differences were found in the ash contents between the two environments. The low-input approach, jointed probably with the autumn sowing, gave an upward trend in lignin productivity in Bologna trials with a mean yield of 0.4 Mg

Table I - Productive parameters and seed oil, protein and glucosinolate yields of *Camelina sativa* cv. Italia, cultivated for two consecutive years (values are averaged over 2014 and 2015 years) in Bologna and Pisa. Mean values ± SD (n=3) are shown.

			Bologna	Pisa
TSW	g	FW*	1.1 ± 0.1	1.1 ± 0.2
Grain yield	Mg ha^{-1}	DW**	0.8 ± 0.2b	1.3 ± 0.2a
Harvest Index			22.5 ± 3.7b	34 ± 2a
Moisture	%		4.0 ± 2.0	5.8 ± 0.5
Oil seed yield	kg ha^{-1}	DW	179.0 ± 6.0b	250.0 ± 41.0a
Seed Protein yield	kg ha^{-1}	DW	127.0 ± 13.0b	205.0 ± 11.0a
Total GSLs yield	kg ha^{-1}	DW	7.8 ± 0.3b	11.7 ± 0.6a

Values followed by different letters are significantly different at $P<0.05$ using LSD.
* FW: Fresh Weight
** DW: Dry Weight

Table II - Glucosinolate content and yield of seeds of *Camelina sativa* cv Italia, cultivated for two consecutive years (values are averaged over 2014 and 2015 years) in Bologna and Pisa. Mean values ± SD (n=3) are shown. The results are expressed as µmoles g^{-1} of seed meal (DW, dry weight) and as open field production considering the mean seed production per hectare of the two years (kg ha^{-1}).

	Content (µmoles g^{-1})		Yield (kg ha^{-1})	
	Bologna	Pisa	Bologna	Pisa
Glucoarabin	9.3 ± 0.8	9.2 ± 0.5	2.2 ± 0.20b	3.6 ± 0.2a
Glucocamelin	20.7 ± 0.8a	19 ± 1b	5.0 ± 0.2b	7.2 ± 0.4a
11-methylsulfinylundecyl Glucosinolate	2.7 ± 0.2a	2.3±0.10b	0.66 ± 0.05b	0.96 ± 0.04a

Values followed by different letters are significantly different at $P<0.05$ using LSD test.

Table III - Fatty acid profile of *Camelina sativa* cv. Italia oil derived from crops cultivated for two consecutive years (mean value of 2014 and 2015) in Bologna and Pisa. Mean values ± SD (n=3) are shown. Data were expressed as % of the total peak area from the gas-chromatography analysis.

Fatty acid composition			
Fatty acid	**Common name**	**Bologna %**	**Pisa %**
C 16:0	Palmitic acid	6.1 ± 0.3b	6.5 ± 0.1a
C 18:0	Stearic acid	2.5 ± 0.1	2.5 ± 0.1
C 18:1	Oleic acid	16.7 ± 0.1b	17.9 ± 0.5a
C 18:2	Linoleic acid	17.4 ± 0.5b	20.4 ± 0.3a
C 18:3	Linolenic acid	36 ± 1a	32 ± 2b
C 20:0	Arachidic acid	0.8 ± 0.7	1.0 ± 0.7
C 20:1	Gadoleic acid	13.2 ± 0.8	12.9 ± 0.2
C 20:2	Cis-11,14-eicosadienoic	1.5 ± 0.6	0.9 ± 0.8
C 20:4	Arachidonic acid	1.4 ± 0.2a	0.96 ± 0.06b
C 22:0	Behenic acid	0.0 ± 0.1	0.1 ± 0.2
C 22:1	Erucic acid	2.5 ± 0.1	2.6 ± 0.2
C 24:0	Lignoceric acid	0.3 ± 0.2a	0.04 ± 0.08b
C 24:1	Nervonic acid	0.55 ± 0.09	0.55 ± 0.04
	SFA	9.8 ± 0.60	10.1 ± 0.8
	MUFA	33.0 ± 0.3b	34.0 ± 0.6a
	PUFA	56.7 ± 0.9a	54 ± 1b
	n6/n3	0.56 ± 0.02b	0.70 ± 0.06a

Values followed by different letters are significantly different at $P<0.05$ by using LSD test. SFA saturated fatty acids, MUFA mono unsaturated fatty acids, PUFA polyunsaturated fatty acids.

ha^{-1}. In Pisa, the cellulose content of crop residues, was quite similar to that reported for rapeseed straw [36], while lower hemicellulose and lignin contents were obtained in both environments. The reduced external input, mainly in terms of nitrogen fertilisers, brought the lower grain yields observed in Bologna in comparison to those achieved in Pisa. Some authors consider the optimal N rate ranges between 44 and 185 kg ha^{-1} [37-39] even if negative effects in terms of plant lodging and pod shattering were observed after a N application greater than 75 kg ha^{-1} [38]. On the other hand, the negative role of N on the qualitative characteristics of Camelina seeds is well known, and several authors have already observed an antagonistic effect of N application on Camelina oil content and the relative fatty acid composition [37, 39]. Similar results were obtained also in this study, thus confirming some previous findings. In fact, the quantitative analyses of seeds (Table I), showed a significant higher oil seed content in the trials in Bologna, reaching a mean oil content of 38.92%, data in accordance to the results obtained in different trials realized in Europe [4]. The reduction of carbohydrates for oil synthesis due to an increased protein synthesis could explain the negative correlation between oil content and N rates. However, in our study protein contents were similar in both cultivation sites, therefore, it could be assumed that the differences in oil yield were mainly due to the different environmental conditions [40]. In fact, the dif-

Table IV - Total phenols (TPC), total flavonoids (TFC), antioxidant activity (AC) and anti-radicalic activity (EC_{50}) of defatted seed meal from *Camelina sativa* cv. Italia, cultivated for two consecutive years (values are averaged over 2014 and 2015 years) in Bologna and Pisa. Mean values ± SD (n = 3) are shown. The results for TPC and TFC are expressed both as content and yield.

		Bologna	Pisa
TPC	Content (mg GAE g^{-1})	6.9± 0.3	7 ± 1
	Yield (kg GAE ha^{-1})	1.9 ± 0.2b	3.4 ± 0.3a
TFC	Content (mg CAE g^{-1})	6.1 ± 0.5	6 ± 1
	Yield (kg CAE ha^{-1})	1.7± 0.3b	2.8 ± 0.5a
AC * EC50	ABTS•+ mg mL^{-1}	2.9 ± 0.5	3.0 ± 0.9
	DPPH• mg mL^{-1}	1.8 ± 0.4	1.9 ± 0.4

Values followed by different letters are significantly different at $P<0.05$ using LSD test.
* EC_{50} = mg of defatted seed meal mL^{-1} of extract required to obtain 50% DPPH• or ABTS•+ scavenging in the assay conditions.

Table V - Lignin, hemicellulose, cellulose and ash content in *Camelina sativa* cv. Italia straw derived from crops cultivated for two consecutive years years (value are averaged over 2014 and 2015 years) in Bologna and Pisa.
Mean values ± SD (n = 3) are shown. Results are expressed both as % on dry matter basis and field production (Mg ha^{-1}).

		Bologna	Pisa
Lignin	%	15.2 ± 0.6a	13.9 ± 0.7b
	Mg ha^{-1}	0.40 ± 0.2 0	0.37 ± 0.01
Hemicellulose	%	9.4 ± 0.8b	19 ± 1a
	Mg ha^{-1}	0.30 ± 0.1b	0.49 ± 0.03a
Cellulose	%	37 ± 2b	44 ± 2a
	Mg ha^{-1}	1.0 ± 0.3	1.17 ± 0.02
Ash	%	5.3 ± 0.6	5.7 ± 0.8
	Mg ha^{-1}	0.14 ± 0.05	0.15 ± 0.02

Values followed by different letters are significantly different at $P<0.05$ by using LSD test.

ferences in sowing time between the two locations and, consequently, the climatic conditions occurred during seed ripening, may influence the oil synthesis phase, which was probably longer in Bologna than in Pisa (see Fig.1).
With specific reference to fatty acid profiles, a positive correlation between N rate and oleic acid and a negative one between N rate and linolenic acid were previously observed, and the inverse relationship between the two fatty acids was partially explained by the known conversion of oleic acid to linolenic acid in angiosperms [37]. The lower PUFA/MUFA ratio registered in Pisa could be due to the higher temperatures at seed ripening phase - mean temperature during the last sixty days before harvest time in Pisa and in Bologna were 22 and 20°C respectively - that interfered with the activity of enzymes responsible for PUFA metabolism [34, 41, 42]. With regard to phenolic and flavonoid contents, reported in Table IV, the results well overlapped recent literature [43], considering that the amount of phenolic compounds mostly accounts free phenols. The overall yields of bioactive molecules that is GSLs and phenols, per hectare were lower in the defatted meals obtained from the Bologna trial, because of lower seed yields if compared to those from Pisa trial, thus Pisa production seemed more convenient for industrial applications. On the other hand, in the face of lower yields, sustainable production methods, even if less profitable, can ensure healthier products for food and pharma applications.
Finally, if we consider the residual above ground biomass, even if lower than those produced by other oilseed crops, these residues could impact on soil fertility and on the economic sustainability of the entire cropping system, especially considering that Camelina requires, during cultivation, low agronomical input. In fact, other authors already observed that, if the fallow period in wheat-based cropping systems is replaced by Camelina as cover crop, the crop residue incorporated into the soil is much greater in Camelina–wheat than in fallow–wheat rotation, which is expected to improve soil quality and productivity on long term [44]. On the other hand, a full biomass exploitation approach could increase the economical sustainability of this crop if Camelina straws could be converted into bioethanol, biogas, biochemicals and nanofibrillated cellulose (NFC). Actually, the low content of hemicellulose found in our experiments could lead to a poor nanofibrillation aptitude of the fibres of Camelina [45]. Recently, the thermochemical valorisation of Camelina straws were investigated with the aim of obtaining bio-oil via thermal or catalytic fast pyrolysis that would be the first stage on a sequence of chemical processes for biofuel production [46]. In our open field trials, Camelina showed interesting characteristics as feedstock for pyrolysis because its low protein content reduces the nitrogen and sulphur content, making the hydro processing required to remove impurities less intensive. At the same time, it showed a good adaptability in Northern and Central Italy pedo-climatic conditions that could guarantee a two-up annual yield that is strongly required for the maintenance of food and pharma industries production.

4. CONCLUSIONS

In this paper, new perspectives for the uses and the valorisation of Camelina in a green chemistry approach based on the integral exploitation of biomasses were pointed out. The presence of many macromolecules and bioactive molecules per hectare opens to several industrial applications for Camelina. While an integrated pest and weed management

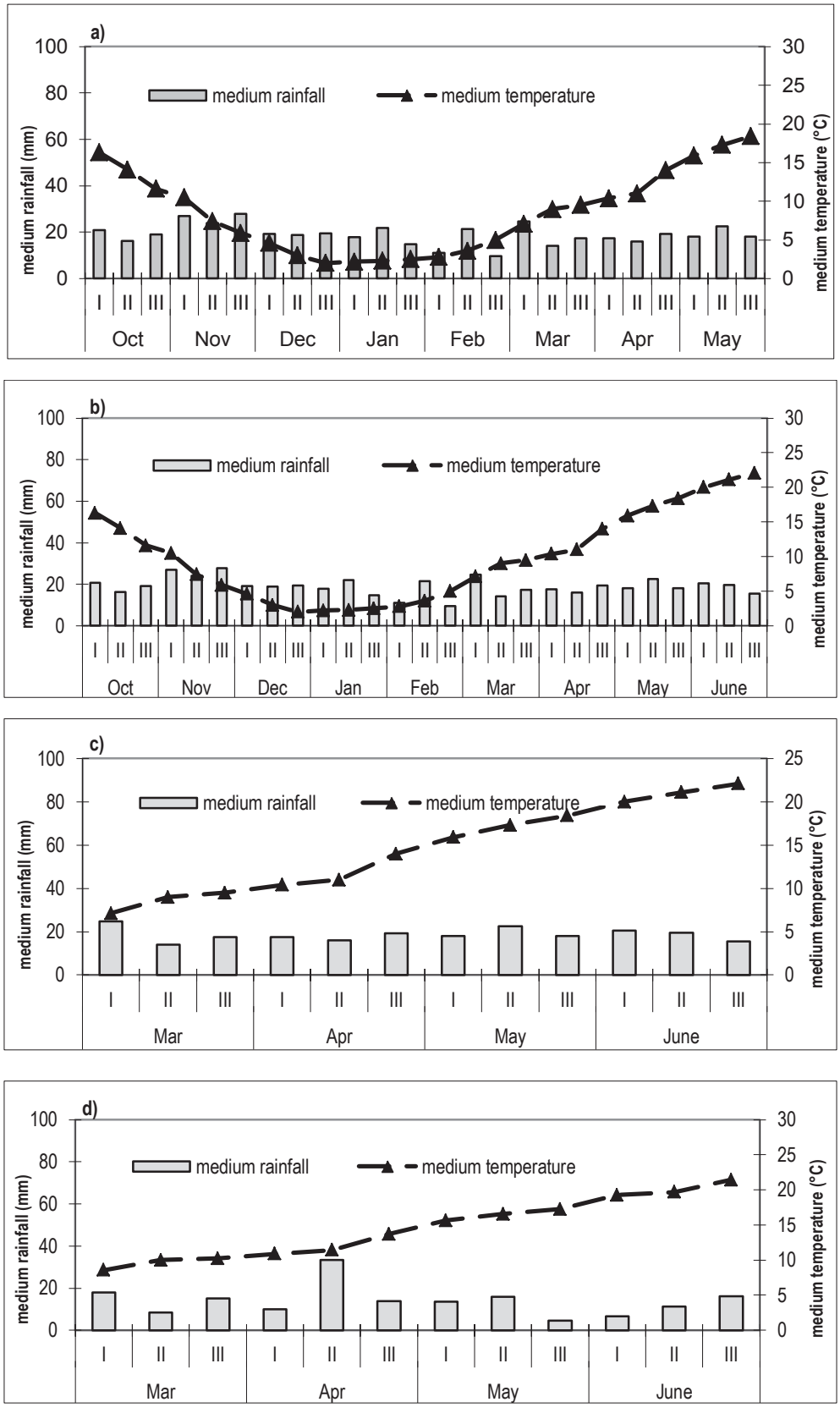

Figure 1 - Meteorological data of growing seasons in Bologna (a,b) and Pisa (c,d). a) from October 2013 to May 2014; b) from October 2014 to June 2015; c) from March 2014 to June 2014; from March 2015 to June 2015. Data represents average maximum and minimum temperatures and total rainfall per decade.

seemed to be more productive and, therefore, more suitable for industrial exploitation, a very low input approach could provide safest and healthiest bio-based products. This is very important when the molecular features of Camelina are examined for their healthy values. In fact, insufficiently validated health claims and aggressive marketing strategies may lead to unsafe fresh or processed agricultural products when the total production chain is not properly controlled.

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REFERENCES

[1] D. Kurasiak-Popowskaa, A. Tomkowiaka, M. Człopińskab, J. Bocianowskic, D. Weigta, J. Nawracałaa. Analysis of yield and genetic similarity of Polish and Ukrainian *Camelina sativa* genotypes. Industrial Crops & Products. Vol. *123*, 667–675 (2018).

[2] M. Leclère, C. Loyce, M.H. Jeuffroy. Growing camelina as a second crop in France: A participatory design approach to produce actionable knowledge. European Journal of Agronomy. Vol. *101*, 78–89 (2018).

[3] M.K. Waliaa, M.S. Wellsa, J. Cubinsa, D. Wysea, R.D. Gardnerb, F. Forcellac, R. Geschc. Winter camelina seed yield and quality responses to harvest time. Industrial Crops & Products. Vol. *124*, 765–775 (2018).

[4] J. Vollmann, C. Eynck. Camelina as a sustainable oilseed crop: contributions of plant breeding and genetic engineering. Biotechnology Journal. Vol. *10*, 525-35 (2015).

[5] J. Zubr, Carbohydrates, vitamins and minerals of *Camelina sativa* seed. *Nutrition & Food Science*. Vol. *40* (5), 523-531 (2010).

[6] E.A. Waraich, Z. Ahmed, R. Ahmad, M. Yasin Ashraf, N.V. Saifullah, M.S. Naeem, Z. Rengel. Camelina sativa, a climate proof crop, has high nutritive value and multiple-uses: A review. Australian Journal of Crop Science. Vol. *7* (10), 1551-1559 (2013).

[7] Official Journal of the European Union 2012: Commission Regulation (EU) No 432/2012 of 16 May 2012 Establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. L136/1-L136/40 (2012).

[8] E.J. Baker, E.A. Miles, G.C. Burdge, P. Yaqoob, P.C. Calder. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. Progress in Lipid Research. Vol. *64*, 30-56 (2016).

[9] G.E. Billman. The effects of omega-3 polyunsaturated fatty acids on cardiac rhythm: a critical reassessment. Pharmacology & Therapeutics. Vol. *140*, 53-80 (2013).

[10] U.S. Schwab, M.A. Lankinen, V.D. de Mello, S.M. Manninen, S. Kurl, K.J. Pulkki, D.E. Laaksonen, A.T. Erkkila. Camelina Sativa Oil, but not Fatty Fish or Lean Fish, Improves Serum Lipid Profile in Subjects with Impaired Glucose Metabolism-A Randomized Controlled Trial. Molecular nutrition & food research. Vol. *62* (4), Article number 1700503 (2018).

[11] I. Herr, M.W. Buchler. Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. Cancer Treatment Reviews. Vol. *36*, 377-83 (2010).

[12] Sulforaphane glucosinolate monograph. Alternative Medicine Review Vol. *15* (4), 352-360 (2010).

[13] Y. Fuke, Y. Haga, H. Ono, T. Nomura, K. Ryoyama,. Anti-carcinogenic activity of 6-methylsulfinylhexyl isothiocyanate-, an active anti-proliferative principal of wasabi (*Eutrema wasabi* Maxim.). Cytotechnology. Vol. *25*, 197-203 (1997).

[14] P. Rose, K. Faulkner, G. Williamson, R. Mithen. 7-Methylsulfinylheptyl and 8-methylsulfinyloctyl isothiocyanates from watercress are potent inducers of phase II enzymes. Carcinogenesis. Vol. *21*, 1983-8 (2000).

[15] N. Das, M.A. Berhow, D. Angelino, E.H. Jeffery. Camelina sativa defatted seed meal contains both alkyl sulfinyl glucosinolates and quercetin that synergize bioactivity. Journal of Agricultural and Food Chemistry. Vol. *62*, 8385-91 (2014).

[16] M. Haack, M. Lowinger, D. Lippmann, A. Kipp, E. Pagnotta, R. Iori, B.H. Monien, H. Glatt, M.N. Brauer, L.A. Wessjohann, *et al.* Breakdown products of neoglucobrassicin inhibit activation of Nrf2 target genes mediated by myrosinase-derived glucoraphanin hydrolysis products. Biological chemistry. Vol. *391* (11), 1281-1293 (2010).

[17] S. Melega, D. Canistro, E. Pagnotta, R. Iori,

A. Sapone, M. Paolini. Effect of sprout extract from Tuscan black cabbage on xenobiotic-metabolizing and antioxidant enzymes in rat liver. Mutation Research - Genetic Toxicology and Environmental Mutagenesis. Vol. *751*, 45-51 (2013).

[18] Y. Zhang, P. Talalay, C.G. Cho, G.H. Posner. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proceedings of the National Academy of Sciences U S A. Vol. *89*, 2399-403 (1992).

[19] T. Sivapalan, A. Melchini, S. Saha, P.W. Needs, M.H. Traka, H. Tapp, J.R. Dainty, R.F. Mithen. Bioavailability of Glucoraphanin and Sulforaphane from High-Glucoraphanin Broccoli. Molecular Nutrition & Food Research. Vol. *62* (18), Article number e1700911 (2018).

[20] T.W. Kensler, J.G. Chen, P.A. Egner, J.W. Fahey, L.P. Jacobson, K.K. Stephenson, L. Ye, J.L. Coady, J.B. Wang, Y. Wu, *et al*. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. Cancer epidemiology, biomarkers & Prevention. Vol. *14*, 2605-2613 (2005).

[21] K. Kumar, R. Pathak. Phytochemical analysis and assessment of in vitro antibacterial activity of non-polar solvent based Camelina seed extracts. Indian Journal of Plant Physiology. Vol. *21* (3), 255-262 (2016).

[22] K. Kumar, S.M. Gupta, M.C. Arya, M. Nasim. In Vitro Antimicrobial and Antioxidant Activity of Camelina Seed Extracts as Potential Source of Bioactive Compounds. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. Vol. *87* (2), 521-526 (2017).

[23] L. Lazzeri, L. Malaguti, S. Cinti, L. Ugolini, G.R. De Nicola, M. Bagatta, N. Casadei, L. D'Avino, R. Matteo, G. Patalano. The Brassicaceae biofumigation system for plant cultivation and defence. An Italian twenty-year experience of study and application. Acta Horticulturae. Vol. *1005*, 375-382 (2013).

[24] ISO 10565:1998. Oilseeds - Simultaneous determination of oil and water contents - Method using pulsed nuclear magnetic resonance spectrometry. International Organization for Standardization (1998).

[25] ISO 659:2009(en) Oilseeds — Determination of oil content (Reference method) (2009).

[26] ISO 12966-4:2015(en). Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 4: Determination by capillary gas chromatography (2015).

[27] ASTM D5373.2016 Standard Test Methods for Determination of Carbon, Hydrogen and Nitrogen in Analysis Samples of Coal and Carbon in Analysis Samples of Coal and Coke. ASTM International, West Conshohocken, PA.

[28] ISO 9167-1:1992/Amd 1:2013. Graines de colza – Dosage des glucosinolates – Partie 1:Methode par chromatographie liquide à haute performance (1992).

[29] E. Pagnotta, N. Agerbirk, C.E. Olsen, L. Ugolini, S. Cinti, L. Lazzeri. Hydroxyl and Methoxyl Derivatives of Benzylglucosinolate in Lepidium densiflorum with Hydrolysis to Isothiocyanates and non-Isothiocyanate Products: Substitution Governs Product Type and Mass Spectral Fragmentation. Journal of Agricultural and Food Chemistry. Vol. *65*, 3167-78 (2017).

[30] R. Russo, I. Galasso, R. Reggiani. Variability in Glucosinolate Content among Camelina Species. American Journal of Plant Sciences. Vol. *5* (3), 294-298 (2014).

[31] V. Dewanto, X. Wu, K.K. Adom, R.H. Liu. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry. Vol. *50*, 3010-4 (2002).

[32] L. Jing, H. Ma, P. Fan, R. Gao, Z. Jia. Antioxidant potential, total phenolic and total flavonoid contents of Rhododendron anthopogonoides and its protective effect on hypoxia-induced injury in PC12 cells. BMC Complementary and Alternative Medicine. Vol. *15*, Article number 287 (2015).

[33] P.J. Van Soest, J.B. Robertson, B.A. Lewis. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science. Vol. *74* (10), 3583-3597 (1991).

[34] J. Vollmann, T. Moritz, C. Kargl, S. Baumgartner, H. Wagentristl. Agronomic evaluation of camelina genotypes selected for seed quality characteristics. Industrial Crops and Products. Vol. *26* (3), 270-277 (2007).

[35] F.H. Chilton, R. Dutta, L.M. Reynolds, S. Sergeant, R.A. Mathias, M.C. Seeds. Precision Nutrition and Omega-3 Polyunsaturated Fatty Acids: A Case for Personalized Supplementation Approaches for the Prevention and Management of Human Diseases. Nutrients. Vol. *9*, Article number 1165 (2017).

[36] A. Chaker, P. Mutje´, M.R. Vilar, S. Boufi. Agriculture crop residues as a source for the production of nanofibrillated cellulose with low energy demand. Cellulose. Vol. *21*, 4247–4259 (2014).

[37] S.D. Urbaniak, C.D. Caldwell, V.D. Zheljazkov, R. Lada, L. Luan. The effect of cultivar and applied nitrogen on the performance of Camelina sativa L. in the Maritime Provinces of Canada.

Canadian Journal of Plant Science. Vol. *88* (1), 111-119 (2008).
[38] A. Solis, I. Vidal, L. Paulino, B.L. Johnson, M.T. Berti. Camelina seed yield response to nitrogen, sulfur, and phosphorus fertilizer in South Central Chile. Industrial Crops and Products. Vol. *44*, 132-8 (2013).
[39] D.J. Wysocki, T.G. Chastain, W.F. Schillinger, S.O. Guy, R.S. Karow. Camelina: Seed yield response to applied nitrogen and sulfur. Field Crops Research. Vol. *145*, 60-66 (2013).
[40] P. Pecchia, R. Russo, I. Brambilla, R. Reggiani, S. Mapelli. Biochemical seed traits of Camelina sativa - an emerging oilseed crop for biofuel: environmental and genetic influences. Journal of Crop Improvement. Vol. *28*, 465-483 (2014).
[41] T.M. Cheesbrough. Changes in the enzymes for fatty acid synthesis and desaturation during acclimation of developing soybean seeds to altered growth temperature. Plant Physiology. Vol. *90*, 760–764 (1989).
[42] J. Zubr, Qualitative variation of Camelina sativa seed from different locations. Industrial Crops and Products. Vol. *17* (3), 161-169 (2003).
[43] M.J. Rahman, A. Costa De Camargo, F. Shahidi. Phenolic profiles and antioxidant activity of defatted camelina and sophia seeds. Food Chemistry. Vol. *240*, 917-25 (2018).
[44] C. Chen, A. Bekkerman, R.K. Afshar, K. Neill. Intensification of dryland cropping systems for bio-feedstock production: Evaluation of agronomic and economic benefits of Camelina sativa. Industrial Crops and Products. Vol. *71*, 114-121 (2015).
[45] A. Chaker, P. Mutje, F. Vilaseca, S. Boufi. Reinforcing potential of nanofibrillated cellulose from nonwoody plants. Polymer Composites. Vol. *34*, 1999-2007 (2014).
[46] H. Hernando, J. Fermoso, I. Moreno, J.M. Coronado, D.P. Serrano, P. Pizarro. Thermochemical valorization of camelina straw waste via fast pyrolysis. Biomass Convers. Biorefinery. Vol. *7* (3), 277-287 (2017).

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