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Oil content, fatty acid and phytosterol compositions of chia seeds cultivated under organic conditions in France☆

Gwendoline Gravé[1], Zephirin Mouloungui[1], Muriel Cerny[1], Eric Lacroux[1], Romain Valentin[1], Jean-François Fabre[1] and Othmane Merah[1,2,*]

[1] Laboratoire de Chimie Agro-industrielle, LCA, Université de Toulouse, INRA, Toulouse, France
[2] Université Paul Sabatier, IUT A, Département Génie Biologique, 32000 Auch, France

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Abstract – Chia (*Salvia hispanica* L.) is a candidate to be included in European diet due to its seed oil content and composition. Its cultivation in Europe has been assessed in several European countries by introducing genotypes from Latin America. The cultivar Oruro (released by Panam Company, France) was used in this study and cultivated at two locations of southern West of France (Lavaur and Samatan). The aims of this study were to investigate the oil content and composition of chia seeds cultivated in two locations under organic conditions in southern West of France. As expected, location factor affected oil content. Moreover, this factor affected also fatty acid, sterols and tocopherols content and composition. Lavaur presented higher oil content than Samatan. This later location presented higher temperature during grain filling period. Polyunsaturated fatty acids were more represented at Lavaur than at Samatan. Saturated and monounsaturated fatty acids showed the opposite trends. Sterols and tocopherols were more accumulated in chia seeds at Lavaur. These results showed that all quality traits of chia seed cultivated in France were affected by climatic conditions prevailing in location of cultivation.

Keywords: Spanish sage / *Salvia hispanica* L. / location / lipid composition / climatic conditions

Résumé – Teneur en huile, composition en acides gras et en phytostérols des graines de chia cultivées en agriculture biologique en France. Le chia (*Salvia hispanica* L.) est un candidat pour être inclus dans le régime alimentaire européen en raison de la teneur en huile de ses graines et de sa composition. Sa culture en Europe a été évaluée dans plusieurs pays européens en introduisant des génotypes d'Amérique latine. Le cultivar Oruro (sélectionné par la compagnie Panam, France) a été utilisé dans cette étude et cultivé sur deux sites du sud-ouest de la France (Lavaur et Samatan). Les objectifs de cette étude étaient d'examiner la teneur en huile et la composition des graines de chia cultivées dans deux sites en agriculture biologique dans le sud-ouest de la France. Comme prévu, le facteur site a affecté la teneur en huile. De plus, ce facteur a également altéré la teneur et la composition en acides gras, stérols et tocophérols. Lavaur a présenté une teneur en huile plus élevée que Samatan. Cette dernière localité présentait une température plus élevée pendant la période de remplissage du grain. Les acides gras polyinsaturés étaient plus importants à Lavaur qu'à Samatan. Les acides gras saturés et monoinsaturés ont montré des tendances opposées. Les stérols et les tocophérols étaient plus accumulés dans les graines de chia à Lavaur. Ces résultats ont montré que tous les traits de qualité des graines de chia cultivées en France étaient affectés par les conditions climatiques prévalant dans le lieu de culture.

Mots clés : sauge espagnole / *Salvia hispanica* L. / site / composition lipidique / conditions climatiques

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*Correspondence: othmane.merah@esiacet.fr

1 Introduction

Among the Lamiaceae family, known for its aromatic species used in both culinary dishes and medicinal purposes, *Salvia hispanica* L. (called chia or Spanish sage), an ancient Central American crop (Capitani *et al.*, 2012), had attracted large considerations. Indeed, this species contains an important oil level which rich in omega-3 and omega-6 (Zettel and Hitzmann, 2018; Knez-Hrnčič *et al.*, 2019). Moreover, this species possesses important concentration in fibers and contains all essential amino acids for the human nutrition (Grancieri *et al.*, 2019; Kulczyński *et al.*, 2019). Spanish sage that can be called a multiuse plant, offers several industrial purposes in functional foods, baked goods, dairy and fish products, pharmaceutics and cosmetics (Zettel and Hitzmann, 2018; Knez-Hrnčič *et al.*, 2019; Muñoz-González *et al.*, 2019; Câmara *et al.*, 2020; Mas *et al.*, 2020). This species have been reported to exhibit also several benefits for human health (Valenzuela *et al.*, 2015). Indeed, numerous studies and reviews have reported the impact of chia consumption on health. Supply of chia in rat feed rich in sucrose, induced a decrease of free triglycerides and hyperglycaemia (Ferreira *et al.*, 2020). Anti-inflammatory effects and osteoarthritis prevention have been shown in albino rats (Arpitha *et al.*, 2019). Antioxidant, anticholesterolemic, antihypertensive, hypoglycemic, effects were also noticed (Grancieri *et al.*, 2019). Chia seeds present a major action on health by increasing the absorption of glucose, its oxidation and the restoration of sensitivity of tissues to insulin supply (Enes *et al.*, 2020). A body weight gain, a bone mineral density and content increase with a diet including chia seeds were reported (Chañi *et al.*, 2018).

Our reports have studied the chemical composition of different oil crops species as Brassicaceae (rapeseed and woad), shell seeds (sunflower and safflower) and oleo-proteaginous and mucilaginous seeds (flax, camelina; Roche *et al.*, 2010a, 2010b, 2016, 2019: Fabre *et al.*, 2015, 2020: Zemour *et al.*, 2021: Cassen *et al.*, 2022). In a first publication, we studied the accumulation of oil and both fatty acid and sterols composition during seed development and filling of chia (Gravé *et al.*, 2019). This species contains high level of oil with polyunsaturated fatty acids, phytosterols and tocopherols (Zettel and Hitzmann, 2018; Grimes *et al.*, 2019). It is well known that these bioactives are dependent on environmental and genotypic factors and crop management (Ayerza, 1995, 2010; Gravé *et al.*, 2019; Grimes *et al.*, 2019). Obviously, these studies concerned South American countries. In European Union, attract for chia seed in human food and health augmented during the last decade (Gravé *et al.*, 2019; Grimes *et al.*, 2019) due mainly to acceptation of chia as novel food ingredient (CE-EU, 2009, 2013). Therefore, studies have been carried out to introduce the cultivation of Spanish sage in Europe (Bochicchio *et al.*, 2015; Karkanis *et al.*, 2018; Grimes *et al.*, 2019) by examining South American cultivars. Oruro, is a first released cultivar (Panam Company, Villemur-sur-Tarn, southern West of France, registration number 20332) adapted to European conditions It is cultivated since 2013 in South of France (Gravé *et al.*, 2019). There is no information concerning effect of location on biochemical traits of Spanish sage, in France. Therefore, this work aims to compare effect of location (climatic conditions) on biochemical traits of the cultivar Oruro cultivated under organic conditions at two locations in southern West of France. This concerns oil, protein, ash, fatty acid, sterol and tocopherol contents.

2 Materials and methods

2.1 Plant material and weather conditions

Two field-based participatory experiments were carried out under organic cultivation at Samatan (53 km southern West of Toulouse, 43° 29′ 37″ N, 0° 55′ 54″ E) in 2017 and Lavaur (44 km East of Toulouse, 43° 41′ 59″ N, 1° 49′ 11″ E) in 2015. Oruro cultivar (Panam Company, France) was used for experiments. It is long days variety with a cycle of 150 days. It exhibits cream–brown seeds (Fig. 1). The trials were carried out on the basis of participatory experimentation. The choice of sites and years was based on the farmers involved with the Panam Company.

Based on Panam recommendations, seeds were sown at a density of 4 kg.ha^{-1} at 1 cm deep. The cultivation characteristics are presented in Table 1. The crops were cultivated under rainfed conditions.

Table 2 presents rainfall and temperatures during the plant cycle. The two cropping seasons were contrasted for their climatic conditions. Samatan was the warmest and rainiest location. Indeed, Samatan presented 2 °C and nearly 59 mm of rainfall more than Lavaur during whole plant cycle. During the grain filling period (August to October), Samatan presented higher temperatures (2 °C more than Lavaur) and quite similar rainfall amount (Tab. 2) as Lavaur.

2.2 Extraction and determination of fatty acids, sterol and tocopherol contents and compositions

The composition of fatty acids was assessed after esterification (Standards ISO 5509:1990). One gram of seeds (containing about 300 mg of oil) were mixed with 10 mL of cyclohexane. The mix was then centrifuged at 10 000 g during 15 min at ambient temperature. The extractions were carried out in triplicates and the organic phases were collected. They were then filtered on anhydrous Na_2SO_4 and dried under nitrogen. The fatty acid profile of oil was revealed after trans-methylation with TMSH (0.2 M trimethylsulfonium hydroxide in methanol) based on AFNOR Method NF EN ISO 12966-3 (ISO 5983-1, 2005). A 15 mg sample of oil was solubilized in 1 mL of tert-butyl methyl ether (TBME). One hundred µL of this solution were then methylated with 50 µL TMSH. The fatty acids methyl esters (FAME) were analysed by gas chromatography (Roche *et al.*, 2010a). The column used was a CP-select CB (50 m long, 0.32 mm i.d., 0.25 µm film thickness); helium was used as the carrier gas, at a flow rate of 1.2 mL/min; split injector (1:100) and FID were maintained at 250 °C; the initial oven temperature was set at 185 °C for 40 min, increased to 250 °C at a rate of 15 °C/min and maintained at this temperature for 10 min. Sterol content was also determined by gas chromatography. Fifty microliters of a 2 mg/mL solution of cholestanol in chloroform were introduced into a 15 mL screw top tube. Chloroform was then eliminated through nitrogen flow and 100 mg of oil then 2 mL

Fig. 1. Plants (A), inflorescences (B) and seeds (C) of chia (cultivar Oruro) cultivated in South-West France.

of 1 M KOH in ethanol were added. The tube was then vortexed and heated at 75 °C (water bath) during 30 minutes. After cooling to ambient temperature, 1 mL of distilled water was added and the mixture was vortexed again. The unsaponifiable material was then extracted with 6 mL of cyclohexane. One hundred sixty microliters of the cyclohexane phase were then silylated with 40 μL of BSTFA/TMCS (99/1 mixture). The sample was quickly heated in an oven at 103 °C and then analysed through gas chromatography. One microliter was injected into the Perkin Elmer device equipped with an Agilent VF-5ms column (30 m long, internal diameter of 0.25 mm, film thickness of 0.25 μm) and coupled to a flame ionization detector operating at 355 °C. The carrier gas was helium, at a column head pressure of 100 kPa. The thermal program was, for the injector: 55 °C for 0.5 min, 55 °C to 340 °C at a rate of 200 °C/min, 340 °C for 30 min; For the oven, it was: 160 °C for 0.5 min, 160 °C to 260 °C at a rate of 20 °C/min, then of 2 °C/min to 300 °C and of 45 °C/min to 350 °C. Commercial standards or reference values were utilized to identify the different sterols and an internal standard was used for quantification.

Content of tocopherol was assessed using high-performance liquid chromatography. Ten milligrams of oil were added to 1 mL of cyclohexane. Twenty microliters of the solution were injected into a Dionex chromatograph equipped with a Kromasil 100 SIL 5 μm column (250 mm long, with an internal diameter of 4 mm). The mobile phase was composed of 99.5% isooctane and 0.5% isopropanol (v/v) and was used with a flow rate of 1.1 mL/min. The detector was a Dionex fluorimeter with an excitation and an emission wavelength of 290 nm of 317 nm, respectively. Reference standards were employed to identify the tocopherols and an internal standard was used for quantification.

Protein and ash contents were evaluated on seed according to ISO 5983-1 (2005) and ISO 749 (1977), respectively. Moreover, the factor of conversion (NPCF) between total *N* and protein was 5.3 calculated according to Mosse (1990) and Gosukonda (2020).

2.3 Statistical data analysis

The statistical analyses were carried out with Sigmastat (Ver. 2.0, USA) package. The analyses of variance to determine the significance between locations for all measured traits. Duncan's test was used to compare means at $p < 0.05$ probability level.

3 Results and discussion

3.1 Oil, protein and ash content

Table 3 presents mean values of fatty acid composition, oil, protein and ash contents of seeds harvested in the two locations. Both ash and protein contents were not affected by location. In contrast, oil content was 2.5% higher in Lavaur (2015) than in Samatan (2017).

The noticed oil content variation is within the range of values already reported in different countries depending on genotype and selection, location, weather conditions, ripening stages of seed, and methods of extraction (Ayerza, 1995, 2010; Ayerza and Coates, 2004; Sandoval-Oliveros and Paredes-López, 2013; Coelho and Salas-Mellado, 2014; Amato *et al.*, 2015; Dąbrowski *et al.*, 2017; Gravé *et al.*, 2019). The range observed with Oruro under our experimental conditions is higher than the one observed in Germany under different agricultural practices (Grimes *et al.*, 2019). These differences may be due to genotype, climatic conditions, cultivation practices or all factors together. Surprisingly, protein content in chia seeds was not significantly affected by location (Tab. 3). Observed values are within the range (12.3–26.0) already reported depending on location, genotype, climatic conditions and agricultural practices (Ayerza, 2010; Ayerza and Coates, 2004; Sandoval-Oliveros and Paredes-López, 2013; Coelho and Salas-Mellado, 2014; Grimes *et al.*, 2019). Nevertheless, it is necessary to notice that, generally, the conversion factor used between *N* content and protein level is 6.25. This value allows for a higher crude protein content and may introduce differences between studies. We have used a factor of 5.3 which is more adapted to high protein level material (Mosse, 1990; Gosukonda, 2020). This fact mirrored that crude protein content observed in our work should be considered to be higher than the apparent results in other studies that have used 6.25 as conversion factor.

3.2 Fatty acid composition

Fatty acid profiles observed in two locations are presented in Table 3. Expectedly, high proportion of polyunsaturated

Table 1. Cultivation characteristics and practices on chia under organic condition at Lavaur and Samatan (South of France).

Location	Year	Sowing	Flowering	Maturity	Cycle (days)	Fertilization	Practice
Lavaur	2015	13th May	18th Aug	13th Oct	154	60 units ha^{-1} of crushed feathers	Weeds were eliminated mechanically
Samatan	2017	25nd May	30th Aug	24th Oct	151		

Table 2. Mean monthly temperatures and rainfall in two locations: Lavaur (East of Toulouse, in 2015) and at Samatan (southern West of Toulouse, in 2017).

Month	Lavaur		Samatan	
	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)
May	64.8	15.8	118.4	17.6
June	65.3	19.7	37.9	22.4
July	35.3	21.6	51.9	22.5
August	35.4	21.8	37.1	28.8
September	60.4	18.4	61.6	17.0
October	17.2	14.9	22.3	16.0
November	43.2	9.1	26.2	7.5
December	78.2	5.6	62.2	6.9
Mean May–Oct		*18.7*		20.7
Sum May–Oct	278.4		332.2	
Mean Aug–Oct		*18.4*		*20.6*
Sum Aug–Oct	*113.0*		*121.0*	

Table 3. Fatty acid composition, oil, protein, and ash contents of chia seeds cultivated at two locations in South of France under organic conditions.

Fatty acid (%)	Lavaur	Samatan	Effect
	2015	2017	Location
C16:0	6.9 ± 0.1b	7.3 ± 0.1a	**
C18:0	3.0 ± 0.1b	3.5 ± 0.2a	***
SFA	**9.8 ± 0.1b**	**10.9 ± 0.2a**	***
C18:1n7	0.8 ± 0.0a	0.8 ± 0.0a	ns
C18:1n9c	5.3 ± 0.2b	5.9 ± 0.2a	*
MUFA	**6.1 ± 0.1b**	**6.7 ± 0.2a**	***
C18: 2n6	18.0 ± 0.2a	17.7 ± 0.1a	ns
C18:3n3	66.1 ± 0.2a	64.7 ± 0.3b	***
PUFA	**84.1 ± 0.1a**	**82.4 ± 0.4b**	***
SFA/MUFA	*1.6*	*1.6*	
SFA/PUFA	*0.12*	*0.13*	
ω6/ω3	*0.27*	*0.27*	
OY (%)	*32.9 ± 0.1a*	*30.4 ± 0.1b*	***
Ash (%)	*4.7 ± 0.1a*	*4.7 ± 0.1a*	ns
Proteins (%)	*19.6 ± 0.1a*	*19.8 ± 0.1a*	ns

Data were means ± S.D of three replicates. C16:0 (palmitic acid); C18:0 (stearic acid); C18:1n7 (vaccenic acid); C18:1n9c (oleic acid); C18: 2n6c (linoleic acid); C18:3n3 (linolenic acid); SFA/UFA: ratio saturated/unsaturated fatty acids; ω3/ω6: ratio linolenic acid/linoleic acid; OY: oil yield. In same line, means with the same letter are not significantly different at 0.05 probability level. *, ** and *** are significant effect of studied factor at 0.05, 0.01 and 0.001 probability levels, respectively. ns: not significant.

Table 4. Sterol and tocopherol content and composition in chia seeds cultivated at two locations in southern West of France.

Sterol (mg/100 g seed)	Lavaur 2015	Samatan 2017	Effect Location
Cycloartenol	6.1 ± 0.3a	2.5 ± 0.2b	**
24 methylene cycloartenol	5.3 ± 0.3a	3.4 ± 0.4b	**
dimethylsterols	**11.4 ± 0.3a**	**5.9 ± 0.4b**	***
Gramisterol	6.7 ± 0.4a	5.8 ± 0.1b	***
Citrostadienol	6.8 ± 0.4a	5.5 ± 0.2b	***
Methylsterols	**13.5 ± 0.6a**	**11.3 ± 0.3b**	***
Campesterol	25.2 ± 0.5a	19.3 ± 5.7b	***
Stigmasterol	11.4 ± 0.8a	8.1 ± 0.7b	***
β-sitosterol	140 ± 1a	98.9 ± 8.6b	***
Δ5-Avenasterol	14.8 ± 1.0a	9.5 ± 0.2b	***
Δ7-stigmastenol	1.0 ± 0.1	nd	*
Δ7-avenasterol	1.5 ± 0.3	nd	*
Desmethysterols	**193 ± 3a**	**136 ± 15b**	***
Total sterols	*218a*	*153b*	***
α tocopherol	0.61 ± 0.09a	0.43 ± 0.07a	ns
γ tocopherol	49.7 ± 0.7a	33.6 ± 0.2b	***
δ tocopherol	2.4 ± 0.1a	1.6 ± 0.2d	**
Total tocopherols	***52.6 ± 0.6a***	***35.6 ± 0.2b***	***

Data were means ± S.D of three replicates. In same line, means with the same letter are not significantly different at 0.05 probability level. *, ** and *** are significant effect of studied factor at 0.05, 0.01 and 0.001 probability levels, respectively. ns: not significant; nd: not detected.

fatty acids (PUFA) was observed. A range of 64.7–66.1% for α-linolenic (C18:3 n-3) and 82.4–84.1% for PUFA were observed. These values are higher than results reported in Germany (Grimes *et al.*, 2019), Canada (Abad and Shahidi, 2020), Poland (Dąbrowski *et al.*, 2017), Argentina, Bolivia and Ecuador (Ayerza, 1995, 2010). Our results are ranged similarly as those reported for stage of seed ripening (Gravé *et al.*, 2019), or locations (Ayerza and Coates, 2004).

The FA profile, at Lavaur, was characterized by 1.1% and 0.6% lower levels of saturated (SFA) and monounsaturated (MUFA) fatty acids than at Samatan. Conversely, PUFA were 2% higher at Lavaur compared to Samatan (Tab. 3), which affected accordingly the ratio of SFA to PUFA (Tab. 3). As we used one genotype, these profile differences may be explained by impact of location or environment parameters (Ayerza, 1995, 2010). This is supported by higher temperatures (2 °C difference during whole grain filling period) at Samatan than at Lavaur (Tab. 1). As already reported in literature, temperatures and rainfall have an important impact on fatty acid composition in several oilseed species (Gouzy *et al.*, 2016; Roche *et al.*, 2016, 2019; Merah *et al.*, 2020; Nguyen *et al.*, 2020; Zemour *et al.*, 2021). In Atlas Pistachio, Labdelli *et al.* (2019) reported differences in oil content and fatty acid composition in four locations. Indeed, both PUFA and MUFA level increased according to a gradient of climatic areas with the high of unsaturated FA was observed under rainy and temperate conditions. The same trend was observed in safflower cultivated different sowing dates. Late sowing date induced a decrease of unsaturated fatty acids (Roche *et al.*, 2019). It is well known that saturated fatty acids are first synthetized. The desaturations, which imply several enzymes, allow transformation of stearic acid into oleic, linoleic and then linolenic acids (Knez-Hrnčič *et al.*, 2019). Increase in PUFA was mainly due to the rise of SFA + MUFA (Tab. 3), which mirrored that climatic conditions affected negatively the desaturases activities which allowed to obtain lower PUFA at Samatan compared to Lavaur. In fact, lower content in PUFA was only due to lower value of linolenic acid which is probably due to the lower Δ*15 desaturase* activity. This results in higher level of linoleic acid (the precursor of the linoenic acid) was observed at Lavaur location (Tab. 3). Even not the case in our study (one cultivar used), similar differences could be observed between genotypes or ecotypes from different origins. In cumin seeds, oil content and fatty acid profile have shown to vary depending the country (Merah *et al.*, 2020). In safflower, Zemour *et al.* (2021) have reported variation in oil content and fatty acid composition according to beth climatic and ecotype factors.

In our study, ω-6/ω-3 fatty acids ratio was 0.27 regardless of location which is similar to what has already been reported elsewhere (Amato *et al.*, 2015). These results confirm that chia a healthy source of equilibrated fatty acids for European countries diet (Amato *et al.*, 2015; Gravé *et al.*, 2019).

3.3 Phytosterols and tocopherols content and composition

Similarly, to fatty acids, sterols content and composition were also impacted by location (Tab. 4). Total sterol content exhibited a wide range of variation (153–218 mg/100 g seed). The lowest values of phytosterols content were observed at Samatan. The desmethylsterols were the main group in chia, at least 88.7% (in 2015) of total sterols. Surprisingly, only few studies have reported sterol content and composition in chia seeds (Dąbrowski *et al.*, 2017). Our results showed lower values than those noticed in already published works. These differences may be due to the used genotypes, climatic conditions during cultivation, stage of seed filling, extraction and measurement methods (Merah *et al.*, 2012; Coelho and Salas-Mellado, 2014; Merah and Mouloungui, 2019).

At Samatan in 2017, climatic conditions were harsh during seed filling duration (Tab. 2). These conditions allowed to decrease accumulation of all sterols (Tab. 4). For example, cycloartenol (the early formed sterol in biosynthesis pathway) content was more than two times lower at Samatan than at Lavaur. The same trend was observed for oil and fatty acid at this location. In fact, probably, lack of water availability and higher temperatures affected lipid synthesis (Tab. 1). Moreover, in the case of phytosterols these conditions affected mainly squalene, precursor of sterols synthesis (Merah *et al.*, 2012) due to down-regulation of enzymes implicated in squalene synthesis and transformation *Squalene Synthetase*, *Squalene Epoxidase*, and *CycloArtenol Synthase*. Unfortunately, squalene was not assessed in our study.

Tocopherol content and composition of chia seeds are given in Table 4. Similarly, to other lipids, lower values of tocopherols were observed at Samatan (Tab. 4). The predominant isomer of tocopherol was β/γ tocopherol and corresponded to more than 94% of total tocopherol content (Tab. 4). These values were comparable to those obtained in previous studies. The level of these compounds is dependent on extraction method, genotype and environment (Dąbrowski *et al.*, 2017).

The cultivation of chia in the European Union has a long-term objective of ω6 balance by using a new source of healthy oil. Hispanic sage has a low ω6/ω3 ratio which surely corresponds to this objective. Our results, based on the use of a variety of chia released for cultivation in Europe. In addition, it has been shown that the intake of chia seeds in the diet (by considering the recommendations of the European Union) leads to a reduction in cardiovascular risks, hepatocellular and intestinal damages, prevention of inflammatory diseases and hypertension (Chañi *et al.*, 2018; Grancieri *et al.*, 2019; Da Silva *et al.*, 2019).

4 Conclusions

This study aimed to evaluated the impact of location on oil content, fatty acid, sterols and tocopherols compositions in Chia (*Salvia hispanica* L.) seed, cultivated in southern West of France. This is a first study that investigated effects of locations on a cultivar released for cultivation in Europe. All seed quality traits have been influenced by the location of cultivation. The obtained results emphasized the impact of locations (and their pedo-climatic conditions) on lipids content and composition of chia. Higher temperatures during grain filling of chia at Samatan, induced lower oil content and modified composition of fatty acids, sterols and tocopherols compared to Lavaur. There results mirrored that even chia can be cultivated in South of France, the climatic conditions could influence its quality content. This fact can help to manage agricultural practices to regulate the quality traits. Further investigations are necessary to explain how the climatic conditions can affect the lipid biosynthesis pathway under a large panel of locations.

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Authors' contributions

Gwendoline Gravé: Methodology; Formal analysis; Writing original draft; **Zephirin Mouloungui:** Conceptualization; Writing – review and editing; Supervision; Project administration; Project management; **Muriel Cerny:** Methodology; Formal analysis; **Eric Lacroux:** Methodology; Formal analysis; Writing original draft; **Romain Valentin:** Methodology; Writing original draft; **Jean-François Fabre:** Methodology; Formal analysis; Software; Writing original draft; **Othmane Merah:** Conceptualization; Software; Writing original draft; Writing – review and editing; Supervision; Project administration.

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