

Accumulation during fruit development of components of interest in seed of Chia (*Salvia hispanica* L.) cultivar Oruro[©] released in France[☆]

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Abstract – This study aimed to examine the accumulation of oil, fatty acids, sterols and tocopherols contents and compositions during fruit development on the new cultivar of Chia Oruro (Panam Cie, France) cultivated in France. This cultivar is the first released genotype in Europe and for cultivation in Europe. The experiment was conducted in 2017, in south-western France at the Regional centre for organic agriculture, at Auch (near Toulouse, South-West of France). Four samplings were made at 17, 24, 27 and 35 days after flowering (DAF). These dates of sampling correspond to stages 7–8 (fruit development and maturity) of BBCH scale. Oil content, fatty acid composition and sterols and tocopherols contents and profiles were determined. The highest levels of oil and tocopherols were reached at 27 DAF. In contrast, phytosterols content was highest at 17 DAF (726.2 mg/100 g of oil) and decreased after this date. β -sitosterol was the main sterol in chia seed (63.4 to 89.1% of total sterols, depending of stage of fruit development). γ -tocopherol represented more than 92% of the total tocopherols present in the seed. High levels of polyunsaturated fatty acids (74.4 to 80% of total fatty acids) were observed in our study confirming those already reported in this species. High levels of saturated and monounsaturated fatty acids were reported at earlier stages and decreased gradually during fruit development. Polyunsaturated fatty acids followed an opposite trend. Their level was lowest at 17 DAF (74.4%) and increased gradually to reach their higher level at 35 DAF (80%). These results suggested clearly the interest to harvest and use of seed before entire maturity of the grain.

Keywords: Chia / fatty acids / phytosterols / tocopherols / fruit development / accumulation

Résumé – **Accumulation des composants d'intérêt durant le développement des fruits dans la graine du cultivar français Oruro[©] de Chia (*Salvia hispanica* L.).** Cette étude a examiné l'accumulation de l'huile, des acides gras, des stérols et des tocophérols durant le développement du fruit du nouveau cultivar de Chia Oruro (Panam Cie, France) cultivé en France. Ce cultivar est le premier génotype sélectionné en Europe et pour l'Europe. L'expérimentation a été réalisée en 2017, dans le sud-ouest de la France, au Centre régional d'expérimentation en agriculture biologique, à Auch (près de Toulouse, dans le sud-ouest de la France). Quatre prélèvements ont été effectués 17, 24, 27 et 35 jours après la floraison (DAF). Ces dates d'échantillonnage correspondent aux stades 7–8 (développement du fruit et maturité) de l'échelle BBCH. La teneur en huile, la composition en acides gras et les teneurs et profils en stérols et en tocophérols ont été déterminés. Les teneurs les plus élevées d'huile et de tocophérols ont été atteintes à 27 DAF. Au contraire, la teneur en phytostérols était la plus élevée à 17 DAF (726,2 mg/100 g d'huile) et a diminué après cette date. Le β -sitostérol a été le principal stérol contenu dans les graines de chia (63,4 to 89,1 % des stérols totaux et en fonction du stade de développement du fruit). Le γ -tocophérol représente plus de 92 % du total des tocophérols présents dans la graine. Des teneurs élevées d'acides gras polyinsaturés (74,4 à 80 % des acides gras totaux) ont été observées dans notre étude, ce qui confirme celles déjà signalées chez cette espèce. Des

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teneurs plus élevées d'acides gras saturés et mono-insaturés ont été observées aux stades précoces du développement du fruit et ont diminué progressivement au cours de la maturation. Les acides gras polyinsaturés ont suivi une tendance opposée. Leur niveau était le plus bas à 17 DAF (74,4 %) et a augmenté progressivement pour atteindre leur niveau supérieur à 35 DAF (80 %). Ces résultats suggèrent clairement l'intérêt de récolter et d'utiliser les graines avant leur maturité complète.

Mots clés : Chia / acides gras / phytostérols / tocophérols / développement du fruit / accumulation

1 Introduction

Chia (*Silvia hispanica* L.), a Lamiaceae species, is an ancient Central American crop (Capitani *et al.*, 2012) recently promoted as a functional food and known as source of omega-3 and omega-6. Chia seeds contain more than 30% of oil (310 g/kg of seeds) and, particularly, a high proportion of unsaturated fatty acids present (PUFA: 82.0% and MUFA 6.9%). Chia oil also contains high levels of tocopherols and sterols (Ciftçi *et al.*, 2012). The proportions of these active compounds in chia seeds depend on several factors. Environmental differences, climatic change, the availability of essential nutrients, genotype, year of cultivation and soil type all play a crucial role in determining seed composition (Ayerza and Coates, 2009). For instance, protein levels decrease with increasing temperature (Ayerza and Coates, 2011). An inverse relationship has also been found between altitude and saturated fatty acid (SFA) levels. At low altitude, the proportion of SFA is higher in areas of higher temperature (Ayerza, 2010). In Argentina, temperature was found to influence greatly the fatty acids composition of chia oil (Ayerza, 1995). Seed coat, growing locations, genotypes were found to influence oil content, fatty acid composition of chia seed (Ayerza, 1995, 2010; Ayerza and Coates, 2009, 2011), which allowed to propose the use of these traits as criteria to determine the commercial origin of seeds of chia (Ayerza and Coates, 2011). Moreover, evolution of the quality of chia during plant growth was studied for its interest as forage in green plant part (Peiretti and Meineri, 2008). During plant development, in South America, from April to May, an increase in ambient temperature triggered a decrease in polyunsaturated fatty acid (PUFA) levels in vegetative plant parts. In Europe, studies performed from May to September, showed a modification of PUFA according to genotypes and location (Grimes *et al.*, 2018). The amount of alpha-linolenic acid (ALA) (ω 3) has been shown to decrease by 23% from early development to the budding stage. This decrease is accompanied by increases in linoleic acid (LA) (ω 6) and lignin levels (Peiretti and Meineri, 2008). No information is available on the variation of fatty acid composition during fruit development of chia. Moreover, all the previous studies were carried out in South America. Few studies have been already performed for Chia cultivation in Europe using some genotypes released in America (Bochicchio *et al.*, 2015; Grimes *et al.*, 2018; Karkanis *et al.*, 2018). These works revealed the possibilities to cultivate, in Europe, some genotypes released in South America. Therefore, the aim of this work is to study the accumulation of oil, fatty acids composition, sterols and tocopherols content and composition during seed filling and maturation of one cultivar of Chia released in France in France.

2 Materials and methods

2.1 Plant material, trial and environmental conditions

Seeds of chia, genotype Oruro®, were provided by PANAM Cie (Villemur, France). The experiment was conducted in 2017, in south-western France at the Regional Centre for Organic Agriculture, at Auch (near Toulouse, south-west France, 43°38'47"N, 0°35'08"E). Field trial was carried out on a deep limestone clay silt soil with high water-holding capacity. The trial was sown at a density of 40 seeds by m² on June 6th, with 0.6 m between rows and at 1 cm of depth. The crops were completely managed under organic and rainfed conditions without any chemical supply. Crushed feathers were applied as an organic N fertiliser at a rate of 60 units ha⁻¹. Weeds were eliminated mechanically. Flowering occurred at end August and maturity took place at the end of September. Four samplings were made at 17 (71–73 BBCH stages), (Hack *et al.*, 1992), 24 (77 and 78 BBCH stages), 27 (80 and 81 BBCH stages) and 35 (83–84 BBCH stages) days after flowering (DAF). Sampling at full maturity was not released.

Table 1 presents rainfall and temperatures during the plant cycle and were compared to the weather data of the last 55 years. This comparison was done in order to explain that climatic conditions prevailed during 2017 were, almost as a "model year" of the last half century. Indeed, amount of rainfall in 2017 was quite similar to those recorded during 55 years in the same area. In contrast, the beginning of the cycle was 0.5 °C warmer than the half century temperature (Tab. 1).

2.2 Extraction and determination of fatty acid and sterol contents and compositions

Fatty acids were determined according to a method based on the oil solubility in cyclohexane and the transformation of fatty acids to their methyl esters (norm ISO 5509: 1990). Whole seeds (1 g) were ground with 10 mL of cyclohexane. After centrifugation at 10 000 g during 15 minutes, the organic phase is set aside and two others extractions are realised. Organic phases are met and filtered on anhydrous Na₂SO₄. Filtrate is dried by nitrogen flow. The fatty acid profile of the triglycerides present in the oil was determined after trans-methylation with TMSH (0.2 M trimethylsulfonium hydroxide in methanol) according to AFNOR Method NF EN ISO 12966-3. The fatty acids methyl esters (FAME) obtained by this transesterification reaction were analyzed with a gas chromatograph (Roche *et al.*, 2006) equipped with a CP-select CB column (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

Table 1. Mean monthly temperatures and rainfall during the cropping season of 2017 and average of 55 years in Auch (near Toulouse, South West of France).

Month	Rainfall (mm)		Temperature (°C)	
	2017	55 years	2017	55 years
June	65,3	61,1	21,8	18,9
July	35,3	47,1	21,7	21,5
August	57,2	58,3	22,1	21,3
September	52,9	55,9	16,7	18,5
October	60,4	56,8	15	14,4
November	17,6	57,2	7,2	9
December	92,9	68,1	6,1	6,2
Mean June-Oct			19,46	18,92
Sum June-Oct	271,1	279,2		
Mean Aug-Sept			19,4	19,9
Sum Aug-Sept	110,1	114,2		

to 250 °C at a rate of 15 °C/min and maintained at this temperature for 10 min. Sterol content was determined by gas chromatography (Roche *et al.*, 2010a). We introduced 50 µL of a 2 mg/mL solution of cholestanol in chloroform in to a 15 mL screw top tube. The chloroform was then eliminated by evaporation. A test run was then carried out with the addition of 100 mg of oil to the tube, followed by 2 mL of 1 MKOH in ethanol. The mixture was vortexed and heated at 75 °C in a water bath for 30 min. It was then allowed to cool to room temperature; 1 mL of distilled water was added and the mixture was vortexed. The unsaponifiable material was then extracted in 6 mL of cyclohexane. We collected 160 µL of the cyclohexane phase, which we then silylated by adding 40 µL of BSTFA/TMCS (99/1 mixture). The sample was heated for a few minutes at 103 °C and then 1 µL was injected into a Perkin-Elmer gas chromatograph 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 conditions were as follows: 55 °C for 0.5 min, an increase in temperature of 200 °C/min to 340 °C, then maintenance at 340 °C for 30 min for the injector; 160 °C for 0.5 min, then an increase of 20 °C/min to 260 °C, then of 2 °C/min to 300 °C and 45 °C/min to 350 °C for the oven. The various sterols were identified by comparing their retention times with those of commercial standards or reference values. The sterols were quantified against an internal standard.

Tocopherol content was determined by high-performance liquid chromatography. We solubilized 10 mg of oil in 1 mL of cyclohexane. We then injected 20 µL of the resulting solution into a liquid chromatography system (Dionex) equipped with a Kromasil 100 SIL 5 µ column (250 mm long, with an internal diameter of 4 mm). A mixture of 99.5% isooctane and 0.5% isopropanol (v/v) was used as the mobile phase, at a flow rate of 1.1 mL/min. Tocopherols were detected with a fluorimeter (Dionex), with an excitation wavelength of 290 nm and an emission wavelength of 317 nm. The tocopherols present in the oil were quantified against an external standard, and were identified by a comparison of retention times with those of reference standards.

2.3 Statistical data analysis

Statistical analyses were carried out on all the plots with three analytical replications. Analyses of variance and Duncan tests for means comparison between seed development stages were applied. The statistical analyses were performed using a statistical package (Sigmastat Version 2.0, USA).

3 Results and discussion

3.1 Oil yield

Table 2 presents oil content and fatty acid composition during seed fruit development of chia. Oil yield of chia seed has been found to vary between 23.3%–28.4% at different stages of seed fruit development (Tab. 2). Oil content increased gradually fruit development and reached the highest value at 27 days after flowering (DAF). Nevertheless, this value decreased slightly at 35 DAF. This result obtained under organic cultivation are quite in the range of values already reported for different genotypes cultivated in South America (Ayerza, 1995, 2010; Ayerza and Coates, 2009, 2011) or obtained by different extraction methods (Sayed-Ahmad, 2018) or by using different solvents (Segura-Campos *et al.*, 2014). Recent study conducted in Germany revealed higher oil yield than in our study (Grimes *et al.*, 2018). This result could be explained by genotypic, environmental factor, as well as method and solvent of extraction. Indeed, the genotype and locations of cultivation differed from their climatic conditions. Moreover, Grimes *et al.* (2018) used hexane while, cyclohexane was used in our study. This difference of chemical structure may induce difference in oil extraction (Oladipo and Betiku, 2019). Since, the seeds were harvested before full maturation, the slight decrease of oil content observed at 35 DAF could be, probably, due to respiration losses (Roche *et al.*, 2016; Belo *et al.*, 2018). Accumulation of oil in oilseed crops is regulated by the “Fatty Acid Synthetase” enzymatic system, which achieves differently during fruit development phases. Thus, at earlier phases, the FA-Synthetase system was induced, and oil content was increased. When oil content reached the plateau phase, these enzymes were less active and

Table 2. Fatty acids (%) and oil (%) accumulation during seed ripening of chia seed in 2017.

Fatty acid (%)	Days after flowering			
	17	24	27	35
OY	23.3d	26.9c	28.4a	27.4b
Saturated fatty acids (SFA)				
C16:0	9.2±0.0a	8.4±0.1b	8.2±0.0b	8.3±0.1b
C18:0	4.0±0.0a	3.4±0.0c	3.7±0.0b	3.4±0.0c
Total	13.2±0.0a	11.8±0.1b	11.9±0.0b	11.6±0.1b
Monounsaturated fatty acids (MUFA)				
C18:1n7	1.1±0.0c	1.0±0.0c	7.7±0.0a	7.4±0.1b
C18:1n9	11.3±0.1a	7.7±0.1b	0.9±0.0c	1.0±0.0c
Total	12.4±0.1a	8.7±0.1b	8.7±0.0b	8.4±0.1c
Polyunsaturated fatty acids (PUFA)				
C18:2n6	22.0±0.1a	19.9±0.1c	20.4±0.1b	20.0±0.1c
C18:3n3	52.4±0.2c	59.6±0.2a	59.0±0.0b	60.0±0.3a
Total	74.4±0.3b	79.5±0.3a	79.4±0.0a	80.0±0.4a
SFA/MUFA	1.06	1.36	1.37	1.38
SFA/PUFA	0.18	0.15	0.15	0.15
ω 6/ ω 3	0.42	0.33	0.35	0.33
Water content (%)	63.1±1.2a	52.7±1.0b	39.0±1.1c	25.2±0.9c

Data were means ± SD 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 a (linolenic acid); SFA/UFA (ratio saturated/unsaturated fatty acids); ω 3/ ω 6 (ratio linolenic acid/linoleic acid); OY (oil yield). In same line, means with the same letter are not significantly different at 0.05 probability.

less efficient; this in turn reduced the rate of oil accumulation (Msaada *et al.*, 2009; Nguyen *et al.*, 2015).

3.2 Fatty acid composition

Two saturated (palmitic and stearic acids), two monounsaturated (vaccenic and oleic acids) and two polyunsaturated (linoleic and linolenic acids) fatty acids were detected during chia fruit development (Tab. 2). Saturated fatty acids (SFA) constituted 13% of total fatty acids at 17 DAF, and decreased to reach 11.6% at 35 DAF. This decrease was more marked for the monounsaturated fatty acids (MUFA). Indeed, this trait decreased by 30% between 17 and 35 DAF (Tab. 2). In contrast, polyunsaturated fatty acids (PUFA) increased from 74% (at 17 DAF) to 80% (at 35 DAF). Linolenic acid, linoleic, oleic stearic and palmitic acids were the major fatty acids (Tab. 2). Nevertheless, the fatty acids accumulation did follow neither the same trend nor the same speed. Indeed, at earlier stages of fruit development (17 DAF), palmitic, stearic, oleic and linoleic acids were at their higher level in the seed. The content of these fatty acids declined to reach its lowest level around the 35th day after flowering. Moreover, while stearic, palmitic and linoleic acids decreased slightly oleic acid declined more than ten times between 17 and 35 DAF (Tab. 2). In the same time, linolenic and vaccenic acids followed the opposite path with a progression of their content to reach the highest content between 27 and 35 DAF (Tab. 2). The ratio of ω 6/ ω 3 followed similar trend. In fact, this ratio was highest at 17 DAF and decreased rapidly to reach a plateau at 24 DAF (Tab. 2). Similar trends have been reported in other species from different botanical families (Hamrouni-Sellami *et al.*, 2007; Msaada *et al.*, 2009; Sriti *et al.*, 2011; Nguyen *et al.*, 2015; Roche *et al.*, 2016). The ratio of unsaturated to saturated

fatty acids increased markedly from 6.6 (17 DAF) to 7.5 (24 DAF) and remained constant until complete maturity. These values are similar to those reported recently in Germany (Grimes *et al.*, 2018).

The fatty acids' accumulation seems to follow the biosynthesis dynamics as described in Kennedy pathway evolved acyl-CoA elongase and desaturases enzymes that could explain the decrease of saturated FA which served to synthesize polyunsaturated fatty acids. Moreover, in our study linoleic acid level was nearly 15% higher than results reported previously (Ayerza, 1995, 2010; Ayerza and Coates, 2009, 2011; Ciftçi *et al.*, 2012). This difference could be due to genotype and climatic conditions. Indeed, Oruro® is newly released genotype which was cultivated in France for the first time. The ratio of SFA/MUFA increased by more 40% (Tab. 2) from 17 to 24 DAF, while SFA/PUFA ratio decreased for the same period from 0.18 to 0.15. Both ratios remained constant until maturity (Tab. 2). These values are similar to those reported recently in Germany (Grimes *et al.*, 2018). Moreover, ω 3/ ω 6 ratio of the chia oils varied from 2.4 (17 DAF) to 3.0 (24–35 DAF) during the fruit development. These values are in lines with those previously reported results even in other growth conditions and other genotypes (Ayerza, 1995, 2010; Ayerza and Coates, 2009, 2011; Grimes *et al.*, 2018; Sayed-Ahmad, 2018). These values are significantly higher than those of most vegetable oils, e.g. canola oil (0.36), olive oil (0.08–0.12), soybean oil (0.15), Safflower (0.01) Atlas pistachio (0.10) and walnut oil (0.25) (Galão *et al.*, 2014; Ojeda-Amador *et al.*, 2018; Rodrigues *et al.*, 2018; Beyzi *et al.*, 2019; Labdelli *et al.*, 2019; Roche *et al.*, 2019). The tocopherol content increased in same way between 17 to 24 DAF (Tab. 3). It is well known that these molecules act as antioxidant in order to protect lipids oxidation. This could explain the concomitant

Table 3. Phytosterol and tocopherols accumulation during seed ripening of chia seed in 2017.

Days after flowering (DAF)				
Phytosterols (mg/100 g of oil)	17	24	27	35
Campesterol	99.7±0.6a	92.2±0.8b	85.7±1.6c	86.3±1.1c
Stigmasterol	42.5±0.3a	37.5±0.9b	34.1±0.6c	34.6±0.7c
β-Sitosterol	460.7±4.3a	454.3±7.6a	431.2±6.1b	429.4±9.7b
Δ-5 Avenasterol	46.8±2.9a	39.7±0.1b	39.9±1.5b	36.6±0.7c
Total Desmethylsterols	649.7±5.9a	623.6±9.5b	590.9±8.7c	586.9±11.6c
Gramisterol	15.2±0.1c	14.1±0.2d	17.2±0.1a	15.9±0.2b
Citrostadienol	16.3±0.3b	14.8±0.0c	20.1±0.6a	18.3±1.5a
Cycloartenol	22.8±1.4a	16.8±1.0b	17.4±0.7b	18.9±1.4b
Methylen Cycloartanol	22.2±1.2a	15.11±0.5d	17.9±0.8b	16.0±0.2c
Total Di and Methylsterols	76.5±2.8a	60.8±1.4c	72.5±1.5a	69.1±1.9ab
Total Sterols	726.2±3.1a	684.4±8.1b	663.5±9.2c	656.0±13.2c
Days after flowering (DAF)				
Tocopherol (mg/100 g of oil)	17	24	27	35
α-tocopherol	1.5±0.1b	1.8±0.1a	1.4±0.0b	1.5±0.1b
γ-tocopherol	38.8±1.8b	45.3±2.7a	47.9±0.6a	47.9±1.7a
δ-tocopherol	1.6±0.0c	1.7±0.2b	2.0±0.0a	1.9±0.1ab
Total tocopherols	42.0±1.9b	48.8±3.0a	51.3±0.7a	51.4±1.9a

In same line, means with the same letter are not significantly different at 0.05 probability.

accumulation of both polyunsaturated fatty acids and tocopherols in same way.

Supply of chia seeds in alimentation on rat blood plasma resulted in significant reduction of triglycerides (TG) and low-density lipoproteins (LDL) were significantly reduced, while, high-density lipoproteins (HLD) and omega-3 (PUFA) have decreased (Ayerza and Coates, 2007; Fernandez *et al.*, 2008). Relationships between fatty acids composition of diet and some diseases, like cardiovascular diseases or diabetes, have been extensively reported (Betti *et al.*, 2009). This also true for chia seed (Nieman *et al.*, 2009; Guevara-Cruz *et al.*, 2012; Jin *et al.*, 2012). Indeed, PUFA are necessary for the proper functioning of the brain, the eyes, and the entire nervous system and to prevent the risk of cardiovascular diseases (Corley and Tinker, 2003; Vedtofte *et al.*, 2011). Mono and polyunsaturated present an effective action in lowering total cholesterol (Gretchen and Heather, 2014; Kratz *et al.*, 2002), and reducing the risk of breast cancer (Kushi and Giovannucci, 2002). Moreover, linoleic acid helps to prevent human diseases, particularly cardiovascular disease, and certain disorders like Alzheimer's disease (Khan *et al.*, 2015). The oil of chia is composed of 75% of mono and polyunsaturated fatty acids and therefore constitutes very healthy oil for human and could increase the lowering of risks of cardiovascular diseases. Furthermore, the recommendations of the FAO as well as Food and Alimentary national Agencies, concerning the ratio saturated to unsaturated and linoleic to linolenic acids, are clearly established (FAO, 2010; Kalonji *et al.*, 2012; Schwingshackl and Hoffmann, 2012). Indeed, minimum intake values PUFA to prevent deficiency symptoms are estimated to be 2.5% of energy for linoleic acid plus 0.5% of energy for linolenic acid (FAO, 2010). For MUFA intakes, international recommendations vary greatly between 12–25%. Moreover, the ratio of linoleic to linolenic must be lesser than five (Kalonji *et al.*, 2012; Ristić-Medić *et al.*, 2013; Labdelli *et al.*, 2019). Our results showed, at 24 DAF, the chia seeds

fulfill the recommendations mentioned above (Tab. 2). This fact reinforced the great interest to harvest and use seed of chia before maturity as reported in other studies (Msaada *et al.*, 2009; Roche *et al.*, 2010b, 2016; Sriti *et al.*, 2011; Nguyen *et al.*, 2015; Belo *et al.*, 2018).

3.3 Sterols and tocopherols contents and compositions

Chia seeds contain between 656 and 726 mg/100 g of oil of total sterols. Eighty-nine percent of them are desmethylsterols represented mainly by β-sitosterol and campesterol at a level of 140 mg/100 g SDM (Tab. 3). Sterols content observed in our study were quite higher than those reported by Ciftçi *et al.* (2012) Dąbrowski *et al.* (2017) and Sayed-Ahmad (2018), who observed a range of 402–508 mg.100 mL⁻¹ of oil. These differences can be explained by both genotypic and environmental effect. As already presented, our study was based on a new cultivar developed in Europe and cultivated in France for the first time.

During seed filling and maturation of chia seeds phytosterols content was higher at 17 DAF and followed the progressive and slight decrease to reach a minimum in the whole seed at 27 and 35 DAF (Tab. 3). Sterol concentration differed according to sterol category. Desmethylsterol concentration followed similar trend that total sterols and was the highest at 17 DAF. This result was expected since desmethylsterols represented more than 89% of total phytosterols (Tab. 3). In contrast, the concentration of dimethyl- and methylsterol content varied during grain filling with the highest values were observed at 17 and 27 DAF and the lowest ones at 24 DAF (Tab. 3). Methylcycloartanol and cycloartenol (dimethylsterol category) showed higher value at 17 DAF followed by a decrease (24 DAF) and an increase after this date. In the methylsterol category, citrostadienol and gramisterol content increased also until 27 DAF then

decreased until harvest (Tab. 3). Nevertheless, the dynamics of these two categories of sterols seems to be linked. Indeed, dimethylsterols serve as precursor for the synthesis of methylsterols (Merah *et al.*, 2012). The decrease of dimethylsterols observed at 24 DAF resulted in the increase of the level of methylsterols at 27 DAF, as reported in other species (Merah *et al.*, 2012; Roche *et al.*, 2016).

Tocopherols content and composition of chia during fruit development is presented in Tab. 3. Content of tocopherols varied slightly. High level of tocopherols was noticed along the fruit development. A slight decrease was observed at 24 DAF to increase again and reaching the highest value at 27 DAF. As for sterols, these results were higher than those reported in other works (Ciftçi *et al.*, 2012; Dąbrowski *et al.*, 2017; Sayed-Ahmad, 2018). The main isomer was γ tocopherol that represented more than 92 of the total tocopherols as already reported by (Ciftçi *et al.*, 2012; Sayed-Ahmad, 2018). In contrast, Dąbrowski *et al.* (2017), who used different methods of extraction, observed nearly 5 to 7% more γ tocopherol than in our study.

The level of these compounds is not only dependent on extraction method (Dąbrowski *et al.*, 2017), but also on genotype and environment (Sriti *et al.*, 2011; Msaada *et al.*, 2009; Roche *et al.*, 2010b).

4 Conclusion

This work aimed to investigate the fatty acid, sterols compositions and oil accumulation in Chia (*Salvia hispanica* L.) seed, cultivar Oruro© released in France, from onset of seed filling until maturation. This was the first report on bioaccumulation of oil and fatty acid in chia seed. Results demonstrated that the highest oil yield was achieved at 27 DAF. Fatty acid profile varied greatly during fruit development. Linolenic acid (60% of total fatty acids at maturity) reached its higher level at 35 days after flowering. In addition, polyunsaturated fatty acids increased, whereas saturated and monounsaturated fatty acids decreased significantly during fruit development. The ratio of $\omega 6/\omega 3$ was highest at 17 DAF and lowest at 35 DAF. Therefore, if the objective is to decrease the $\omega 6/\omega 3$ ratio of it will be preferable to harvest at 35 DAF (which is not the full maturity in our study). In contrast, if the aim is to harvest higher oil content and higher tocopherol level, seed should be harvest at 27 DAF or at full maturity. Our results highlighted the possibility to seeds of chia according their components concentration. Moreover, this requires the development of techniques for harvesting, storing and probably drying of fruits. These results need to be ascertained by other field experiments.

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Author contributions

Z.M. is the project Manager. Z.M. and O.M. conceptualization. G.G., M.C., C.P. and O.M developed the methodology

and performed the experiments and the measurements. M.C., G.G. and C.P. assisted with measurements. O.M., G.G. and Z.M. contributed to the analysis and interpretation of the data. G. G. Z.M. and O.M. contributed to data collection and to the writing of the manuscript.

Conflicts of interest. The authors declare that they have no conflicts of interest in relation to this article.

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