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Shelf-life evaluation and nutraceutical properties of chia seeds from a recent long-day flowering genotype cultivated in Mediterranean area

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ABSTRACT

Chia seeds (*S. hispanica* L.) are a good source of nutrients like essential fatty acids, fiber and antioxidant compounds; for this reason they are considered as functional foods. Chia seeds are usually cultivated in Central America, while a few reports about their growing outside of this area are reported. In this paper seeds obtained from a recent G8 genotype, a long-day flowering line of chia, grown in Mediterranean area have been analyzed during storage time to evaluate their oxidative stability and nutraceutical compounds. Seeds were grown with two different levels of irrigation and two levels of sowing density. No significant influence of these factors on chemical composition of seeds has been found. Seed yield was comparable with the one from native area as well as fatty acid composition and antioxidant compounds content, even if a higher content of peroxides and a shorter predicted shelf life was recorded.

1. Introduction

Salvia hispanica L. is an annual herbaceous specie belonging to *Lamiaceae* or *Labiateae* family traditionally cultivated in pre-Columbian age in Central America (Muñoz, Cobos, Diaz, & Aguilera, 2013).

Nowadays there is a growing interest in chia seeds and oil for their nutraceutical and technological characteristics. The oil content of chia seeds ranges from 20 to 35% (Porras-Loaiza, Jiménez-Munguía, Sosa-Morales, Palou, & López-Malo, 2014); chia seeds are a valid vegetable source of ω-3 fatty acids, being α–linolenic acid the most representative (~60%) (Bochicchio et al., 2015). Chia seeds are a good source of vitamins, minerals, antioxidants and fiber both soluble and insoluble (Amato et al., 2015; Marineli et al., 2014; Muñoz et al., 2013) and they are also a good source of proteins. Unlike most of vegetable protein sources, chia seeds contain all the essential amino acids, so they have a better proteic quality than cereals and the other oily seeds (Nitrayová et al., 2014).

Furthermore, chia could be a food resource for reducing cardiovascular risk factors including diabetes, hypertension and inflammation (Cooper, 2015). Seeds are very rich in antioxidant compounds; tocopherols are the most abundant ones, but can be also found phenolic acids, flavonols (Ayerza, 2013; Capitani, Spotorno, Nolasco, & Tomás, 2012; Marineli et al., 2014; Reyes-Caudillo, Tecante, & Valdivia-López, 2008; Taga, Miller, & Pratt, 1984) and isoflavones (Martínez-Cruz & Paredes-López, 2014).

The interest of this functional food is also due to the possible application of chia seeds mucilage and soluble proteins as encapsulation material for probiotics (Bustamante, Oomah, Rubilar, & Shene, 2017), to produce edible films (Dick et al., 2016), to replace fats in cakes (Ramos, Fradinho, Mata, & Raymundo, 2017) and as health-promoting ingredient in frankfurter (Pintado, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2016). Moreover, chia seeds do not contain gluten, so they can be consumed by people affected by celiac disease (Martínez-Cruz & Paredes-López, 2014; Menga et al., 2017; Reyes-Caudillo et al., 2008). Chia is mostly requested in USA, Japan and Europe; a growing demand in chia is foreseen in next years, especially in EU where chia seeds are becoming extremely popular despite their recent introduction. Chia seeds in Europe are part of the novel food catalogue; their placing on the market was authorized since 2009 (Commission EU, 2009) and 2013 (Commission EU, 2013), while chia oil placing was authorized in 2014 (European Commission, 2014).

Chia crops are common in Bolivia, Paraguay, Argentina, Mexico,

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Australia and Central America, but there is a growing interest in cultivating chia in new areas. There are few reports of chia crops outside areas of origin (Amato et al., 2015).

Since chia is a short day flowering plant it is not able to complete the set of the flower at every latitude; usually domesticated chia plant produces flowers buds when the photoperiod is around 12 h, in temperate areas that leads to the kill of the flowers by the frost. Some researchers started breeding to develop new varieties able to grow in different climatic regions. Jamboonsri, Phillips, Geneve, Cahill, and Hildebrand (2012) developed lines of chia able to grow in different areas, specifically a long-day flowering genotype (early flowering) able to flower with more than 14 h of photoperiod. This important characteristic allows a better growth of chia in temperate areas.

Agronomical conditions generally affect seeds quality; in literature there are different studies involving the influence of the growing locations on chia seeds characteristics and on agronomical performance (Ayerza, 2010).

Chia seeds analyzed in the present work for their chemical and nutraceutical characteristics have been cultivated in Basilicata, a region of South Italy, taking into account also the effect of irrigation and sowing density on seed quality. They belong to a recent long-day flowering genotype (G8 genotype) that should be more suitable to the growth in the Mediterranean area with respect to the genotype studied by Amato et al. (2015). Furthermore, the oxidative stability of seeds during storage time has been also evaluated.

2. Materials and methods

2.1. Sampling

Seeds from G8, a long-day flowering chia genotype developed at the University of Kentucky and obtained through an agreement between the University of Kentucky and the University of Basilicata, were grown in Southern Italy (Atella, Potenza, Italy), Lat. N 40°51′37.59″, Long. E 15°38′49.43″, in the period June–November 2014, on a Luvi-vertic Phaeozeum loam soil (IUSS Working Group, 2006). The soil was amended with 25 t/ha of the solid fraction of biogas digested materials with the following characteristics: dry matter 8.5%; carbon 20.4 kg/t; nitrogen (N) 2.8 kg/t; ammonium ($N-NH_4$) 0.6 kg/t; P_2O_5 1.4 kg/t; K_2O 2.5 kg/t. After sowing (48 days) a foliar fertilizer NPK 20-20-20 and micronutrients at 1.25 kg/ha was applied. Precipitation was 197 mm during the plant cycle.

Two agronomic factors were tested in a randomized block design with three replications: Sowing density with two levels (d1: 40 plants/m^2 and d2: 13 plants/m^2) and irrigation with two levels (I: irrigation applied at 100% of evapotranspiration with a drip irrigation system or NI: not irrigated). The single and combined effects of treatments were studied.

The seeds characteristics were evaluated after 0 (t1), 5 (t2) and 10 (t3) months of storage. Seeds were stored in glass jars in the dark at room temperature during the whole experimentation time.

2.2. Extraction of oil and analysis

Lipids were extracted from seeds according to Amato et al. (2015) with slight modifications. Seeds were milled for 10 s in a coffee mill (Moulinex Type a591, Groupe SEB Italia SPA, Milan, Italy) and then the oil was extracted through Soxhlet method; the extraction was carried out with petroleum ether as solvent and lasted 6.5 h. The oil content was gravimetrically determined and expressed as percentage weight. For further analyses, oil samples and defatted flour were stored in amber bottles and glass jars respectively at 13 °C. The oil fatty acid composition was determined as methyl esters (FAMEs), according to the procedure of Ichihara, Shibahara, Yamamoto, and Nakayama (1996), using an Agilent 7890A GC (Varian-Agilent, Milan, Italy), equipped with a SLB®-IL111 Capillary GC Column (100 m × 0.25 mm x 0.20 μm). The separation was carried out at 90/240 °C with helium as carrier gas and a flame ionization detector (FID) at 300 °C. FAMEs were identified by comparison of retention times with FAME standard mixture under the same conditions (Supelco 37 Component FAME Mix analytical standard, Sigma-Aldrich, Milan, Italy). Acidity and peroxide value (P.V.) were determined according to Reg. UE 2568/91 and Reg. UE 299/2013. *p*-Anisidine value was determined according to the AOCS Official Method Cd 18–90 (AOCS, 2009). Tocopherol oil content was analyzed by HPLC using an Adsorbil column 5 μm 250*4.6 mm (Grace-Alltech, Milan, Italy) and a Varian Chromatography System comprising a 9012 pump and a 9070 fluorescence detector (Varian-Agilent, Milan, Italy) according to Amato et al. (2015).

2.3. Extraction of phenolic compounds and analysis

The extraction of phenolic compounds was performed on the defatted flour. Five grams were extracted with 20 ml of the acetonitrile/acetic acid (10%) solution at room temperature and in the dark under orbital shaking (140 rpm) for 3 h. The mixture was stored at 4 °C overnight in a Falcon test tube; the upper part was filtered on paper filter (Whatman N.4) and then with PTFE syringe filter (0.45 μm). This extract has been used to determine total phenolic content, antioxidant activity, flavonols and hydroxycinnamic acids content. The obtained data were referred to the weight of the whole seed.

Total phenolic content was determined on the extract according to Folin-Ciocalteu method. One hundred μl of Folin Ciocalteu reagent, 1775 μl of water and 25 μl of extract were mixed and incubated at room temperature in the dark for 2 min. Then 300 μl Na_2CO_3 were added; the sample was mixed again for 1 min and incubated at 40 °C for 30 min. The absorbance was read at 765 nm (Cary 1E UV-VIS spectrophotometer, Varian – Agilent, Milano, Italy). Results were expressed as gallic acid equivalent (mg GAE/g seed).

With the attempt to determine flavonol aglycones and free hydroxycinnamic acids, crude extracts were subjected to acid hydrolysis before injection according the procedure to Caruso et al. (2015). Flavonols were determined as aglycons, in particular myricetin, quercetin and kaempferol on the hydrolysed extract by HPLC using a Varian Chromatography System comprising a 9012 pump and a 9050 UV-Vis detector (Varian-Agilent, Milan, Italy) at 360 nm. Twenty μl of sample were injected in Gemini NX-C18 column (250 × 4.60 mm, 5 μm, 110 Å; Phenomenex, Bologna, Italy) at controlled temperature (40 °C) using water: formic acid 90:10 (A), acetonitrile: water: formic acid 50:40:10 (B) as mobile phases at flow rate 0.5 ml/min. Flavonol aglycones were quantified using a 5-point external standard curve. Caffeic and chlorogenic acids were determined by HPLC at 280 nm on the same hydrolysed extract used to determine flavonol aglycones. Twenty μl of the sample were injected in Gemini C18 column (250 × 4.60 mm, 5 μm, 110 Å; Phenomenex, Bologna, Italy) using phosphoric acid 0.1% and methanol as mobile phase at flow rate of 0.8 ml/min. Caffeic and chlorogenic acids were quantified using a 5-point external standard curve. All chemicals were purchased from Sigma-Aldrich (Milan, Italy). All the analytical determinations were made in triplicate.

2.4. Antioxidant activity

Antioxidant activity was quantified according to Re et al. (1999) by a decolorization assay of the ABTS radical cation solution, measuring decrease of its absorbance at 734 nm (Cary 1E UV-VIS spectrophotometer, Varian–Agilent). The results were expressed

as μmol TROLOX equivalent (TE)/g seed.

2.5. Oxidative stability

Oxidative stability was evaluated on whole seeds by Oxitest (Velp Scientifica, Usmate, MB, Italy) at 90 °C and oxygen pressure of 6 bar. For the analysis, 10 g of milled seeds were distributed on a sample holder, in each reaction chamber. The response was evaluated as Induction Period (I.P.), expressed as a "stability time" before the beginning of fat oxidation. I.P. value was automatically calculated from oxidation curves by graphical method. A prediction of the shelf life was also performed following an appropriate procedure and testing the sample at three different temperatures (80, 90 and 100 °C) (Caruso, Galgano, Colangelo et al., 2017). Therefore, it was possible to extrapolate and estimate the oxidative stability of seeds at room temperature. All the results were elaborated by using OXISoft™ software (Velp Scientifica).

2.6. Statistical analysis

Data were subjected to PCA (Principal Component Analysis) and ANOVA (analysis of variance). Differences between means were compared with LSD test (Least Significant Difference) with a 95% confidence interval. Data were analyzed with the statistical packages Unscramble Version 10.4 a - Camo and SYSTAT, Version 10 – Spss Inc.

3. Results and discussion

3.1. Influence of sowing density, irrigation rate and storage time on chia seeds characteristics

Seed yield was remarkable and comparable to that of the best areas of origin (Bochicchio et al., 2015) and it was significantly higher ($p < 0.05$) in d1 (18.5 q/ha) than in d2 (13.3 q/ha). Furthermore, seed yield was affected by irrigation (18.8 q/ha in irrigated vs. 13.1 q/ha in not irrigated at $p < 0.05$). Score plot after PCA analysis showed that is possible to group the samples according to storage time; in this case the explained variance (35% of which along PC1 and 15% along PC2) represented 50% of the total variance (Fig. 1). The time t1 differed mostly from the others storage times; a three way ANOVA analysis confirmed that time is the factor that affects significantly all parameters, except for fatty acids, while sowing density and irrigation had an influence only on a few chemical parameters (Table 1). The combined effects of treatments was not significant.

There are no evidence in other studies of the influence of time storage on chia seeds composition, but some researches stated that there is an effect of the growing area on seed and oil yield and on α-linolenic acid content; also the ecosystem has an influence on some seed characteristics (protein content and fatty acid composition) (Ayerza, 2010).

The evolution of chemical composition of chia seeds is reported in Table 2. The average fat content was 32.55 at t1; the results are similar to those reported for chia oil content in areas of origin (Ayerza & Coates, 2011; Ayerza, 2010; Porras-Loaiza et al., 2014). In this study neither the sowing density nor the irrigation effect significantly influenced the oil yield; a study indicated an influence of irrigation and row spacing on oil content of soybeans (Boydak, Alpaslan, Hayta, Gerçek, & Simsek, 2002). In our case the high precipitation (99 mm) during grain filling and ripening between September and October probably may justify the lack of significant differences in seed composition.

Table 1
Three way ANOVA analysis – p values.

	Factors		
	t	d	i
Fat	0.003	ns	ns
Palmitic acid	ns	ns	ns
Stearic acid	ns	ns	ns
Oleic acid	ns	ns	ns
Linoleic acid	ns	ns	ns
α-linolenic acid	ns	ns	ns
Acidity	0.000	ns	0.002
P.V.	0.000	ns	ns
p-Anisidine value	0.000	ns	ns
Total phenols	0.001	ns	ns
ABTS	0.000	0.000	ns
Myricetin	0.000	0.000	0.008
Kaempferol	0.013	ns	ns
Caffeic acid	0.000	ns	ns
Chlorogenic acid	0.005	0.005	ns
α-tocopherol	0.000	0.005	0.008
δ- tocopherol	0.000	0.019	ns
γ-tocopherol	0.000	ns	ns
I.P.	0.013	0.004	ns

t = time, d = sowing density, i = irrigation.
95% confidence interval.
ns: not significant.

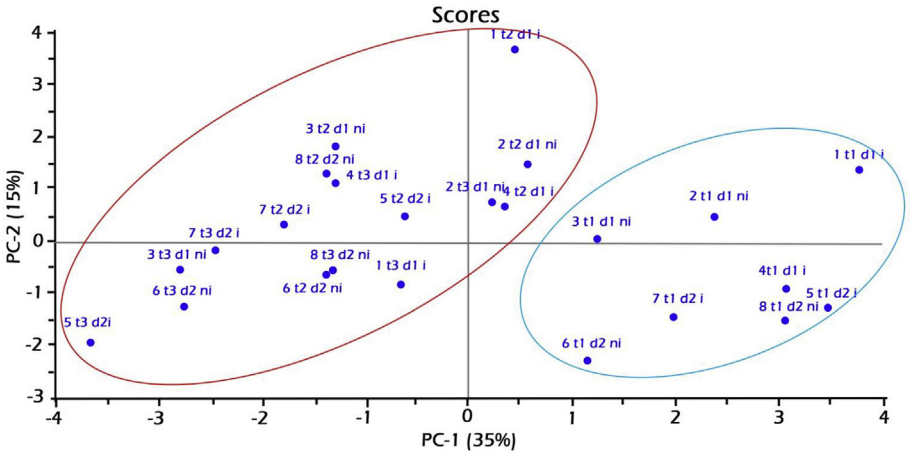

Fig. 1. Score plot of the first (PC1) and second (PC2) principal components obtained by PCA analysis of chemical composition and oxidative stability of chia seeds grown in different conditions and analyzed at different times. (1–8 = sample number; t1-t3 = analysis time; d1 = 40 plants/m^2, d2 = 13 plants/m^2, i = irrigated; ni = not irrigated).

Table 2
Chemical composition of *Salvia hispanica* L. seeds grown in Basilicata during time (average values of all analyzed samples).

	t1	t2	t3
Fat (%)	32.55[a]	30.01[b]	31.19[b]
Palmitic acid (%)	6.47[a]	6.18[a]	6.17[a]
Stearic acid (%)	2.74[a]	2.62[a]	2.63[a]
Oleic acid (%)	5.90[a]	5.81[a]	5.82[a]
Linoleic acid (%)	18.71[a]	18.61[a]	18.61[a]
α-linolenic acid (%)	65.42[a]	65.83[a]	65.67[a]
Acidity (%)	0.60[a]	1.10[b]	1.14[b]
P.V. (meq O_2/kg oil)	11.82[a]	16.05[b]	26.09[c]
p-Anisidine value	0.45[a]	0.97[b]	1.41[c]
Total phenols (mg GAE/g seed)	1.77[a]	1.66[b]	1.78[a]
ABTS (μmol TE/g seed)	93.9[a]	82.2[b]	79.3[b]
α-tocopherol (mg/kg oil)	4.52[a]	3.62[b]	3.64[b]
δ-tocopherol (mg/kg oil)	16.3[a]	14.1[b]	14.4[c]
γ-tocopherol (mg/kg oil)	393[a]	359[b]	364[c]
Myricetin (mg/g seed)	0.076[a]	0.070[a]	0.062[b]
Kaempferol (mg/g seed)	0.054[a]	0.053[a]	0.048[b]
Caffeic acid (mg/g seed)	0.482[a]	0.380[b]	0.249[c]
Chlorogenic acid (mg/g seed)	0.128[a]	0.113[b]	0.085[b]
IP (hours)	10.28[a]	9.53[b]	9.49[b]

Different letters indicate statistically significant differences according to LSD test at $p < 0.05$.
t1 = 0 months; t2 = 5 months; t3 = 10 months.

Unsaturated fatty acids are the most representative fatty acids present in chia seeds; in fact the average content in α-linolenic acid was of 65.42%, followed by linoleic acid (18.71%) and oleic acid (5.90%); the other fatty acids present in significant amounts were palmitic acid (6.47%) and stearic acid (2.74%). Chia seeds represent a valid vegetable source of ω-3 and ω-6 fatty acids. Together with other oily seeds they can be an alternative source of essential fatty acids for people who don't eat fish. Other eight fatty acids have been detected in traces (data not shown). Results are in line with the literature, even if the determined α-linolenic acid content was slightly higher, while linoleic acid content was slightly lower than the value reported in literature (Amato et al., 2015; Ayerza, 2010; Dąbrowski, Konopka, Czaplicki, & Tańska, 2016). During storage time, the fatty acid profile of chia seeds did not show great changes and the sowing density and irrigation technique did not affect the composition of the main fatty acids. Also Silva et al. (2016) reported no influence of irrigation on linoleic acid and omega 3 content of two phenotypes of chia.

The oil extracted from all samples had a low acidity at each analysis time; the mean values at t1 and t3 were 0.60% and 1.14%, respectively. The acidity was comparable with the one of chia grown in typical areas of cultivation (Vázquez-Ovando, Rosado-Rubio, Chel-Guerrero, & Betancur-Ancona, 2009), while it is 3-fold less than the acidity of commercial seeds from Peru grown in Basilicata (Amato et al., 2015).

Peroxide value of chia seeds increased during storage, as well as *p*-Anisidine value. The mean P.V. was 11.82 meq O_2/kg oil at t1 and 26.09 meq O_2/kg oil at t3. Chia seeds P.V. usually ranges from 0.6 meq O_2/kg to 3.9 meq O_2/kg in oils extracted with different extraction methods (Ayerza, 2010; Dąbrowski et al., 2016; Guiotto, Ixtaina, Nolasco, & Tomás, 2014). Commercial chia seeds from Peru grown in Basilicata (Amato et al., 2015) had a P.V. of 11.05 meq O_2/kg, while the same seeds grown in Peru had a P.V. of 4.14 meq O_2/kg. The growing ecosystem could have an influence on chia seeds P.V., but Ayerza and Coates (2004) found no differences among chia seeds grown in six tropical and subtropical ecosystems. The higher P.V. of seeds analyzed in the present work could be related to the specific genotype cultivated or to the interaction between genotype and soil and climate characteristic of the growing area.

The mean total phenolic content was 1.77 mg GAE/g seed. The phenolic content was higher than that reported in other studies in which this chemical parameter ranged from 0.53 mg GAE/g seed to 0.94 mg GAE/g seed (Amato et al., 2015; Coelho & Salas-Mellado, 2014; Marineli et al., 2014; Reyes-Caudillo et al., 2008). Beside different agronomic conditions and genotype of seed analyzed, this difference among data could be related to the different analysis method adopted and to the extraction method selected. The highest phenolic content is reported for chia seeds from Brazil and Mexico with a mean content ranging from 4.79 to 7.89 mg GAE/g seed (Dick et al., 2016).

In this study ABTS assay has been adopted to evaluate the antioxidant activity of chia seeds; the comparison among the obtained data and the values from other studies showed a high variability among different results. The average antioxidant activity was 93.9 μmol TE/g seed at t1 and 79.8 μmol TE/g seed at t3; these results are in line with ABTS values of Mexican chia (84.5 ± 6.38 μmol TE/g) reported by Dick et al. (2016). However, these values were much lower than that reported by the same authors for Brazilian chia seeds (256 ± 16.94 μmol TE/g) and than 489 μmol TE/g, value recorded for chia grown in Mexico (Vázquez-Ovando et al., 2009).

The average content of caffeic acid was 0.482 mg/g seed; this value is among the highest ones reported in different studies concerning the chemical composition of chia seeds. Although the caffeic acid content significantly decreased over the entire storage period, its value was still higher after 10 months of storage (0.249 mg/g) than what reported in other studies: 0.0274 mg/g (Martínez-Cruz & Paredes-López, 2014) and 0.0136 mg/g (Ayerza, 2013).

The average content of chlorogenic acid was 0.128 mg/kg; Reyes-Caudillo et al. (2008) reported a mean value of 0.102 mg/g, while Ayerza (2013) reported a content of 0.226 mg/g. These phenolic acids were determined after acid hydrolysis, while most of the results reported in literature for these compounds in chia seeds are referred to non-hydrolysed extracts. Myricetin, quercertin and kaempferol are flavonol aglycones usually found in chia seeds; in this study quercetin was unquantifiable, while amounts of 0.076 mg myricetin/g seed and 0.054 mg kaempferol/g seed have been found; these compounds slightly decreased during time. Ayerza (2013) reported a higher amount of myricetin (0.115 mg/g seed) and a smaller amount of kaempferol than the values for seeds analyzed in this experimentation. No correlation between measured phenolic compounds and antioxidant activity has been found; however, chia seeds contain other phenolic compounds (i.e. protocatechuic ethyl ester 0.7471 mg/g seed, rosmarinic acid 0.9267 mg/g seed) that might have a major influence on ABTS assay (Martínez-Cruz & Paredes-López, 2014).

Chia seeds from native areas and chia grown in Basilicata too can be considered a good source of flavonols and phenolic acid for the human diet, retaining their nutraceutical properties over time. Phenolic acids, of which are part hydroxycinnamic acid derivatives, and flavonols have anti-carcinogenic and anti-mutagenic effects; flavonols and L-ascorbic acid have a synergistic protective effect towards oxidative damages of DNA in lymphocytes. Both chlorogenic and caffeic acid are antioxidants in vitro, and they are potential inhibitors for the formation of mutagenic and carcinogenic N-nitroso compounds in vitro (Ozcan & Delikanli, 2014).

Tocol content (tocopherol fundamental unit) and the degree of unsaturation of an oil have an high impact on oxidative stability (Shahidi & Shukla, 1996). Chia is a good source of tocopherols; γ-tocopherol (393 mg/kg oil) was predominant in chia seeds, followed by δ- (16.3 mg/kg oil) and α- (4.52 mg/kg oil). Tocopherol content is in line with other reports of chia seeds (Guiotto et al., 2014). Capitani et al. (2012) determined the tocopherol content of oils recovered from defatted bran after solvent and physical extraction, reporting an high tocopherol value (500–600 mg/kg), so even by-products from chia seed could be a source of healthy

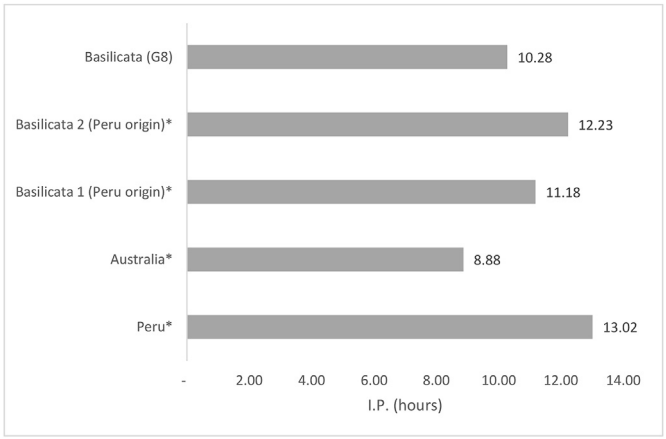

Fig. 2. Induction period (calculated by Oxitest) of chia seeds from different geographical cultivation areas. I.P. of G8 genotype refers to t1. *Amato et al. (2015).

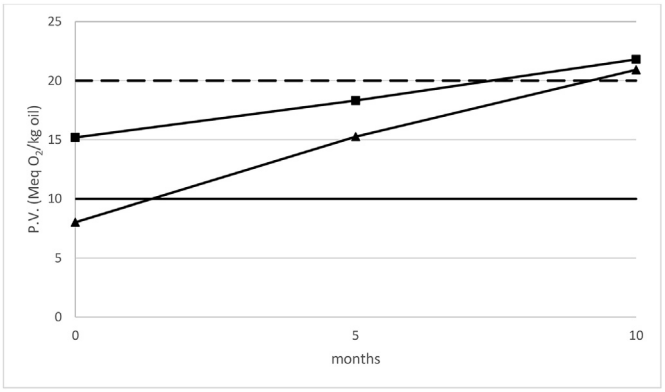

Fig. 4. P.V. Trends of sample 1 (■) and 2 (▲) during time. The continuous line indicates the P.V. limit for chia seeds oil according to decision 2014/890/EU, the dotted line indicates P.V. limit for extra virgin olive oil. (Sample 1 = 13 plants/m^2, irrigated; sample 2 = 13 plants/m^2, not irrigated).

compounds that may be used in different applications.

3.2. Evaluation of oxidative stability by Oxitest

The I.P. mean value at t1 was 10.28 h and differed significantly from I.P. values at t2 and t3. A decrease of I.P. value was recorded during storage time. No correlation was found between I.P. value and pro-oxidant or antioxidant factors. An influence of free fatty acid content on the oxidation of vegetable oils has been found (Frega, Mozzon, & Lercker, 1999). Dąbrowski et al. (2016) showed a significant correlation between induction time and acidity value (0.84) and between induction time and peroxide value (−0.74) in chia seeds, while Caruso, Galgano, Scarpa, Ornaghi, and Favati (2017) indicated a correlation between extra virgin olive oil I.P. and total phenolic content.

I.P. values of chia seeds grown in different geographical areas and analyzed in our laboratory are reported in Fig. 2. It is possible to compare these different I.P. since they have all been calculated in the same conditions. G8 genotype seeds grown in Basilicata showed a lower I.P. than commercial seeds from Peru cultivated in Peru and Basilicata, but had a higher I.P. than commercial seeds from Australia. There is a lack of other reports for chia seeds analyzed by Oxitest (Amato et al., 2015). A shelf life test was made on two seeds samples, having the same sowing density. The estimated oxidative stability was 225 days (7.5 months) for the irrigated sample (Fig. 3) and 239 (7.9 months) for the not irrigated one at 20 °C. Limiting irrigation volume has been reported to optimize the yield and nutritional quality of vegetables (Favati et al., 2009). Resellers usually indicated for chia seeds a shelf life of 2–3 years. Amato et al. (2015) conducted a shelf life test by Oxitest on commercial chia seeds from Peru; they had an oxidative stability of 848 days (28.8 months); this value was over 3 fold than the predicted shelf life for seeds from Basilicata. As stated previously, the seeds analyzed in the present experimentation had a higher peroxide value than that found from other researchers. The shorter shelf life could be related to this parameter. However, Amato et al. (2015) did not report P.V. during storage time, so is not possible to make a complete comparison and to state with certainty that this difference is related to P.V. trend.

There are no guidelines for chia seeds to be sold in Europe regarding their chemical characteristics, while chia oil has to contain a minimum 60% of α-linolenic acid and a maximum of P.V. of 10 meq O_2/kg oil (Commission EU, 2014) and it has to be extracted by physical method.

An oil extracted from our seeds would be too close to the P.V. limit or even higher, so its selling would not be allowed in the European market. The end of shelf of life for the two samples predicted with Oxitest seems to coincide with the reaching of 20 meq O_2/kg that is the P.V. limit value for extra virgin olive oil (Fig. 4).

4. Conclusions

There is an increasing use of both oil and chia seeds in human diet, animal feed, drying oil in paints, cosmetic field. Therefore, there is an interest to generate new chia strains that would allow the cultivation of chia seeds also in temperate regions and to mature before the frost, such as long-day flowering chia genotypes.

In this context the proposed research reports the first results of

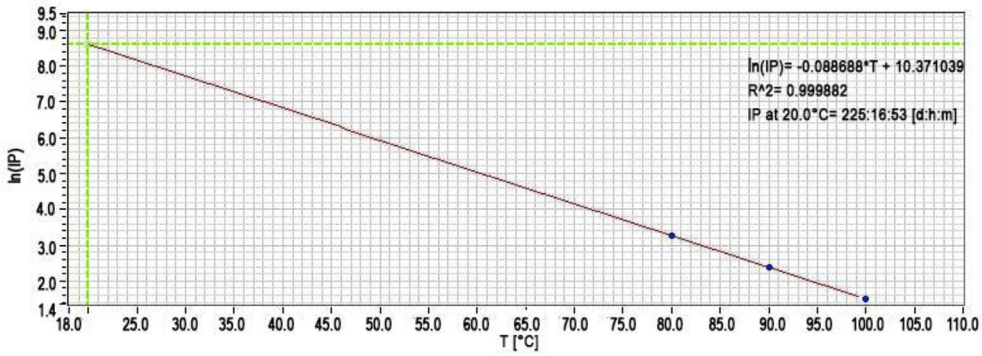

Fig. 3. Results of shelf life test on a chia sample (sowing density: 13 plants/m^2, irrigated).

the oxidative stability and nutraceutical characteristics of a new long-day flowering line of chia seeds grown in Europe and the effect of sowing density and irrigation rate on their chemical parameters. No relevant influence of sowing density and irrigation rate has been found on seeds characteristics. The quality of seeds for acidity, fatty acid composition, phenolic content and antioxidant activity was comparable with the one of chia seeds grown in their native area. This result allows to state that in Mediterranean area the chia seeds production is possible. However, a higher content of peroxides and a shorter predicted shelf life has been recorded. This could make not marketable in Europe an oil extract from these seeds, according to 2014/890/EU decision. This aspect could require further studies to understand if the high P.V. is a characteristic of this genotype or is due to soil and to climate conditions or to an interaction of both factors. The chemical characteristics of seeds were monitored for 10 months; the results showed that seeds retain pretty well their nutraceutical compounds, preserving their richness in tocopherol, phenolic acid and flavonols for several months.

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