CHARACTERIZATION OF PRESS AND SOLVENT EXTRACTION OILS FROM NEW SUNFLOWER SEEDS WITH MODIFIED PHYTOSTEROL COMPOSITIONS

RUNNING TITLE: Sunflower oils with modified phytosterol compositions

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ABSTRACT

BACKGROUND: Phytosterols are plant components with health benefits. Oleaginous seed hybridization can be relevant to increase phytosterols in diet through enriched oils. Sunflower oils obtained by press (PO) and subsequent solvent extraction (SO) from three types of phytosterol-enriched seeds were characterized. One presented a phytosterol composition of common sunflower seeds, whereas the others were rich in Campesterol and Δ7-Estigmastenol, respectively. Seeds from two different harvests, 2015 and 2017, were studied.

RESULTS: The type of extraction did not have a significant influence on the fatty acid (FA) composition. However, considerable differences were found between harvests. The oleic-to-linoleic ratio decreased from 0.71 in 2015 to 0.47 in 2017. The phytosterol compositions of the PO were similar to their SO homologues and no substantial differences were found between harvests. However, the SO presented higher total contents of phytosterols (4849-9249 mg kg^{-1}) than the PO (2839-5284 mg kg^{-1}) and the oils of 2017 showed higher levels (4476-9249 mg kg^{-1}) compared to 2015 (2839-5754 mg kg^{-1}). Unlike phytosterols, no significant differences were found in the tocopherol contents between the PO and SO or between harvests. The PO met Codex specifications for edible oils, except for trace metals, with concentrations close or above the limits for Cu, Fe, Pb and As.

CONCLUSION: Differences in environmental and/or cultivation conditions between harvests may result in substantial differences in the FA composition and phytosterol content in oils from the new sunflower seeds. Rigorous measures and controls to avoid metal-trace contamination are required so that the PO can be considered edible virgin oils.

Keywords: phytosterols; sunflower oil; press oil; solvent-extracted oil; virgin oil; heavy metals

INTRODUCTION

Today, consumers are increasingly demanding foods enriched in bioactives to improve health and reduce risk factors associated with ailments. Phytosterols are bioactive components present in plants with different health benefits, such as their ability to reduce cholesterol in plasma. There are numerous foods in the market that have been enriched in phytosterols by simple addition.[1]

Vegetable oils are principal sources of phytosterols.[2] Oleaginous seed hybridization can be a relevant alternative to increase phytosterols in diet through oils enriched in a natural way. In fact, new sunflower seed lines with increased phytosterol content and modified phytosterol compositions have recently been developed.[3,4] An in-depth study of extraction and composition of the oils from these new seeds is required to examine their feasibility in market.

The industrial production of sunflower oils is carried out by press followed by subsequent solvent extraction. In general, both oils are blended and the resulting oil is refined for commercialization. However, the great interest of consumers for minimally processed foods makes it appealing to consider the two oils separately so that the press oils can be commercialized as non-refined or virgin oils.[5]

The production of virgin oils from new cultivars with modified phytosterol contents would provide higher quality oils, since part of the compounds of nutritional interest, such as phytosterols, are lost in the refining process.[6] The Codex Alimentarius[7] classifies *virgin oils* as those that are obtained by mechanical procedures without modifying the oil and by application of heat only, without applying refining. The elements Ca, Mg, Cu, Mn, Fe, Zn, Co, Mo, Se and I are regarded as important trace elements from a nutritional point of view, whereas Cd and Pb, incorporated by environmental pollution,[8] are considered highly toxic and their levels are limited by Codex specifications. [7]

The influence of the oil extraction procedures separately, press followed by subsequent solvent extraction, on the chemical composition of oils from three types of sunflower seeds obtained from hybrids enriched in phytosterols were studied.[3] The phytosterol composition of one of them was that found in common sunflower seeds, whereas the other two presented high concentrations of Campesterol (CAMP) and Δ7-Estigmastenol (Δ7-STIG), respectively. Seeds from two different harvests, 2015 and 2017, were evaluated. The results of the press oils were compared to the Codex specifications for edible virgin oils.

MATERIALS AND METHODS

Seeds

The seeds were provided by the *Institute for Sustainable Agriculture (CSIC)* (Córdoba, Spain). They were developed by Fernández-Cuesta *et al* [3] using recombination of seeds from germplasm. The seeds with conventional phytosterol composition had been used as a control sample in the genetic studies and it was also considered as such in the present work, since their total content of phytosterols was within the range established for non-modified sunflower seeds.[7]

The two harvests examined were different in terms of sowing time. The sowing of 2015 took place on March 27th. The flowering occurred on June 2nd, with 50% of the plants in bloom, while ripening took place on July 17th, taking into account that 50% of the plants were ripen. In 2017, planting was later, on April 13th. Flowering occurred on June 6th and ripening on July 15th.

Extraction processes

Sunflower seeds were subjected to press followed by solvent extraction. The oil extraction processes were replicated in selected samples. The CAMP sample of 2015 was divided into two portions and Δ7-STIG of the same crop in three. The oils obtained from each portion were treated as independent samples. Previous to the extraction, 10% (w/w) distilled water was added to the seeds and then these were conditioned in an oven at 60°C for 1 h.

Extraction by press (Expeller). The device used was a 40a Täby Pressen (Skeppsta Maskin AB, Örebro, Sweden) with a capacity of 3.5 kg h^{-1}. The Expeller comprised a 12-mm nozzle (i.d.) and operated with a screw rotation speed of 70 rpm. The amounts of seeds processed in 2015 were 1 kg control, 3 kg CAMP and 5 kg Δ7-STIG, whereas those in 2017 were 13 kg control, 3 kg CAMP and 5 kg Δ7-STIG.

Solvent extraction. The residual oil in collets was further extracted in a Soxhlet (2-kg capacity). An amount of 1 or 2 kg pellets and 2.5 L hexane were used in each extraction. This was performed for 6 h. The solvent was removed in a rotary evaporator at 60°C under vacuum.

For comparative purposes, the total oil obtained by direct Soxhlet extraction of ground seeds was also evaluated. The extraction was performed in a Soxhlet (250-g capacity) for 4 h according to UNE-EN ISO 659:2010. An amount of 10-15 g ground seeds and 150 mL hexane were used.

Analytical methods

The moisture in the seeds was determined according to official method UNE-EN ISO 665:2001. The total content of oil was performed with hexane according to UNE-EN ISO 659:2010. Acidity was determined following method UNE-EN ISO 660:2010.

The FA composition and the levels of phytosterols were determined by using a recent method developed in our lab.[9] Briefly, an aliquot of 50-mg oil was trans-methylated with 5 mL of 3% sodium methoxide solution in methanol at 80°C for 30 min. Then for the methylation of the free FA, a solution of 4% sulfuric acid in methanol was added and the reaction was run at 80°C for 30 min. The lipid fraction was extracted three times with n-hexane, washed two times with water and dried with anhydrous sodium sulfate. Finally, the solvent was evaporated under nitrogen and the sample was re-dissolved in hexane for SPE fractionation (NH_2-500 mg/3 mL). Two fractions comprising the FA methyl esters and the phytosterols, respectively, were obtained and analyzed by GC.[9]

Tocopherols were determined by HPLC with fluorescence detection following IUPAC Standard Method 2.432.

The analysis of metal and non-metal traces, i.e. Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, V and Zn, were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) at the *Institute of Natural Resources and Agrobiology (CSIC)* (Seville, Spain). SRM 1573a (Tomato Leaves) and an inter-laboratory sample (SE 2016 IPE-WEPAL) from the University of Wageningen were used as reference materials. Briefly, an aliquot of 0.25 g of each oil sample, weighed with an accuracy of 0.1 mg, was digested with a mixture of 4 mL of nitric acid and 2 mL of hydrogen peroxide in hermetically sealed Teflon vessels in a microwave oven (MILESTONE mod. START D). The digested sample was dissolved in 25 mL of purified water and filtered for further analysis. The measurements were performed on a Varian ICP 720-ES axial configuration device.

Statistical analyses

A full factorial design of three factors, type of seed (Control, GF-2 and GF-7), harvest (2015 and 2017) and extraction process (press, solvent extraction of the oil remaining after press and solvent extraction of total oil) was considered. The statistical program used was SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) and significance was considered at $p < 0.05$.

Univariate analysis using a generalized linear model (GLM) was applied to assess the influence of the type of seed and harvest on the moisture content of seeds and oil yield. *Student's t-test* was used for comparison between the crops. Analysis of variance (ANOVA) and *Tukey's test* were applied for comparison between the three types of seeds.

For the FA composition data, a GLM was used applying multivariate analysis of three factors. The type of seeds, harvest and extraction process were the independent factors, while the dependent variables were each of the FAs. The acids C16:1, C18:3 and C20:1 were not taken into account, since their quantities were so low that in certain samples they could not be determined for presenting levels below the quantification limits. *Levene's test* was not met for any of the FAs ($p = 0.000$) and, therefore, non-parametric analysis for independent samples was applied to each of the three factors. The significance tests applied were the *Kruskal-Wallis and Mann-Whitney U tests* for 3 and 2 levels, respectively. Because substantial differences between harvests were found, the same non-parametric analysis was applied to the data obtained for each crop separately. The comparison between crops was carried out by applying the *paired-samples T-test* and also a non-parametric test of related samples. Both tests provided equal results.

The same statistical analysis described above for the FAs was used for the composition of phytosterols, but, for obvious reasons, the statistical analysis was applied separately to each type of sample (Control, CAMP and Δ7-STIG). Again, *Levene's test* was not fulfilled. Non-parametric analysis for independent samples was applied to evaluate the influence of the extraction process, whereas a non-parametric analysis of related samples was used to assess the influence of the harvest year.

The total phytosterols content was analysed using a three-factor GLM. Again, it was necessary to apply non-parametric tests because *Levene's test* was not met. Non-parametric analysis of independent samples was used to evaluate the influence of the type of seeds and the extraction process, whereas non-parametric analysis of related samples was applied to evaluate the harvest year. Due to the large differences found between crops, the statistical analysis was applied to data of each crop separately. To establish differences between the average values of oils obtained by different extraction processes, for each type of seeds and year of harvest, one-factor ANOVA was used applying *Tukey's test* for equal variances or Games-Howell for unequal variances.

The tocopherol contents and the metal trace contents were analysed with the *paired-samples T-test* to assess the influence of the extraction process (press and solvent) and the harvest (2015 and 2017). ANOVA and *Tukey's test* were used to compare the three types of seeds.

RESULTS AND DISCUSSION

Characteristics of the seeds

The seed samples presented moisture contents that were between 45 and 67 g kg^{-1} (Table 1). These values were similar to those found in the literature.[10]

The press extraction gave rise to yield values that were between 213 and 312 g kg^{-1}, expressed on seed weight (Table 1). Slightly higher values were obtained for the Δ7-STIG samples, with average of approximately 300 against 230 and 250 g kg^{-1} obtained in the Control and CAMP, respectively. According to Gunstone,[11] the mechanical extraction of sunflower oil can reach yields between 700 and 800 g kg^{-1} of total oil. In the present study, the hybrids CAMP and Δ7-STIG presented values between 710 and 730 g kg^{-1} of total oil. No significant differences were found for the press performance between the two harvesting periods.

The amounts of oil obtained by solvent extraction in the resulting pellets were similar for the Control and Δ7-STIG samples, with average values of 160 g kg^{-1}, and slightly lower for the CAMP sample, with 130 g kg^{-1}.

With respect to the mass balance, the total amount of oil obtained in the two extraction procedures as a whole was consistent with the total oil content in the seeds. In fact, the meal residual oil was very low, ranging between 1.2 and 3.0 g kg^{-1}, which corresponded to 2-5 g kg^{-1} of the total oil extracted.

Acidity

Acidity did not exceed 2% in all cases (Table 1S). The oils obtained by press (PO) showed lower values (≤1%) compared to the oils obtained by solvent extraction (SO) (0.7-1.6%). The results for the PO were in agreement with previous studies.[12,13] In this regard, Bendini *et al*.[13] analyzed regular cold-pressed sunflower oils manufactured by different European countries and found acidity values of 0.65-1.59%, figures that are characteristic of virgin oils (unrefined) of good quality.

Fatty acid composition

The analysis of FAs for the three hybrids showed similar compositions and characteristic of conventional high linoleic sunflower oils (Table 2). As expected, C18:1 and C18:2 constituted 87-89% of all FAs, which was consistent with CODEX standards.[7]

No significant differences were found in the composition of fatty acids between the PO and SO. Nor were significant differences found when the oils obtained by the three extraction procedures were compared, press, solvent extraction of the remaining oil after press and solvent extraction

of total oil. Therefore, the results showed that the extraction processes did not have a significant influence on the fatty acid composition.

However, substantial differences were found between oils from different seasons. The C18:1 content was higher for the 2015 oils, with an average of 36.6% (± 2.6) compared to 28.1% (± 3.2) in 2017. The opposite occurred for the C18:2, with an average of 51.8% (± 2.8) in 2015 compared to 60.2% (± 2.7) in 2017. Since the harvest factor presented a high variability, statistical analysis was applied to the data of each harvest separately. The results of this analysis showed slight differences for all FAs when the three oil samples were compared in both the 2015 and 2017 harvests. Again, no substantial differences were found when the extraction methods were compared in each season separately. Only slight but significant (p <0.05) differences were observed and these were sample and harvest dependent (Table 2).

The substantial differences found between oils from different harvesting periods could be related to differences in environmental conditions during seed development. Differences in temperature and water stress can have consequences in the physiological accumulation of FAs.[14-17] According to Lajara *et al*.,[14] the average temperature from flowering to ripening seems to be an important factor that affects the FA composition of sunflower oil. They observed that an increase in temperature resulted in a decrease of linoleic acid and an increase of oleic acid. The effect of these factors, however, seemed to depend on the type of genotype studied, i.e. sunflower *versus* high oleic sunflower. In high oleic sunflower oils, only temperature affected the oleic-to-linoleic ratio.[15]

In the present study, temperatures were recorded during the two growth periods. The 2017 harvest was exposed to higher temperatures, with an average of 29.0ºC compared to 27.5ºC in 2015. The maximum temperature was also higher, 37.7ºC compared to 36.6ºC, and especially the minimum temperatures, 20.0°C versus 17.4°C. Figure 1 shows the distribution of the temperatures recorded during the growing cycle. The main differences took place in the initial period, where temperatures in 2017 were significantly higher. However, unlike other studies,[15] the oleic-to-linoleic ratio decreased markedly in the 2017 harvest, from 0.71 in 2015 to 0.47.

Differences in rainfall between the harvests were also observed. Although there were no substantial changes in the total amount between 2015 (0.47 mm day^{-1}) and 2017 (0.48 mm day^{-1}), there were differences in the distribution over time (Figure 1). Rainfall was less numerous in 2017. These differences could have had an impact on the FA composition and have contributed differently in different stages of seed development. In this regard, Roche *et al*.[17] observed in safflower seeds that a decrease in rainfall resulted in a decrease in C18:2 level, while no significant differences were observed in C18:1 level.

Phytosterols

As remarked earlier, the Control sample presented a phytosterol composition similar to that of conventional or non-modified sunflower seeds (Table 3). Fernández-Cuesta *et al.*[18] reported values of 54.0-60.6% for β-Sitosterol, 13.3-19.1% for Δ7-Stigmastenol, 7.9-10.9% for Campesterol, 9.0-10.5% for Stigmasterol and 2.8-4.2% for Δ7-Avenasterol.

The CAMP sample presented 27.9% (± 2.5) of Campesterol *versus* 7.2% (± 0.7) found on average in the other two samples (Table 3). The Δ7-STIG sample showed 26.4% (± 2.4) of Δ7-Stigmastenol *versus* 7.4% (± 0.6) and 16.6% (± 1.7) found in the CAMP and Control samples, respectively. The increase in the levels of these two types of phytosterols in the seeds resulted in a decrease in the levels of the majority sterol, β-Sitosterol. While the average level of β-Sitosterol in the Control was 62.2% (± 2.2), the relative amounts found in CAMP and Δ7-STIG were 49.7% (± 1.1) and 51.0 % (± 2.5), respectively.

As in previous studies,[12] no substantial differences were found in the composition of phytosterols between the PO and SO. Only, slight differences were observed and these were both sample and harvest dependent (Table 3). Likewise, slight but significant (p <0.05) differences were also found in the phytosterol compositions between oils from different harvests. These were sample dependent except for stigmasterol, whose levels were greater for 2017.

Regarding the total content of phytosterols, the Δ7-STIG sample showed higher concentrations than the Control and CAMP samples, whereas no significant differences were found between the last two samples, neither for the 2015 harvest (p = 0.443) nor for that of 2017 (p = 0.912) (Table 3).

The SO showed higher total phytosterol contents (4849-9249 mg kg^{-1}) than their PO homologues (2839-5284 mg kg^{-1}). These results are in agreement with previous studies on sunflower seeds [3,12] and also in other oleaginous seeds.[19] The higher concentrations of phytosterols in the SO can be attributed to the higher extractive capacity of the solvent, despite its lower polarity compared to the oil. In the PO, the concentration of phytosterols depends on the amount of oil released, which acts as a solvent. Once the oil is saturated in phytosterols, this cannot incorporate more amount. However, from a material that has already lost a large amount of oil in the previous press extraction, the phytosterols are concentrated in the solvent extraction by continuous contact with fresh solvent.

No significant differences were found in the total content of phytosterols between the PO and the total oils, neither in the 2015 harvest (p = 0.111) nor in 2017 (p = 0.406). Even when the phytosterol contents were higher in the SO, their contribution to the total oil was not sufficient

to significantly increase the levels of the PO. In addition, the oil that would result from the blend between the PO and SO must be refined, giving rise to partial losses of phytosterols.[6] Therefore, cold press oils are a good alternative to the oils obtained from the mixture in terms of the total content of phytosterols.

The oils of the 2017 harvest clearly showed higher total contents of phytosterols than those of 2015. The concentrations found for the PO were between 2839 and 4290 mg kg^{-1} in 2015 and between 4476 and 5284 mg kg^{-1} in 2017. Likewise, the concentrations in the SO were between 4849 and 5754 mg kg^{-1} and between 6678 and 9249 mg kg^{-1} in 2015 and 2017, respectively. These remarkable differences could also be attributed to differences in environmental conditions during seed development. The higher temperatures recorded in 2017, in combination with the differences remarked above for the rainfall, may explain the greater concentrations of total phytosterols in the oils of this harvest. In this regard, Schaller[20] suggested that a greater accumulation of phytosterols in sunflower seeds may be a response of the seeds to high temperatures, due to their role in regulating fluidity and permeability of the cell membranes. Later, Roche *et al*.[14] observed that high levels of phytosterols were related to warm temperatures and severe water deficits. In another study, Roche *et al*.[21] stated that higher levels of total phytosterols were obtained with planting late, with higher average temperatures being recorded during flowering and seed maturation. These results are also in agreement with other studies on safflower seeds [17] and on soybeans subjected to high temperatures in greenhouse[22] and also in the field.[23] In contrast, no temperature effect has been observed in canola seed.[24] In addition, different authors have reported that water stress during seed development improves the accumulation of phytosterols in sunflower seeds.[25]

Tocopherols

Both the composition and contents of tocopherols found in the oils were characteristic of conventional sunflower oils. In agreement with reported data, the oils comprised α-tocopherol (96% of total tocopherols) and minor amounts of β-tocopherol.[26] The CAMP oils exhibited the highest levels of tocopherols (922 ± 71 mg kg^{-1}), followed by Δ7-STIG oils (762 ± 28 mg kg^{-1}) and the Control sample (512 ± 14 mg kg^{-1}) (Table 2S). The total tocopherol contents were in the range reported in other studies.[27] The results obtained in the PO were similar to their SO counterparts. Unlike the total content of phytosterols, no significant differences in the total content of tocopherols were found between oils from the two harvests studied.

Studies on soybean[28] and sunflower[18,26] oils have clearly shown that tocopherol levels depended on genotype and weather conditions, in particular, temperature. A reduction in the total content of tocopherols took place when the plants were exposed to higher temperatures during the seed development.[26] The results of the present study are however in agreement with studies on

soybean, where the total levels of tocopherols were not affected by different temperatures during the seed growth.[29]

Heavy metals and other elements

Table 4 shows the contents of heavy metals and other elements found in the oils. The major elements were Ca, Fe, K, Mg, Na, P and S. The levels of Cu and Fe were consistent with those found in other studies,[30] but Cd, Pb and Zn levels were below those reported by Ansari *et al*.,[31] whose ranges were 1.7-6.2 mg kg^{-1}, 0.7-4.4 mg kg^{-1} and 2.7-7.7 mg kg^{-1}, respectively.

Significant differences in the contents of Ca, K, Mg and P were found between the three oils studied, whereas no significant differences were detected for the rest of the elements analyzed. With the exception of Ca in the 2015 harvest, the levels of these four elements were higher in the Δ7-STIG sample, followed by the Control and CAMP.

The PO clearly showed lower levels of Ca, K, Mg and P than their SO homologues. Specifically, the PO showed P levels between 9 and 36 mg kg^{-1}, whereas the levels ranged from 263 to 585 mg kg^{-1} in the SO. Levels of P as high as those of the present work have also been reported by other authors.[30,32] These results can be attributed to the greater extraction capacity of hexane than of the oil itself, as previously remarked for phytosterols.

As to the minerals of nutritional interest, such as Cu, Fe and Zn, no substantial differences were found between the PO and SO, except for Fe in the 2017 harvest, where the SO clearly presented higher levels.

The oils from the 2017 harvest exhibited higher levels of Ca compared to their counterparts in 2015. The same was also observed for Mg and P, with the exception of the Control SO. Likewise, the Fe concentrations in the SO were also higher in the 2017 harvest, whereas similar results were found between harvests for the PO.

Comparison with the characteristics required in the Codex Alimentarius for seed virgin oils

The press oils met the definition of seed virgin oil, since they were obtained without modifying the oil by mechanical procedures and by application of heat only after seed conditioning. They can even be considered *cold pressed oils*, since the seeds were not roasted before extraction.[33] The color, smell and taste were characteristic of sunflower oil, and free of strange flavors.

The contents of sterols and tocopherols were within the ranges of specifications for sunflower oil (Table 3S). However, except the Control, the oils had special compositions of sterols. Therefore, an adaptation of the regulation would be required to include these new cultivars.

The oil samples met the quality specifications for volatile matter at 105°C (< 0.2%) and insoluble impurities (< 0.05%) (data not shown), as well as acidity, which was less than 1% in all cases.

The greatest difficulties to market these virgin oils were found in the content of trace metals, specifically Fe, Cu, Pb and As (Table 3S). The contents of Fe, Cu and Pb were close to the limit or exceeded the maximum levels established in the Codex Alimentarius. Arsenic showed rather high levels in 2015 (0.7-1.0 mg/kg) compared to the limit established (≤0.1 mg/kg), whereas it was not detected in 2017. In this respect, high levels of heavy metals in oils, such as Pb, Ni, Cd and Mn have been attributed to their presence in soil and in wastewater used sometimes for agricultural irrigation.[8]

CONCLUSIONS

Differences in environmental and/or cultivation conditions between harvest seasons may result in substantial differences in the FA composition and the total content of phytosterols in the oils from the three new sunflower seed hybrids studied.

Through rigorous measures and controls to avoid metal-trace contamination from fertilizers and plant-protection products, the PO could be commercialized as edible virgin oils and so be considered a good alternative to their blend with SO, which necessarily have to be refined losing significant amounts of phytosterols and other bioactive components.

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FIGURE CAPTIONS

Figure 1.- Temperatures and rainfall during the growing cycle of seeds. Solar radiation was 23.30 and 27.45 MJ m^{-2} day^{-1} in 2015 and 2017, respectively.

Table 1. Characteristics of the seeds and yield values for the oil extraction processes.

	Control		CAMP		Δ7-STIG	
	2015	**2017**	**2015**	**2017**	**2015**	**2017**
Moisture (g kg^{-1})	67±1.5bC	55±1.8aC	62±1.3bB	51±1.8aB	45±1.2aA	46±1.7aA
Fat Content (g kg^{-1})	327±8aA	375±4bA	351±8aA	326±2bA	420±1aB	376±3bB
Oil yield (g kg^{-1})						
Press	213±11aA	247±12aA	268±13aB	245±12aB	312±16aC	282±14aC
Solvent †	164±16aB	148±15aB	141±14aA	123±12aA	182±18bB	148±15aB
Meal oil ‡ (g kg^{-1})	1.7±0.5aAB	2.4±0.2aAB	2.0±0.1aA	1.2±0.04aA	2.4±0.7aB	3.0±0.9aB

† Values expressed on pellets. ‡ Residual oil in meal. The results are expressed as mean values ± standard deviation (n=3). Different lowercase letters show significant differences between harvests (*Student's t-test*) and different capital letters indicate significant differences between seed samples (*Tukey's test*) (p<0.05).

Table 2.- Main fatty acid composition (%) of the oils.

		Control			CAMP			Δ7-STIG		
		Total †	**Press**	**Solvent**	**Total †**	**Press**	**Solvent**	**Total †**	**Press**	**Solvent**
2015										
	C16:0	7.4±0.03aB	7.3±0.05aB	7.7±0.05bB	7.0±0.07aB	7.0±0.22aB	7.3±0.29aB	5.9±0.10aA	6.0±0.04aA	6.4±0.013bA
	C16:1	0.2±0.01A	nd	nd	nd	nd	nd	nd	nd	nd
	C18:0	4.3±0.04aB	4.3±0.02aB	4.3±0.01aB	2.5±0.06aA	2.5±0.16aA	2.6±0.15aA	3.9±0.07aA	3.9±0.03abA	4.0±0.03bA
	C18:1	35.2±0.20bB	35.3±0.08bB	34.3±0.08aB	34.5±0.12aB	34.4±1.40aB	33.7±1.41aB	40.8±0.35bB	39.2±0.58abB	38.3±0.73aB
	C18:2	51.4±0.21aA	51.8±0.13bA	52.4±0.04cA	54.8±0.21aA	55.0±1.33aA	55.5±1.29aA	47.9±0.39aA	49.6±0.58abA	49.9±0.65bA
	C20:0	0.4±0.02aB	0.4±0.01aB	0.4±0.02aB	0.2±0.03aA	0.2±0.02aA	0.2±0.01aA	0.3±0.03aA	0.3±0.01abA	0.4±0.02bA
	C18:3	0.2±0.01	nd	nd	0.2±0.01	nd	nd	0.2±0.02	nd	nd
	C20:1	nd	nd	nd	nd	nd	nd	nd	nd	nd
	C22:0	0.7±0.02aB	0.7±0.01aB	0.7±0.02aB	0.5±0.03aA	0.6±0.03aA	0.5±0.01aA	0.7±0.08aB	0.7±0.02aB	0.7±0.02aB
	C24:0	0.3±0.02aA	0.3±0.02aA	0.3±0.01aA	0.2±0.01a	0.3±0.02bB	0.2±0.01aA	0.3±0.03aA	0.3±0.01aA	0.4±0.02bA
2017										
	C16:0	6.9±0.06aA	7.0±0.01aA	7.3±0.02bA	6.6±0.11aA	6.5±0.01aA	6.8±0.03bA	7.1±0.15aB	7.1±0.01aB	7.4±0.00bB
	C16:1	0.2±0.00aA	0.2±0.00a	0.2±0.00a	nd	nd	nd	nd	0.1±0.00a	0.1±0.00a
	C18:0	3.6±0.01aA	3.6±0.01aA	3.6±0.01aA	3.0±0.05aB	3.0±0.01aB	3.2±0.01bB	4.0±0.02aB	4.1±0.03bB	4.1±0.01bB
	C18:1	30.8±0.08cA	30.0±0.02bA	29.0±0.03aA	31.0±0.17cA	30.0±0.08bA	29.6±0.04aA	23.9±0.15bA	23.8±0.02bA	23.6±0.02aA
	C18:2	57.2±0.10aB	57.9±0.05bB	58.5±0.06cB	58.7±0.23aB	59.5±0.09bB	59.3±0.07bB	64.0±0.10cB	63.6±0.02bB	63.3±0.02aB
	C20:0	0.3±0.00aA	0.3±0.00aA	0.3±0.00aA	0.2±0.01aA	0.3±0.04bA	0.2±0.00aA	0.3±0.02aA	0.3±0.00aA	0.3±0.00aA
	C18:3	nd	0.1±0.00a	0.1±0.00a	nd	nd	nd	nd	0.1±0.00a	0.1±0.00a
	C20:1	0.2±0.00a	0.2±0.00a	0.2±0.00a	nd	0.1±0.02a	0.2±0.00b	nd	0.2±0.00b	0.2±0.00b
	C22:0	0.6±0.00aA	0.6±0.00aA	0.6±0.01aA	0.5±0.02bB	0.4±0.05aB	0.5±0.01bB	0.5±0.02aA	0.6±0.01bA	0.6±0.00bA
	C24:0	0.3±0.00aA	0.3±0.00aA	0.3±0.00aA	nd	0.1±0.02aA	0.2±0.00bA	0.3±0.03aA	0.3±0.00aA	0.3±0.00aA

† Total oil obtained by Soxhlet extraction from whole seeds. nd, not detected. The results are expressed as mean values ± standard deviation (n=4). Different lower case letters show significant differences between the oils obtained from a given seed cultivar for each harvest separately (*Tukey's test/Games-Howell*) ($p<0.05$). Different capital letters denote significant differences between harvests (student's *t* test) ($p<0.05$).

Table 3.- Composition and total concentration of major phytosterols in the oils.

		Control			CAMP			Δ7-STIG		
		Total †	**Press**	**Solvent**	**Total †**	**Press**	**Solvent**	**Total †**	**Press**	**Solvent**
2015										
	Composition (%)									
	Campesterol	7.6±0.10aB	7.8±0.52aB	8.4±0.15bB	29.0±1.37cB	31.2±1.10bB	26.7±0.62aB	6.8±0.15aA	6.9±0.26aB	8.0±0.37bA
	Stigmasterol	7.4±0.35bA	6.7±0.11aA	10.4±0.32cA	7.4±0.19aA	7.5±0.40aA	9.7±0.24bA	9.2±0.09aA	8.9±0.26aA	10.5±0.26bA
	β-Sitosterol	63.6±0.47abB	65.2±0.87bB	62.6±1.14aB	48.8±0.53aA	49.7±1.18abB	50.9±0.37bA	49.5±0.65aA	52.9±0.96bB	53.5±0.73bB
	Δ5-Avenasterol	2.9±0.28B	nd	nd	3.5±0.51A	nd	nd	3.9±0.20B	nd	nd
	Δ7-Stigmastenol	15.0±0.90abA	16.6±0.44bA	14.6±0.75aA	7.3±0.72aB	7.2±0.59aB	8.3±0.91aB	25.8±0.65abA	26.8±0.72bA	24.0±1.04aA
	Δ7-Avenasterol	3.6±0.06aA	3.7±0.05aA	4.0±0.14bA	3.9±0.08aA	4.4±0.18bA	4.4±0.22bB	4.6±0.20bB	4.4±0.18abA	4.0±0.24aA
	Total (mg kg^{-1})	3644±230bA	3246±35aA	5065±10cA	4008±454bA	2839±109aA	4849±439cA	4415±341aA	4290±232aA	5754±388bA
2017										
	Composition (%)									
	Campesterol	6.3±0.13aA	6.2±0.06aA	7.3±0.15bA	26.7±0.33bA	28.2±0.1cA	23.4±0.19aA	7.0±0.04bB	6.2±0.06aA	8.3±0.04cA
	Stigmasterol	8.9±0.3bB	7.8±0.08aB	11.4±0.14cB	9.0±0.13aB	8.9±0.29aB	11.0±0.11bB	10.9±0.05bB	9.4±0.19aB	12.6±0.25cB
	β-Sitosterol	61.7±0.98bA	61.2±0.4bA	57.9±0.2aA	49.7±0.17bB	47.5±0.07aA	50.9±0.35cA	49.2±0.14bA	46.6±1.1aA	48.1±0.09bA
	Δ5-Avenasterol	1.5±0.03aA	1.6±0.04b	1.7±0.04c	3.3±0.04aA	3.7±0.07b	3.3±0.03a	0.9±0.05aA	0.9±0.03a	1.5±0.05b
	Δ7-Estigmastenol	17.8±1.4aB	18.8±0.51aB	17.3±0.46aB	7.1±0.45aA	6.7±0.18aA	7.7±0.64bA	27.5±0.20bB	32.5±1.58cB	24.8±0.48aA
	Δ7-Avenasterol	3.8±0.12aB	4.5±0.1bB	4.4±0.19bB	4.1±0.11bA	4.9±0.12cB	3.6±0.05aA	4.4±0.04aA	4.3±0.28aA	4.6±0.22aB
	Total (mg kg^{-1})	5370±347bB	4476±136aB	7272±343cB	4666±124aB	5225±203bB	6678±387cB	7177±214bB	5284±200aB	9249±669cB

† Total oil obtained by Soxhlet extraction from whole seeds. nd, not detected. The results are expressed as mean values ± standard deviation (n=4). Different lower case letters show significant differences between the oils obtained from a given seed cultivar for each harvest separately (*Tukey's test/Games-Howell*) (p<0.05). Different capital letters denote significant differences between harvests (student's *t* test) (p<0.05).

Table 4.- Elemental analysis (mg kg^{-1}) of the oils (n=2).

		Control		CAMP		Δ7-STIG	
		Press	**Solvent**	**Press**	**Solvent**	**Press**	**Solvent**
2015							
	As	0.9	1.2	1.0	0.8	0.7	1.1
	Ca	31.8	202.8	24.1	99.6	21.9	158.9
	Cd	nd	nd	0.1	nd	nd	nd
	Cu	0.4	0.4	0.5	0.3	0.4	0.4
	Fe	7.0	4.5	3.1	2.6	4.4	4.3
	K	7.6	75.6	7.2	38.9	4.8	95.0
	Mg	6.7	134.7	5.8	60.4	4.6	107.6
	Na	3.0	2.5	7.6	2.9	nd	11.6
	P	11.7	511.5	9.3	262.8	11.9	513.7
	Pb	nd	0.2	0.2	0.1	0.1	nd
	S	11.4	11.5	11.6	13.4	7.3	19.0
	Sr	0.1	0.4	0.1	0.2	0.1	0.3
	V	0.1	0.2	0.1	0.1	0.1	0.1
	Zn	4.7	1.9	0.8	1.9	1.4	2.9
2017							
	As	nd	nd	nd	nd	nd	nd
	Ca	78.5	217.0	58.6	173.6	106.9	305.4
	Cd	nd	nd	0.1	nd	nd	0.1
	Cu	1.1	0.5	0.2	0.5	0.3	1.1
	Fe	6.0	22.7	4.6	23.5	5.8	32.0
	K	6.7	61.0	7.3	31.0	6.4	95.4
	Mg	11.6	114.1	6.3	63.7	14.4	158.2
	Na	4.0	6.6	2.9	9.8	6.1	9.2
	P	27.3	404.2	15.4	295.6	36.4	585.1
	Pb	nd	0.2	0.2	0.2	0.4	0.4
	S	13.0	17.9	6.6	12.3	14.3	15.0
	Zn	1.0	2.5	0.6	1.5	1.5	4.6

Coefficient of variation ≤ 7%

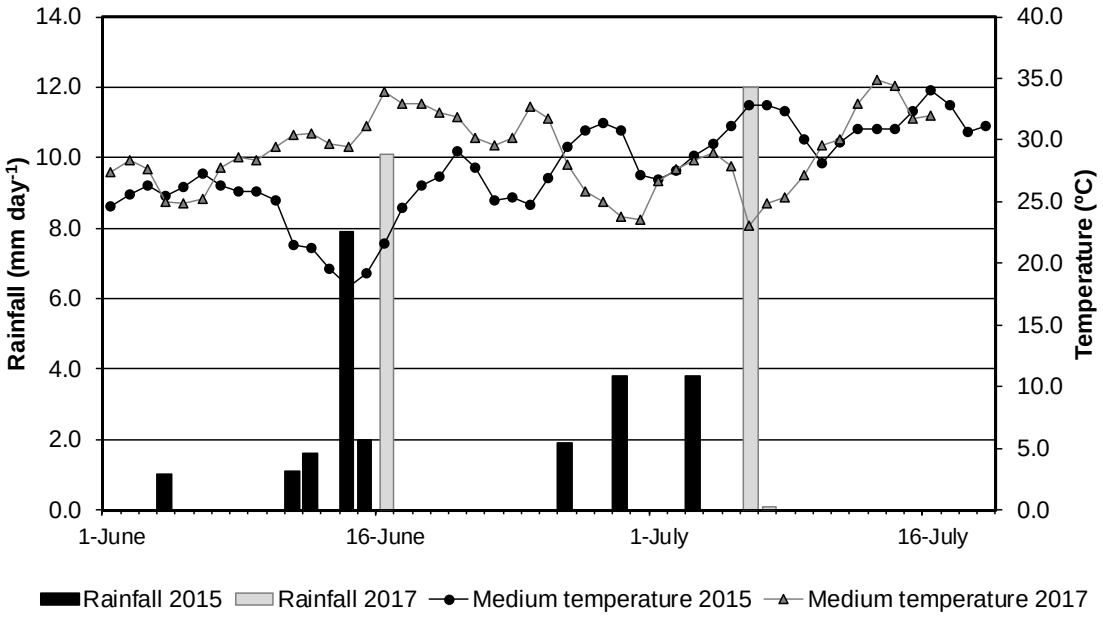
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Rainfall (mm day-1)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Temperature (ºC)
1-June
16-June
1-July
16-July
Rainfall 2015
Rainfall 2017
Medium temperature 2015
Medium temperature 2017

Figure 1

Table 1S.- Acidity (% on oleic acid) of the oils.

	Control		CAMP		Δ7-STIG	
	2015	**2017**	**2015**	**2017**	**2015**	**2017**
Press	0.15aA	1.00aB	0.26aA	0.99aB	0.10aA	1.00aB
Solvent	0.71bA	1.42bB	0.84bA	1.64bB	0.59bA	1.55bB

The results are expressed as mean values (n=2). The variation coefficient was ≤5%. Different lower case letters denote significant differences (p <0.05) between extraction methods (*Mann-Whitney U test*) and different capital letters between harvests (*Mann-Whitney U test*).

Table 2S.- Total tocopherol content (mg kg^{-1}) in the oils.

	Control		CAMP		Δ7-STIG	
	Press	**Solvent**	**Press**	**Solvent**	**Press**	**Solvent**
2015	524aA	492aA	870aA	1025aA	777aA	764aA
2017	515aA	517aA	881aA	912aA	722aA	784aA

The results are expressed as mean values (n=2). The variation coefficient was ≤5%. Different lower case letters denote significant differences (p <0.05) between extraction methods (*Mann-Whitney U test*) and different capital letters between harvests (*Mann-Whitney U test*).

Table 3S.- Main compositional characteristics and quality parameters for virgin sunflower oils according to Codex Alimentarius and average values of the two harvests found in the press oils.

	CODEX STAN 210-1999		**Samples**		
	Sunflower	**High Oleic**	**Control**	**CAMP**	**Δ7-STIG**
Fatty Acids (%)					
C16:0	5.0-7.6	2.6-5.0	7.2±0.2	6.8±0.4	6.6±0.8
C16:1	nd-0.3	nd-0.1	0.1±0.1	nd	0.1±0.1
C18:0	2.3-4.0	2.0-4.0	4.0±0.5	2.8±0.4	4.0±0.1
C18:1	14.0-39.4	75.0-90.7	32.5±3.8	32.2±3.1	31.1±10.9
C18:2	48.3-74.0	2.1-17.0	54.8±4.4	57.3±3.2	56.6±9.9
C18:3	nd-0.3	nd-0.3	0.03±0.04	nd	0.1±0.1
C20:0	0.1-0.5	0.2-0.5	0.4±0.1	0.3±0.0	0.3±0.0
C20:1	nd-0.3	0.1-0.5	0.1±0.1	0.1±0.1	0.1±0.1
C22:0	0.3-1.5	0.5-1.6	0.7±0.1	0.5±0.1	0.7±0.1
C24:0	nd-0.5	nd-0.5	0.3±0.0	0.2±0.1	0.3±0.0
Sterols (%)					
Campesterol	6.5-13.0	5.0-13.0	7.0±1.2	29.7±2.1	6.5±0.5
Stigmasterol	6.0-13.0	4.5-13.0	7.2±0.8	8.2±1.0	9.1±0.4
β-Sitosterol	50-70	42.0-70	63.2±2.8	47.6±1.5	49.7±4.4
Δ5-Avenasterol	nd-6.9	1.5-6.9	0.8±1.2	1.8±2.6	0.4±0.7
Δ7-Stigmastenol	6.5-24.0	6.5-24.0	17.7±1.6	6.9±0.3	29.6±4.0
Δ7-Avenasterol	3.0-7.5	nd-9.0	4.1±0.5	4.6±0.4	4.3±0.1
Total Sterols (mg kg^{-1})	2400-5000	1700-5200	3861±870	4032±1687	4787±702
Tocopherols (mg kg^{-1})	450-1120	450-1120	520±6	876±8	750±39
Quality Characteristics					
Acidity (% on oleic)	≤2	≤2	≤1	≤1	≤1
Fe (mg kg^{-1})	≤5	≤5	≤7	≤4,6	≤5,8
Cu (mg kg^{-1})	≤0.4	≤0.4	≤1,1	≤0,5	≤0,4
Pb (mg kg^{-1})	≤0.1	≤0.1	nd	≤0,2	≤0,4
As (mg kg^{-1})	≤0.1	≤0.1	n.d./≤0,9	n.d./≤1.0	n.d./≤0,7

nd, not detected