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TEMPERATURE EFFECT ON TRIACYLGLYCEROL SPECIES IN OILSEED FROM HIGH-STEARIC SUNFLOWER LINES WITH DIFFERENT GENETIC BACKGROUNDS

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Highlights

- Temperature modified the triacylglycerol species in high stearic sunflower lines.
- Triacylglycerol symmetry was higher in lines with high linoleic than high oleic background.
- Increasing temperature produced a more symmetrical distribution of saturated fatty acids.
- This effect was additional to the effect of temperature on fatty acid composition.

Abstract

The objective of this work was to characterize the influence of temperature during grain filling on the distribution of saturated fatty acids in triacylglycerol molecules in high-stearic and high stearic-high oleic sunflower lines with different genetic backgrounds. Two experiments were conducted in growth chambers with the following day-night temperatures during grain filling: 16-16°C, 26-16°C and 26-26°C (Exp. 1) and 16-16°C, 26-16°C, 26-26°C and 32-26°C (Exp. 2). Plants were harvested after physiological maturity and oilseed was characterized in terms of fatty acid and triacylglycerol compositions and solid fat content profile. High stearic lines in high linoleic background presented a more symmetrical distribution of stearic acid than those with high oleic background. Regardless of the genetic background, increasing night temperature produced an increase in saturated-unsaturated-saturated species, indicating a more symmetrical distribution of saturated fatty acids. The solids fat content was more affected by temperature during grain filling in high stearic lines with high linoleic than high oleic background, mainly explained by a higher variability of fatty acid and triacylglycerol compositions. The higher variations in symmetry among night temperatures were observed in lines with high oleic background, which are more stable in fatty acid composition than lines with high linoleic background. So, the effect of temperature on triacylglycerol composition is not completely explained by its effect on fatty acid composition. These results indicate that night temperature during grain filling affects oil properties via two different effects i) its well known effect on fatty acids synthesis ii) affecting the distribution of fatty acids in the triacylglycerol. This last effect could be further characterized by identifying the different acyltransferase genes expressed during sunflower seed development and characterizing their expression profiles under different day/night temperatures

Keywords: high stearic mutation, temperature, triacylglycerol composition, oil quality

1. Introduction

The physical, chemical, and nutritional properties of oils are determined not only by its fatty acids composition but also by the distribution of fatty acids in the triacylglycerol (TAG) molecules. In sunflower (*Helianthus annuus* L.), TAGs are mainly synthesized by ER-membrane-bound enzymes in the glycerol-3-phosphate pathway or Kennedy pathway (Banaś et al., 2013), starting with the acylation of glycerol 3-phosphate at the *sn*-1 and *sn*-2 positions with acyl-coenzyme A (acyl-CoA) esters. These are mediated in first place by the enzyme glycerol-3-phosphate acyltransferase (GPAT) followed by the production of a phosphatidate by the activity of lysophosphatidate acyltransferase (LPAAT). The phosphatidate is then hydrolyzed to diacylglycerol by the phosphatidate phosphatase. Subsequently, the diacylglycerol can be further acylated by the diacylglycerol acyltransferase (DAGAT) in the *sn*-3 position to yield TAG. This last enzyme is specific to TAG biosynthesis. Indeed, these acyltransferases regulate the stereochemical distribution of the fatty acids; therefore, the acyltransferase specificity and the acyl-CoA pool control the proportion of the different TAG species in the oil. In vegetable oils most of saturated fatty acids are found in the external TAG positions, *sn*-1 and *sn*-3, for that reason, trisaturated TAGs are only found in high saturated plants oils, like palm or cacao. Symmetrical disaturated triacylglycerols with the structure saturated-unsaturated-saturated, are important components providing to margarines and spreads properties such as spreadability, resistance to water/oil loss, and melting at body temperature (O'Brien 1998). A coefficient of asymmetry, denominated α , has been proposed to calculate the saturated fatty acids distribution between the *sn*-1 and *sn*-3 TAG positions (Martínez-Force et al., 2004). Thus, α can range between 0 and 0.5; $\alpha = 0.5$ indicates

a symmetrical distribution of saturated fatty acids in accordance with the Vander Wal theory (Vander Wal, 1960).

Sunflower lines with oilseed with increased saturated fatty acid concentrations have been developed as an alternative to the hydrogenation process. These lines produce >20% of saturated fatty acids (Fernández-Moya et al., 2000; Osorio et al., 1995) versus <10% in traditional cultivars. For human consumption, high stearic oils are preferred since this fatty acid does not increase blood cholesterol, as other saturated fatty acids, such as palmitic or myristic, do (Crupkin and Zambelli, 2008). The higher stearic acid percentage in the oil of these genotypes is determined by a reduction in stearoyl-ACP desaturase activity and associated with higher levels of the acyl-ACP thioesterase activity on stearoyl-ACP when compared to traditional sunflower cultivars (Cantisán et al., 2000). The genetic background of these genotypes can be high linoleic (traditional) or high oleic (with the Pervenets mutation, which reduces the activity of the oleoyl-PC desaturase enzyme increasing the concentration of oleic acid compared to traditional genotypes, Garcés and Mancha, 1989). So, high stearic-high oleic genotypes carrying both mutations present an increased concentration of stearic and oleic acid compared to traditional genotypes (Fernández-Moya et al., 2005).

It is known that sunflower oil fatty acid composition is affected by night temperature during plant growing, mainly by modifying the oleic/linoleic acid ratio. For example, oleic acid percentage in a traditional sunflower cultivar increased from 17% up to 59% when night temperatures during grain filling increased 12°C (Izquierdo et al., 2006). High-oleic cultivars also increased the oleic acid percentage when night temperature increased but its variation was lower than the observed in traditional genotypes (<7 versus almost 40 percentage points of oleic acid; Izquierdo and Aguirrezábal, 2008). The increase in the oleic acid percentage is mainly associated

with a reduction in linoleic acid percentage, but in some cases reductions in total saturated fatty acids were also observed (Izquierdo and Aguirrezábal, 2008). This variability may be also associated with the genetic background of the cultivar. Cultivars with increased stearic acid concentration also modified its oil fatty acid composition when plants were exposed to different night temperatures during grain filling (Fernández-Moya et al., 2002). Increasing temperature reduced the palmitic and stearic acid percentage and increased the oleic/linoleic acid ratio (Martínez-Force et al., 1998) and these effects depended on the genetic background. High stearic genotypes with high linoleic background were more sensitive to temperature than high stearic genotypes with high oleic background, which carry the Pervenets mutation that provides stability to fatty acid composition (Izquierdo et al., 2013).

Moreover, it has been shown in sunflower that, in the presence of a higher content of oleic acid, the distribution of saturated fatty acids in TAG molecules is modified, increasing the asymmetry in the external positions of the TAG (*sn*-1 and *sn*-3) and, accordingly, decreasing the content of disaturated species of TAGs in the oil (Martínez-Force et al., 2009). Therefore, it could then been expected that genotypes with backgrounds potentially expressing different fatty acid composition as well as different sensitivity of this composition to night temperature during grain filling present variations in the distribution of saturated fatty acids in the TAG species.

As described above, the distribution of different fatty acids in TAGs depends on the affinities of the different acyltransferases for them, as well as the levels of expression and maximum activity of each one, not being able to rule out an effect of the temperature in these two last parameters. Moreover, it is also expected that different genetic backgrounds will have variability with respect to these acyltransferases, showing different levels of expression and activity. So, it is important to investigate if variations in TAGs composition in sunflower lines

expressing different saturated fatty acids concentrations are only accounted for by variations in fatty acid concentrations or there is also a direct effect of temperature on TAG synthesis, independent from the effect of this factor on fatty acid synthesis. The aim of this work is to characterize the influence of the temperature on the distribution of saturated fatty acids in TAGs in high-stearic and high stearic-high oleic sunflower lines with different genetic backgrounds grown under controlled conditions (growth chambers) at different temperatures during grain filling stage.

2. Materials and methods

2.1. *Plant material*

Inbred lines (Table 1) were the high stearic-high linoleic lines ADV-2504, ADV-2505, ADV-3512, and CAS-3 (named hereafter HSHL) which carry the CAS-3 mutation (Osorio et al., 1995), and the high stearic-high oleic lines ADV-2802, ADV-2803, ADV-3807, ADV-3816 and ADV-3817 (named hereafter HSHO) which carry both, the Pervenets and CAS-3 mutations.

Table 1

2.2. *Growth conditions*

Seeds from the described inbred lines were sown in 10L pots filled with soil. Each pot was fertilized with N, P, S y B according to Izquierdo et al. (2002). Pots were irrigated every 12 h to avoid water stress. Pests and diseases were adequately controlled if necessary. Phenology was registered according to Schneiter and Miller (1981). Plants were kept under greenhouse conditions until treatments were applied. To prevent cross pollination capitula were covered with pollination bags.

For treatment application pots were placed in 2.0 x 2.7 x 2.4 m growth chambers (Refrimax S.R.L., Mar del Plata, Argentina) 5 days after flowering (R5.1; Schneiter and Miller, 1981). Plants were exposed to 4 different day-night temperatures treatments during grain filling (Table 1). Temperatures in each environment were 16-16°C, 26-16°C and 26-26°C (Exp. 1) and 16-16°C, 26-16°C, 26-26°C and 32-26°C (Exp. 2). The photoperiod was 12 h and the incident radiation at the top of the plants was $690 \pm 75 \text{ mmol m}^{-2} \text{ s}^{-1}$ at the top of the plants. Shorter plants were raised in order to receive the same incident radiation. Air temperature of the growth chambers was recorded every 60 s and the hourly average was recorded with

data loggers (Cavadevices, Buenos Aires, Argentina). Physiological maturity was estimated visually from the hard yellow color of the capitulum back face and from the brown color of its bracts (Farizo et al., 1982). Grains were harvested after physiological maturity. Around 20 grains were harvested from each plant.

2.3. Grain oil extraction

Oil from grains was extracted as described previously (Ruiz-López et al., 2003) by heating the samples at 80 °C for 1 h in 3 mL of a mixture containing 1 mL of NaCl (0.17 M) in methanol and 2 mL of heptane. The TAG composition was analyzed by GLC (gas-liquid chromatography) as described below, and the remaining TAGs in the heptane solution were transmethyated with a solution containing methanol/toluene/dimethoxy propane/sulfuric acid (39:20:5:2). Fatty acid methyl esters (FAMES) were analyzed by GLC.

2.4. Fatty acid methyl ester analysis

The fatty acid methyl esters were quantified using an Agilent 6890 gas chromatography system (Palo Alto, CA) with a Supelco SP-2380 capillary column of fused silica (30 m length, 0.25 mm i.d., 0.20 µm film thickness; Bellefonte, PA). Hydrogen was used as the carrier gas, and the linear gas rate was 28 cm s⁻¹. The detector and injector temperatures were 200 °C, and the oven temperature was kept at 170 °C. Fatty acids were identified by comparison with known standards (Sigma, St. Louis, MO).

2.5. TAGs Analysis by GLC

TAG species were determined by GLC using an Agilent 6890 gas chromatography system (Palo Alto, CA) and hydrogen as the carrier gas (Fernández-

Moya et al., 2000). The injector and detector temperatures were 360 and 370 °C, respectively, the oven temperature was maintained at 335 °C, and a head pressure gradient from 100 to 180 kPa was applied. The gas chromatography column was a Quadrex Aluminum-Clad 400-65HT (30 m length, 0.25 mm i.d., 0.1 µm film thickness; Woodbridge, CT), using a linear gas rate of 50 cm s⁻¹, a split ratio 1:80, and a flame ionization detector (FID). The TAGs were identified and the data corrected for the relative response of the FID (Fernández-Moya et al., 2000).

2.6. *Solids fat content profile by DSC*

To measure changes in the physico-chemical properties of the different oils associated with the variations in fatty acid composition and TAG species content, melting profiles of the fractions were determined by differential scanning calorimetry (DSC) using Q2000 V23.5 calorimeter (TA instruments, New Castle, Delaware, USA) with a refrigerated cooling system. The results were processed using the TA analysis software provided by the manufacturer. The instrument was calibrated prior to use with indium, azobenzene and undecane purchased from Sigma–Aldrich (Madrid, Spain). Nitrogen was used to purge the system. About 6–8 mg of each melted sample was weighed and hermetically sealed into an aluminium pan with an empty pan serving as reference. To study the melting profiles, samples were heated to 80°C with a rate of 20°C/min to delete the previous crystalline structure. Then the temperature was decreased to -40°C followed by heating from -40°C to 50°C at 5°C/min to generate melting curves. The amount of solids as a function of temperature was determined by continuous integration of the DSC melting curves using the TA universal analysis software.

2.7. *Data analysis*

Each triacylglycerol species was expressed as a percentage of the total triacylglycerol species identified in the oil. The distribution of saturated fatty acids between the *sn*-1 and *sn*-3 external positions of TAGs was calculated using the coefficient of asymmetry α as the quotient between subclasses Saturated-Unsaturated-Saturated and Saturated-Unsaturated-Unsaturated (α SUS/SUU), proposed by Martinez-Force et al., (2004). Total remodelled fatty acids between different growth temperatures, [REM (%)], was calculated as the sum of positive increments (Martínez-Force et al., 1998). Sigmaplot software (Sigmaplot 8.0, SPSS Inc., Chicago, IL) was used to establish the relationships.

3. Results

3.1. TAG species in high stearic sunflower lines with high linoleic (HSHL) or high oleic (HSHO) background

The compositions of TAG species of plants grown at 16-16°C are presented in Figure 1 (HSHL lines) and Figure 2 (HSHO lines). All lines carrying the same mutations presented a similar TAGs profile, differing slightly according to the variations in fatty acid composition. In HSHL lines, the most abundant species was SLL, representing >30% of total TAGs. The species including the major fatty acids, stearic, oleic and linoleic (SLS, SOL, OLL and LLL) ranged between 5 and 18% (Figure 1). These five species, plus those including palmitic acid (PLS and PLL) represented more than 85% of the TAGs. The species including only stearic and oleic acids (i.e. SOS and SOO) represented between 0.6% and 1.7%. The other identified species were OOO, OOL and those including arachidic and/or behenic acids (OOA, SOA, SLA, OOB) with concentrations <2%. In the HSHO lines the most abundant species was SOO representing more than 30% of the TAGs, followed by the OOO with 14-26% of total TAGs. These two species represented 50-60% of total TAGs. The species POO, SOS, SOL and OOL ranged between 5% and 11%. Other minor identified species were POS, POL, OOA, SOA, SOB and OOB with concentrations between 0.9 and 3.9%.

Figures 1 and 2

3.2. Temperature effect on TAG species of high stearic sunflower lines with high linoleic (HSHL) or high oleic (HSHO) background

Temperature during grain filling modified both oil fatty acid and TAG composition. In all genotypes, independently of the genetic background, higher temperatures increased palmitic and oleic acid and reduced linoleic acid concentrations (Table 2). In HSHL lines the stearic acid concentration decreased when temperature increased while HSHO lines showed a variable behavior for this fatty acid. Thus, stearic acid increased in ADV-3817 and ADV-2802, decreased in ADV-2803 and ADV-3807 or remained unchanged in ADV-3816 when temperature during grain filling increased. The stability in fatty acid composition provided by the high oleic trait when changing growth temperature was confirmed with the lower REM (% total remodelled fatty acids between different growth temperatures) values of HSHO lines compared to HSHL lines (Table 2).

Table 2

When temperature increased HSHL lines reduced the concentration of the most abundant TAG species (SLL) by 20 percentage point (Table 3). This effect was mainly observed with variations in night temperature (16 °C versus 26). In these lines, the concentration of the species LLL and SLS were also reduced when night temperature increased, probably due to the reductions in stearic and linoleic acids concentrations mentioned above. These variations were at expense of higher concentrations of the TAG species including oleic acid as POO, POL, OOO and OOL. In spite of the reductions in stearic acid concentration, the specie SOO was also increased when temperature increased. In general, increasing temperature during grain filling increased TAG species with at least two oleic molecules (POO, SOO, LOO, AOO, BOO and OOO) and reduced those species with no oleic acid molecules and at least two linoleic acid molecules (PLL, SLL and LLL) (Table 3). So,

the stearic acid that is combined in TAG species with linoleic acid at low temperature (SLL, SLS) becomes part of TAG species with oleic acid (SOO, SOS) at high temperature (Table 3).

Table 3

In HSHO lines, the response of TAG species distribution to temperature variations was different among genotypes (Table 4). In line ADV-2802 the concentration of the most abundant TAG species SOO was increased when temperature increased but remained stable in the other HSHO lines. In lines ADV-2802, ADV-3817 and ADV-3816 the species SOS increased when temperature increased. The concentration of the species SOL and OOL were reduced in all HSHO lines when temperature increased. The species OOO increased in lines ADV-3816, ADV-3807 and ADV-2803 when temperature increased (Table 4). In all HSHO lines, the POL species and those with at least two linoleic molecules in the TAG were reduced when temperature increased, as observed for HSHL lines. Thus, the concentration of TAGs with stearic and linoleic acids was reduced in all HSHO lines when temperature increased. However, the concentration of TAGs with stearic and oleic acid only increased in lines ADV-2802 and ADV-3817, in agreement with increase of stearic acid when night temperature increased observed in these two materials (Table 2).

Table 4

3.3. *Temperature effect on saturated fatty acid distribution in TAG species:*
coefficient of asymmetry α

The content of the TAG species of the high stearic lines grown at different day-night temperatures is presented in Figure 3. These data show the highest saturated fatty acids content in lines with a linoleic background (open symbols) at night temperatures around 16°C and a more symmetrical distribution of these fatty acids in the TAG external positions *sn*-1 and *sn*-3 (lower content in SUU) when compared with lines with high oleic background (closed symbols) (Fig. 3 A and B). On the other hand, the night temperature increase to 26 °C resulted in a decrease of the saturated fatty acids concentration in lines with high linoleic background and a more symmetrical distribution in lines with high oleic background, increasing the content of SUS TAG subclass (Fig. 3C and D).

A more symmetrical distribution of saturated fatty acids (in particular the stearic acid) at high night temperatures is also evidenced when plotting the alpha value (α SAT symmetry coefficient) vs. the stearic acid content (Figure 4). When increasing the night temperature lines improved the saturated fatty acids distribution, measured as the coefficient α increase (higher levels of SUS TAG subclass) mainly in lines with high oleic background. This effect of temperature on the distribution of saturated fatty acids in the triacylglycerol was higher in HSHO than in HSHL lines. On the other hand, regardless of night temperature high stearic lines with high oleic backgrounds presented a greater asymmetry and therefore lower α value than those in high linoleic backgrounds (Figure 4).

Figure 3 and 4

3.4. *Modification of physicochemical properties of the oilseed of high stearic sunflower lines with high linoleic (HSHL) or high oleic (HSHO) background*

As mentioned in the introduction, physicochemical properties of oils are mainly determined by fatty acid composition as well as by TAG composition. The changes produced by temperature during grain filling on oil composition of HSHL and HSHO lines was reflected when solids fat contents were determined. Some examples are presented in Figure 5, where the higher stability of oils from high oleic background lines is observed. The HSHL lines presented differences in the solid fat content by effect of temperature changes correlated to their response at level of TAG composition. The line CAS-3, which strongly modified the stearic acid content and SUS TAGs when temperature increased, presented higher differences in the solid fat content curves than the ADV-2504 line which presented a more stable oil composition. In fact, the solid fat content in CAS-3 was reduced at higher temperature during grain filling (26°C), but it was slightly increased in line ADV-2504. These responses of HSHL lines are different than those observed in HSHO lines, which presented minor differences among solid content curves at different temperatures (Figure 5B).

Figure 5

4. Discussion

Temperature during grain filling modified TAG composition of the oilseed of high stearic sunflower genotypes. This effect was additional to the effect on TAG composition that occurs via variations in fatty acids contents. Accordingly, the effect of temperature during grain filling on oil quality is the result of its effect on fatty acid composition and TAG composition. The suitability of vegetable oils developed to substitute hydrogenated oils basically depends on the concentration of saturated fatty acids, mainly stearic, but also on the distribution of those fatty acids molecules in the TAGs since these factors impacts on its oxidative stability and solids fat content and therefore on its functional properties. From a practical point of view, sunflower oils from seeds developed under warmer nights will have higher content of SUS TAGs giving better plasticity properties to the oil as observed with the solids fat curves and, if they are fractionated for food applications, they will yield bigger stearin fractions (solid fraction with high concentrations of stearic acid).

The effect of temperature on fatty acid composition has been widely studied in traditional and mutated sunflower genotypes as high oleic, high stearic and high stearic-high oleic (e.g. Harris et al., 1978, Izquierdo et al., 2002, 2013, Pleite et al., 2008, Rochester and Silver, 1983). Increasing temperature increases the oleic/linoleic acid ratio via its effect on the activity of the oleoyl-CoA desaturase enzyme (OLD), which catalyzes the first extraplastidial desaturation in plants and converts oleic acid esterified to phosphatidylcholine, mainly in the *sn*-2 position, to linoleic acid (Garcés and Mancha 1991; Garcés et al., 1992). This effect is less evident in genotypes with the *Pervenets* mutation as high oleic and high stearic-high oleic in which the enzyme activity is inhibited (Izquierdo et al., 2002 and 2013, Izquierdo and Aguirrezábal, 2008; Triboï-Blondel et al., 2000). Temperature can also

increase palmitic acid and reduce stearic acid concentrations both in traditional and in high stearic genotypes. As for oleic/linoleic acid ratio, variations in stearic acid concentration are less pronounced in high stearic genotypes with high oleic than with high linoleic genetic background, since the *Pervents* mutation provides higher stability to fatty acid composition (Izquierdo et al., 2013).

These variations in fatty acid composition are naturally reflected in variations in TAG composition (e.g. Fernández-Moya et al., 2000). Since oleic and palmitic acids concentrations increased with temperature in HSHL lines, the TAG species including these fatty acids also increased, at expense of the TAG species with linoleic acid. These effects are lower in HSHO lines because variations in fatty acids are minor. However, a direct effect of temperature on TAG composition also occurs, since the α coefficient increased with night temperature, indicating a more symmetrical distribution of saturated fatty acids, even when the concentration of saturated fatty acids was reduced.

The distribution of fatty acids in the TAG is controlled by the acyltransferases system which together with fatty acids content determines the distribution of TAG subclasses: trisaturated (SSS), disaturated (SUS), monosaturated (SUU) and unsaturated (UUU). It is known that the distribution of fatty acids in the TAG molecule is not at random (Martínez Force et al., 2004; Reske et al., 1997). Saturated fatty acids in general are located in the extreme positions of the molecule (*sn-1* and *sn-3*) and that distribution depends mainly on the Kennedy pathway enzymes. The more symmetric distribution of saturated fatty acids in the TAG, even when oleic acid concentration is increased at high temperature, could be a consequence of a different expression profile of glycerol-3-phosphate acyltransferases, increasing the use of non-preferred fatty acids, as for example, stearic acid (Aznar-Moreno et al., 2013).

Interestingly, variations in TAGs composition were mainly associated with variations in night temperature in agreement with the results reported for the effect of temperature on fatty acid composition (Izquierdo et al., 2002 and 2006). Pleite et al. (2008) reported that the association between night temperature and oleic acid concentration is explained because the desaturation of oleate to linoleate via the oleoyl-PC desaturase enzyme increases during the night period. However, it is still unknown why TAG composition is also better associated with night temperature than others.

In all genotypes, night temperature influenced the α coefficient of asymmetry. However, at any temperature, lines with high oleic background presented lower symmetry than lines with high linoleic background as observed by Martínez-Force et al. (2009) for traditional and high oleic sunflower genotypes. The effect of the background was mainly associated with the presence or absence of the Pervenets mutation. Lines with this mutation produced more asymmetry in TAG composition compared to lines with high linoleic background. Differences among lines with the same mutations were minor, and probably due to allelic variations in the expression of the acyltransferases system.

The effect of temperature on TAG symmetry was more evident in lines with high oleic background than in those with high linoleic background. Since genotypes carrying the Pervenets mutation showed a fatty acid composition more stable under temperature variations, (Izquierdo et al., 2013) results presented in this work show that the effect of temperature on TAG composition is only partially explained by the well-known effect of this environmental factor on fatty acid composition (e.g. Martínez et al., 2012; Izquierdo et al., 2013). As the best of our knowledge, results presented in this work indicate that a separate effect of temperature on the acyltransferases system also exists. This last effect could be further characterized by identifying the

different acyltransferase genes expressed during sunflower seed development (for example glycerol-3P acyltransferases, diacylglycerol acyltransferases, glycerolcholine acyltransferases, phospholipid:diacylglycerol acyltransferases) and the characterizing of their expression profiles under different day/night temperatures.

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6. References

- Aznar-Moreno, J.A., Martínez-Force, E., Venegas-Calcrón, M., Garcés, R. and Salas, J. 2013. Changes in acyl-coenzyme A pools in sunflower seeds with modified fatty acid composition. *Phytochemistry* 87: 39-50.
- Banaś, W., Sánchez-García, A., Banaś, A. and Stymne, S. 2013. Activities of acyl-CoA:diacylglycerol acyltransferase (DGAT) and phospholipid:diacylglycerol acyltransferase (PDAT) in microsomal preparations of developing sunflower and safflower seeds. *Planta* 237: 1627-1636.
- Cantisán, S., Martínez-Force, E. and Garcés, R. 2000. Enzymatic studies of high stearic acid sunflower seed mutants. *Plant Physiol. Biochem.* 38: 377-382.
- Crupkin, M. and Zambelli, A. 2008. Detrimental Impact of Trans Fats on Human Health: Stearic Acid-Rich Fats as Possible Substitutes. *J Comp Rev Food Sci Food Safety* 7: 271-279.
- Farizo, C.L., Pereyra, V.R., Cardinali, F. and Orioli, G. 1982. Determination of physiological and harvest maturity in sunflower. *Proceedings of the Tenth International Sunflower Conference 10th*, Surfers Paradise, Australia. Pp. 42-44.
- Fernández-Moya, V., Martínez-Force, E. and Garcés, R. 2000. Identification of triacylglycerol species from high-saturated sunflower (*Helianthus annuus*) mutants. *J. Agric. Food Chem.* 48: 764-769.
- Fernández-Moya, V., Martínez-Force, E. and Garcés, R. 2002. Temperature effect on a high stearic acid sunflower mutant. *Phytochemistry* 59: 33-37.
- Fernández-Moya, V., Martínez-Force, E. and Garcés, R. 2005. Oils from improved high stearic acid sunflower seeds. *J. Agric. Food Chem.* 53: 5326-5330.
- Garcés, R. and Mancha, M. 1989. Oleate desaturation in seeds of 2 genotypes of sunflower. *Phytochemistry* 28: 2593-2595.

- Garcés, R. and Mancha, M. 1991. In vitro oleate desaturase in developing sunflower seeds. *Phytochemistry* 30, 2127–2130.
- Garcés, R., Sarmiento, C., Mancha, M. 1992. Temperature regulation of oleate desaturase in sunflower (*Helianthus annuus* L.) seed. *Planta* 186, 461–465.
- Harris, H., McWilliam, R., Mason, W. 1978. Influence of temperature on oil content and composition of sunflower seed. *Aust. J. Agric. Res.* 29:1203–1212.
- Izquierdo, N.G., Aguirrezábal, L.A.N., Martínez-Force, E., Garcés, R., Paccapelo, V., Andrade, F., Reid, R. and Zambelli, A. 2013. Effect of growth temperature on the high stearic and high stearic-high oleic sunflower traits. *Crop Pasture Sci.*: 18-25.
- Izquierdo, N., Aguirrezábal, L., Andrade, F. and Cantarero, M. 2006. Modeling the response of fatty acid composition to temperature in a traditional sunflower hybrid. *Agronomy J.* 98: 451-461.
- Izquierdo, N., Aguirrezábal, L., Andrade, F. and Pereyra, V. 2002. Night temperature affects fatty acid composition in sunflower oil depending on the hybrid and the phenological stage. *Field Crops Res.* 77: 115-126.
- Izquierdo, N.G. and Aguirrezábal, L.A.N. 2008. Genetic variability in the response of fatty acid composition to minimum night temperature during grain filling in sunflower. *Field Crops Res.* 106: 116-125.
- Martínez-Force, E., Álvarez-Ortega, R., Cantisan, S. and Garcés, R. 1998. Fatty acid composition in developing high saturated sunflower (*Helianthus annuus* L.) seed: maturation changes and temperature effect. *J. Agric. Food Chem.* 46: 3577-3582.
- Martínez-Force, E., Ruiz-López, N. and Garcés, R. 2004. The determination of the asymmetrical stereochemical distribution of fatty acids in triacylglycerides. *Anal. Biochem.* 334: 175–182.

- Martínez-Force, E., Ruiz-López, N. and Garcés, R. 2009. Influence of specific fatty acids on the asymmetric distribution of saturated fatty acids in sunflower (*Helianthus annuus* L.) triacylglycerols. J. Agric. Food Chem. 57: 1595-1599.
- O'Brien, R. D. 1998 Fats and Oils, Formulating and Processing for Application, Technomic Publishing: Lancaster, PA, USA; pp 437-458.
- Osorio, J., Fernandez-Martínez, J., Mancha, M. and Garcés, R. 1995. Mutant sunflowers with high concentration of saturated fatty acids in the oil. Crop Sci. 35: 739-742.
- Pleite, R., Rondanini, D., Garcés, R. and Martínez-Force, E. 2008. Day/night variation in fatty acids and lipids biosynthesis in sunflower (*Helianthus annuus* L.) seeds. Crop Sci. 48: 1952–1957.
- Reske, J., Siebrecht, J. and Hazebroek, J. 1997. Triacylglycerol composition and structure in genetically modified sunflower and soybean oils. J. Am. Oil Chem. Soc. 74: 989–998.
- Rochester, C., Silver, J. 1983. Unsaturated fatty acid synthesis in sunflower (*Helianthus annuus* L.) seeds in response to night temperature. Plant Cell Rep. 2:229–231.
- Ruiz-López, N., Martínez-Force, E. and Garcés, R. 2003. Sequential one step extraction and analysis of triacylglycerols and fatty acids in plant tissues. Anal. Biochem. 317: 247-254.
- Schneider, A.A. and Miller, J.F. 1981. Description of sunflower growth stages. Crop Sci. 21: 901–903.
- Triboï-Blondel, A., Bonnemoy, B., Falcimagne, R., Martignac, M., Messaoud, J., Philippon, J. and Vear, F. 2000. The effect of temperature from flowering to maturity on seed composition of high oleic sunflower inbreds and mid oleic

hybrids. Proceedings 15th International Sunflower Conference. Toulouse, France, I: A-67.

Vander Wal, R. J. 1960. Calculation of the distribution of the saturated and unsaturated acyl groups in fats, from pancreatic lipase hydrolysis data. J. Am. Oil Chem. Soc. 37: 18-20.

Figure legends

Figure 1. Triacylglycerol species composition in high stearic-high linoleic sunflower oils from grains developed at 16-16°C (day-night temperatures). Data are the average of all analyzed experiments. P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; A, arachidic acid; and B, behenic acid.

Figure 2. Triacylglycerol species composition in high stearic-high oleic sunflower oils from grains developed at 16-16°C (day-night temperatures). Data are the average of all analyzed experiments. P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; A, arachidic acid; and B, behenic acid.

Figure 3. Content of triacylglycerol subclasses (mol%) as a function of the total saturated fatty acids content (mol%) from high stearic-high linoleic (open symbols) and high stearic-high oleic (filled symbols) sunflower lines growing at different day-night temperatures (A: 16-16°C; B: 26-16°C; C: 26-26°C; and D: 32-26°C).

Disaturated TAG, SUS (□■ and dotted line); monounsaturated TAG, SUU (○● and dashed line); and fully unsaturated TAG, UUU (△▲ and solid line). Adjusted lines (second degree polynomial) represent the behavior of high linoleic background lines.

Figure 4. Alpha value (α SAT symmetry coefficient) plotted against stearic acid content in oils from high stearic-high linoleic (circles) and high stearic-high oleic (triangles) sunflower lines growing at 16°C (open symbols) or 26°C (filled symbols) night temperature.

Figure 5. Solid content curves corresponding to the melting of different oils from HSHL (A) and HSHO (B) sunflower lines. A dashed line, corresponding to 30% solid

fat content, is shown as reference to compare between sunflower lines in panels A and B.

Tables

Table 1. Genotypes and day-night temperature treatments during grain filling periods.

Experiment	HSHL [†]	HSHO [‡]	Treatments
Expt. 1	CAS-3	ADV-2802	16-16°C
	ADV-2505	ADV-3807	26-16°C
		ADV-3817	26-26°C
Expt. 2	CAS-3	ADV-2803	16-16°C
	ADV-2504	ADV-3807	26-16°C
	ADV-3512	ADV-3816	26-26°C
			32-26°C

[†] High stearic-high linoleic

[‡] High stearic-high oleic

Table 2. Fatty acid composition observed in oils from HSHL and HSHO sunflower lines cultivated at 16-16°C (day-night), changes when increasing the temperature to 26-26°C (*) or 32-26°C (**), and the total remodeled fatty acids between both temperatures, [REM (%)], as the sum of positive increments. P, palmitic acid; S, stearic acid, O, oleic acid; L, linoleic acid; and Others, arachidic and behenic acids.

		Fatty acid composition (%)					
		P	S	O	L	Others	REM (%)
High stearic-high linoleic							
ADV-2505	16-16°C	4.8	30.6	6.7	53.6	4.3	
	Δ↑T*	0.7	-14.4	35.9	-22.0	-0.2	36.6
ADV-3512	16-16°C	5.1	28.1	8.8	56.7	1.3	
	Δ↑T*	0.6	-7.0	26.8	-20.4	0.1	27.5
	Δ↑T**	0.7	-8.5	29.0	-21.2	0.0	29.7
ADV-2504	16-16°C	6.0	23.7	9.0	60.3	1.0	
	Δ↑T*	2.7	-3.2	28.1	-27.6	0.0	30.8
	Δ↑T**	4.2	-2.2	27.6	-29.8	0.2	32.0
CAS-3	16-16°C	4.5	23.2	12.0	58.3	2.0	
	Δ↑T*	1.7	-4.9	16.7	-13.8	0.3	18.7
	Δ↑T**	2.2	-7.1	13.9	-8.0	-1.0	16.1
High stearic-high oleic							
ADV-2803	16-16°C	5.1	23.5	61.6	8.4	1.3	
	Δ↑T*	1.3	-5.6	7.9	-3.6	0.0	9.2
	Δ↑T**	1.2	-6.7	10.4	-5	0.0	11.6
ADV-3817	16-16°C	3.9	22.7	63.9	5.7	3.8	

	$\Delta\uparrow T^*$	1.4	2.8	-1.8	-4.0	1.6	5.8
ADV-3816	16-16°C	5.1	22.5	60.1	10.8	1.5	
	$\Delta\uparrow T^*$	1.8	-0.8	5.8	-7.9	0.2	7.8
	$\Delta\uparrow T^{**}$	2.7	-0.8	5.8	-7.9	0.2	8.7
ADV-3807	16-16°C	4.6	22.3	59.6	10.3	3.2	
	$\Delta\uparrow T^*$	1.0	-2.6	8.9	-7.5	0.2	10.1
	$\Delta\uparrow T^{**}$	2.2	-5.0	11.7	-7.2	-1.7	13.9
ADV-2802	16-16°C	2.2	20.9	63.9	9.0	4.0	
	$\Delta\uparrow T^*$	0.9	3.7	2.2	-6.8	0.1	6.9

1 Table 3. Triacylglycerol species composition (mol%) in oils from high stearic-high linoleic sunflower lines (HSHL) grown at different
2 day-night temperatures during the grain filling period. Data are the average values from both experiments. P, palmitic acid; S,
3 stearic acid, O, oleic acid; L, linoleic acid; A, arachidic acid; and B, behenic acid.

Line	Treatment	TAG species composition (%)																		
		POS	POO	PLS	POL	PLL	SOS	SOO	SLS	OOO	SOL	OOL	SLL	OLL	LLL	SOA	OOA	SLA	OOB	Rest
ADV-2505	16-16 °C	0.6	0.1	5.4	1.3	4.6	1.4	0.5	15.4	0.0	7.7	0.2	35.7	5.6	12.2	0.2	0.1	1.9	0.0	7.1
	26-16 °C	0.8	0.2	7.1	1.9	6.5	1.4	0.6	13.1	0.2	7.2	0.2	32.5	6.2	11.4	0.3	0.1	2.0	0.1	8.2
	26-26 °C	2.2	3.8	1.9	4.3	2.0	3.3	10.2	2.7	11.1	12.9	14.1	7.5	9.6	3.6	0.9	0.9	0.5	2.2	6.3
ADV-3512	16-16 °C	0.7	0.1	4.7	1.3	4.2	1.7	0.8	14.0	0.4	10.0	0.9	34.4	5.1	13.5	0.3	0.0	1.8	0.0	6.1
	26-16 °C	0.7	0.2	5.6	1.4	5.1	1.5	0.8	13.0	0.2	8.6	0.6	34.9	5.4	14.2	0.2	0.0	1.7	0.0	5.9
	26-26 °C	2.3	2.2	2.5	3.5	2.0	4.7	10.2	5.1	5.3	17.3	10.0	11.8	9.6	4.3	0.9	1.3	0.7	1.2	5.1
	32-26 °C	2.3	2.6	2.3	3.7	1.9	4.3	11.1	4.1	6.9	16.5	12.0	9.7	10.2	3.3	0.8	1.6	0.5	1.3	4.9
ADV-2504	16-16 °C	0.6	0.3	5.1	1.7	5.3	1.2	1.2	11.1	1.0	8.2	1.9	33.3	6.8	14.1	0.1	0.0	1.4	0.0	6.7
	26-16 °C	1.2	0.3	9.1	2.2	8.0	1.7	1.0	13.0	0.3	7.0	0.8	30.1	4.3	11.0	0.2	0.0	1.8	0.0	8.0
	26-26 °C	4.3	3.8	3.8	4.4	2.4	6.2	12.9	5.2	5.8	14.5	7.2	9.0	5.6	3.0	1.3	1.3	0.8	1.8	6.7
	32-26 °C	4.8	4.4	4.0	4.6	2.7	6.5	13.1	4.9	5.9	12.7	6.7	8.3	5.5	3.2	1.4	1.2	0.7	2.3	7.1
CAS-3	16-16 °C	0.7	0.3	3.6	1.8	4.6	1.8	1.4	8.5	0.2	10.3	1.7	30.1	10.3	18.9	0.4	0.0	1.1	0.0	4.3

26-16 °C	1.0	0.4	5.7	2.2	6.1	1.8	1.5	10.2	0.1	10.0	1.1	31.5	7.5	13.4	0.3	0.0	1.2	0.0	6.0
26-26 °C	2.0	2.2	3.2	3.7	3.9	3.3	7.4	4.7	6.1	12.2	7.4	15.3	11.9	9.0	0.7	0.7	0.6	0.7	5.0
32-26 °C	1.4	1.7	3.6	4.4	5.0	1.9	5.0	4.2	3.3	11.6	8.5	17.1	15.3	11.6	0.5	0.4	0.5	0.4	3.6

1 Rest: POP, PLP, OLA, LLA, EOB, ELB, OLB and LLB.

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1 Table 4. Triacylglycerol species composition (mol%) in oils from high stearic-high oleic sunflower lines (HSHO) grown at different
2 day-night temperatures during the grain filling period. Data are the average values from both experiments. P, palmitic acid; S,
3 stearic acid, O, oleic acid; L, linoleic acid; A, arachidic acid; and B, behenic acid.

Line	Treatment	TAG species composition (%)															
		POP	POS	POO	PLS	POL	SOS	SOO	SLS	OOO	SOL	OOL	SOA	OOA	SOB	OOB	Rest
ADV-2802	16-16°C	0.1	1.6	3.5	0.0	0.9	6.2	33.4	0.6	26.4	7.6	9.3	0.9	3.2	1.1	3.7	1.5
	26-16 °C	0.3	2.5	5.3	0.0	0.6	7.1	39.1	0.3	24.3	4.1	3.7	1.1	3.4	1.3	5.5	1.4
	26-26 °C	0.3	3.3	4.5	0.0	0.2	12.5	41.2	0.4	22.9	1.9	1.5	1.8	3.8	1.6	4.0	0.1
ADV-3817	16-16 °C	0.4	3.2	6.4	0.2	0.9	7.9	39.5	0.5	20.7	5.7	4.7	1.2	3.5	0.9	3.2	1.1
	26-16 °C	0.6	3.5	9.6	0.0	0.7	5.2	36.7	0.0	27.7	2.6	3.0	1.1	3.6	0.9	4.6	0.2
	26-26 °C	0.7	5.7	7.3	0.0	0.4	13.9	38.5	0.0	16.0	1.8	1.0	2.7	4.1	2.6	5.3	0.0
ADV-3816	16-16 °C	0.4	3.9	6.2	0.4	1.8	8.9	35.1	1.0	15.4	10.0	6.5	1.3	2.7	1.0	2.7	2.7
	26-16 °C	0.7	5.1	9.3	0.2	1.1	8.2	41.1	0.2	17.3	4.8	3.6	1.4	3.1	0.5	3.3	0.1
	26-26 °C	0.9	5.7	9.2	0.2	0.8	9.9	37.1	0.2	20.3	2.8	2.4	1.8	3.2	1.6	3.9	0.0
	32-26 °C	1.1	6.8	10.4	0.1	0.5	10.6	36.7	0.1	19.4	2.0	1.7	2.1	3.2	1.6	3.5	0.2
ADV-3807	16-16 °C	0.4	3.4	5.7	0.5	1.8	8.3	33.9	1.2	13.7	11.0	6.7	1.3	3.1	1.3	3.6	4.1
	26-16 °C	0.7	4.2	9.1	0.3	1.7	6.7	35.5	0.4	18.3	6.1	4.9	1.0	3.4	1.0	4.9	1.8

	26-26 °C	0.6	4.4	8.4	0.0	0.6	8.2	36.0	0.1	24.8	2.2	2.4	1.6	3.5	1.9	5.3	0.0
	32-26 °C	0.9	4.9	10.3	0.0	0.5	7.3	35.1	0.0	27.8	1.7	1.8	1.3	3.1	1.3	4.1	0.0
ADV-2803	16-16 °C	0.3	3.3	5.9	0.3	1.2	8.6	35.7	0.6	20.2	6.9	6.7	1.1	2.8	0.9	3.5	2.0
	26-16 °C	0.5	3.8	7.7	0.2	0.7	7.1	39.5	0.2	22.3	3.6	3.1	1.2	3.1	1.1	4.7	1.2
	26-26 °C	0.6	4.4	8.7	0.2	0.7	7.4	34.3	0.3	27.0	2.9	3.1	1.4	3.0	1.2	4.2	0.6
	32-26 °C	0.6	4.1	8.9	0.2	0.5	6.7	34.4	0.3	31.0	1.9	2.0	1.3	3.0	1.1	4.0	0.0

1 Rest: PLL, ELL, OLL, OLA and OLB.

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1 Table 5. Night temperature influence on triacylglycerol subclasses content (mol%) in HSHL
2 and HSHO oils from different genetic backgrounds. Data correspond to both experiments.

Night Temperature		16°C			26°C		
		SUS	SUU	UUU	SUS	SUU	UUU
HSHL lines	CAS-3	19.5	53.9	26.6	14.9	48.5	36.6
	ADV-2504	25.7	54.1	20.2	25.5	53.1	21.4
	ADV-2505	27.2	54.8	18.0	13.3	48.2	38.5
	ADV-3512	24.4	55.4	20.2	17.0	52.2	30.7
HSHO lines	ADV-2802	11.6	55.7	32.7	19.8	55.8	24.4
	ADV-2803	14.7	58.6	26.7	15.0	53.4	31.6
	ADV-3807	15.4	61.9	22.8	16.2	55.4	28.4
	ADV-3816	16.6	61.4	22.0	21.4	56.7	21.9
	ADV-3817	12.8	58.8	28.4	25.7	57.3	17.1

5 SUS, disaturated TAG; SUU, diunsaturated TAG; and UUU, triunsaturated TAG.

1 Figure 1

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1 Figure 2

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1 Figure 3

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1 Figure 4

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1 Figure 5

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