Research Article

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Temperature effect on triacylglycerol species in seed oil from high stearic sunflower lines with different genetic backgrounds

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Abstract

BACKGROUND: This study characterized the influence of temperature during grain filling on the saturated fatty acid distribution in triacylglycerol molecules from high stearic sunflower lines with different genetic backgrounds. Two growth chamber experiments were conducted with day/night temperatures of 16/16, 26/16, 26/26 and 32/26 °C.

RESULTS: In all genotypes, independently of the genetic background, higher temperatures increased palmitic and oleic acid and reduced linoleic acid concentrations. Increasing night temperature produced an increase in saturated-unsaturated-saturated species, indicating a more symmetrical distribution of saturated fatty acids. The solid fat index was more affected by temperature during grain filling in lines with high linoleic than high oleic background. Higher variations in symmetry among night temperatures were observed in lines with high oleic background, which are more stable in fatty acid composition.

CONCLUSION: The effect of temperature on triacylglycerol composition is not completely explained by its effect on fatty acid composition. Thus night temperature affects oil properties via its effects on fatty acid synthesis and on the distribution of fatty acids in the triacylglycerol molecules.

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

INTRODUCTION

The physical, chemical and nutritional properties of oils are determined not only by their fatty acid composition but also by the distribution of fatty acids in the triacylglycerol (TAG) molecules. In sunflower (*Helianthus annuus* L.), TAGs are mainly synthesized by endoplasmic reticulum (ER) membrane-bound enzymes in the glycerol 3-phosphate pathway or Kennedy pathway,[1] starting with the acylation of glycerol 3-phosphate at the *sn*-1 and *sn*-2 positions with acyl-coenzyme A (acyl-CoA) esters. This step is sequentially mediated 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 phosphatidate phosphatase. Subsequently, the diacylglycerol can be further acylated by diacylglycerol acyltransferase (DAGAT) in the *sn*-3 position to yield TAG, this enzyme being specific to TAG biosynthesis. Thus these three 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 saturated fatty acids are located in the external TAG positions *sn*-1 and *sn*-3; for that reason, tri-saturated TAGs are only found in high saturated plant oils such as palm or cacao. Symmetrical di-saturated triacylglycerols with saturated-unsaturated-saturated structure are important components of margarines and spreads, providing properties such as spreadability, resistance to water/oil loss, and melting at body temperature.[2] A coefficient of asymmetry, denoted αSAT, has been proposed to calculate the saturated fatty acid distribution between the *sn*-1 and *sn*-3 TAG positions.[3] Thus αSAT can range between 0 and 0.5; $\alpha = 0.5$ indicates a symmetrical distribution of saturated fatty acids in accordance with the theory of Vander Wal.[4]

Sunflower lines with seed oil with increased saturated fatty acid concentrations have been developed as an alternative to the hydrogenation process in order to avoid *trans* fatty acids.[5] For human consumption, high stearic oils are preferred, since this

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fatty acid does not increase blood cholesterol as other saturated fatty acids such as palmitic or myristic acid do.[6] 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 acyl-ACP thioesterase activity on stearoyl-ACP when compared with traditional sunflower cultivars.[7] The genetic background of these genotypes can be high linoleic (traditional) or high oleic (due to the presence of Pervenets mutation).[8] In these high oleic mutants the activity of the oleoyl-PC desaturase enzyme is dramatically reduced, thus increasing the concentration of oleic acid compared with traditional genotypes.[9] Therefore high stearic-high oleic genotypes carrying both mutations present an increased concentration of stearic and oleic acids compared with traditional genotypes.[10]

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.[11,12] Consequently, variations in oil quality among locations, sowing dates and years have been reported. For example, oleic acid percentage in a traditional sunflower cultivar increased from 17 to 59% when night temperature during grain filling increased by 12 °C.[11] High oleic cultivars also increased their oleic acid percentage when night temperature increased, but the variation was lower than that observed in traditional genotypes.[12] Increasing temperature increases oleic acid concentration and reduces linoleic acid concentration and, in some cases, total saturated fatty acids also. The variability in the response of fatty acid composition to temperature may be associated with the genetic background of the cultivar.[12] Cultivars with increased stearic acid concentration also modified their oil fatty acid composition when plants were exposed to different night temperatures during grain filling.[13] Increasing temperature reduced the palmitic and stearic acid percentage and increased the oleic/linoleic acid ratio,[14] and these effects depended on the genetic background. High stearic genotypes with high linoleic background were more sensitive to temperature than those with high oleic background, which carry the Pervenets mutation that provides stability to fatty acid composition.[15]

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 di-saturated TAG species in the oil.[16] Therefore it could be expected that genotypes with different genetic background as well as different sensitivity of fatty acid composition to night temperature during grain filling present differences 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 catalytic efficiencies of the different acyltransferases to them, as well as on the levels of expression and maximum activity of each enzyme, and probably also on an effect of the temperature on these two last parameters. Moreover, it can also be expected that different genetic backgrounds have variability in acyltransferase functionality due to different levels of expression and/or activity. Thus it is important to investigate if variations in TAG composition in sunflower lines expressing different saturated fatty acid concentrations are only accounted for by variations in fatty acid concentrations or if there is also a direct effect of temperature on TAG synthesis, in addition to its known effect on fatty acid synthesis. The aim of this work was to characterize the influence of temperature on the distribution of saturated fatty acids in TAGs in high stearic sunflower lines with different genetic backgrounds grown under controlled conditions at different temperatures during the grain-filling stage.

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

Experiment	HSHL	HSHO	Treatment (°C)
1	CAS-3	ADV-2802	16/16
	ADV-2505	ADV-3807	26/16
		ADV-3817	26/26
2	CAS-3	ADV-2803	16/16
	ADV-2504	ADV-3807	26/16
	ADV-3512	ADV-3816	26/26
			32/26

HSHL, high stearic-high linoleic; HSHO, high stearic-high oleic.

MATERIALS AND METHODS

Plant material

Inbred lines (Table 1) were the high stearic-high linoleic (HSHL) lines ADV-2504, ADV-2505, ADV-3512 and CAS-3, which carry the CAS-3 mutation,[5] and the high stearic-high oleic (HSHO) lines ADV-2802, ADV-2803, ADV-3807, ADV-3816 and ADV-3817, which carry both the Pervenets and CAS-3 mutations.

Growth conditions

Seeds from the described inbred lines were sown in 10 L pots filled with soil. Each pot was fertilized with N, P, S and B according to Izquierdo *et al.*[17] 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.[18] 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 m × 2.7 m × 2.4 m growth chambers (Refrimax SRL, Mar del Plata, Argentina) 5 days after flowering (R5.1).[18] Plants were exposed to four different day/night temperatures treatments during grain filling (Table 1). Temperatures in each environment were 16/16, 26/16 and 26/26 °C (experiment 1) and 16/16, 26/16, 26/26 and 32/26 °C (experiment 2). Three plants per genotype and treatment were used. The photoperiod was 12 h and the incident radiation at the top of the plants was 690 ± 75 mmol m^{-2} s^{-1}. 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.[19] Grains from three plants per genotype and growth chamber were harvested after physiological maturity. Around 20 grains were harvested from each plant.

Seed oil extraction

Oil from seeds was extracted as described previously by heating the samples at 80 °C for 1 h in 3 mL of a mixture containing 1 mL of NaCl (0.17 mol L^{-1}) in methanol and 2 mL of heptane.[20] The TAG composition was analyzed by gas–liquid chromatography (GLC) and the remaining TAGs

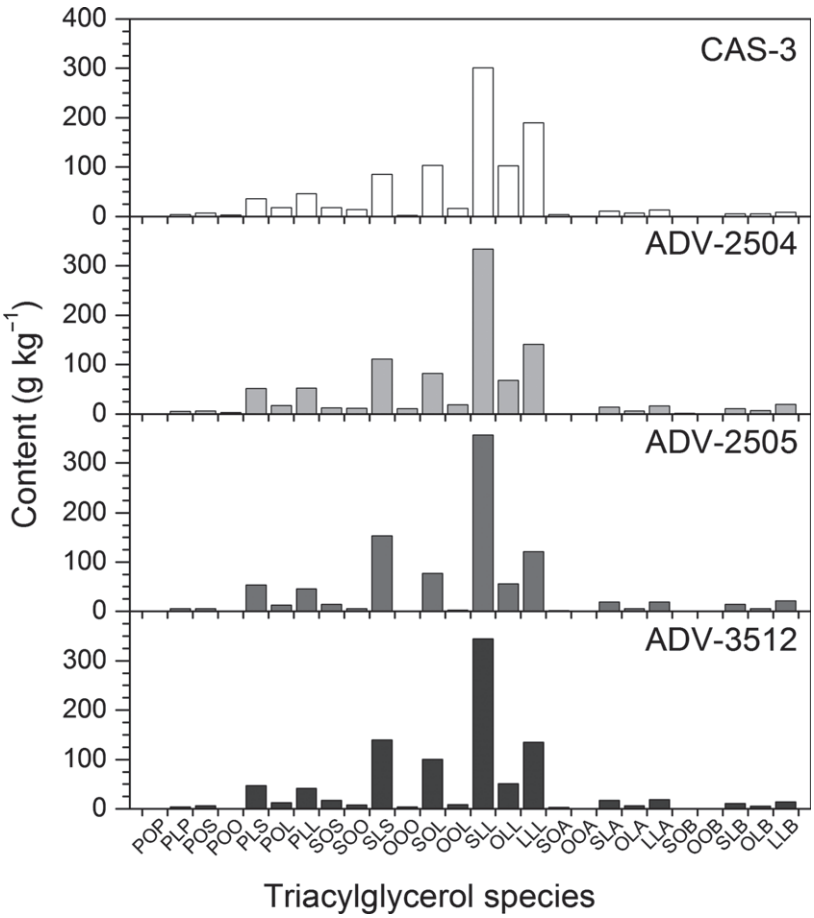

Figure 1. Triacylglycerol species composition in HSHL sunflower oils from grains developed at 16/16 °C (day/night temperatures). Data are the average of all analyzed experiments. P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; A, arachidic acid; B, behenic acid.

in the heptane solution were transmethylated with a solution containing methanol/toluene/dimethoxypropane/sulfuric acid (39:20:5:2 v/v/v/v).[21] Fatty acid methyl esters (FAMEs) were analyzed by GLC.

FAME analysis

FAMEs were quantified using an Agilent 6890 GC system (Palo Alto, CA, USA) with a Supelco SP-2380 fused silica capillary column (30 m length, 0.25 mm i.d., 0.20 µm film thickness; Bellefonte, PA, USA). Hydrogen was used as the carrier gas at a linear gas rate of 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, USA). The average of two fatty acid analyses per plant was calculated.

TAG analysis by GLC

TAG species were determined by GLC using an Agilent 6890 GC system and hydrogen as the carrier gas.[22] 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 GC column was a Quadrex Aluminum-Clad 400-65HT (30 m length, 0.25 mm i.d., 0.1 µm film thickness; Woodbridge, CT, USA), using a linear gas rate of 50 cm s^{-1}, a split ratio of 1:80 and a flame ionization detector (FID). The TAGs were identified and the data corrected for the relative response of the FID.[22]

Solid fat index profile by differential scanning calorimetry (DSC)

To measure changes in the physicochemical 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 DSC using a Q2000 V23.5 calorimeter (TA Instruments, New Castle, DE, 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 aluminum pan, with an empty pan serving as reference. To study the melting profiles, samples were heated to 80 °C at a rate of 20 °C min^{-1} to delete the previous crystalline structure. Then the temperature was decreased to −40 °C followed by heating from −40 to 50 °C at 5 °C min^{-1} 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.

Data analysis

The distribution of saturated fatty acids between the *sn*-1 and *sn*-3 external positions of TAGs was calculated using the coefficient of asymmetry αSAT as the quotient between subclasses saturated-unsaturated-saturated and saturated-

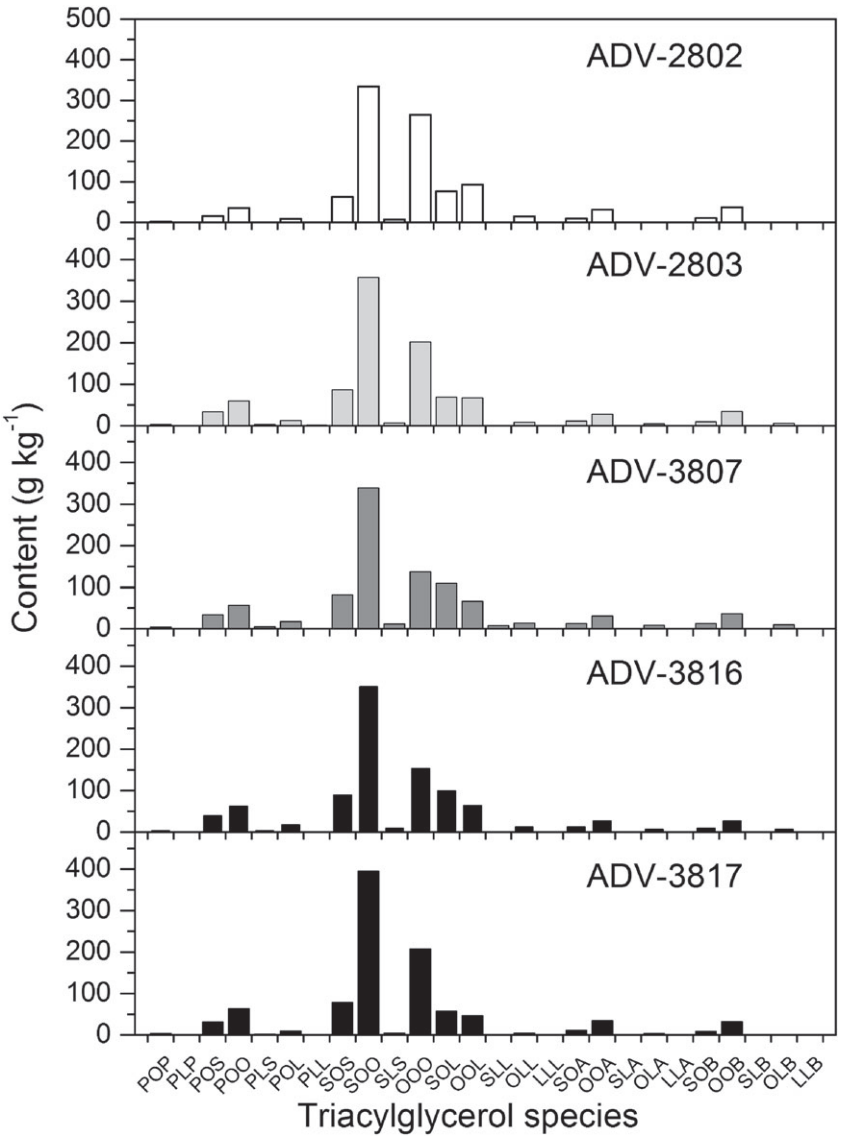

Figure 2. Triacylglycerol species composition in HSHO sunflower oils from grains developed at 16/16 °C (day/night temperatures). Data are the average of all analyzed experiments. P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; A, arachidic acid; B, behenic acid.

unsaturated-unsaturated (α SUS/SUU) proposed by Martínez-Force *et al.*[3] and the *sn*-2 saturated fatty acid content calculated as in Martínez-Force *et al.*[16] The total remodeled fatty acids between different growth temperatures, REM (%), was calculated as the sum of positive variations.[14] SigmaPlot 8.0 software (SPSS Inc., Chicago, IL, USA) was used to establish the relationships.

RESULTS

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 Fig. 1 (HSHL lines) and Fig. 2 (HSHO lines). All lines carrying the same mutations presented a similar TAG profile, differing slightly according to the variations in fatty acid composition. In HSHL lines the most abundant species was SLL with concentrations >300 g kg^{-1}. The species including the major fatty acids stearic, oleic and linoleic (SLS, SOL, OLL and LLL) ranged between 51 and 189 g kg^{-1} (Fig. 1). These five species plus those including palmitic acid (PLS and PLL) represented more than 85% of total TAGs. The species including only stearic and oleic acids (i.e. SOS and SOO) presented concentrations between 5 and 18 g kg^{-1}. The other identified species were OOO, OOL and those including arachidic acid and/or behenic acid (OOA, SOA, SLA and OOB) with concentrations <20 g kg^{-1}. In the HSHO lines the most abundant species was SOO with concentrations >330 g kg^{-1}, followed by OOO with 137–264 g kg^{-1}. These two species represented 50–60% of total TAGs. The species POO, SOS, SOL and OOL ranged between 35 and 110 g kg^{-1}. Other minor identified species were POS, POL, OOA, SOA, SOB and OOB with concentrations between 9 and 39 g kg^{-1}.

Temperature effect on TAG species and fatty acid composition 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

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 (Δ↑*T*) to *26/26 °C or **32/26 °C, and total remodeled fatty acids between both temperatures, REM (%), as the sum of positive increments

Line	*T* (°C) (day/night)	Fatty acid composition (g kg^{-1})					*REM*(%)
		P	S	O	L	Others	
HSHL							
ADV-2505	16/16	48.0	306.1	67.0	536.3	42.6	
	Δ↑*T**	6.8	−143.9	359.0	−220.1	−1.8	36.6
ADV-3512	16/16	50.9	281.0	87.8	567.0	13.4	
	Δ↑*T**	5.8	−70.4	268.0	−204.0	0.6	27.5
	Δ↑*T***	7.4	−84.5	289.8	−212.2	−0.4	29.7
ADV-2504	16/16	60.4	236.8	89.6	602.8	10.4	
	Δ↑*T**	26.8	−31.9	280.6	−275.8	0.4	30.8
	Δ↑*T***	42.2	−21.9	275.8	−298.0	1.9	32.0
CAS-3	16/16	45.3	232.1	120.0	583.0	19.6	
	Δ↑*T**	16.9	−49.1	167.4	−138.2	3.1	18.7
	Δ↑*T***	21.9	−70.8	138.7	−80.1	−9.7	16.1
HSHO							
ADV-2803	16/16C	51.5	235.2	616.1	84.0	13.2	
	Δ↑*T**	12.6	−55.9	78.8	−35.6	0.1	9.2
	Δ↑*T***	12.2	−66.9	104.0	−49.6	0.3	11.6
ADV-3817	16/16	38.9	226.6	639.4	57.2	38.0	
	Δ↑*T**	14.4	28.3	−18.5	−40.3	16.1	5.8
ADV-3816	16/16	50.9	225.5	600.9	107.6	15.2	
	Δ↑*T**	17.5	0.4	54.2	−73.7	1.7	7.3
	Δ↑*T***	27.2	−8.0	58.4	−79.5	2.0	8.7
ADV-3807	16/16	45.8	222.7	596.3	103.3	32.0	
	Δ↑*T**	10.2	−26.3	89.5	−74.9	1.6	10.1
	Δ↑*T***	22.3	−49.6	116.9	−2.4	−17.2	13.9
ADV-2802	16/16	21.8	209.4	638.7	89.8	40.3	
	Δ↑*T**	8.7	36.9	21.8	−68.4	1.0	6.9

P, palmitic acid; S, stearic acid, O, oleic acid; L, linoleic acid; Others, arachidic and behenic acids.

Table 3. Triacylglycerol species composition in oils from HSHL sunflower lines grown at different day/night temperatures during grain-filling period

Line	Treatment (°C)	TAG species composition (g kg^{-1})																		
		POS	POO	PLS	POL	PLL	SOS	SOO	SLS	OOO	SOL	OOL	SLL	OLL	LLL	SOA	OOA	SLA	OOB	Rest
ADV-2505	16/16	6.0	1.0	54.0	13.0	46.0	14.0	5.0	154.0	0.0	77.0	2.0	357.0	56.0	122.0	2.0	1.0	19.0	0.0	71.0
	26/16	8.0	2.0	71.0	19.0	65.0	14.0	6.0	131.0	2.0	72.0	2.0	325.0	62.0	114.0	3.0	1.0	20.0	1.0	82.0
	26/26	22.0	38.0	19.0	43.0	20.0	33.0	102.0	27.0	111.0	129.0	141.0	75.0	96.0	36.0	9.0	9.0	5.0	22.0	63.0
ADV-3512	16/16	7.0	1.0	47.0	13.0	42.0	17.0	8.0	140.0	4.0	100.0	9.0	344.0	51.0	135.0	3.0	0.0	18.0	0.0	61.0
	26/16	7.0	2.0	56.0	14.0	51.0	15.0	8.0	130.0	2.0	86.0	6.0	349.0	54.0	142.0	2.0	0.0	17.0	0.0	59.0
	26/26	23.0	22.0	25.0	35.0	20.0	47.0	102.0	51.0	53.0	173.0	100.0	118.0	96.0	43.0	9.0	13.0	7.0	12.0	51.0
	32/26	23.0	26.0	23.0	37.0	19.0	43.0	111.0	41.0	69.0	165.0	120.0	97.0	102.0	33.0	8.0	16.0	5.0	13.0	49.0
ADV-2504	16/16	6.0	3.0	51.0	17.0	53.0	12.0	12.0	111.0	10.0	82.0	19.0	333.0	68.0	141.0	1.0	0.0	14.0	0.0	67.0
	26/16	12.0	3.0	91.0	22.0	80.0	17.0	10.0	130.0	3.0	70.0	8.0	301.0	43.0	110.0	2.0	0.0	18.0	0.0	80.0
	26/26	43.0	38.0	38.0	44.0	24.0	62.0	129.0	52.0	58.0	145.0	72.0	90.0	56.0	30.0	13.0	13.0	8.0	18.0	67.0
	32/26	48.0	44.0	40.0	46.0	27.0	65.0	131.0	49.0	59.0	127.0	67.0	83.0	55.0	32.0	14.0	12.0	7.0	23.0	71.0
CAS-3	16/16	7.0	3.0	36.0	18.0	46.0	18.0	14.0	85.0	2.0	103.0	17.0	301.0	103.0	189.0	4.0	0.0	11.0	0.0	43.0
	26/16	10.0	4.0	57.0	22.0	61.0	18.0	15.0	102.0	1.0	100.0	11.0	315.0	75.0	134.0	3.0	0.0	12.0	0.0	60.0
	26/26	20.0	22.0	32.0	37.0	39.0	33.0	74.0	47.0	61.0	122.0	74.0	153.0	119.0	90.0	7.0	7.0	6.0	7.0	50.0
	32/26	14.0	17.0	36.0	44.0	50.0	19.0	50.0	42.0	33.0	116.0	85.0	171.0	153.0	116.0	5.0	4.0	5.0	4.0	36.0

Data are average values from both experiments. P, palmitic acid; S, stearic acid, O, oleic acid; L, linoleic acid; A, arachidic acid; B, behenic acid; Rest, POP, PLP, OLA, LLA, EOB, ELB, OLB and LLB.

Table 4. Triacylglycerol species composition in oils from HSHO sunflower lines grown at different day/night temperatures during grain-filling period

Line	Treatment (°C)	TAG species composition (g kg^{-1})														
		POP	POS	POO	PLS	POL	SOS	SOO	SLS	OOO	SOL	OOL	SOA	OOA	SOB	OOB	Rest
ADV-2802	16/16	1.0	16.0	35.0	0.0	9.0	62.0	334.0	6.0	264.0	76.0	93.0	9.0	32.0	11.0	37.0	15.0
	26/16	3.0	25.0	53.0	0.0	6.0	71.0	391.0	3.0	243.0	41.0	37.0	11.0	34.0	13.0	55.0	14.0
	26/26	3.0	33.0	45.0	0.0	2.0	125.0	412.0	4.0	229.0	19.0	15.0	18.0	38.0	16.0	40.0	1.0
ADV-3817	16/16	4.0	32.0	64.0	2.0	9.0	79.0	395.0	5.0	207.0	57.0	47.0	12.0	35.0	9.0	32.0	11.0
	26/16	6.0	35.0	96.0	0.0	7.0	52.0	367.0	0.0	277.0	26.0	30.0	11.0	36.0	9.0	46.0	2.0
	26/26	7.0	57.0	73.0	0.0	4.0	139.0	385.0	0.0	160.0	18.0	10.0	27.0	41.0	26.0	53.0	0.0
ADV-3816	16/16	4.0	39.0	62.0	4.0	18.0	89.0	351.0	10.0	154.0	100.0	65.0	13.0	27.0	10.0	27.0	27.0
	26/16	7.0	51.0	93.0	2.0	11.0	82.0	411.0	2.0	173.0	48.0	36.0	14.0	31.0	5.0	33.0	1.0
	26/26	9.0	57.0	92.0	2.0	8.0	99.0	371.0	2.0	203.0	28.0	24.0	18.0	32.0	16.0	39.0	0.0
	32/26	11.0	68.0	104.0	1.0	5.0	106.0	367.0	1.0	194.0	20.0	17.0	21.0	32.0	16.0	35.0	2.0
ADV-3807	16/16	4.0	34.0	57.0	5.0	18.0	83.0	339.0	12.0	137.0	110.0	67.0	13.0	31.0	13.0	36.0	41.0
	26/16	7.0	42.0	91.0	3.0	17.0	67.0	355.0	4.0	183.0	61.0	49.0	10.0	34.0	10.0	49.0	18.0
	26/26	6.0	44.0	84.0	0.0	6.0	82.0	360.0	1.0	248.0	22.0	24.0	16.0	35.0	19.0	53.0	0.0
	32/26	9.0	49.0	103.0	0.0	5.0	73.0	351.0	0.0	278.0	17.0	18.0	13.0	31.0	13.0	41.0	0.0
ADV-2803	16/16	3.0	33.0	59.0	3.0	12.0	86.0	357.0	6.0	202.0	69.0	67.0	11.0	28.0	9.0	35.0	20.0
	26/16	5.0	38.0	77.0	2.0	7.0	71.0	395.0	2.0	223.0	36.0	31.0	12.0	31.0	11.0	47.0	12.0
	26/26	6.0	44.0	87.0	2.0	7.0	74.0	343.0	3.0	270.0	29.0	31.0	14.0	30.0	12.0	42.0	6.0
	32/26	6.0	41.0	89.0	2.0	5.0	67.0	344.0	3.0	310.0	19.0	20.0	13.0	30.0	11.0	40.0	0.0

Data are average values from both experiments. P, palmitic acid; S, stearic acid, O, oleic acid; L, linoleic acid; A, arachidic acid; B, behenic acid; Rest: PLL, ELL, OLL, OLA and OLB.

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 lower REM (%, total remodeled fatty acids between different growth temperatures) values of HSHO lines compared with HSHL lines confirm the stability in fatty acid composition provided by the high oleic trait (Table 2).

When temperature increased, HSHL lines reduced the concentration of the most abundant TAG species SLL (Table 3). This effect was mainly observed with variations in night temperature (16 *vs* 26 °C). In these lines the concentrations of the species LLL and SLS were also reduced when night temperature increased, probably owing to the reductions in stearic and linoleic acid concentrations mentioned above. These variations were at the expense of higher concentrations of the TAG species including oleic acid (POO, POL, OOO and OOL). In spite of the reductions in stearic acid concentration, the species 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). Thus the stearic acid that is combined in TAG species with linoleic acid at low temperature (SLL and SLS) becomes part of TAG species with oleic acid (SOO and SOS) at high temperature (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 concentrations 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 species POL and those species 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 acids only increased in lines ADV-2802 and ADV-3817, in agreement with the observed increase in stearic acid in these two genotypes when night temperature increased (Table 2).

Temperature effect on saturated fatty acid distribution in TAG species: coefficient of asymmetry αSAT

The content of the TAG subclasses in the high stearic lines grown at different day/night temperatures is presented in Fig. 3. These data show the highest saturated fatty acid 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 of SUU) when compared with lines with high oleic background (full symbols) (Figs 3A and 3B). On the other hand, the night temperature increase to 26 °C resulted in a decrease in the saturated fatty acid 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 (Figs 3C and 3D, Table 5).

The more symmetrical distribution of saturated fatty acids (particularly stearic acid) at high night temperatures is also evidenced when plotting the αSAT symmetry coefficient *versus* the stearic acid content (Fig. 4). When increasing the night temperature, lines improved the saturated fatty acid distribution, measured as the

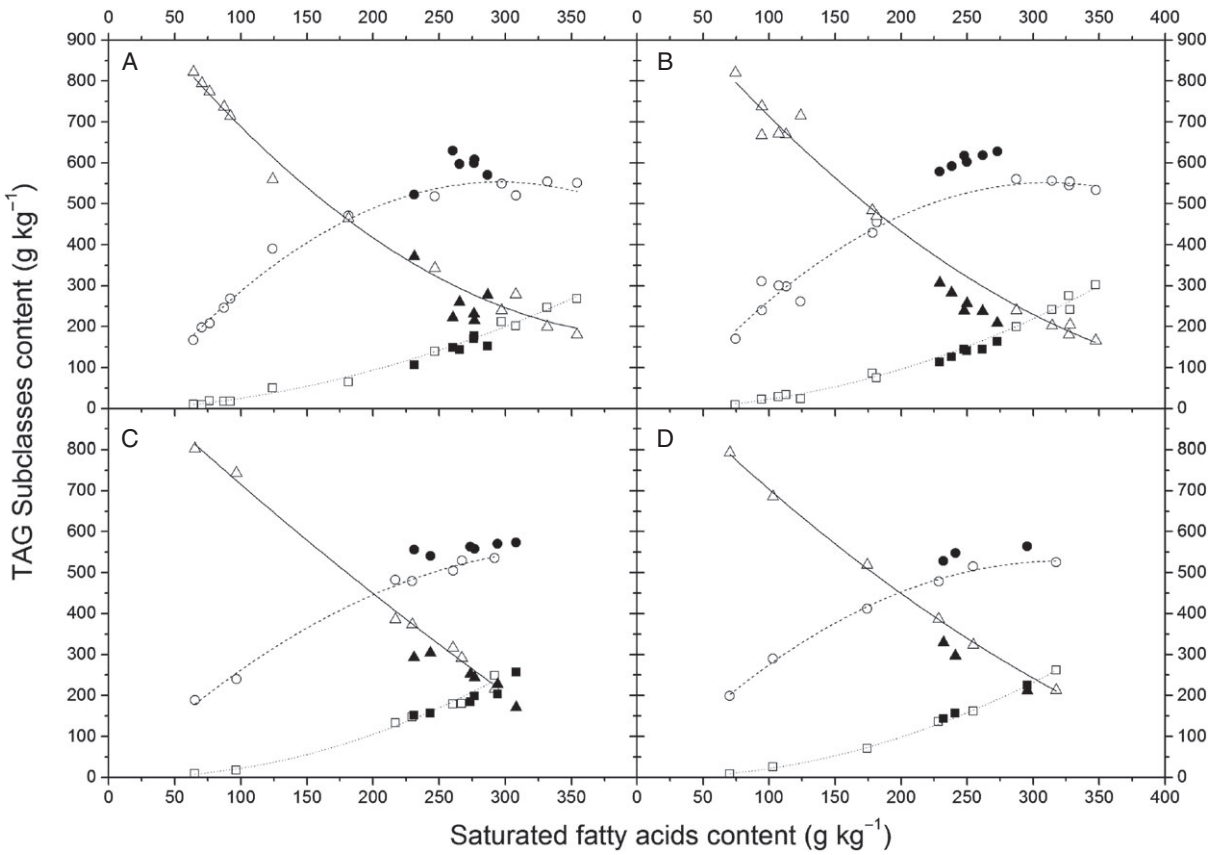

Figure 3. Content of triacylglycerol subclasses (di-saturated TAG (SUS), squares and dotted lines; di-unsaturated TAG (SUU), circles and broken lines; tri-unsaturated TAG (UUU), triangles and full lines) as a function of total saturated fatty acid content from HSHL (open symbols) and HSHO (full symbols) sunflower lines growing at different (A, 16/16; B, 26/16; C, 26/26; D, 32/26 °C) day/night temperatures. Adjusted lines (second-degree polynomial) represent the behavior of high linoleic background lines.

Table 5. Night temperature influence on triacylglycerol subclass content (g kg^{-1}) in HSHL and HSHO oils

Line	Night temperature 16 °C			Night temperature 26 °C		
	SUS	SUU	UUU	SUS	SUU	UUU
HSHL						
CAS-3	195	539	266	149	485	366
ADV-2504	257	541	202	255	531	214
ADV-2505	272	548	180	133	482	385
ADV-3512	244	554	202	170	522	307
HSHO						
ADV-2802	11.6	557	327	198	558	244
ADV-2803	147	586	267	150	534	316
ADV-3807	154	619	228	162	554	284
ADV-3816	166	614	220	214	567	219
ADV-3817	128	588	284	257	573	171

Data correspond to both experiments. SUS, di-saturated TAG; SUU, di-unsaturated TAG; UUU, tri-unsaturated TAG.

coefficient αSAT increase (higher levels of SUS TAG subclass), this effect being more accentuated in lines with high oleic background. This effect of temperature on the distribution of saturated fatty acids in the TAG 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

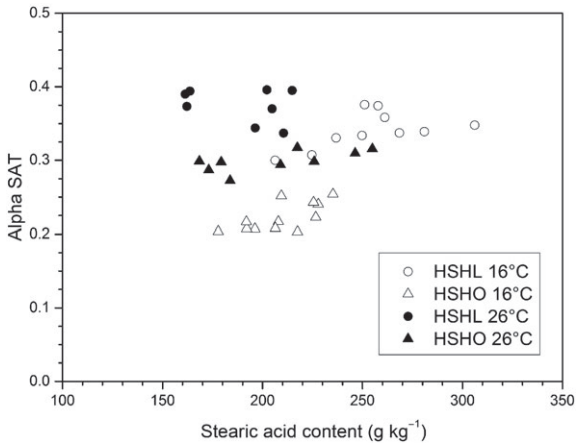

Figure 4. αSAT symmetry coefficient plotted against stearic acid content in oils from HSHL (circles) and HSHO (triangles) sunflower lines growing at 16 °C (open symbols) or 26 °C (full symbols) night temperature.

therefore lower αSAT coefficient than those with high linoleic backgrounds (Fig. 4).

Modification of physicochemical properties of seed oil of high stearic sunflower lines with high linoleic (HSHL) or high oleic (HSHO) background

The changes produced by temperature during grain filling in oil composition of HSHL and HSHO lines was reflected when solid

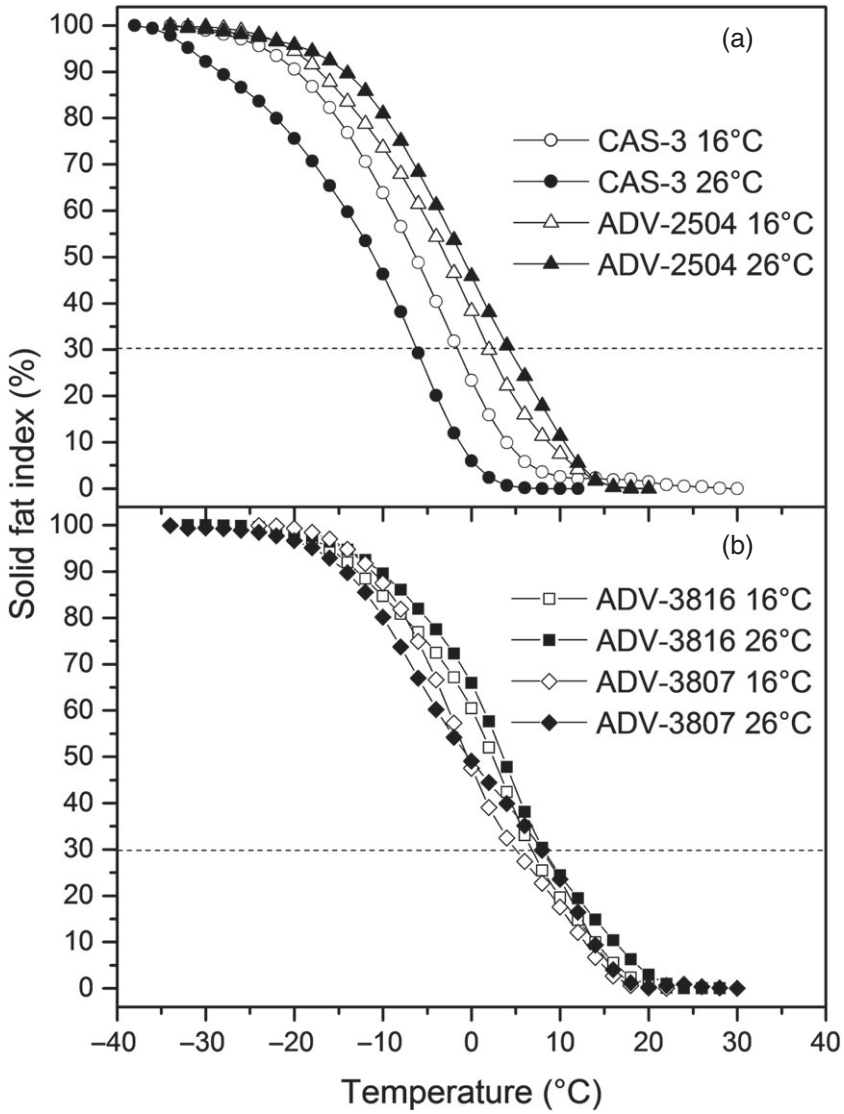

Figure 5. Solid content curves corresponding to melting of different oils from (A) HSHL and (B) HSHO sunflower lines. A broken horizontal line, corresponding to 30% solid fat index, is shown as reference to compare between sunflower lines in A and B.

fat indices were determined. The HSHL lines presented differences in the solid fat index by effect of temperature changes correlated with their response at level of TAG composition (Fig. 5A). The line CAS-3, which strongly modified the stearic acid content and SUS TAGs when temperature increased, presented higher differences in the solid fat index curves than the ADV-2504 line, which presented a more stable oil composition. In fact, the solid fat index 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 from those observed in HSHO lines, which presented minor differences among solid content curves at different temperatures (Fig. 5B).

DISCUSSION AND CONCLUSIONS

Temperature during grain filling modified TAG composition of the seed oil of high stearic sunflower genotypes. This effect was additional to the effect on TAG composition that occurs via variations in fatty acid contents. Accordingly, the effect of temperature during grain filling on oil quality is the result of its effect on fatty acid composition and on TAG composition. From a practical point of view, sunflower oils from seeds developed under warmer nights will have higher contents of SUS TAGs giving better plasticity properties to the oil, as observed with the solid fat curves, and, if they are fractionated for food applications, they will yield bigger stearin fractions (solid fraction with high concentrations of stearic acid).[23] Knowing these effects is important to predict oil composition according to the temperature during grain filling of the crop, mainly defined by location and sowing date but also when the impact of global climate change on product quality is analyzed.

The effect of temperature on fatty acid composition has been widely studied in traditional and mutated sunflower genotypes such as high oleic, high stearic and high stearic-high oleic.[15,17,24–26] Increasing temperature increases the oleic/linoleic acid ratio via its effect on the activity of the oleoyl-PC desaturase (ODS) enzyme, which catalyzes the first extraplastidial desaturation in plants and converts oleic acid esterified to phosphatidylcholine, mainly in the *sn*-2 position, to linoleic acid.[27,28] This effect is less evident in genotypes carrying the Pervenets mutation, such as high oleic and high

stearic-high oleic, in which the enzyme activity is inhibited.[12,15,17,29] 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 stability to fatty acid composition.[15]

These variations in fatty acid composition are naturally reflected in variations in TAG composition.[22] Since oleic and palmitic acid concentrations increased with temperature in HSHL lines, the TAG species including these fatty acids also increased, at the 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 αSAT 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, which together with fatty acid content determine the distribution of TAG subclasses tri-saturated (SSS), di-saturated (SUS), di-unsaturated (SUU) and tri-unsaturated (UUU). It is known that the distribution of fatty acids in the TAG molecule is not random.[3,30] 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. In sunflower it is known that stearic acid is mainly incorporated in the *sn*-3 position, *sn*-1 being defective in this fatty acid.[30] 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 such as stearic acid.[31]

Interestingly, variations in TAG composition were mainly associated with variations in night temperature, in agreement with the results reported for the effect of temperature on fatty acid composition.[11,17] Pleite *et al.*[25] 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 unknown why TAG composition is also better associated with night temperature than with other (i.e. mean or daily) temperatures.

In all genotypes, night temperature influenced the αSAT 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.*[16] 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 than 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 acyltransferase 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, 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.[15,32] To the best of our knowledge, the results presented in this work indicate that a separate effect of temperature on the acyltransferase system also exists. This last effect could be further elucidated by identifying the different acyltransferase genes expressed during sunflower seed development (e.g. glycerol-3-phosphate acyltransferases, diacylglycerol acyltransferases, glycerolcholine acyltransferases and phospholipid:diacylglycerol acyltransferases) and characterizing their expression profiles under different day/night temperatures.

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