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Oleic conversion effect on the tocopherol and phytosterol contents in sunflower oil

Efecto de la conversión oleica sobre la cantidad de tocoferoles y fitoesteroles en aceite de girasol

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Abstract. In sunflower, conventional breeding is widely used for the modification of traits such as the fatty acid composition, disease resistance, and mainly for obtaining commercial oil with high oleic acid content. There is a growing interest on tocopherols and phytosterols present in sunflower, due to their human health benefits. This emphasizes the need of studies on breeding programs for these bioactive components. A hundred of isogenic pairs of classic and its oleic version of hybrids and parental lines were cultivated in different locations in France between 2003 and 2006. The results indicated that sunflower oil is rich in α-tocopherol and β-sitosterol. However, there was little correlation between traditional linoleic and oleic sunflower oils for the total tocopherol content, and no correlation for the total phytosterol content. Additionally, there was little or no effect of the oleic conversion for the tocopherol and phytosterol contents. Nevertheless, tocopherol content was significantly lower in the oleic sunflower than in the classic genotypes, but it was function of the year.

Keywords: Sunflower; Oil; Oleic conversion; Phytosterols; Tocopherols.

Resumen. En girasol, la selección convencional es ampliamente utilizada para la modificación de caracteres como la composición en ácidos grasos, la resistencia a enfermedades, y especialmente la obtención de aceites comerciales con un alto contenido en ácido oleico. Hay un creciente interés en los tocoferoles y en los fitoesteroles, presentes en el girasol, por sus propiedades benéficas sobre la salud humana. Esto enfatiza la necesidad de estudios de programas de selección por estos componentes bioactivos. Un centenar de parejas isogénicas de híbridos y líneas parentales de girasol clásico y su versión oleica fueron cultivados entre 2003 y 2006 en diferentes lugares en Francia. Los resultados indicaron que el girasol es rico en α-tocoferol y en β-sitoesterol. Sin embargo, hubo muy poca correlación entre el aceite de girasol tradicional linoléico y el rico en ácido oleico, en lo que respecta la a cantidad total de tocoferoles, y ninguna correlación con respecto a la cantidad de fitoesteroles totales. Por otra parte, no hubo casi o ningún efecto de la conversión de ácido oleico sobre las cantidades de tocoferoles y de fitoesteroles. No obstante, la cantidad de tocoferoles fue significativamente inferior en el girasol oleico que en el clásico, pero solo en algunos años.

Palabras clave: Girasol; Aceite; Conversión oleica; Fitoesteroles, Tocoferoles.

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INTRODUCTION

The Mediterranean diet, with a high intake of oleic acid (mainly from olive oil consumption), has a proven benefit on human health. Many studies have demonstrated that a continuous intake of polyunsaturated fatty acids (PUFA) can reduce cardiovascular problems, dyslipidemias, diabetes and some cancers (Reaven et al., 1993; Zock & Katan, 1998; Benatti et al., 2004; Brouwer et al., 2004). In the 90's, sunflower and canola breeders became interested in oleic acid, and started research programs to develop new varieties with a high content on this fatty acid. Sunflower breeders also generated hybrids with a very high oleic acid content (more than 90%). A variety is considered as oleic when its oleic acid content is over 75% of the total fatty acids. Similarly, hybrids with a middle oleic acid content (Mid oleic) have also been developed to maintain an optimal proportion of linoleic acid in the final oil composition. Moreover, in 2012, more than 60% of the French cultivated sunflower was high in oleic acid content (CETIOM, 2013).

At the same time, it appeared an increasing interest for some minor components present in sunflower seeds. For example, tocopherols (Vitamin E) and phytosterols are bioactive compounds with health benefits present in the oil fraction. Tocopherols are lipid antioxidants, known to have a protective effect against many diseases. They include cancer, cardiovascular diseases, cell and DNA damage by free radicals, Alzheimer disease and oxidation of LDL (Bramley et al., 2000; Morris et al., 2005; Richey, 2005). The recommended dietary allowance for α-tocopherol in adults is 15 mg/day (U.S. Department of Agriculture, 2011). Phytosterols are plant hormones with anticholesterolemic properties, which have been studied since long ago (Normen et al., 2000; Kritchevsky, 2002; Trautwein et al., 2003; Richelle et al., 2004). Phytosterols are currently used to reduce cholesterol levels in humans' blood. They are added in products such as margarines and yoghurts. Moreover, plant sterols also have other interesting properties like anticancer dietary components (Awad & Fink, 2000), and anti-inflammatory (Bouic et al., 1999) and anti-atherosclerosis properties (Patel & Thompson, 2006).

Sunflower tocopherol content is affected not only by genotype (Velasco et al., 2002; Ayerdi Gotor et al., 2006a) but also environmental conditions (Nolasco et al., 2004; Izquierdo et al., 2007). Its content varies between 300 to 1800 mg/kg of oil. Alpha-tocopherol is the major homologous representing more than 95% of the total tocopherol content. The phytosterol content in sunflower oil also varies among genotypes (Vlahakis & Hazebroek, 2000; Ayerdi Gotor et al., 2006b) and environmental conditions (Roche et al., 2006; Ayerdi Gotor et al., 2008), but less than tocopherols.

The difference in tocopherol content between oleic and classic cultivars has been observed in canola (Goffman & Becker, 2002), peanut breeding lines (Jonnala et al., 2006) and in sunflower hybrids (Nolasco et al., 2006). However, these studies did not compare the original lines with the converted ones. We found no reference on phytosterols in the literature.

Breeding programs are used mostly to modify yield, fatty acid composition or disease resistance. The objective of this research was to evaluate the influence of breeding for high oleic acid content on the tocopherol and phytosterol contents in sunflower oil.

MATERIALS AND METHODS

Plant material. Isoline pairs of sunflower hybrids and parental classic lines (low oleic acid content and their oleic equivalents -- oleic acid content higher than 75% of the total fatty acids) were cultivated between 2003 and 2006. Plants were generated by the different breeder partners of our project (Table 1).

Table 1. Genetic origin of the pairs used each year obtained from different breeders and locations.
Tabla 1. Origen genético de las parejas usadas cada año obtenidas de diferentes mejoradores y localidades de cultivo.

Year	Number of pairs	Genetic origin of Oleic *vs*. Classic pairs
2003	7	Isogenic parental lines
	4	Self fecundated hybrids
	4 x 6	Self fecundated hybrids cultivated in 6 locations
2004	3	Isogenic parental lines
	4 x 6	Self fecundated hybrids cultivated in 6 locations
2005	5	Isogenic parental lines
	5	Self fecundated hybrids
2006	28	Isogenic parental lines, couples were close by cultivated

Chemical analysis of achenes

Solvent extraction of lipids. The analysis of the total oil content was performed by hexane extraction using a soxhlet extractor apparatus. Fifty grams of dry-freeze achenes were ground with a sample mill (KnifeTec 1095; Foss Tecator AB, Sweden) and placed in cellulose cartridges (Whatman ø = 30 mm, h = 100 mm, Prolabo-Subra, France). Cartridges were subjected to hexane (n-hexane, Prolabo-Subra, France) extraction for 4 hours in six soxhlet-ramp (AFNOR, 1998). Then, the solvent was removed from the extracts under low pressure evaporation with a rotavapor (HS 40 Huber, Bioblock Scientific, Heildolph). Lipid extracts were weighed and conserved at -18 °C to minimize oxidative reactions before analysis.

Tocopherol determination. Complete separation of all native tocopherols was achieved using a high-performance

liquid chromatography (HPLC) (SpectraPhysics; Thermo Separation Products, USA) and detection was determined with a fluorescence detector (excitation wavelength = 298 nm and emission wavelength = 344 nm; Waters 2475 multi λ, France) (ISO, 1997). A normal-phase LiChrosorb Si60 column (250 cm x 4 mm x 5 µm, CIL Cluzeau, France) was used. The mobile phase was hexane/isopropanol (99.7:0.3 v/v HPLC grade, SDS, France) and the solvent flow was 1 mL/min. One gram (precisely weighed: Sartorius Analytical balance Precisa 205 A, Italy) of oil sample was diluted in 25 mL of hexane and 20 µL of this dilution were injected into the HPLC. Tocopherols were identified by comparison of retention times with respective standards (Tocopherol Kit; ChromaDex, USA). Standards were prepared in a methanolic solution (0.1 mg/mL). Standard solutions were checked with an UV spectrophotometer (Hitachi U-1100 photometer). Total tocopherol content was calculated as the sum of α- , β-, γ- and δ-tocopherol contents, and they were expressed in mg/kg oil (Ayerdi Gotor et al., 2006a).

Sterol determination. The oil (250 mg) with 1 mL of a freshly prepared solution of betulin (1mg/mL acetone), as internal standard, was saponified with 5 mL of an ethanolic solution of KOH 5% (w/v) during 15 minutes. Then the sample was purified in an aluminium oxide column (pH 9-10, Panreac Química, Spain), and the unsaponifiable fraction was recovered after complete evaporation of solvents. The total and the individual sterol content were analyzed by Gas Chromatography following the international norm (AFNOR, 1999), after a silylation with trimethylsylil (TMS) ester derivatives [1-methylimidazole and N-methyl-N-(trimethylsilyl) heptafluorobutyramide reagent 5:95 v/v Sigma, France] during 15 minutes in a 105 °C oil bath. One µL of the TMS solutions was injected into a silica capillary column (ZB-5, 30 m x 0.25 mm x 0.25 µm, Phenomenex, France) in the gas chromatography (Fisons GC 8000 series MMFC 800 Multi-function controller, Italy) fitted with a flame ionization detector. Sterols were identified using the ratio obtained between betulin (Internal standard) and sterol standards (Sigma-Aldrich, France). Sterols were expressed in mg/100 g oil.

Statistical Analysis. The data were statistically analyzed using SPSS V16 (SPSS Inc, USA). Student's t tests were performed for paired samples for each variable and year.

RESULTS AND DISCUSSION

A total of 100 isogenic pairs were cultivated in France in different places between 2003 and 2006. Total tocopherol content varied from 233 to 1016 mg/kg of oil. Alpha-tocopherol was the major homologous representing more than 95% of the total tocopherol content. These values were in accordance with the literature (Velasco et al., 2002; Ayerdi Gotor et al., 2006a; Ayerdi Gotor et al., 2007). Total phytosterol varied from 179 to 467 mg/100g of oil; total phytosterol corresponds to the sum of 7 sterols: β-sitosterol was the major one (40-50%) followed by campesterol (15-25%) and stigmasterol (15-25%). Several authors reported similar values for phytosterol content and composition in sunflower oil (Vlahakis & Hazebroek, 2000; Ayerdi Gotor et al., 2008; Roche et al., 2010). Mean values of total tocopherol and phytosterol contents for high oleic *versus* classic parental lines and hybrids are presented in Table 2.

Tocopherols. The comparison of the total tocopherol content between classic *versus* its oleic isogenic lines showed that there were little differences (Fig. 1). In general, total tocopherol content was higher in classic hybrids or classic parental lines than in their corresponding oleic lines; this difference was statistically significant ($p<0.05$) in 2003 and 2004, but not during 2005 or 2006 ($p>0.05$; Table 2).

Table 2. Mean values of total tocopherol and phytosterol contents for the parent lines and hybrids as a function of their oleic acid content (oleic or classic) from 2003 to 2006. A Student's t test was made for paired samples. Mean values within the same row with $p<0.05$ are significantly different.

Tabla 2. Medias del contenido de tocoferoles y fitosteroles totales de las líneas parentales y los híbridos en función de su contenido en ácido oleico (alto oleico y clásico) entre 2003 y 2006. Se efectuó el test de Student para las muestras apareadas. Los valores promedio en una misma fila con $p<0,05$ son diferentes significativamente.

	Year	df*	Oleic Mean ± sd*	Classic Mean ± sd*	p-value* (Sig 2-tail)
Phytosterols	2003	32	323.2 ± 7.1	331.0 ± 6.6	0.451
	2004	24	501.4 ± 13.8	494.0 ± 18.3	0.720
Tocopherols	2003	33	435.7 ± 15.8	465.9 ± 20.8	0.032
	2004	23	492.9 ±12.5	552.9 ± 16.0	< 0.001
	2005	20	486.2 ± 31.8	514.0 ± 17.8	0.212
	2006	25	700.6 ± 59.2	641.5 ± 40.8	0.292

*df = degree of freedom; sd = standard deviation; α=0.05.

*df = grados de libertad; sd = desviación estándar; α=0,05.

Phytosterol. Figure 2 shows the absence of correlation for the phytosterol content between classic and oleic hybrids and parental lines. Table 2 shows the absence of significant differences ($p>0.05$) between oleic and classic parental lines or hybrids for their total tocopherol contents in 2003 and 2004.

CONCLUSIONS

The contents of tocopherol were significantly lower in the oleic hybrids and parental lines than in the classic ones in 2003 and 2004; phytosterol contents did not vary inside the couples studied (oleic-linoleic couples). The tocopherol antioxidant function has been largely reported in oils (Evans et al., 2002; Psomiadou & Tsimidou, 2002; Quiles et al., 2002). It has been proved that the more unsaturations the fatty acid contained, the more easily it was oxidized. Also, the more tocopherols the oil contained, the more resistant it was to oxidations. However, when we observed the quasi isogenic pairs used in this report, there was no difference in tocopherol content, even if there were changes in the fatty acid profile, preserving their nutritional properties. There is no evidence of any effect on the tocopherol and phytosterol contents when the fatty acid composition in sunflower oil is modified by breeding programs. Nevertheless, during seed development, regulatory mechanisms (e.g., enzyme activity) of these components (fatty acids, tocopherols and phytosterols) could be altered by environmental factors (e.g., temperature) to protect the plant against oxidations, altering in this way its final oil composition.

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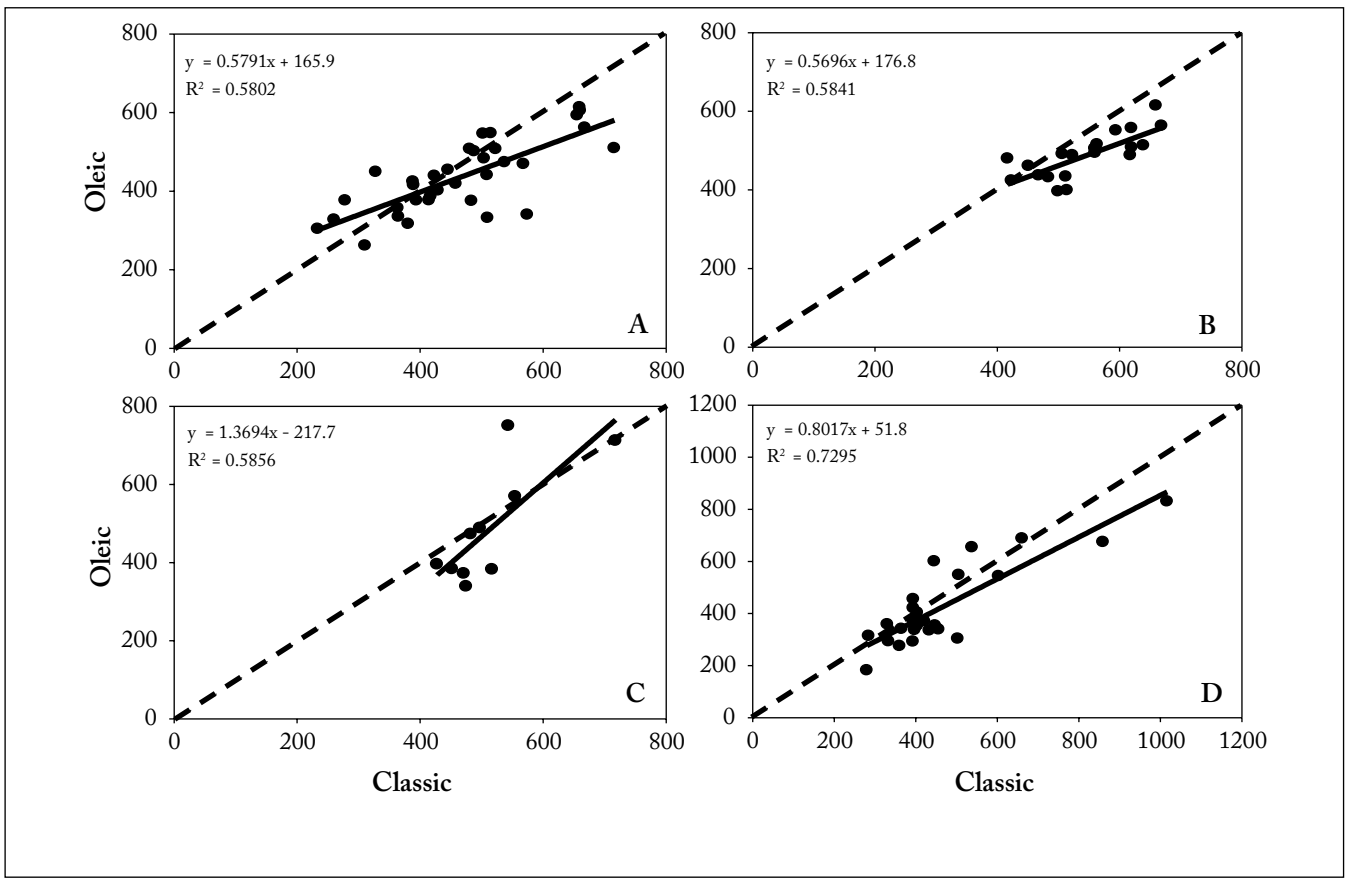

Fig. 1. Correlation between classic and oleic sunflower for the total tocopherol content (mg/kg oil) during (A) 2003; (B) 2004; (C) 2005; (D) 2006. The 1:1 relationship is shown by the dashed line.

Fig. 1. Correlación entre los girasoles oleicos y clásicos según la cantidad total de tocoferoles (mg/kg de aceite) en (A) 2003; (B) 2004; (C) 2005; (D) 2006. La relación 1:1 se muestra con la línea cortada.

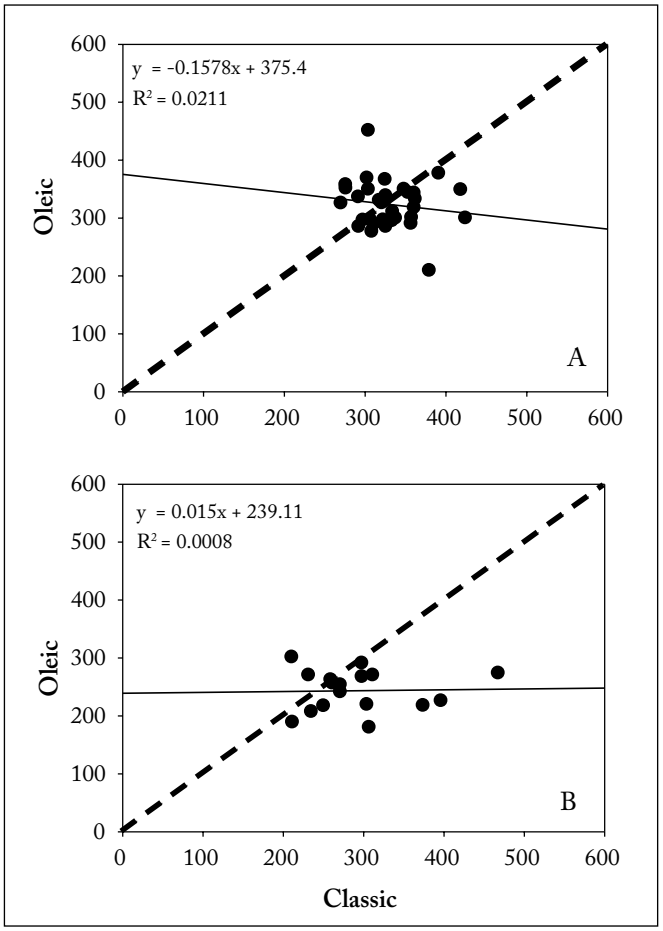

Fig. 2. Correlation between classic and oleic sunflower for the total phytosterol content (mg/100 g oil) during (A) 2003; (B) 2004. The 1:1 relationship is shown by the dashed line.

Fig. 2. Correlación entre los girasoles oleicos y clásicos según la cantidad total de fitoesteroles (mg/100 g de aceite) en (A) 2003; (B) 2004. La relación 1:1 se muestra con la línea cortada.

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