BREEDING FOR SPECIALTY OIL TYPES IN SUNFLOWER

José M. Fernández-Martínez, Begoña Pérez-Vich, Leonardo Velasco,
Instituto de Agricultura Sostenible, Apartado 4084, Córdoba, E-14080, Spain
E-mail: cs9femaj@uco.es; bperez@uco.es; ia2veval@uco.es

Juan Domínguez, CIFA Alameda del Obispo, IFAPA, Junta de Andalucía, Apartado 3092, Córdoba, E-14080, Spain Introduction Station, Iowa State
E-mail: juan.dominguez.jimenez@juntadeandalucia.es

Abstract

Oil quality in vegetable oils is a relative concept that depends on the end-use of the oil. Vegetable oils are intended for food applications (salads and cooking oils, margarines, shortenings, etc) and nonfood industrial applications (biodiesel, lubricants, surfactants etc). Sunflower (*Helianthus annuus* L.) oil has been traditionally appreciated in the world oil market. However, new emerging markets are demanding changes in the oil quality for both food and nonfood applications. Nutritional and functional properties determining oil quality are primarily determined by the fatty acid composition of the oil and the total content and composition of natural antioxidants, especially tocopherols. During the last 30 years these components have been extensively modified in sunflower through conventional selection from naturally occurring variation and trough mutagenesis. As a result, together with the standard sunflower oil, with a fatty acid profile made up of 11% saturated fatty acids, 20% oleic acid, and 69% linoleic acid, there is currently available a vast diversity of other sunflower oil types, for example low saturated (<7%), high palmitic (>25%), high stearic (>25%), high oleic (>85%), high linoleic (>75%) as well as a number of oils with intermediate levels and combinations among them. Similarly, the standard sunflower oil with 95% of the tocopherols in the alpha-tocopherol form has been modified to produce oils with high levels of beta-tocopherol (>75%), gamma-tocopherol (>95%), and delta-tocopherol (>65%). The novel fatty acid and tocopherol traits are in all cases governed by a reduced number of genes, which facilitates considerably their management in plant breeding programs aimed to develop cultivars with improved oil quality.

Introduction

Sunflower is the second source of vegetable oil in Europe after rapeseed and the fourth largest source at a world level after soybean, palm and rapeseed. Sunflower oil has been traditionally appreciated as a high quality commodity in the world oil market. However, new emerging markets together with high concern about health risks of foods and an increasing request of vegetable oils for specific nonfood uses are demanding changes in the oil quality

Traditionally, the concept of oil quality has been almost exclusively associated with the fatty acid composition of the oil. Recently, however, this concept has evolved and oil

chemists and nutritionists are emphasizing other components of vegetable oils that influence their nutritional and technological properties. As in other vegetable oils, the objective of modifying sunflower seed oil quality has been to develop oils with enhanced nutritional and functional properties and oils that require little, if any, processing for specific end-use markets. Oil processing results in many cases in detrimental implications for human health. For example, common uses of sunflower oil such as deep frying or margarine production requires previous hydrogenation or hardening of the oil. This process produces *trans* and positional isomers which have been proved to be negatively related to heart disease. Similarly, standard sunflower oil is not optimally suited to some potential uses, for example those requiring a high oxidative stability. In this paper we report the development by plant breeding of novel sunflower oil types with improved properties for food and nonfood applications.

Components of oil quality and breeding objectives

Vegetable oils for human consumption are included in the diet as visible fat (butter, margarine, salad oil, cooking oil) and invisible fat (milk, meat, cheese, pastry, snacks, bread, nuts). Apart from food uses, large quantities of vegetable oils are directed to nonfood applications. They are used as motor fuels (biodiesel) and lubricants as well as for many applications in the oleochemical industry (detergents, soaps, surfactants, emulsifiers, cosmetics, etc.).

Nutritional and functional properties of the oils are determined by the fatty acid composition of the oil, the distribution pattern of the fatty acids within the triacylglycerol molecule and the total content and composition of natural antioxidants especially tocopherols.

From a nutritional point of view, saturated fatty acids, especially palmitic acid, are regarded as undesirable for human consumption because they have a detrimental atherogenic effect, mainly by rising serum cholesterol levels as compared with mono and polyunsaturated fatty acids (Mensink and Katan, 1989). Conversely, oleic and linoleic acids are hipocholesterolemic but, although linoleic acid is an essential fatty acid, oils rich in oleic acid are preferred as it combines the hypocholesterolemic effect (Mensink and Katan, 1989) and a greater oxidative stability than linoleic acid (Yodice, 1990). However, high linoleic acid oils have alternative nutritional advantages such as the production of conjugated linoleic acid (CLA), associated with a wide range of positive health benefits (Belury, 1995; Ip, 1997). Another important parameter playing an important role in lipid nutritional value is the stereochemical position of the three fatty acids in the triacylglycerol (TAG) molecule as the absorption rate of the fatty acids is higher when they are sterified at the central *sn*-2 TAG position than when they are at the external *sn*-1 and *sn*-3 positions (Bracco, 1994). Thus, vegetable oils having undesirable saturated fatty acids at the *sn*-2 position, such as palm oil or partially hydrogenated fats, are more atherogenic than those having a similar total saturated fatty acid content but distributed at the external positions, as is the case of high palmitic acid sunflower (Alvarez-Ortega et al., 1997).

Oil oxidation involves the production of free radicals which are implicated in a number of human diseases. Tocopherols are fat soluble compounds that exert an antioxidant action both *in vivo* (vitamin E activity) and *in vitro*. Among the four tocopherol derivatives (alpha-, beta-, gamma-, and delta-tocopherol) alpha-tocopherol has the highest vitamin E activity as compared with the other tocopherol derivatives, which are more powerful *in vitro* antioxidants than alpha-tocopherol (Kamal-Eldin and Appelqvist, 1996). Standard sunflower oil has an average fatty acid composition of 11% saturated fatty acids (palmitic -16:0- and stearic acid -18:0-), 20% oleic acid (18:1), and

69% linoleic acid (18:2) and a total tocopherol content in standard refined sunflower oil of around 700 mg kg^{-1}, with about 95% of the total tocopherols in the alpha-tocopherol form. Therefore, applications demanding a high nutritional value of the oil (e.g. salad oil) will require a reduction of saturated fatty acids and enhancement of the vitamin E (=alpha-tocopherol) content of the oil.

Some other breeding objectives are associated with the desired technological properties for the food industry such as plasticity and resistance to oxidation, particularly at high temperatures. For example, the manufacture of margarines and other specialty products requires solid or semi-solid fats, but standard sunflower oil, mainly made up of unsaturated fatty acids, is liquid at room temperature. Accordingly, its utilization by the food industry usually requires a previous chemical hardening to change to a solid or semi-solid state, usually conducted by hydrogenation or transesterification. However, during these processes are produced *trans*-fatty acids which are harmfully related to heart disease (Ascherio and Willett, 1997). These uses demand sunflower oils with high concentration of saturated fatty acids, preferably stearic acid which has a neutral effect on serum lipoprotein cholesterol (Pearson, 1994). For other food applications including high temperature processes (e.g. frying oil industry) sunflower oils with high concentration of oleic acid and the *in vitro* antioxidants gamma- and/or delta-tocopherols are much more appropriate.

For industrial nonfood uses, oils with a maximum concentration of the desired fatty acid are demanded in order to reduce the amount of waste and the processing costs (Lühs, and Friedt, 1998). For example, for some uses requiring high oxidative stability at high temperatures (biodiesel and biolubricants) high or very high levels of oleic acid and gamma- and/or delta tocopherols are needed. Conversely, the lower oxidative stability associated with high and stable concentrations of linoleic acid, is a desirable property for drying oils that are used in coatings applications, such as paints, inks and varnishes.

Genetic improvement of sunflower oil quality traits

A great deal of genetic variation in the content of all fatty acids and tocopherols in sunflower oil has been developed by searching of natural variation in world collections and to more extent by the use of mutagenesis. A key factor for this success was the utilization of fast, cheap, reliable and non-destructive methods of analysis using the half seed and the near-infrared spectroscopy (NIRS) techniques.

Mutagenesis has been successfully used for developing variation in the fatty acid profile of sunflower. The following mutants with altered fatty acid content have been developed (Table 1): The high oleic acid mutant “Pervenets” (Soldatov, 1976); Mutants 275HP and CAS-5 with high palmitic acid content (Ivanov, 1988; Osorio et al., 1995), CAS-12 with high palmitic acid and increased oleic acid content (Fernandez-Martinez et al., 1997) and CAS-37 with high palmitic acid and medium content of palmitoleic acid (Salas et al., 2004); Mutants CAS-8, CAS-4 and CAS-3 with medium to high stearic acid content (Osorio et al.,1995) and CAS-14 with very high stearic acid content (Fernández-Moya et al., 2002); Two mutants with low stearic acid content LS-1 and LS-2 and one mutant LP-1 with low palmitic acid content (Miller and Vick, 1999). Mutagenesis was also effective for developing the mutants IAST-1 and IAST-540 with increased levels of gamma tocopherol (>95%) (Velasco et al., 2004) and mutants with increased levels of saturated fatty acids almost exclusively at the *sn-1* and *sn-3* TAG positions (Alvarez-Ortega et al., 1997). Germplasm evaluation and recombination has been also effective to develop variation for fatty acid content, for example lines with low total saturated fatty acids (RS1 and RS2, Vick et al., 2002 and NMS 2229, Seiler, 2004), with high stearic acid on high oleic acid background (CAS-15, Fernández-Moya et al., 2005) or with high

linoleic acid content (2698-L, Miller and Vick, 2001) (Table 1) as well as lines with increased levels of beta-tocopherol (>30-50%) (LG-15, Demurin et al., 1996 and T589, Velasco et al., 2003) and high gamma-tocopherol (>95%) (LG-17, Demurin et al., 1996 and T2100, Velasco et al., 2003). Lines IAST-5 with high beta-tocopherol content (>75%) and IAST-4 with delta- tocopherol (>65%) were further developed by recombination (Velasco et al., 2004).

Table 1. Fatty acid composition (%) of the seed oil of the principal induced and natural mutants of sunflower, in comparison with the standard type. The concentrations of the most altered fatty acids are printed in bold.

Mutant	Fatty acid *composition (%)				
or line	16:0	16:1	18:0	18:1	18:2
Standard	5,7	----	5,8	20,7	64,5
	6,5	----	3,0	40,9	49,6
Low content in saturated fatty acids					
LS-1	5,6	----	**4,1**	20,2	67,4
LS-2	8,6	----	**2,0**	10,8	75,0
LP-1	**4,7**	----	5,4	23,8	63,7
RS1	**3,9**	----	**2,6**	40,1	51,8
RS2	**4,4**	----	**3,2**	42,9	47,7
NMS 2229	**3,9**	----	**1,9**	----	----
High content in palmitic acid					
275HP	**25,1**	6,9	1,7	10,5	55,8
CAS-5	**25,2**	3,7	3,5	11,4	55,1
CAS-12	**30,7**	7,6	2,1	**56,0**	3,1
HP line	**23,9**	3,4	2,0	20,4	50,7
CAS-37	**29,5**	**12,3**	1,4	5,4	38,7
High content in stearic acid					
CAS-3	5,1	----	**26,0**	13,8	55,1
CAS-4	5,4	----	**11,3**	34,6	48,0
CAS-8	5,8	----	**9,9**	20,4	63,8
CAS-14	8,4	----	**37,3**	12,4	38,0
CAS-19	6,8	----	**15,3**	21,5	56,4
CAS-20	5,7	----	**7,7**	35,9	50,5
CAS-15	6,1	----	**24,9**	**57,8**	8,2
High content in oleic acid					
Pervenets	----	----	----	**79,3**	14,8
M-4229	3,4	----	4,1	**86,1**	3,9
M- 3067	3,9	----	5,2	**54,6**	33,9
High content in linoleic acid					
F6 sel.	----	----	----	----	**77,3**
2698-1	----	----	----	----	**78,0**

*16:0=palmitic acid; 16:1= palmitoleic acid;18: 0=stearic acid; 18:1=oleic acid; 18:2=linoleic acid.
Source: Adapted from Fernández-Martinez et al. (2004) and Fernández-Moya et al., 2005

All these traits are usually under embryonic control by a reduced number of genes (see review in Fernández-Martínez et al., 2004) which facilitate their management in breeding programs. For example, three genes are involved in the control of high levels of palmitic (P1, P2, P3) and high stearic acid content (Es1, Es2, Es3) and one or two genes in the control of low levels of these fatty acids. One principal gene Ol1 and several modifier genes are involved in the control of high oleic acid content. Similarly, two

unlinked genes, Tph1 and Tph2, control altered tocopherol composition. Moreover, in recent years have been developed molecular markers for some of the traits, for example for high stearic and high oleic acid contents (Pérez-Vich et al., 2002), or high beta- and gamma-tocopherol contents (Vera-Ruiz et al., 2006; Garcia-Moreno et al., 2006). The use of these molecular markers will contribute to improve breeding efficiency.

Concluding remarks

A wide range of sunflower lines with contrasting fatty acids profiles have been developed, for example with high and low levels of saturated fatty acids, mid and high levels of oleic acid, high concentration of linoleic acid, as well as different ranges of intermediate levels of the different fatty acids and combinations among them. Similarly, lines with high levels of beta-, gamma-, and delta-tocopherol have been developed providing more variability for tocopherol profiles in sunflower oil than in any other oilseed crop. The combination of several quality traits in a single phenotype will enable the tailoring of specialty oils for specific uses in the food and nonfood industry. The novel fatty acids and tocopherol traits are in all cases governed by a reduced number of genes and can be easily managed in breeding programs aimed to develop cultivars incorporating these traits.

The following types of sunflower oils are currently commercialized or are foreseeable in a few years:

-Low saturated oils both in standard and high oleic acid backgrounds suitable for salads and cooking.

-High stearic acid oils, on standard and high oleic acid backgrounds suitable for the production of more healthy margarines.

-Mid and high oleic acid oils combined with high content of *in vitro* antioxidants usable for biodiesel and applications requiring high temperature processes (deep frying, biodegradable lubricants).

-High palmitic acids oils in high oleic background as an alternative for high performance frying operations without hydrogenation.

-High and stable linoleic acids oils with applications in the coating industry and for producing conjugated linoleic acid (CLA), a novel therapeutic nutrient.

Some of these types of oils have special characteristics which differentiate them from similar types in other oilseed crops. For example, sunflower oil with low saturates and very high oleic acid, has the highest oleic acid levels (>92%) of any vegetable oil currently in the market. Other oil types, such as high stearic or high palmitic acids on high oleic background, are also available in other oilseeds, as cottonseed, but they are genetically-modified (GM) products while in the case of sunflower have been obtained trough conventional plant breeding. The possibility of tailoring different specialty oils for food and nonfood applications guarantees a promising future to sunflower in the global world market.

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