From the lab to the market: new challenges for sunflower oil quality research

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ABSTRACT

- Conventional sunflower oil is regarded as a healthy oil by consumers around the world. Its main characteristics are a fatty acid profile rich in polyunsaturated linoleic acid, a moderate to high content of alpha-tocopherol, with high vitamin E activity, and a moderate to high content of phytosterols, which play an important role in reducing blood cholesterol and subsequently the risk of coronary heart disease. Notwithstanding these properties, sunflower breeders have been extraordinarily successful in developing new traits that either confer improved nutritional and technological properties to the oil for conventional uses or have opened up new applications for sunflower oil.

- Most of the genetic modifications of sunflower oil quality have affected the fatty acid and tocopherols profiles. New traits such as mid and high oleic acid content, both low saturated and high saturated fatty acid content, and different combinations of increased levels of beta-, gamma-, and delta-tocopherol have been developed. Some of them such as mid and high oleic acid content are playing an important role in the market, particularly in the US, where the industry has completely shifted to mid oleic acid sunflower oil. Others such as the combination of high stearic acid and high oleic acid, particularly well suited for the industry of margarines and spreads, and the combination of low saturate and mid or high oleic acid, with enhanced nutritional properties, are close to the commercial level. Germplasm with enhanced vitamin E (alpha-tocopherol) content has recently being developed and breeding research on phytosterols, just started, will probably throw a new set of traits in coming years.

- Despite most of the novel oil quality traits are qualitatively inherited, the integration of several traits in a breeding program requires strong support of molecular tools. There have been important advances in recent years in mapping and tagging genes underlying modified oil quality traits as well as identifying their role in the biosynthetic pathway through candidate gene approaches. However, a deeper understanding of the genetic and molecular bases of these traits is required, particularly in relation to the generalized presence of modifying genes affecting their expression.

Key words: Fatty acids – modifying genes – molecular markers – oil quality – phytosterols – tocopherols

INTRODUCTION

Sunflower oil is the fourth vegetable oil produced in the world (FAOSTAT, 2011). Conventional sunflower oil is characterized by a fatty acid profile made up of saturated palmitic acid (6-7%) and stearic acid (3-5%), monounsaturated oleic acid (17-41%), and polyunsaturated linoleic acid (50-72%). It contains moderate to high levels of tocopherols (700 mg kg^{-1}) mainly in the alpha-tocopherol form (> 90%), which is the tocopherol derivative with the highest vitamin E activity. Sunflower oil is also a rich source of phytosterols (3,900 mg kg^{-1}) largely made up of beta-sitosterol (60%) and to lesser extent campesterol (8%), stigmasterol (8%), delta-5-avenasterol (4%), delta-7-stigmastenol (15%), delta-7-avenasterol (4%), and also minor amounts of other phytosterols such as delta-campesterol, clerosterol, and delta-5,24-stigmastadienol (Padley et al., 1994; Fernández-Martínez et al., 2004). Even though conventional sunflower oil has been traditionally regarded as a high-quality salad and cooking oil (Fernández-Martínez et al., 2009), the quality of sunflower oil can be modified in different manners to produce healthier oils as well as oils with better technological properties for a number of uses. The most relevant breeding achievements and their commercial implications, the future breeding objectives, and the foreseeable problems derived from the discovery of novel traits of value for improving sunflower oil quality are discussed below.

DISCUSSION

Fatty acids: Sunflower breeders met oil quality

After the early discovery of the first modified fatty acid traits in rapeseed in the early sixties, Russian breeder K.I. Soldatov identified in 1970 a sunflower mutant with elevated levels of oleic acid and produced the first high oleic acid variety developed in any major oilseed crop, Pervenets (Soldatov, 1976). The trait was found to be controlled by one major gene with dominant gene action designated *Ol*, though modifying genes that reduced the expected high oleic acid content were also identified (Urie, 1985). Even though the novel high oleic acid trait did not attract an immediate commercial interest, high-yielding hybrids producing high oleic acid oil (>80%) were available in the early 90s (Fernández-Martínez et al., 1993). However, a patent awarded in 1986 in the US to the high oleic acid trait (Fick, 1986) precluded the widespread use of high oleic acid hybrids. An alternative strategy based on the development of hybrids with mid oleic acid content (55-75%), which did not infringe the existing patent, was launched by the US National Sunflower Association in 1995. Mid-oleic acid oil was commercialized under the NuSun trade mark (Kleingartner, 2002). Mid-oleic acid hybrids were produced following different breeding strategies. The most common one consisted in crossing a high-oleic female parent with a low-oleic male parent carrying a dominant modifying gene, which resulted in hybrids with average oleic acid content between 60 and 70% (Vick and Miller, 2002). By 2007, close to 90% of the oilseed sunflower planted in the US was NuSun. After the patent on high oleic sunflower oil has expired, high oleic acid hybrids are gaining market share in some countries, particularly in France where they currently represent around 50% of the sunflower acreage (Kleingartner, 2011).

A second landmark in the genetic improvement of sunflower oil quality was the discovery of mutants with increased levels of saturated fatty acids, either with high palmitic acid (>25%) or high stearic acid (>25%) content (Osorio et al., 1995). Sunflower oil with high saturated fatty acid content is semisolid at room temperature and therefore suitable for producing a wide range of margarine and spread products without need for chemical transformations such as partial hydrogenation, which produces *trans* fatty acids that are expected to raise the risk of coronary heart disease (Katan, 1998). For these applications, high stearic acid is preferred over high palmitic acid as the latter has a hypercholesterolemic effect whereas the former has a neutral effect on serum cholesterol (Pearson, 1994). Sunflower hybrids with high stearic (18%) and high oleic (70%) acid content, marketed as HSHO Nutrisun (http://ww.nutrisun.com) are expected to be released for commercial production in 2013, with an estimated production of 100.000 t in 2015 (A. León, personal communication).

Reduction of saturated fat intake is a priority of public health bodies to decrease risk for cardiovascular disease (USDA, 2011). Accordingly, the development of sunflower oil with low saturated fatty acid content has been a breeding priority in recent years particularly in the US. A novel sunflower oil with very low saturated fatty acid content (< 3% of total combined palmitic and stearic acid) and high oleic acid content has been recently obtained (Gerdes et al., 2011) and is currently being marketed as Omega-9 Sunflower Oil (http://www.omega-9oils.com/).

Tocopherols: The second wave

Sunflower seeds contain most of the tocopherols (>90%) in the alpha-tocopherol form. Russian breeder Y. Demurin reported in 1993 the discovery of germplasm containing two new traits: increased beta-tocopherol content (50%) and high gamma-tocopherol content (95%), which were recessive and monogenically inherited (Demurin, 1993). Germplasm with similar characteristics was later identified by Velasco and Fernández-Martínez (2003). Velasco et al. (2004) produced two additional modified tocopherol traits: high beta-tocopherol content (75%) and high delta-tocopherol content (65%). Technological studies with sunflower oils having similar fatty acid profiles and contrasting tocopherol composition, with either high alpha- (>95%) or high-gamma-tocopherol content (>95%), demonstrated that the replacement of alpha-tocopherol by gamma-tocopherol had a marked beneficial effect on oil oxidative stability (Demurin et al., 1996; Warner, 2007; Marmesat et al., 2008). Breeding for seed tocopherols in sunflower has not only been directed towards modification of the tocopherol profile in search for more oxidative stability, but also to increasing total content in the alpha-tocopherol form to enhance the vitamin E properties of the oil (Velasco et al., 2010). Because of the beneficial properties that the modified tocopherol traits confer to sunflower oil, both from a technological (modified profiles) and nutritional (increased levels) perspective, it is expected that seed companies will incorporate some of the novel traits into their breeding programs, though there is no information available on the current status of breeding for tocopherols in the private sector.

Phytosterols: The next generation?

Phytosterols are important traits for human nutrition. Because of their close structural similarity to cholesterol, dietary phytosterols compete with cholesterol for absorption in the intestine, which contributes to reduce blood cholesterol levels (Plat and Mensink, 2005). There is also emerging evidence supporting the inhibitory action of phytosterols on several types of cancer (Woyengo et al., 2009). The health-promoting effect of phytosterols has stimulated a growing market for functional food enriched in phytosterols (Zawistowski, 2010). Sunflower oil naturally enriched in phytosterols might have a niche within the healthy oils market. Also, some phytosterols such as delta-5-avenasterol and delta-7-avenasterol, present in low amounts in sunflower seeds, are effective antioxidants at high temperatures. They act protecting the oil against polymerisation and retard the loss of tocopherols (Rossell, 2001). Breeding for increased levels of these compounds and combination with other traits that retard oil oxidation at high temperatures such as high saturate fatty acids, high oleic acid, and high gamma- and delta-tocopherol content might produce unique oil types for high temperature applications. One of the bottlenecks for breeding for modified phytosterol content and composition in sunflower is the lack of suitable methods for screening for this trait within breeding programmes. A method well suited to breeding research, based on direct analysis of sunflower seeds instead of sunflower oil, has been recently validated (Fernández-Cuesta et al., 2012a). Its application to the analysis of single sunflower half seeds is presented within this conference (Fernández-Cuesta et al., 2012b).

Modifiers: Friends and foes

Modifying genes, also known as modifier genes or simply modifiers, are minor genes having no known effect except to intensify or diminish the expression of a major gene (Briggs and Knowles, 1967). For sunflower oil quality traits, modifiers were first reported for the high oleic acid trait (Urie, 1985). As mentioned above, breeders are making a beneficial use of modifiers to break complete dominance of high oleic acid in order to develop hybrids producing mid-oleic acid oil. However, modifiers may become a serious trouble when the objective is producing hybrids with high oleic acid content and these minor genes get involved (Fernández-Martínez et al., 1989). Modifiers are not a phenomenon specific to high oleic acid, but they have been found to influence most of the oil quality traits developed thus far such as high palmitic acid content (Velasco et al., 2008), high stearic acid content (L. Velasco et al., unpublished results), low saturated fatty acid content (J.T. Gerdes, personal communication), and modified tocopherol profiles (Velasco et al., 2004b). The presence of modifiers hinders breeding for oil quality traits. Accordingly, identification of modifiers in the initial breeding material is mandatory and germplasm containing them must be discarded to avoid unnecessary problems at the final steps of breeding. Fig. 1 shows two examples of the genetic action of modifiers on high oleic acid content and high gamma-tocopherol content.
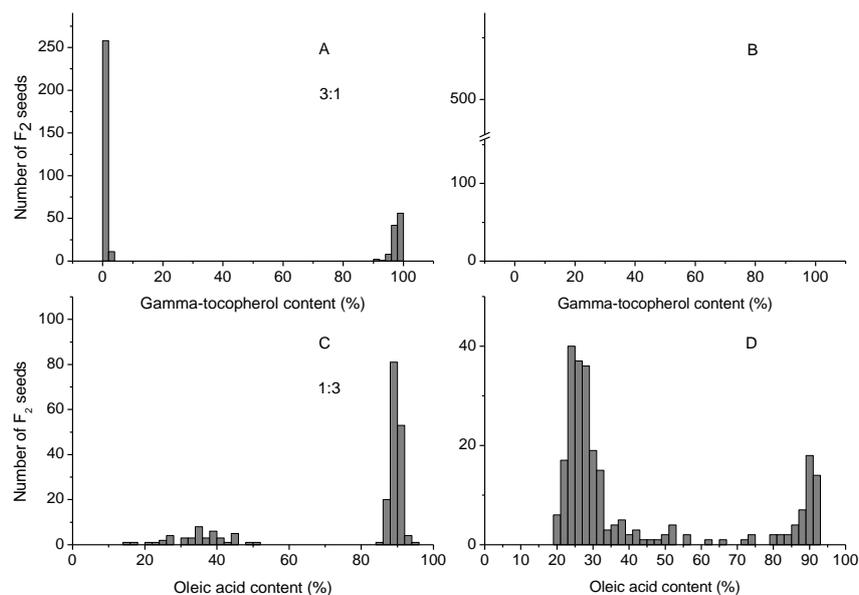
Fig. 1. F₂ segregation for gamma-tocopherol content in absence (A) or presence (B) of modifiers from a cross Low gamma-tocopherol x High gamma-tocopherol. F₂ segregation for oleic acid content in absence (C) or presence (D) of modifiers from a cross Low oleic acid x High oleic acid.

As shown in Fig. 1, modifiers exert a dramatic effect upon the expression of alleles controlling modified oil quality traits such as dominant *Ol1* that determines high oleic acid content and recessive *tph2* responsible for high gamma-tocopherol content. The genetic bases underlying modifiers action are poorly understood. Recently, García-Moreno et al. (unpublished results) identified four modifiers of *tph2*, which underlies a gamma-tocopherol methyltransferase (gamma-TMT) enzyme, on four different linkage groups of sunflower genome and demonstrated that in most cases they corresponded to duplicated copies of gamma-TMT. This is the first evidence of the molecular bases of modifiers, but much more research is needed to understand their mode of action.

Future prospects: The challenge of managing many qualitative traits
Without taking into consideration the role of modifying genes, sunflower oil quality traits are controlled by a reduced number of genes. For modified fatty acid traits, genetic studies have identified three genes involved in high palmitic acid content (Pérez-Vich et al., 1999a), two genes underlying high stearic acid content (Pérez-Vich et al., 1999b), one gene for each of the low palmitic and low stearic acid traits (Gerdes et al., 2011), and one gene for high oleic acid content (Urie, 1985). For modified tocopherol traits, one and two genes are responsible for mid and high beta-tocopherol content, respectively (Demurin, 1993; Velasco and Fernández-Martínez, 2003; Hass et al., 2006), one gene controls high gamma-tocopherol content (Demurin, 1993; Velasco and Fernández-Martínez, 2003), three genes are involved in high delta-tocopherol content (Hass et al., 2006), and one predominant gene underlies increased total tocopherol content (Del Moral et al., 2011). Even though most of the traits are recessively inherited, individual traits can be managed with relative facility in a breeding programme. However, the availability of so many complementary traits makes it attractive to combine several of them within the same genotype. For example, oil from a sunflower line incorporating high palmitic acid, high oleic acid, and high gamma-tocopherol content had 40-fold higher oil stability index than conventional sunflower oil (Marmesat et al., 2008). In that case, at least five genes were involved. Producing a combination of high palmitic acid, high oleic acid, and high delta-tocopherol content will involve at least seven main genes plus an undefined number of modifiers. Managing such a high number of genes in a backcross programme is unrealizable without the help of reliable molecular markers.

Ideally, molecular markers should provide 100% accurate prediction of the phenotype in all genetic backgrounds. This is achieved with functional markers based on the gene mutation responsible for the trait of interest (Andersen and Lübberstedt, 2003; Pérez-Vich and Berry, 2010). Very robust markers can be also developed from the gene(s) underlying the novel trait, even though the mutation is not detected. A number of genes associated with modified oil quality traits have been identified through candidate gene approaches. A stearoyl-acyl carrier protein desaturase gene was found to underlie the

major gene *Es1* involved in high stearic acid content (Pérez-Vich et al., 2002). Gerdes et al. (2011) demonstrated the co-localization of a major QTL for low palmitic acid content with a beta-ketoacyl-acyl carrier protein synthase III gene. For high oleic acid, the seed-specific oleoyl phosphatidyl-choline desaturase *FAD2-1* gene has been unequivocally associated with the *Ol1* gene (Hongtrakul et al. 1998; Pérez-Vich et al., 2002). Schuppert et al. (2006) determined the physical structure of the *FAD2-1* locus and developed polymorphic sequence-tagged-site (STS) DNA markers diagnostic for the *Ol1* mutation. For modified tocopherol profiles, Tang et al. (2006) determined that two 2-methyl-6-phytyl-1,4-benzoquinone/2-methyl-6-solanyl-1,4-benzoquinone methyltransferase (MPBQ/MSBQ-MT) loci *MT-1* (=*Tph1*) and *MT-2* were underlying the high beta-tocopherol phenotype. In the case of *MT-1*, the authors found that the mutation was caused by the insertion of a 5.2 kb Ty3/gypsy-like retrotransposon, and developed functional STS markers diagnostic for wildtype and mutant *MT-1* alleles. Hass et al. (2006) demonstrated the co-segregation of two gamma-tocopherol methyltransferase gene paralogs with the *Thp2* locus underlying high gamma-tocopherol content and developed STS markers diagnostic for *Tph2*, though none of them were based on sequence motifs affecting phenotypic variation as the mutation could not be detected.

Even though there have been important advances in the identification of genes responsible for altered oil quality traits and development of diagnostic markers reliable for marker-assisted selection, additional research is still required for a more complete genetic and molecular characterization of the broad set of innovative traits related to oil quality already developed in sunflower. Basic research on the genetic nature of modifiers and their mode of action is particularly required, together with more applied studies directed to mapping and tagging modifiers for individual traits. As stated above in this review, modifiers are not an isolate phenomenon affecting only high oleic acid, but they have been found to affect every oil quality trait developed so far in sunflower. Therefore successful incorporation of novel oil quality traits into elite lines by backcrossing will require early identification of potential sources of modifiers, which should be discarded at early breeding stages. However, molecular characterization of modifying genes is not an easy task. Because their weak individual effects are largely masked by the highly significant effect of the major gene(s) responsible for the trait, the power of QTL analyses to identify modifiers is low. Optimal phenotyping through the development of populations segregating for modifiers but not for the main gene(s), and the advent of new genomic tools and an accurate reference genome for sunflower (Kane et al., 2011) will contribute to the identification of these elusive genes.

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