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SUNFLOWER: SOME EXAMPLES OF CURRENT RESEARCH
TOURNESOL : EXEMPLES DE TRAVAUX DE RECHERCHE

DOSSIER

Effects of refining process on sunflower oil minor components: a review

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Abstract – Sunflower oil is well known because of its diversity of fatty acids profiles which allow different uses (food: dressing salads, margarines; nonfood: agrofuel, lubricants). Besides, crude oil contains high amounts of desirable minor components (tocopherols, phytosterols, polyphenols, phospholipids…) that present important nutritional features with a positive impact on human health. The different steps of the refining process have as main objective to remove contaminants and other compounds that could hamper the continuity of the process or alter oil during storage. An indirect consequence of this treatment used to preserve food safety is that micronutriments of interest are also partially eliminated reducing the nutritional quality of the oil. This review describes in the first part the chemical composition of sunflower oil focusing on desirable and undesirable components. In the second part the refining process is detailed following the losses of micronutriments at each step of the process and the elimination of unwanted compounds.

Keywords: Sunflower oil / minor components / tocopherols / sterols / refining

Résumé – Effets du procédé de raffinage sur les composants mineurs de l'huile de tournesol : revue. L'huile de tournesol est bien connue pour la diversité de ses profils d'acides gras qui permettent des usages variés (alimentaires – huile de table et margarines – ou non alimentaires – agrocarburants et lubrifiants). En outre, l'huile brute contient aussi de grandes quantités de composés mineurs souhaitables (tocophérols, phytostérols, polyphénols, phospholipides…) aux caractéristiques nutritionnelles importantes et à impact positif sur la santé humaine. Les différentes étapes du raffinage ont pour principal objectif d'éliminer les contaminants et les composants susceptibles d'entraver la suite du process ou de provoquer une altération de l'huile au stockage. La conséquence indirecte de ce traitement destiné à préserver la sécurité alimentaire est que les micronutriments d'intérêt sont partiellement éliminés réduisant ainsi la qualité nutritionnelle de l'huile. Dans une première partie, cette revue décrit la composition chimique de l'huile de tournesol en distinguant les composés recherchés et indésirables. La seconde partie examine le procédé de raffinage en suivant les pertes de micronutriments à chaque étape du procès ainsi que l'élimination des produits indésirables.

Mots clés : Huile de tournesol / composants mineurs / tocophérols / stérols / raffinage

1 Introduction

Sunflower (*Helianthus annuus* L.) seeds have been object of research to study its nutritional characteristics because of its fatty acids and minor components composition. Sunflower oil represented almost 13% of the total oilseeds consumed in the world with near 9.5 Millions of tons in 2011 (FAO, 2015) being the 4th more consumed oil. Sunflower oil has an interesting composition in fatty acids (Tab. 1) on its regular form with high content in linoleic acid (Regular SO) but also with the other four variants in fatty acids composition: High Oleic Sunflower Oil (HOSO), Mid Oleic Sunflower Oil (MOSO), High Steraric High Oleic Sunflower Oil (HSHOSO) and High Palmitic High Oleic Sunflower Oil (HPHOSO). These four other compositions are interesting for different usages like: biodiesel (Del Gatto *et al.*, 2015), lubricant (Al Mahmud *et al.*, 2013) or its ability to replace hydrogenated fats (Salas *et al.*, 2014). Its oil also contains an important unsaponifiable fraction mainly composed of tocopherols (Vitamin E) and phytosterols, but that also has polyphenols and carotenoids.

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Table 1. Fatty acid composition (%) of regular sunflower compared to high oleic, mid oleic and high stearic sunflower.

Fatty acid	Regular SO	HOSO	MOSO	HSHOSO	HPHOSO
C16:0	5.0–7.6	2.6–5.0	4.0–5.5	6	29
C18:0	2.3–4.0	2–4.0	3.0–5.0	21	1.7
C20:0	0.1–0.5	0.2–0.5	0.2–0.4	0.2	/
C22:0	0.3–1.5	0.5–1.6	0.6–1.1	0.4	/
C24:0	ND–0.5	ND–0.5	0.3–0.4	ND	/
C18:1	14.0–39.4	75.0–90.7	43.1–71.8	69	53–57
C18:2	48.3–74.0	2.1–17.0	18.7–45.3	4	2.1–4.3

Data from Fernández-Moya *et al.*, 2006; Ghazani and Marangoni, 2013; Gunstone, 2011; Marmesat *et al.*, 2008; O'Brien, 2009; ND = Non Detectable; SO = Sunflower Oil; HOSO: High Oleic Sunflower Oil, MOSO = Mid Oleic Sunflower Oil; HSHOSO = High Stearic High Oleic Sunflower Oil; HPHOSO = High Palmitic High Oleic Sunflower Oil.

Table 2. Tocopherol contents and composition of several contrasted sunflower oils (mg/kg oil).

	α-toco	β-toco	γ−toco	δ-toco	Total toco
Regular SO	153–957	ND–45	ND–34	ND–7	176.9–1872
HOSO	400–1090	10–35	3–30	ND–17	450–1120
MOSO	488–668	19–52	2–19	ND–2	509–741
HPHOSO- α	826	/	/	/	/
HPHOSO- γ	/	/	808	/	/
IAST-4	4 (%)	3 (%)	34 (%)	58 (%)	/
IAST-5	30(%)	0–77(%)	/	/	/
T2100	/	/	19–97 (%)	/	/

Data from Ayerdi Gotor *et al.*, 2007, 2014; Gunstone, 2011; Marmesat *et al.*, 2008; Velasco *et al.*, 2002, 2004; ND = Non Detectable; SO = Sunflower Oil; HOSO: High Oleic Sunflower Oil, MOSO = Mid Oleic Sunflower Oil; HPHOSO = High Palmitic High Oleic Sunflower Oil.

1.1 Minor components with a nutritional interest

1.1.1 Tocopherols

Tocopherols, also known as Vitamin E, are well known antioxidants molecules naturally found in vegetal oils. There are four forms α-, β-, γ- and δ-tocophérol. γ-tocopherol has showed the highest *in vitro* antioxidant activity followed by δ-tocopherol (Kamal-Eldin and Appelqvist, 1996; Seppanen *et al.*, 2010). Whereas α-tocopherol presents the highest *in vivo* activity (Traber and Atkinson, 2007) and it exists daily recommended intakes of up to 16 mg/d (Institute of Medicine, 2011). Sunflower oil contains a considerable amount of tocopherols but is the oil having the highest amount in α-tocopherol (Gunstone, 2011). This content can vary within genotypes (Ayerdi Gotor *et al.*, 2006; Velasco *et al.*, 2002) and environmental conditions during cultivation (Ayerdi Gotor *et al.*, 2006, 2015). The tocopherols composition could also be modified (Velasco *et al.*, 2004) (Tab. 2) but is not linked with the composition of fatty acids (Ayerdi Gotor *et al.*, 2014).

1.1.2 Phytosterols

Phytosterols, also known as plant sterols, are a family of compounds which have been studied largely because of their property to reduce the level of cholesterol in blood, but also because of the reduction of the incidence of some cancers (Kritchevsky, 2002). The American National Cholesterol Education Program (Expert panel on detection evaluation and treatment of high blood cholesterol in adults 2001) recommended a daily intake of 2 g of phytosterol to reduce the low density lipoproteins (LDL cholesterol) in blood. The development of food with added phytosterol has led to regulations on the labeling of these products to avoid an excessive consumption (European Commission, 2013) and a maximum intake of 3 g/day it has been suggested (European Food Safety Authority, 2008). Sunflower oil has a high content in phytosterols (Gunstone, 2011; Vlahakis and Hazebroek, 2000) (Tab. 3) being β-sitosterol the main sterol. Phytosterol content is mainly affected by environmental conditions during plant growth (Ayerdi Gotor *et al.*, 2015) and genetics (Aguirre *et al.*, 2014; Fernández-Cuesta *et al.*, 2011) but there is no effect of the modification of the fatty acids profile (Ayerdi Gotor *et al.*, 2014).

1.1.3 Others terpenoids

Together with the phytosterols, there are two other terpenoids in sunflower oil, the squalene also a triterpenoid, as the sterols, and the family of carotenoids that are tetraterpenoids. Rao *et al.* (1998) concluded that the squalene reduces the risk of colon cancer and the serum cholesterol level. Few studies have focused in the variability of squalene in sunflower oil, Merah *et al.* (2012) reported a variation from 10 to 202 mg/kg

Table 3. Phytosterols contents and composition of several contrasted sunflower oils (mg/100 g oil).

	Campesterol	Stigmasterol	β-sitosterol	Δ^5-Avenasterol	Δ^7-Stigmasterol	Total phytosterol
Regular SO	15.6–65.0	14.4–65.0	120.0–350.0	ND-34.5	15.6–120	125–765
HOSO	8.5–67.6	7.7–67.6	71.4–364.0	2.6–358.8	11.1–124.8	170–520
MOSO	9.1–9.6(%)	9.0–9.3(%)	56.0–58.0(%)	4.8–5.3(%)	6.5–24(%)	–
IASP-18	–	–	–	–	–	1370

Data from Aguirre *et al.*, 2007, 2014; Gunstone, 2011; ND = Non Detected; SO = Sunflower Oil; HOSO: High Oleic Sunflower Oil, MOSO = Mid Oleic Sunflower Oil.

on a collection of inbred lines. Otherwise, sunflower oil it is not particularly rich in carotenoids with only 1–1.5 ppm of carotenoids (Gunstone, 2011). The major carotenoids in sunflower oil are xantophylls which reach up to 81% of the total (Rade *et al.*, 2004).

1.1.4 Phenolic acids

Phenolic acids have largely been studied because of their antioxidants and neuroprotectives properties (Stevenson and Hurst, 2007). Sunflower oil presents two major polyphenols, namely, vanilic acid with 6.9 µg/100 g oil and caffeic acid with 4.9 µg/100 g oil. Moreover, there are also small amounts (each one around 1.5 µg/100 g) of p-hydroxybenzoic, p-coumaric, ferulic and sinapic acid (Siger *et al.*, 2008).

1.1.5 Coenzymes Q9 and Q10

Coenzymes are isoprenoid chains with 6 to 10 isoprenoid units (number indicated after the Q letter) attached to substituted benzoquinone moiety (Pravast *et al.*, 2010). Few studies have evaluated the content on coenzyme Q9 (CoQ9) and CoQ10 in oils. Rodríguez-Acuña *et al.* (2008) developed a new method by HPLC MS/MS. They found that sunflower oil had mainly CoQ9 with 101.3 mg/kg and only 8.7 mg/kg of CoQ10 in refined oil whereas other studies showed higher amounts of CoQ10 up to 15 mg/kg in crude oil (Pregnolato *et al.*, 1994). These coenzymes are interesting because they have antioxidant and anti-inflammatory activities (Yang *et al.*, 2015).

1.2 Other constituents with undesirable functions

1.2.1 Phospholipids

Crude sunflower oil presents high content of phospholipids, which are the major constituents of the biological membranes. The main families of phospholipids found in sunflower oil are phosphatidylcolines, phosphatidylethanolamides, phosphatidylinositols and phosphatidic acids (Gupta, 2002). Sunflowers has mainly hydratable phospholips (Zufarov *et al.*, 2008) but also non hydratable phospholipids, which content could vary in function of the activity of the D phospholipase who is able to convert hydratable onto non hydratable phospholipids in presence of water (Haraldsson, 1983). These molecules have unfavorable effects during the refining process as they can saturate bleaching earths (Taylor, 1993) or induce browning during deodorization (Zamora *et al.*, 2004). This affects the flavor, odor and appearance of the oil, they have, therefore, to be removed during the refining process at the degumming stage (Verleyen, Sosinska *et al.*, 2002). However, polyphenols have a role during oil storage increasing the oxidation stability (Poiana *et al.*, 2009) and could have beneficial effects on human health (Küllenberg *et al.*, 2012).

1.2.2 Free fatty acids

The presence of free fatty acids (FFA) in oils may promote oxidation (Frega *et al.*, 1999). The presence of high levels of free fatty acids, or free acidity, is due to wet harvest conditions which promote the action of lipases generating these molecules, as well as moist grains during storage (Beratlief and Iliescu, 1997). In sunflower oil FFA varied from 1.19 to 1.35% (w/w) in regular sunflower oil (Kreps *et al.*, 2014), from 0.76 to 1.13% in HOHPSO (Marmesat *et al.*, 2008) and can reach up to 4% in HOSO (Moschner and Biskupek-Korell, 2006). FFA are neutralized during the refining process in order to reduce their undesirable effects as undesirable flavor.

1.2.3 Colorants

The two most common pigments present in vegetable oils are carotenoids and chlorophylls. Few studies have evaluated the content of those two families of molecules in sunflower oils as they are present in small quantities and they are eliminated during the refining process (at the bleaching step). Topkafa *et al.* (2013) found that chlorophyll varied from 403 to 1021 ppb in four crude sunflower oils and β-carotene varied from 1692 to 2803 ppm. The refining process reduces the chlorophylls content up to 96% and 80% the β-carotene content (Kreps *et al.*, 2014).

1.2.4 Wax

Sunflower seeds contain around 0.9% of waxes (Carelli *et al.*, 2002) that are present in the hulls. Only a part of the total wax content is eliminated during the winterization step of the refining process, only the small wax chains with less than

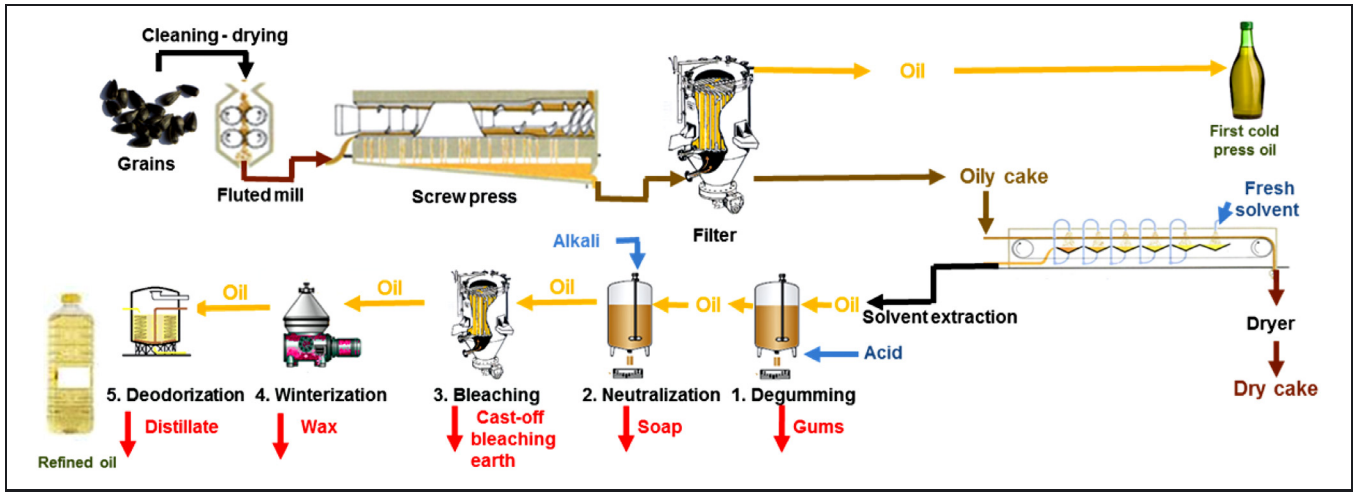

Fig. 1. Extracting and refining steps of sunflower oil.

42 carbons are found in the final product (Carelli *et al.*, 2002). Sunflower has mainly C40 and C41 waxes in cold pressed oil (26.8 and 30.2 mg/kg) for a total of 205 mg/kg, whereas in pressed oil C44 was the most abundant (20.3 mg/kg) followed by C41, C46 and C40 for a total of 409 mg/kg (Brevedan *et al.*, 2000), in both cases oil has a wax diversity from C36 to C48.

1.2.5 Trace metals

Trace metals can act as pro-oxidants wherefore the refining process should diminish their content. In sunflower crude oil we can found Iron at 8.37 mg/kg, cupper at 3.41 mg/kg, calcium at 95.34 mg/kg and magnesium at 1505.04 mg/kg (Lamas *et al.*, 2014), and traces of other heavy metals as cobalt, cadmium, chrome, lead; nickel or zink (Pehlivana *et al.*, 2008).

Preserving oil minor components during the refining process is a major issue in world nutrition. In the present review we focus on the resistance of these minor components at each step of the refining process.

2 Sunflower oil refining

Sunflower oil mechanical extraction is the first step to obtain crude oil, after filtration cold press oil is obtained. On the other hand the residual oily cake is going to be submitted to a solvent extraction and after five successive stages refined oil is obtained. Figure 1 represents schematically the main steps of sunflower oil extraction and refining. There are five main stages where there is a potential leak of minor components: Degumming, neutralization, bleaching; winterization and deodorization. In industries we can find several adaptations of this simplified schema. When the degumming step or the neutralization step are absent they are called physical or solvent refining respectively.

2.1 Refined *vs.* crude oil

The purpose of refining sunflower oil is to convert the crude oil, which has a high acidity level and/or organoleptic defects, to make it suitable for human consumption. The refining is also aimed at removing contaminants as pesticides and process solvents. Therefore, several refining processing steps are used to remove all undesirable molecules (Fig. 1). Nevertheless, valuable minor components are destroyed and or eliminated with by-products. Generally, several studies have discussed the influence of refining process on the reduction of some minor components present in olive, soybean and rapeseed oil. However, few reports have investigated the effect of each refining step on the total and individual content of each bioactive minor component.

Few studies have evaluated the content of polyphenol and coenzymes in refined oils and none of them have studied the impact of the refining process on all minor components in sunflower oil. The global impact of the refining process observed in different oil matrix was a reduction in these families of molecules. In sunflower oil, we observed a reduction of the total phenolic content from 19.23 to 1.82 mg of gallic acid equivalent/10 g of oil (Kostadinovic-Velickovska and Mitrev, 2013). In rapeseed oil (Kraljić *et al.*, 2015), the loss of polyphenolic compounds was of 63% during the neutralization, 16% during the bleaching and was of 67% during the deodorization step.

In chemical refining process, total tocopherol content gradually declined during overall of process. The reduction in total tocopherols content ranged from 14 to 34% with an average loss of about 27% (Alpaslan *et al.*, 2001; Ergönül and Köseoğlu, 2014; Karaali, 1985; Talal *et al.*, 2013; Tasan and Demirci, 2005). Ergönül and Köseoğlu (2014) showed the lowest diminution as they found that the level of total tocopherols decreased from 737 mg/kg in crude sunflower oil to 633.8 mg/kg in refined sunflower oil. The highest decline was reported by Tasan and Demirci (2005).

Physical refining process induced a higher reduction of the total tocopherol content compared to chemical refining process. Total tocopherols losses during the physical process varied from 24.6 to 54.8% with an average loss of 41% (Alpaslan *et al.*, 2001; Kreps *et al.*, 2014; Tasan and Demirci, 2005). Differences in the amount reduction depend on the nature of sunflower cultivars (*i.e.*: HOSO or regular SO) and the severity of the process conditions such as temperature and steam flow.

Phytosterol content and composition changes during the hole refining process were evaluated by Verleyen, Forcades *et al*. (2002) who found that the process modified the phytosterol composition by increasing the esterified sterols passing from 28.6% in bleached oil to 40% in the refined oil (after the deodorization) of the total sterol content. Karaali (1985) determined in chemically refined sunflower oil a decrease of 60.3% on total phytosterols, with the highest loss during the physical neutralization step.

The level of squalene was found to decrease continuously during all refining steps (Nergiz and Çelikkale, 2011). Total average reduction for sunflower seed oil samples during refining process was 32.9%. The level was about 13.8 mg/100 g in crude sunflower oil and about 9.2 mg/100 g in refined sunflower oil.

2.2 Degumming

The main objective of the degumming step is to remove phospholipids (Segers and van de Sande, 1990) as they lead to a dark oil color and promote off-flavors in processed oils. The degumming could be made with water when oil contains hydratable phospholipids or with a previous treatment with an acid like phosphoric or citric acid when oil contains nonhydratable phospholipids (Segers and van de Sande, 1990; Zufarov *et al.*, 2008). Both treatments convert hydratable and nonhydratable forms into hydrated gums. The water degumming process permits to remove about 83% of total phospholipids from solvent extracted sunflower oil (Brevedan *et al.*, 2000). During the refining process metals are removed, acid degumming reduces the content of calcium and magnesium of 88 and 90% (Zufarov *et al.*, 2008), and the iron content is reduced from 4.4 to 3.3 ppm (Karaali, 1985). FFA content is reduced up to 82% passing from 1.24 to 0.23% (w/w) in a combined degumming-neutralization step (TOP, also called total degumming process) (Kreps *et al.*, 2014). But it decreased only 20% from 1.03 to 0.83 (% g oleic acid) in a classic degumming step (Karaali, 1985). Chlorophylls are reduced from 5.62 to 4.63 (mg/kg pheophytin a) in HOSO and from 4.32 to 3.56 in regular SO in acid degumming (Kreps *et al.*, 2014).

In addition, the degumming step could be done using enzymatic treatment (Carelli *et al.*, 2002; Lamas *et al.*, 2014). Phospholipid content is reduced up to 97.8% while phosphorus content is decreased up 99.4% (Lamas *et al.*, 2014). About 81.2% and 93% of the calcium and magnesium content respectively, is removed from the crude sunflower oil during enzymatic degumming (Lamas *et al.*, 2014).

This degumming step also affects the content of desirable molecules, tocopherol content is reduced between 6.6 to 8.4% of the total content in regular SO and HOSO respectively (Kreps *et al.*, 2014) but there is no modification in the composition (Tab. 4). These reductions are in agreement with other reported losses (Tasan and Demirci, 2005). These authors found losses of 6% in total tocopherol content during degumming stage of the physical refining process. Karaali (1985) showed a lower reduction in the total tocopherol content, in about 4%. Karaali (1985) also studied the effect of chemical degumming step on sterol content in sunflower oil and found a decrease of 22.4% in total sterol content with equivalent results on individual sterols, except for Δ7-Avenasterol which increased its relative content 50%.

Reduction in minor components contents during degumming stage could be attributed to acid catalyzed hydrolysis (Verleyen *et al.*, 2001). No data of the content of these components in the produced gum have been reported. Therefore, losses could not be attributed to liquid-solid partitioning of minor components into the gum.

2.3 Neutralization

Oil alkali neutralization is also known as deacidification. It is an important step in the edible oil refining process and aims to remove FFA in oil. It neutralizes free fatty acids in the oil using caustic soda and potash, also decomposes pigments, it eliminates phosphorus compounds, trace metals, proteins and oxidizing materials. Alkali treatment converts the acids into soaps (Hendrix, 1990). These soaps are easily removed by decantation or by centrifugation. This step is only used in the chemical refining process.

Few investigations have discussed the effect of oil neutralization step on minor constituents of refined sunflower oil. Four to 16% losses in total tocopherol level were observed between crude and neutralized oil. Alpasan *et al.* (2001) analyzed total and individual tocopherol contents of sunflower oil, processed either by chemical refining or physical refining methods. About 4.7% of loss in total tocopherol content occurred during chemical neutralization (Tab. 4). They reported a significant decrease of all tocopherols but 63% of the reduction was due to α-tocopherol. Higher elimination of the total tocopherol content has been obtained during chemical neutralization step (Karaali, 1985; Naz *et al.*, 2011). Karaali (1985) showed a significant loss of 11.8% while Naz *et al*. (2011) presented losses of 16% in the total sunflower tocopherol content. More recently, Talal *et al*. (2013) showed a significant decrease in the level of total tocopherols content in sunflower oil during the degumming and alkali neutralizing step. Results of their study indicated a loss of about 10% in total tocopherol amount.

The reduction in the total tocopherol content during the oil neutralization is in agreement with the results of several reported studies on vegetable oils, according to which caustic soda treatment affected the tocopherol level of oils (Ferrari *et al.*, 1996; Karaali, 1985; Tasan and Demirci, 2005). The decline of tocopherols may be due to the fact that tocopherols are unstable in the presence of long contact time with air and alkali and are oxidized to tocoquinones (Tasan and Demirci, 2005).

In addition, chemical treatment do not have similar impact on individual tocopherol content since a loss of α-, β-, γ- and δ-tocopherols of 3, 26, 24, 16%, respectively, was observed (Alpaslan *et al.*, 2001).

A significant decline in the total sterol content in vegetable oil has been reported during the chemical neutralization step (Gutfinger and Letan, 1974), which is attributed to a liquid–liquid partitioning of phytosterols into the soapstock (Serani and Piacenti, 1992). Neutralization process caused in oils 29.3% losses of total sterols. Results of Ruiz-Méndez *et al.* (2011) confirm that an important quantity of removed sterols

Table 4. Losses (%) of minor components during each step pf the refining process.

Processing stages	Total Tocopherols	Total Phytosterols	Squalene	Polyphenols
Chemical refining process				
Degumming	4	22.4	ND*	ND
Neutralization	3.9–14.7	29.3	ND	ND
Bleaching	5.85–7.9	ND	ND	ND
Winterization	0.6–4.6	5.2	ND	ND
Deodorization	3.6–15.7	3.4	ND	ND
Total loss	14–34	60.3	ND	90.5%
Physical refining process				
Degumming	6–8.4	ND	6.9	ND
Bleaching	3.5–15.8	ND	5.3	ND
Winterization	0.8–5.8	ND	4	ND
Deodorization	20.2–25.7	ND	16.9	ND
Total loss	24.6–54.8	ND	33.1	ND

*ND = Not available data. Data from: (Alpaslan *et al.*, 2001; Ergönül and Köseoğlu, 2014; Karaali, 1985; Kreps *et al.*, 2014; Nergiz and Çelikkale, 2011; Talal *et al.*, 2013; Tasan and Demirci, 2005; Verleyen, Forcades *et al.*, 2002).

is found in soapstocks obtained from sunflower oil. Authors demonstrated the influence of alkali concentration and temperature used during neutralization on the total sterol content.

A significant reduction of the total polyphenol content which represents a loss of near 25% of the initial content during this neutralization step was found by Karaali (1985).

Nergiz and Çelikkale (2011) have evaluated the effect of the refining steps on the squalene content of some vegetable oils and found significant reduction in the squalene content after the neutralization/physical refining step. The reduction during this stage of refining was found to be 7% as compared to crude sunflower oil.

2.4 Bleaching

This third step of the oil refining process has as main objective to remove coloring pigments from carotenoids, chlorophylls, and related compounds that catalyze the oxidation of the oil by reacting with light, by using bleaching earths that can be acid activated or coupled with activated carbon to increase the adsorption power and diminish the quantities used (Anderson, 2005; Topkafa *et al.*, 2013). Bleaching earth are also adsorbing residual soaps, phospholipids and other polar lipids (Anderson 2005). Although bleaching generally improves crude oil quality with respect to color, initial and aged flavor, along with oxidative stability, this refining step also has other effects. Some of them are desirable; others are undesirable as the isomerization of the triglycerides (Anderson, 2005). FFA content do not vary significantly during this step in sunflower oil (Kreps *et al.*, 2014) but this step could lead to an increase in FFA in other oils. Chlorophylls are eliminated up to 96% in both HOSO and regular SO where the bleaching conditions were: temperature 90 °C, 0.59% w/w bleaching clay (TAIKO, type: Classic 1G, Malaysia) with mechanical stirring was used at vacuum 13×10^3 Pa for 30 min (Kreps *et al.*, 2014) and carotenoids around 77% of the initial value (Rade *et al.*, 2004). The decreases in total tocopherol contents due to bleaching step have been largely studied (Alpaslan *et al.*, 2001; Ergönül and Köseoğlu 2014; Kreps *et al.*, 2014; Tasan and Demirci, 2005). The average reduction is approximately 8% (Alpaslan *et al.*, 2001; Kreps *et al.*, 2014; Tasan and Demirci, 2005). Tasan and Demirci (2005) showed declines of 7.2 and 7.6% in total tocopherol contents for chemical and physical refining process respectively but there is no indication of the bleaching conditions used. Alpaslan reported the lowest percentage of losses during physical process but also the bleaching conditions were no described. Ergönül and Köseoğlu, 2014 (90 °C, 20 min, 1% of activated earth w/w) showed 6% losses. Kreps *et al.* (2014) reported losses of 14.9% in HOSO and 15.8% in regular sunflower oil during this refining step without change in the composition of tocopherol isomers. Naz *et al.* (2011) reported the highest losses of 38.2% of the total tocopherol content (with unspecified bleaching conditions). These reductions during the process of bleaching are due to the complexation of tocopherols with molecules of bleaching clay. The alkalinity and acidity of bleaching clay may damage tocopherol molecules. It has been demonstrated that acid activated bleaching clay can catalyze tocopherol esterification. The acid can also play a role in protonation of tocopherol to produce oxonium ions (McMurry, 2004; Taylor, 2005).

No study has evaluated the impact of bleaching stage on the sunflower's sterols. In other oils total sterol content was slightly reduced in cotton seed oil (8.7% according to El-Mallah *et al.* (2011). In other study authors observed a reduction of 1.3, 8 and 18.5% in corn, soybean and rapeseed respectively in the total sterol content (Ferrari *et al.*, 1996). A deeper analysis on sterol evolution during the refining process was conducted by Verleyen, Sosinska *et al.* (2002). They showed a differentiated behavior depending on the oil matrix for the evolution of esterified and free sterols. Corn oil presented an increase of up to 3% of free sterols and a decrease

of 5% of esterified sterols. In palm oil they observed no difference on esterified sterols and a decrease of 16% on free sterols.

Last, 6% of the squalene content is lost during this bleaching step (Nergiz and Çelikkale, 2011).

2.5 Winterization

Winterizationalso called dewaxing has as main objective to eliminate long chain waxes (with carbon number higher than C42) (Brevedan *et al.*, 2000) and saturated triglycerides by reducing the oil's temperature to 6–8 °C and eliminating the solid particles, for instance, by filtration (Ruiz Mendez *et al.*, 2013). Winterization is the step that reduces the least the minor compounds and particularly tocopherols. In sunflower oil, the reduction in total tocopherol content range from 0.6 to 5.8% with an average of about 2.4% and a variation coefficient between studies of about 76%. These variations are due to the refining process used and the nature of sunflower. Kreps *et al.* (2014) found a loss of 5.8% in HOSO and of 2.9% in regular SO during the process of winterization. In that study, authors explained the decline of tocopherols in winterized oil by interactions between detergent and tocopherols and their removal with waxes.

The winterization has no impact on chlorophylls and reduces 18% of the content of β-carotene in both regular and HOSO (Kreps *et al.*, 2014). Karaali (1985) found that the total sterol content diminish in 5.2% that came from an equivalent reduction of each individual sterol except campesterol, its content was reduced 11% and Δ7 avenasterol which increased nearly 35%.

Nergiz and Çelikkale (2011) investigated the changes in the amount of squalene in different vegetable oils during the refining process and found that the smallest reduction of squalene was caused during the winterization. The average reduction was found to be 4%.

2.6 Deodorization

In the edible oil processing, deodorization is the final key step used to remove the off flavors generated during the previous refining steps as oxidation products such as: hydroperoxides, aldehydes, ketones and epoxides, and pulling out volatile components as FFA or contaminants (Akterian, 2009). At the end of this stage, the taste, odor, flavor, color and stability of the oil is improved. During deodorization oil is subjected to high temperatures, to a stripping stream and pressure for a given duration aimed to the optimization of the elimination of undesirable substances, but preserving minor components (leaving at least 80% of them) and reducing the creation of trans fatty acids or oxidation products (Martinčič *et al.*, 2008).

Changes in the content of total tocopherols during this stage are tightly related to the conditions of oil treatment. Physical refining process causes the highest decline. Therefore, losses in total tocopherols content range from 3.7 to 15.7% during chemical refining process (Alpaslan *et al.*, 2001; Ergönül and Köseoğlu, 2014; Karaali, 1985; Talal *et al.*, 2013; Tasan and Demirci, 2005) while the reported values of reductions during physical refining process are between 21.2% and 25.7% (Alpaslan *et al.*, 2001; Kreps *et al.*, 2014; Tasan and Demirci, 2005). According to these reports, the deodorization stage is the highest reducing stage for tocopherols. The percentages of losses due to this step range from 26.2% (Ergönül and Köseoğlu, 2014) to 86.3% (Alpaslan *et al.*, 2001) with an average of about 48.6%. In order to decrease the effect of this step on minor components, studies have been done to optimize deodorization conditions. To reduce the losses and preserve up to 90% of the total tocopherol and phytosterol content, Martinčič *et al.* (2008) found in HOSO that the conditions during this deodorization step should be: temperature under 235 °C, with a pressure of 3 mbar and a sparge steam between 2.25 and 1%. Reducing the temperature during the process reduces the losses, but increases the FFA content and reduces the organoleptic value of the oil. During the deodorization step in the oil there is formation of steradienes, that are the dehydrated form of sterols caused by high temperatures and acid conditions (in physical refining) (Verleyen, Szulczewska *et al.*, 2002) reducing the content of free sterol. In sunflower oil Karaali (1985) observed a reduction of 3.4% of total sterol content with higher losses of campesterol and stigmasterol (12% and 15% respectively) and just 3% in β-sitosterol, relatively Δ5-avenasterol and Δ7-stigmasterol increased their content to 9 and 7% respectively. Squalene is lost up to 7% in the deodorization process (Nergiz and Çelikkale, 2011). Carotenoids suffer a supplementary reduction of between 8% and 28% of the initial content during this step, reaching a final content equivalent to 15–20% of the initial value in regular SO and 25% in HOSO (Kreps *et al.*, 2014; Rade *et al.*, 2004).

Chlophylls are slightly reduced during this step (Kreps *et al.*, 2014) to a minimal value of 0.18–0.21 mg/kg pheophytin a (regular and HOSO respectively) that represented 4% of the initial value on crude sunflower oil which represented a supplementary elimination of 15% during this step.

Deodorization step also influence the content of squalene in sunflower oil (Nergiz and Çelikkale, 2011). The last report showed that most of the losses of squalene (17%) occurred during this refining step.

Decline of minor components in deodorized oil is in part due to removal of these molecules during injection of heated steam. Therefore, valuable minor components such as tocopherols, tocotrienols, phytosterols, squalene and hydrocarbons are stripped. Then, they are found in the deodorizer distillate which is the most important by-product of edible oil refining. Naz *et al.* (2011) studied the chemical characterization of sunflower oil deodorizer distillates and found significant amounts of sterols, squalene and tocopherols. They showed that total sterols in deodorized distillate sunflower were present at the concentration of 13.9–14.2% and total tocopherols and hydrocarbons were estimated at average percentages of 6.5 and 16.5, respectively.

3 Conclusions

During the refining process sunflower oil loses considerable amounts of minor components that have interesting nutritional and health related characteristics. Several studies have been done to reduce the loss of micronutriments and preserve final oil quality and nutritional characteristics,

for example by using pre heating with microwave (Veldsink *et al.*, 1999; Zacchi and Eggers, 2008) which increases the final tocopherol and polyphenols content; or, modifying the temperatures during the deodorization step (Martinčič *et al.*, 2008) that reduces the losses of phytosterols and tocopherols. However losses persist, but the residual products of this refining process are partially recovered to be used in other industries like cosmetic industry (tocopherols and phytosterols), gums in the form of lecithin is included in several food products instead of soybean lecithin. Challenges are: (i) to find cultivars with high levels in these minor components, as in the case of sterols (Velasco *et al.*, 2014), (ii) To evaluate the possibility and developing new commercial varieties with these new traits continuing efforts already done (Ayerdi Gotor *et al.*, 2008), (iii) To evaluate the impact at each step of the refining process of minor nutriments like coenzymes or β-carotene, (iv) To find the optimal refining conditions at each step, to preserve the maximum of all these nutriments. The last goal of this essential research is to increase the amount of the mentioned minor components after the refining process which has an important repercussion in the nutritional and economical value of the obtained oil.

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