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Effect of Refining Process and Use of Natural Antioxidants on Soybean Oil

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1. Introduction

Soybean (*Glycine max* L. Merril.) is an annual plant of Asian origin, adapted to temperate climates. This plant is a member of the family *Fabaceae*, subfamily *Papilionoideae*, tribe *Phaseoleae*, genus *Glycine* and subgenus *Soja* (Hymowitz, 2004). One of the most important agronomic characteristics of soybean is that it can take nitrogen from the air and fix it to be used as nutrient by the plant. The symbiotic relationship between the soybean plant and its modulation by bacterium (*Rhixobium japonicum*) is responsible for the conversion of atmospheric nitrogen into plant-available nitrogen. This also makes to soybean a good rotational plant for use with high nitrogen-consuming crops. Another important benefit of this nitrogen fixation is that it helps to keep the production cost of soybeans relatively low compared to other crops that competing for the same land area (Erickson, 1995).

Among the factors that allow the soybean to be a dominant crop could be mentioned some of the most important agronomic traits: good profits for producers and processors, as well as the possibility of obtaining high-protein quality meal, which are used as ingredient in food animal. These characteristics have allowed the soybean oil is consumed more than other vegetable oils.

Soybean is the most important crop in the world because of its high quality protein and edible oil products. World production of oilseeds during 2009-2010 was 403.58 million metric tons (tons), from which 60% corresponded to soybean, 13.74% to rapeseed, 10.23% to cottonseed, 8.30% to peanut and 7.87% to sunflower (Soya & Oilseed Bluebook, 2010).

Others factors that have contributed to soybean worldwide importance are the high demand of meal and oil. The main consumer of these meals is the agricultural sector. World production of vegetable meals during 2009-2010 was 237 million tons; soybean meal represented 67%, rapeseed 13.6% and cotton 6.2% (Soya & Oilseed Bluebook, 2010). Oil is a byproduct of meals production, which has a great demand.

In this situation of soybean oil domain in fat total supplies, is extremely important to take advantage of its nutritional properties, such as its high content of polyunsaturated fatty acids essential for humans(n-6 and n-3), and their contents of tocopherols (vitamin E) (Emken, 1995).

2. Importance

The high polyunsaturated fatty acids (PUFA) content in soybean oil is very attractive for meeting the essential fatty acid requirements in human nutrition; however, they are highly

susceptible to oxidative reactions. Therefore, appropriate processing conditions should be used to eliminate or reduce impurities such as phospholipids, gums, metals, free fatty acids (FFA), oxidation products (peroxides, aldehydes and ketones) and pigments, in order to ensure the best oil quality. The impurities are eliminated through degumming (Farr, 2000), chemical or physical refining (Kellens & De Greyt, 2000), bleaching (Ortega-García et al., 2005; González-Tovar et al., 2005), and deodorization (Zehnder, 1995; Medina-Juárez et al., 2000). During this process the modification of essential fatty acids to *trans* isomers, and the loss of tocopherols and sterols, should be monitored (Medina-Juárez et al., 2000). To follow up this process, oils should be physicochemically characterized using the official methodology. By other hand, it is important to use additives such as natural antioxidants that ensure the stability and shelf life of PUFA (Gámez-Meza et al., 1999; Gámez-Meza et al., 2009).

This chapter presents relevant bibliographic information about soybean oil. It highlights the impact of stages of refining process and the importance of natural antioxidants to avoid oxidation. Also the official methods recommended for the physicochemical characterization of this oil, with a special emphasis on the determination of *trans* isomers, omega 3 and 6 and other bioactive compounds naturally found in this vegetable oil as tocopherols and sterols are discussed.

The authors consider these issues as the major areas of research for the present and future knowledge of vegetable oils.

3. Processing

3.1 Oil extraction

Two products are mainly obtained in the milling of soybean: meal and oil.

Soybean meal. The soybean meal is mainly used as ingredient of formulated feed for animal nutrition. This meal is the most extensively used among the oilseed meals and the most economic high-quality protein available for animal feed; hence it assumes a dominant role.

Soybean meal contains from 44 to 50% crude protein and from 2500 to 2800 kcal of metabolizable energy per kilogram, depending of amount of hull present and the species of animal to fed (Smith & Baldwin, 1986). Its nutritional quality and acceptance are widely known.

The process for producing meal for livestock feeding includes three steps: desolventizing-toasting, drying and cooling, and grinding and sizing.

Soybea oil. The three most common procedures to extract oil from oilseeds are: mechanical pressing, pressure-solvent and direct solvent extraction. The application of the extraction process depends primarily on the content of oil in the seed, the content of remaining oil, denatured protein allowed and environmental restrictions for the emissions of volatile organic compounds. Mechanical pressing is generally used for materials exceeding 20% oil content. In the case of soybean oil, the solvent direct extraction is more commonly used, because it is more efficient (<1% residual oil) that when mechanical pressing is used (>6 % residual oil) (Williams & Hron, 1996).

Solvent extraction processes include basically three steps: preparation, extraction, and desolventizing. The major differences in soybean oil solvent extraction processes occur in the preparation steps. One variation in the conventional system is the introduction of an expander after flaking or other method of size reduction (grinding).

The hexane is currently used as solvent, although others solvents have been evaluated (ethanol, isopropanol, acetone, isopentane, isohexane and trichloroethylene). The

advantages of hexane are the high oil solubility and low price. Some disadvantages include the regulation of their emissions and flammability (Johnson, 2000).

3.2 Chemical refining of soybean oil

The purpose of refining of soybean oil is to convert the crude oil that is not adequate for human consumption in a healthy and nutritious food. The crude oil obtained from seeds by mechanical pressing or solvent extraction has many undesirable components. The refining process removes some of those impurities from the oil, such as: free fatty acids (FFA), phospholipids, pigments and other minor impurities. After the refining, a pure oil with desirable properties for the consumers, such as odor, taste, light color, and stability is obtained. The refining term may means different processing steps. We will consider degumming, neutralization, bleaching and deodorizing, as steps of the refining process.

Degumming. Degumming may be considered the first step in the refining process. This process is designed to remove the phosphatides that interfere with subsequent processing and especially for processors with an integral disposal option of gums. The primary reasons for degumming are: to provide a crude-degummed oil suitable for storage or long transportation, to prepare oil for physical refining, or to produce lecithin. In the case of soybean oil, high quality, food grade lecithin can be produced. Lecithin is a product of commercial interest that permits cost reduction by wastewater treatment (Farr, 2000).

Crude soybean oil has high levels of phosphorus, which can be removed in degumming step. The oil has two kinds of gums, hydratable (phospholipids) and non-hydratable (partially hydrolyzed phospholipids, which form salts of calcium, magnesium and iron). The hydratables are easily separated by treating of crude oil with hot water (deionized water at 75°C, at a rate of 1-3%, of oil weight), followed by centrifugation. Contrarily, non-hydratable gums are more soluble in oil than the first and require an additional treatment with phosphoric acid (0.1-0.3% of 85% solution) and sometimes with citric acid (0.1-1.0% of 30% solution) in order to convert them to hydratable gums. During this operation, phosphorus levels are reduced from 800-1200 to 100 ppm when hydratable gums have been removed and they can be reduced to 30 ppm if non-hydratables are efficiently removed (Farr, 2000). It is important to know the amount of tocopherols that are lost with gums in order to maintain the nutritional quality of oil in this operation (Medina-Juarez et al. 2000).

Neutralization. The neutralization operation is usually performed by chemical method, with caustic soda; or by physical method, refining by steam distillation. The aim of this operation is to eliminate the free fatty acids (FFA) through the saponification reaction. To carry out this reaction can be used several alkaline compounds such as sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3). However, in the refining of soybean oils is sodium hydroxide the most widely used. In some cases, despite being more expensive, in order to use the soaps as fertilizer potassium hydroxide is used (Erickson, 1995).

The reversibility of the saponification reaction by pressure and temperature is possible, therefore at atmospheric pressure and temperatures between 60 and 80°C the reaction is from left to right (Egan & Kirk, 1991).

$$R - COOH + NaOH \longrightarrow R - COONa + H_2O$$

Once the oil has been treated with caustic soda, to remove the soap to levels below 5 ppm it is washed with water (two washes with 10% water each). At this point the oil must have

levels of phosphorus not greater than 5 ppm and 0.05% FFA (Farr, 2000). Quantification of tocopherols should be made further, in order to determine the amount reduced during this operation.

Studies of the soybean oil neutralization with sodium silicate show an improved yield and quality, as well as, an efficient removal of soap and trace metals. This allows an efficient filtration to separate the gums and free fatty acids in the form of sodium salts. The advantage of this process is that the amount of soap after filtration can be reduced to 80 ppm without washing with water (Hernández & Rathbone, 1999; Farr, 2000).

Bleaching. The purpose of this operation is to remove all products of oxidation and oxidation promoters to obtain a stable oil to oxidation. The main oxidation products are: hydroperoxides (non-volatile compounds), aldehydes and ketones (volatile compounds). The promoters of oxidation are chlorophyll, phospholipids and traces of metal particles. The loss of oil at this stage is related to the amount of clays used.

The bleaching operation which is done with activated acidic clays (aluminum silicate) eliminates or reduces color besides primary products (peroxides) and secondary oxidation products (aldehydes and ketones), metals, gums and soap traces (Mag, 1990). Peroxides are chemically discomposed during bleaching (Kellens, 1997). The metals form ionic bonds with bleaching clays surface. Also, bleaching clays through forces of Van Der Waals absorbs colorful compounds as chlorophylls and carotenoids. The levels of peroxides, chlorophyll, and soap in bleached oil, should be zero. The *p*-anisidine value (presence of aldehydes and ketones) will be not greater than 5 mmol/kg, the iron content must be less than 0.1 ppm and the copper less than 0.05 ppm (Erickson, 1995; Zschau, 2000). It is very important to know the variation in the content of *trans* isomers and tocopherols in the bleached oil.

To understand the process of bleaching oils is required to know the bleaching clay properties and the variety of impurities present in oils. This process uses aluminum silicates, which are activated clays (bentonite, attapulgite, montmorillonite), containing a high amount of magnesium, calcium or iron. In the case of clays non-activated, they do not adsorb natural pigments (O'Brien, 2000; Mag, 1990; Bockisch, 1998). The granule size of the clays and the specific surface are parameters of great importance for the process (Bockisch, 1998).

The theories that have been established on the bleaching process are based on the Freundlich adsorption isotherms, which are an adaptation of the Langmuir equation. These equations are valid for constant temperatures and describe the dependence of the adsorbed amount of a substance (k) in relation to the residual amount (c) in the solvent (in this case oil).

$$k/k_0 = \alpha\ (c/c_0)^b \tag{1}$$

Taking these considerations into account equation (1) is represented in simplified form as follows:

$$k_r = \alpha\ (c_1)^b \tag{2}$$

That is equivalent to:

$$\log k_r = \log \alpha + (b)\ (\log c_1) \tag{3}$$

Where the indices 0 and 1 from equation (1) indicate the relative amounts of initial and final respectively, k_r is the ratio of adsorbed components, c is the number of components not

adsorbed and b are constants specific system. If an amount of adsorbent m (bleaching agent) is include then the result is:

$$k = (m)(\alpha) + (c^{b}) \quad (4)$$

which would be equal to:

$$\log k - \log m = \log \alpha + (b)(\log c) \quad (5)$$

With the development of various types of bleaching processes using different types of clays, α is a measure of the relative amount of bleaching clays that will be used to obtain a specific result. Keeping the product of *m* and α constant, for example α = 0.25, the amount of clays must be four times as higher as at α = 1.0. Many studies which have developed different values for α and *b*, depending on the equipment used in the process, type of clays and basically type of oil to be bleached (Bockisch, 1998).

Bleaching clay load (typically 0.1-2.0 percent) and temperatures depend of type and quality of oil processed. To minimize the oil oxidation during bleaching a vacuum of 50 mm Hg is recommended. Ortega-García et al. (2005), reported the following optimal bleaching conditions for soybean oil: temperature, 96°C; time, 23 min; clay amount (Tonsil Optimum 320 FF), 1.4% w/w oil; stirring, 250 rpm, and partial vacuum, 60 mmHg. Under these conditions a bleached soybean oil with 0.1 meq/kg of PV, 91.74% of TOCR, and a colour of 1.53 Lovibond red value units was obtained Table 1. A response surface methodology (RSM) was used to find the parameters that produce bleached oil with minimum peroxide value (PV), maximum tocopherol retention (TOCR) and light colour.

Analysis	Neutralized soybean oil	Bleached soybean oil
Free fatty acids (% as oleic acid)	0.456±0.001	0.562±0.002
Peroxide value (meq/kg)	0.292±0.023	0.1±0.04
Colour	70Y 8.3R	1.53R
Tocopherol (ppm)	925.27	848.84

Table 1. Chemical analysis and colour of neutralized and bleaching soybean oil.

During bleaching process, suspended matter in the oil is adsorbed on clays at different adsorption rates. These adsorption rates are important for design of processes. For this reason, knowledge of bleaching kinetics is requires. Gonzalez-Tovar et al., (2005), reported the adsorption kinetic of pigments, peroxides and tocopherols during bleaching process of soybean oil. In this work, both empirical and theoretical mathematical models were applied (Table 2), in order to predict the adsorption kinetic parameters for tocopherols, peroxides and pigments. The experimental part consisted of mixing neutralized soybean oil with different concentrations of bleaching clays (0.16, 1.0 and 2.0% w/w) in a laboratory reactor at 96°C for 64 min. The results showed that the first order theoretical model predicted better (R^2>0.93) the adsorption kinetic for tocopherols, peroxides and pigments than the empirical model. The determined kinetic parameters suggest that these bleaching clays (Tonsil Optimum 320 FF, Süd-Chemie) presented a high capacity for pigment and peroxide adsorption. Adsorption occurred mostly at the first minutes (20 min) of the process. The tocopherols losses were directly related to the bleaching clay dosage: 16.16, 16.56 and 19.98% for bleaching clays concentration of 0.16, 1.0 and 2.0%, respectively.

Model	Equation
First Order	$q = 1 - e^{\left(\frac{k_0}{k_d}\left(e^{-k_d t} - 1\right)\right)}$
Nicol	$r = k_5(KC - q)$
Fleming	$q = k_6 C_0 t^n$
La Brooy	$q = k_7 C t^n$

q=amount of adsorbed material/g adsorbent; C = adsorbate concentration; r = adsorption rate; C_0 = initial concentration of adsorbate, t=time, k_0=initial velocity, k_d = equilibrium constant; K, k_5, k_6, and k_7 = characteristic constants of the models.

Table 2. Kinetics adsorption empirical and theoretical mathematical models.

Deodorization. Deodorization is the final step in the production of edible oils and fats. This step is basically a vacuum-steam distillation at high temperature and very low absolute pressures, where free fatty acids (FFA) and volatile odiferous components (aldehydes and ketones) are removed to obtain bland and odorless oil (Table 3). The deodorization process requires less stripping steam, and shorter residence time (Kellens & De Greyt, 2000). This process is feasible because of the oil low volatility (i.e. triacylglycerides) and the relative volatility of the odiferous compounds in the oil (Mattil et al., 1964; Zehnder, 1995).

Additionally during desodorization, certain carotenoids are reduced, because of their instability at temperatures in which the process is made (Mattil et al., 1964). Therefore, the purpose of deodorization is to obtain an oil with a free fatty acid content of lesser than 0.03%, maximum values of Lovibond color must be 10 Yellow, 1 Red, odorless and essentially bland-tasting (Erickson, 1995).

It is important to consider that during the deodorization, can occur other reactions such as thermal decomposition of triglycerides into free fatty acids, formation of *trans* isomers (Kellens, 1997) and loss of tocopherols and sterols (Zehnder & McMichael, 1967; Medina-Juárez et al., 2000). Therefore, it is very important to establish the optimal conditions to avoid formation of *trans* isomers and the loss of tocopherols.

Absolute pressures	1 – 6 mm Hg (1.3 – 8 x 102 Pa)
Temperature	250-260°C 482-500°F
Time	20 – 40 min
Sparge steam	0.5 – 2.0 %

Kellens and De Greyt, 2000.

Table 3. Deodorization conditions for soybean oil.

The fats, oils and volatile compounds that contain them, by the laws of Dalton partial pressures and Raoult vapor-liquid equilibrium are governed (Zehnder & McMichael, 1967). Dalton's law states pronounce that the molar ratio of volatile compounds is proportional to the ratio of partial pressure. In the case of the deodorizing system, the molar ratio of steam

(S) and molar ratio of volatile compounds such as free fatty acids and carboxylic compounds (V) are proportional to their partial pressures (p_s, p_v').

$$dS/dV = p_s/p_v' \quad (6)$$

Where:
p_s = partial vapor pressure
p_v '= partial volatile compounds pressure
But assuming that pv' is very small compared to p_s, then p_s will be similar to the total pressure (P). Therefore an approximation of equation 6:

$$dS/dV = P/ p_v' \quad (7)$$

Where: P= total pressure or $p_v' + p_s$
Raoult's Law states that the vapor pressure of volatile compounds is equal to its vapor pressure in pure form, multiplied by its molar concentration in the oil.

$$p_v = P_v (V/(O + V)) \quad (8)$$

Where: O = moles of oil
Since V is small compared to O, the equation can be written:

$$p_v = P_v (V/O) \quad (9)$$

Using the equation 10 to calculate the efficiency of vaporization

$$E = p_v '/ p_v \quad (10)$$

Where p_v is the pressure equilibrium, then combining equations 9 and 10, equation 11 is represent as follows:

$$P_v' = (E P_v V)/O \quad (11)$$

From equations 6 and 11 gives

$$dS/dV = PO/(E P_v V) \quad (12)$$

If it is integrate equation 12, it has obtain the amount of steam for deodorization

$$S = PO/E P_v (\ln (V_1 / V_2) \quad (13)$$

Where V_1 is the initial concentration of volatile compounds in the oil and V_2 the final concentration.
This equation applies only to ideal systems. However, in the practice, the system vegetable oil-fatty acid has a non-ideal behavior. Therefore, an activity coefficient (A) is introduced to the equation 13 for the case of non-ideal systems (Mattil et al., 1964), leaving this equation as follows:

$$S = PO/E P_v A (\ln (V_1/V_2) \quad (14)$$

Worldwide the deodorizing equipments can be classified into three groups: batch, semi-continuous and continuous. The selection of the most appropriate depends on several factors, such as: capacity, required investment, and operating costs.

The batch deodorizer equipment is simple and low cost construction. However, operating costs are high (high steam consumption) and long processing times (more than 8 hours). Other disadvantages include its small capacity (50 tons per day), and irregular production (Kellens & De Greyt, 2000).
Semi-continuous system is basically a semi-batch system, designed for larger capacities. The advantage of this system is the good quality oil it can produce and the possibility of frequent feedstock changes of oil per day. However, it requires a large investment, high operating costs and relatively long residence times (Zehnder, 1992).
The continuous deodorizer system is the most preferred by high capacity plants with few stock changes. The main advantages are the moderate investment cost, the short residence times (20-60 minutes), the possible heat recovery and easy maintenance (Kellens & De Greyt, 2000).
There are certain critical steps that's should be carried out during neutralized, bleaching and deodorization, in order to obtain a good-quality soybean oil for edible uses. For example, heating of soybean oil, after neutralized with alkali is an important step in the inactivation of trace metals (Pryde et al., 1980). Also, in order to improve the flavor stability, bleaching can be made more selectively for increase the ratio of carotenoids over chlorophylls. To reduce the problem caused by certain polymers, deodorization of soybean oil should only carryout after ensuring that the oil is free of peroxides or after their removal.

3.3 Physical refining of soybean oil

Another way to remove FFA from vegetable oil is by physical refining. This is not a new process; it was firstly used in the 30´s in order to process oils with low gums content (coconut oil, palm oil and animal fats) and high FFA content (> 1%). The physical refining process can offer important advantages to the refiner, including the use of fewer chemicals, reduction of handling of by-products (soaps, and acidified washed waters), and minimal loss of neutral oil. The physical refining of oils such as canola, sunflower and especially soybean oils has gained increasing interest (Zehnder, 1992). However, in the case of these oils, physical refining is suitable only for crude oils of a good quality (low level of oxidation and low phosphatides content after degumming). Chemically refined oils usually require less steam, shorter residence time, high pressure and low temperature than physically refined oil (Kellens & De Greyt, 2000).

4. Chemistry of soybean oil and its nutritional significance

Vegetable oils are insoluble in water but soluble in organic solvents such as hexane, chloroform and petroleum ether. They consist predominantly of triglycerides, which are characterized by three monocarboxylic fatty acids esterified to a glycerol molecule. These fatty acids generally have an even number of carbon atoms (C14-C22) and can be saturated and unsaturated.

4.1 Fatty acids

The most common saturated fatty acids are palmitic acid (C16) and stearic acid (C18), monounsaturated is oleic acid (C18:1), and the most common polyunsaturated are linoleic acid (C18:2) and α-linolenic acid (C18:3). Unsaturated fatty acids have geometric isomerism, *cis* and *trans* isomers.

Some studies have examined the effect of fatty acids (saturated and unsaturated) on health, concluding that there is sufficient evidence that lauric acid (C12), myristic acid (C14) and palmitic acid (C16), increase serum cholesterol concentration but not stearic acid (C18). In contrast, the consumption of mono and polyunsaturated fatty acids (*cis*), reduce serum cholesterol and low-density lipoprotein (Connor, 2000; Stanley, 2000).

Caggiula & Mustad (1997) reported the results of an epidemiological survey conducted in seven countries, in order to identify dietary factors that could be considered etiologic in the development of coronary heart disease. The study found a high correlation between intake of saturated fatty acids and the incidence of coronary heart diseases. With respect to mono and polyunsaturated fatty acids, they found a negative correlation with these diseases. Given this situation, is recommendable to reduce the intake of saturated fatty acids (animal fats) and *cis* fatty acids substituting them by mono and polyunsaturated (vegetable oils) (Eritsland, 2000).

All fatty acids are necessary for the normal physiological functions of human. The saturated are involved in producing and storing energy in lipid transport and synthesis of phospholipids. Monounsaturated fatty acids are also involved in these processes; however, the term "essential" does not apply to these saturated and monounsaturated fatty acids. This designation is reserved for polyunsaturated fatty acids that are not synthesized by the human body; therefore, they should be in the diet because they are required to maintain good health (Spector, 1999).

Among the polyunsaturated fatty acids, are especially important the linoleic acid (n-6) and α-linolenic acid (n-3). These fatty acids are considered essential for growth and development of the human body. They are precursors of prostaglandins and hormones that play an important activity in the regulation of some physiological and biochemical functions of the human body. When there is a deficiency of essential fatty acids, the growth, and synthesis of prostaglandins is reduced, and damage to the skin can occur. Soybean oil is a major source of these fatty acids, contains about 56% of n-6 linoleic acid and 8% α-linolenic acid, n-3 (Bockisch, 1998).

It has been suggested that an increasing intake of n-3 fatty acids produce beneficial physiological effects such as: a decrease on concentration of triglycerides in the blood, modulation of blood pressure, reduction on the risk of heart and kidney disease, regulation of neuromuscular disorders, control of hyperglycemia, and a beneficial influence on the immune system, allergies, cancer and growth (Chavarro et al., 2007; Sun et al., 2008; Ahren et al., 2009; Barbosa-Tinoco et al., 2009; Sioen et al., 2009; Yongsoon, 2009).

Trans fatty acids. The refining process applied to soybean oil to improve flavor and oxidative stability provokes chemical changes to some compounds with nutritional importance, mainly on deodorizing stage. These nutritional modifications include tocopherols (vitamin E) loss and *trans* isomerization of polyunsaturated fatty acids (Medina-Juarez et al., 2000). Highly polyunsaturated oils such as soybean and rapeseed oils are particularly sensitive to *trans* isomerization during deodorization. Medina-Juarez et al., (2000) evaluated the effects of different conditions of deodorization process (temperature, pressure, time, and steam) on the formation of *trans* fatty acids and tocopherols loss during chemical refining of soybean oil. The results showed that formation of *trans* fatty acids was significatively favored by temperature and time ($P<0.05$). Additionally, this work reported the optimal conditions in lab-scale batch deodorizing for soybean oil (temperature 220°C and 5 mm Hg pressure). Under these conditions, was obtained a deodorizing soybean oil with 1.0% of *trans* fatty acids, 70% of tocopherols retention, 0.03 % of FFA, color 0.7R, and 6

hours Rancimat (110°C) of oxidative stability (Medina-Juarez, 2000). For these reasons the modern deodorizers are operated at low temperatures 220-235/428-455°F, and vacuums of 2 mbar. The highest isomerization rate has been observed for linolenic acid and the lowest for oleic acid (Kellens & De Greyt, 2000).

Several population studies have examined the relationship between *trans* fatty acids intake and coronary heart disease risk. These studies show that high *trans*-fat intake has been linked to coronary heart disease, in which fatty plaques build up in the heart arteries, provoking heart attacks (Hunter, 2006; Mozaffarian et al., 2006; Chiuve et al., 2009). In addition, *trans* fatty acids inhibit desaturation of essential fatty acids (Phivilay et al., 2009).

The average intake of *trans* fatty acids in industrialized countries is estimated as 6-8 g/day, corresponding from 1.5 to 3% of the calories in the diet. The intake of *trans* acids in the diet of Americans is estimates that exceed 10 g/day, being the major source of intake of such products from fast food and snacks. Recent studies showed that the average intake of *trans* fatty acids in women from Turkey was 2.13 +1.03% of the calories in the diet, being the main source, margarine and butter (37.0%), bakery products and confectionery (29.6%) (Samur et al., 2009).

The reason because of *trans* fatty acids, as well as saturated fatty acids are involved in increasing coronary heart disease (Khosla & Hayes, 1996) is that both *trans* fat and saturated fatty acids produce similar effects on plasma proteins, increasing the cholesterol-low density lipoprotein (LDL-C) (Hunter, 2006; Mozaffarian, et al., 2006; Chiuve et al., 2009).

Similar results was reported by Caggiula & Mustad (1997), in a study conducted in seven countries with over 12,000 men from 16 towns, where they found a high correlation (r=0.81) between intake of saturated fatty acids and increased artery disease. Also, they found a strong correlation between the content of *trans* fatty acids in the diet and mortality from coronary artery disease (r=0.78).

Based on these facts and the scientific evidences about the negative effects of *trans* fatty acids on human health, the Food and Drug Administration (FDA) U. S., issued a regulation where, from January 2006 must be included on food labels the content of these *trans* fatty acids.

4.2 Phospholipids

Phospholipids are diacylglycerides (R1, R2) containing a phosphoric acid molecule attached to the glycerol (C3) by an ester bond. These compounds are part of biological membranes and have an active participation in biological processes such as cell permeability and transport of lipids and cholesterol. In vegetable oils are mainly three phospholipids: phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol. These phospholipids are the main components of lecithin, which are used in the food industry as emulsifiers, due to the ability to blend with the lipophilic hydrophilic phases.

Lecithin is a byproduct of the refining process of soybean oil. During the stage of degumming (before neutralization), phospholipids must be removed because the lecithin or phospholipids (gums) increase refining losses, form precipitates in storage tanks or pipes and they make more difficult the filtrate processes (Kellens, 1997).

4.3 Tocopherols

Tocopherols are the most important natural antioxidants in vegetable oils, and they have an important activity as vitamin E. Only plants synthesize these compounds, which are

important nutrients for humans and animals. Tocopherols are present in oilseeds, leaves and other green parts of higher plants. The content of tocopherols in vegetable oils is show in Table 4.

Oil	Tocopherols (ppm)	Iodine value	Unsaponifiables (%)
Soybean	1700-2000	120-143	0.6-1.6
Rapeseed	700-1200	110-126	0.5-1.2
Corn	1000-1500	127-133	0.4-1.2
Sunflower	600-800	110-144	0.3-1.3
Olive	150-200	75-94	0.4-1.1

Kellens, 1997.

Table 4. Tocopherols content in crude vegetable oils.

Tocopherols are found in four forms: α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol. There is another series of compounds with similar chemical structure to tocopherols; the tocotrienols, which differ structurally from tocopherols by having three double bonds in the aliphatic chain. Tocotrienols are found primarily in corn germ, rice bran, palm oil and coconut (Eitenmiller, 1997; Shahidi, 1997).

Vitamin E is known as mixed isomers of tocopherols and tocotrienols with a recognized efficient to inhibit lipid oxidation in foods and biological systems. Tocopherols are found in oilseeds, leaves and other green parts of higher plants. A marked variation on the content of tocopherols can be found in vegetable oils. Kamal-Eldin & Andersson (1997) evaluated oils from 14 plant species, and found a possible correlation between the content of linoleic acid (C18: 2) and α-tocopherol as well as between linolenic acid (C18: 3) and γ-tocopherol. Therefore, it is difficult to establish a correlation between tocopherol content and fatty acid composition.

The antioxidant activity of tocopherols depends on its chemical structure, which defines the ability to donate hydrogen from phenolic group of free radicals. The α-tocopherol isomer has a higher antioxidant activity, followed in descending order by the γ-tocopherol, and δ-tocopherol, in living systems. Contrarily, in the case of fats, the increased activity is δ-tocopherol followed by γ-tocopherol and finally α-tocopherol. The change in the antioxidant activity of tocopherol isomers *in vitro*, not only depends on its chemical reactivity, but also because of the effect of temperature, light and the presence of other compounds that act as pro-oxidants (Kamal & Appelqvist, 1996).

4.4 Sterols

Sterols are the largest proportion of unsaponifiable matter in vegetable oils. The basic structure of these compounds is alcohols derived from polycyclic ciclopentanofenantrene (Bockisch, 1998). These compounds are constituted mainly by β-sitosterol, and in lower concentrations campesterol, stigmasterol and brassicasterol (Frandsen, 1996). The ratio of sterols is specific for each oil. The molecular structure of sterols is similar to the structure of cholesterol. The minor components are used to determine the purity of oil (olive oil "virgin oil") (Gutierrez et al., 2000) or identify edible oil mixtures (Gordon & Miller, 1997, Alonso et al., 1997).

Sterols are also used in the cosmetics industry as emulsifiers. A 75% of the sterols that are currently marketed in the world come from soybean oil. These sterols are obtained from the

distilled products of deodorization of soybean oil and other vegetable oils (Clark, 1996). It has been demonstrated that the sterol consumption may benefit the human health. Recent studies have shown that sterols help in the reduction of LDL-cholesterol in human serum. This reduction is the result of a decrease in cholesterol absorption in the small intestine and an increasing of bile acid excretion (Normén et al., 2000; Vissers et al., 2000; Sanclemente et al., 2009).

4.5 Reactions of soybean oil

The terms for the types of characteristics reactions of oils, reported in the literature are: acidolisis, alcoholysis, esterification, interesterification and transesterification. However, the meaning of these terms has not been consistent in the literature.

Acidólisis. Reaction between an ester (triacylglycerides) and a fatty acid. The result is an exchange of acyl groups.

$$R_1COOR_2 + R_3COOH \longrightarrow R_2OH + H_2O \longrightarrow R_3COOR_2 + R_1COOH$$

Alcoholysis. Reaction between an ester and an alcohol, which can be a glycerol or methanol, resulting an exchange of alcoholic fractions.

$$R_1COOR_2 + R_3OH \longrightarrow R_1COOH + H_2O \longrightarrow R_1COOR_3 + R_2OH$$

Esterification. Reaction between a fatty acid and alcohol, or among compounds containing carbonyl and hydroxyl groups.

$$R_1COOH + R_2OH \longrightarrow R_1COOR_2 + H_2O$$

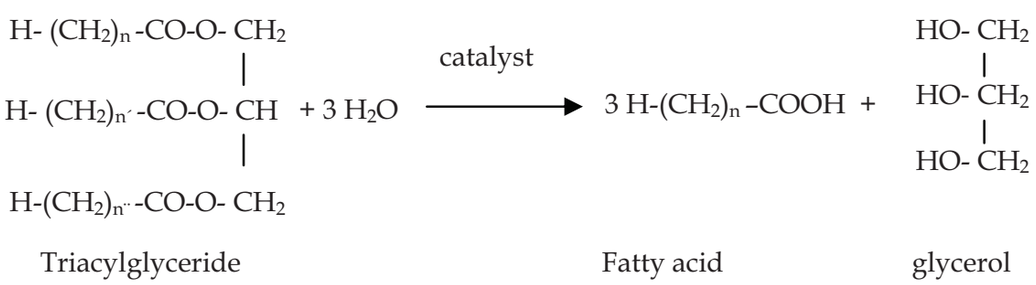
Transesterification. Term commonly used for the exchange of an ester (triacylglycerol) to another ester can be another triacylglycerides or ethyl ester. The result is an exchange of acyl groups between two esters.

$$R_1COOR_2 + R_3COOR_4 \longrightarrow R_1COOH + R_2OH + R_3COOH + R_4OH + H2O \longrightarrow R_1COOR_4 + R_3COOR_2$$

Interesterification. A general term used for the reactions between an ester and a fatty acid, an ester alcohol or other (includes acidólisis, alcoholysis and transesterification). Hydrolysis. Reaction between an ester (triacylglycerides) and a water molecule in the presence of a chemical catalyst or enzyme.

$$R_1COOR_2 + H_2O \longrightarrow R_1COOH + H_2O \longrightarrow R_1COOH + R_2OH$$

The following diagram shows the hydrolysis of a fat.



During these reactions, water is consumed (hydrolysis) or generated (esterification) or consumed and released at once (interesterification).

5. Digestion and absorption of triacylglycerides

Pancreatic lipase preferentially hydrolyzes ester linkages of triacylglycerides 1-3, producing free fatty acids and 2-monoacylglycerols, which are absorbed through the intestinal wall cells (Ikeda et al., 1991). The fatty acids released during digestion, are metabolized more rapidly if they are short and medium chain (<C10: 0), while long-chain fatty acids are absorbed directly as monoacylglycerols. The long-chain triglycerides are not effective as an energy source because they are metabolized more slowly than medium chain triglycerides. The short-chain fatty acids are incorporated into chylomicrons and transported by the lymphatic system to the liver to provide the necessary energy or adipose tissue for storage. The medium chain triglycerides are absorbed by enterocytes of the intestinal wall and enter the portal vein, they not depend of carnitine for transport through the mitochondria, and are not incorporated into chylomicrons, and therefore, they are not easily stored in adipose tissue (Mascioli et al., 1987). In people with malabsorption, essential fatty acids are used more efficiently in the form of 2-diacyl-glycerol (Xu, 2000).

6. Modulation of eicosanoid biosynthesis by n-3 and n-6 dietary fatty acids

Once the digestion, absorption and transport in the human body are done, polyunsaturated fatty acids n-3 and n-6 are introduced to fat cell in non-esterified form, and are used as substrates for the synthesis of eicosanoids (leukotrienes, prostaglandins and thromboxanes). Eicosanoids are synthesized by stimulation. The class of eicosanoids produced varies with the type of tissue. For example, thromboxane A_2, is an agglomerating agent of platelet, which is synthesized in platelets; whereas prostaglandin I_2, which is an inhibitor of the agglomeration of platelets, are synthesized in endothelial cells. Eicosanoids are not stored in the cells; their effects are manifested locally and perform various actions on the cardiovascular, reproductive, respiratory, renal, endocrine, nervous and immune systems. Excessive inhibition of eicosanoid synthesis involves a number of pathological conditions including thrombosis, inflammation, asthma, ulcers and kidney disease. Some studies have suggested that the amount and type of eicosanoids produced in the tissues can be modulated by manipulation of dietary fatty acids, since the type of eicosanoids produced depends on the substrate available.

The n-6 fatty acids such as linoleic acid (LA) or arachidonic acid (AA) act as substrates for the synthesis of prostaglandins of the series-2 (PCI_2thromboxane A_2 and prostacyclins), and leukotrienes of series-4; while, prostaglandin series-3 (PCI_3thromboxane A_3 and prostacyclins), and leukotrienes of series-5 are derivatives of n-3 fatty acids. Depending of enzyme activity in the tissue, the availability of cofactors, the specificity of each enzyme for fatty acid and the concentration of each acid on the value of Michaelis constant (K_m), there are three possible metabolic pathways PUFA: the way of acetyl-CoA, the route of cyclo-oxygenase and route of the lipo-oxygenase (Lands & Bimbo, 1983).

The route of acetyl-CoA regulates oxidation and esterification of fatty acid within lipidic cells, depending on energy demand.

The route of cyclo-oxygenase provides to human organism of prostaglandins, thromboxanes and prostacyclin, by oxidative cyclization of polyunsaturated fatty acids. The prostacyclins

are hormones that play an important role in the regulation of some physiological and biochemical functions of the body. The thromboxanes and prostacyclins, transformation products of prostaglandins, have the function of regulating the activity of platelets (Scott et al., 1982).
The route of the lipo-oxygenase provides to human organism of leukotrienes, which are potent eicosaenoics, which cause contractions and increase the permeability of smooth muscle (Lands & Bimbo, 1983).
Dietary fatty acids may modulate the biosynthesis of eicosanoids in two stages. The first is the desaturation and elongation of fatty acids. There is a competitive inhibition between linoleic acid (LA, n-6) and linolenic acid (n-3). n-3 PUFAs may reduce the conversion of LA to AA, modulating the biosynthesis of eicosanoids and consequently modifying the physiological response(Machlin, 1962). The second stage is the path of cyclo-oxygenase, the n-3 fatty acids competitively inhibit the oxidation of AA. Competitive inhibition between n-3 fatty acids and n-6 by desaturases and cyclo-oxygenases suggests that an increase in dietary n-3 fatty acids may reduce eicosanoids derived from AA (Hwang, 1992).

7. Physical and chemical analysis of soybean oil

Crude vegetable oils should be subjected to refining process in order to improve taste, appearance and oxidative stability. During this process color, free fatty acid, oxidation products (peroxides and aldehydes and ketones), the modification of essential fatty acids to *trans* isomers, and the loss of tocopherols and sterols, should be monitored.
The official methods used for physical-chemical analysis of oils and fats are containing in "Official Methods and Recommended Practices" published in 2009 by the American Oil Chemists' Society. This manual includes the following methods:

7.1 Humidity

The presence of moisture in oil samples indicates that they were inadequately processed (washing in the neutralization) or the oil containers were contaminated. The moisture determination is made in a vacuum oven, calculating the moisture and volatile material by difference in weight (AOCS, Ca 2d-25).

7.2 Color

The method determines color by Lovibond. This equipment uses color filters calibrated in accordance whit the Lobivond tintometer color scale (AOCS, Cc 13e-92).

7.3 Soap

This determination indicates the efficiency of soap removal (washing the oil with water) after neutralization operation. The method determines the alkalinity of the oil with sodium oleate (AOCS, Cc 17-79). The content of soap should be less than 30 ppm after neutralization and less than 1 ppm after the bleaching (Erickson, 1995).

7.4 Conjugated dienes

In the structure of polyunsaturated fatty acids is not common to find conjugated dienes. Therefore, conjugated dienes, are the result of a structural modification of polyunsaturated fatty acids during autoxidation (Banni & Martin, 1998). Quantification of conjugated dienes

is performed by a spectrophotometric method (AOCS, Ti la-64). Polyunsaturated fatty acids containing conjugated dienes have their maximum absorbance at 234 nm. This index is useful for determining the advance of the start of oxidation, since the increase is proportional to oxygen consumption and the concentration of hydroperoxides formed in the early stages of the reaction.

7.5 Free fatty acids

Is the number of milligrams of potassium hydroxide necessary for saponify free fatty acids from a sample of one gram of oil or grease and is expressed as percentage of oleic acid. This method is a measure of the degree of decomposition of the oil triglycerides (AOCS, Ca 5a-40).
This analysis is performed to determine the effectiveness of chemical neutralization in the refining process and to obtain rapid information of loss of oil in the same operation.

7.6 Peroxide value

The formation of primary products of oxidation (hydroperoxides) can be measured by peroxide value. This method quantifies the primary compounds of oxidation reaction in oils and fats (AOCS, Cd 8b-90).

7.7 *p*-anisidine value

Is a colorimetric measurement (350 nm) based on the color development produced by the reaction between *p*-anisidine reagent and the secondary oxidation compounds (aldehydes, ketones, alcohols and acids) (AOCS, Cd 18-90).
The use of peroxide value (PV) and *p*-anisidine value (AV) together provides a comprehensive overview of the oxidation process in oils. This is a mathematical prediction of oxidative stability and the value is calculated as: TOTOX = VA + 2VP (AOCS Cg 3-91).
Unfortunately, none of these methods can characterize the real oxidative grade of oil. A more effective method for understanding the oxidative state of oils is the determination of the composition and amount of volatile materials by solid-phase microextraction (SPME) and gas chromatography (Ho et al., 1996; Hinshaw, 2003). Among the main components, 2-pentilfurane, is a main product of auto-oxidation of linoleic acid and responsible of flavor reversion of soybean oil.

7.8 Oxidative stability

The Rancimat method (AOCS, Cd 12 b-92) has been used successfully to measure the oxidative stability index (OSI) of oils with synthetic and natural antioxidants (Hasenhuettl & Wan 1992; Gamez-Meza et al. 1999). The method measures the increases of the electric conductivity that arises when fats and oils are oxidized to short free fatty acids (chiefly formic acid) under accelerated conditions of heat and aeration (Kolb et al. 2002; Anwar et al. 2003).
OSI values of 5.24 (Anwar et al. 2003), 6.63 (Gámez-Meza et al., 2009) and 7 h (Frank et al. 1982; Judde et al. 2003) for soybean oil without antioxidant at 110ºC have been reported.
Lopez-Aguilar et al., (2006) found that differences in *p*-anisidine value and Rancimat were correlated to the difference in rancidity of soybean oils.

7.9 Minerals

The properly processed soybean oils must provide calcium and magnesium levels below 1 ppm and concentration of iron lower than 0.1 ppm (Erickson, 1995). These trace metals can

be analyzed by atomic absorption spectroscopy or optical emission spectroscopy inductively coupled to plasma (ICP), the latter method is recommended for use in edible oils with less than 1 ppm of trace metals (Perkin-Elmer, 2010).
Copper is not normally found in vegetable oils, however, it is recommended monitor it because it is a potent prooxidant (Coppin & Pike, 2001) and oils can be contaminated easily during processing or storage. The limit values for soybean oil must be below 0.05 ppm (Erickson, 1995).
Ahmad et al. (1983) found that the oxidative stability index of refine soybean oil without antioxidants (6.63 h) was significantly lower ($p< 0.05$) than soybean oil with grape peel extracts and citric acid at all concentrations tested. This suggests that citric acid chelated the iron and inhibited its prooxidant effect.

7.10 Fatty acid profile

The composition of fatty acids is one of the most important chemical characteristics to identify the oils, since each one has a distinctive profile. The method AOCS Ce 1h-05 provides a gas-liquid chromatography procedure for the determination of the fatty acid profile, including the *trans* fatty acids isomers of vegetable oils and fats. The samples are saponified and methylated according to AOCS procedure (Ce 2-66). Fatty acid methyl esters (FAME) are analyzed in a gas chromatograph equipped with flame-ionization detector. A fused-silica capillary column coated with 100% biscyanopropylpolysiloxane as the stationary phase (SP-2560, 100 m × 0.25 mm i.d. 20 µm thickness, Supelco, Bellefonte, PA), and an oven temperature of 180°C are recommended. FAME peaks are identified by comparison with the retention time of the respective standards. *Trans* isomers are identified from a methyl ester isomer mix of linoleic acid and linolenic acid (Figure 1). C21:0 or C23:0 as internal standard is used (Medina-Juarez et al., 2000). Results are expressed as weight percentage of oil. The specific conditions of the method not determined oxidized fatty acids or polymerized.

7.11 Quantification of glycerides

The quantification of mono-, di-and triacylglycerides is done through an equipment of high performance liquid chromatography (HPLC) with ELSD detector (Evaporative Light Scatering Detector) and a Lichrosorb Si60 column (250 x 4.6 mm, 50 um of particle size, Supelco, Inc.). The temperature must be maintained at 40°C, using a column jacket. The solvent system used is hexane (solvent A) and a mixture of hexane:2-propanol:ethyl acetate (80:10:10) with 0.1% of formic acid solution (10% in 2-propanol) (solvent B) (Liu et al., 1993).

7.12 Tocopherols

The quantification of tocopherols by HPLC with UV detector and a Lichrosorb Si60 column (25 cm x 4 mm, 5µ particle size, Supelco) is recommended. The mobile phase for this determination is a mixture of hexane: isopropanol (99.5:0.5) (Medina-Juárez et al., 2000, Ortega-Garcia et al., 2006). The analysis of tocopherols may also be performed with a reverse phase C18 column and an isocratic elution with 100% methanol, using a mass detector (Deselet al., 2007). The wavelength of detector should be set at 292 nm. It must be define the purity of the standards of tocopherols using their extinction coefficients (E 1%) (AOCS Ce 8-89).

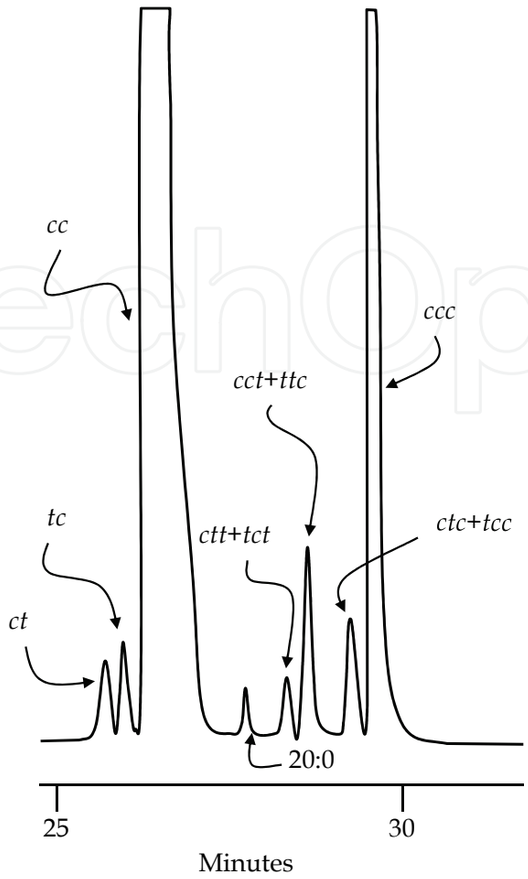


Fig. 1. Partial chromatogram of fatty acid methyl esters of refining soybean oil using a SP-2560 (Supelco, Belloefonte, PA) capillary column with a temperature program, and nitrogen as carrier gas at a flow rate of 20 cm/min. ct, 18:2Δ9c,12t; tc, 18:2Δ9t,12c; cc, 18:2Δ9c,12c; ctt + tct, 18:3Δ9c,12t,15t + 18:3Δ9t,12c,15t; cct + ttc, 18:3Δ9c,12c,15t + 18:3Δ9t,12t,15c; ctc + tcc, 18:3Δ9c,12t,15c + 18:3Δ9t,12c,15c; ccc, 18:3Δ9c,12c,15c. (Medina-Juárez et al, 2000).

7.13 Sterols

The first part of this technique consists to separate the saponified fatty acids from unsaponified fraction (mainly sterols and tocopherols) (Gutierrez et al., 2000). One gram of sample with 100 mL of 0.8 M KOH in methanol, is saponified for 30 min at 80°C. The unsaponified fraction with two fractions of 100 mL of ethyl ether is extracted. The ethereal fraction is washed with water and then this fraction in a rotary evaporator at 60°C and vacuum is concentrated. The sample is brought in ethanol (25 mL) and injected to an equipment of high performance liquid chromatography (HPLC) with a UV detector and a C18 column (15 cm x 4.6 mm, 5µ particle size, Varian) reverse phase. Methanol (100%) is the mobile phase. The measurement is carried out at 206 nm (Ortega-Garcia et al., 2006).

7.14 Sensory evaluation

The flavor characteristic of soybean oil and other linolenate-containing oils at early stages is "beany and grassy" and "fishy or painty" at the more advance stages and at low levels of oxidation (i.e., the peroxide value is 10 or below). The oxidation degree is so low that often it

cannot be measured chemically (Frankel, 1998). Flavor reversion, in contrast to oxidative rancidity is observed at lower levels of oxidation.

According to Sensory Evaluation Division of Institute of Food Technologists (IFT), the sensory evaluation is defined as a scientific discipline, which is responsible to measure, analyze and interpret results of characteristics of food and materials, in how they are perceived by the senses of sight, smell, taste, touch and hearing (Pedrero & Pangborn, 1989). The official method (Cg 2-83), is based on attribute intensity scales. Some researchers have shown that these techniques can generate biased answers due to forgetfulness of intensity from sample to sample, because the assessment is based on the memory of the judges (Kim & O'Mahony, 1999). In recent years, it has been increased research in this area, proposing innovative methods. One of these techniques is called n-AFC (n Alternative Forced Choice). This is a discriminatory technique that has shown to have higher probability of success than traditional discriminatory techniques, duo-trio and triangular (Angulo et al., 2007).

Medina-Juárez, et al., (1998) evaluated the oxidative stability and the sensory characteristics (Cg 2-83) of some samples of soybean oil, produced in Mexico, U.S. and Costa Rica. These authors found that 41% of the samples analyzed from 18 main Mexican refineries had similar flavor to the samples produced in the U.S. and Costa Rica. However, in 2006, Lopez-Aguilar et al. reported that Mexican soybean oils met quality control criteria and correlated with sensory evaluation by two methods (official method of AOCS, Cg 2-83 and forced-choice test of two alternatives, 2-AFC). The method most effective in determining small differences in rancidity was 2-AFC.

8. Oxidative stability of soybean oil: antioxidants

Refined soybean oil has been found to be one of the most unstable product, not only due to its high unsaturated fatty acids content but also due to the absence of natural compounds capable of provide a protective antioxidant effect. By other hand, the types of volatile products depend of triacylglyceride precursors. A complex mixture of volatile products is expected from decomposition of each one of the many of hydroperoxides found in oxidized soybean oil. Due to the improved analytical methodology, the number of flavor and odor compounds identified by different authors is continuously increasing. Therefore, this information should permit better understanding of influence of triacylglycerol structure on the relative oxidative stability of unsaturated glycerides. In spite of many studies in the control of oxidation of refining soybean oil, problems caused by light and thermal instability still await solution.

The effect of trace metals and light on flavor stability is well recognized. Treatment with citric acid is an effective practice to improve flavor stability.

Antioxidant addition during industrial food formulations is one of the most effective means to retard fat oxidation. It is a popular method for increasing the shelf life of oils and oils-containing foods (Halliwell et al., 1995). Synthetic antioxidants, such as butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are widely used in many oils. However, their use has been questioned because of issues related to toxicity and carcinogenicity (Branen, 1975; Imaida et al., 1983). For this reason, considerable attention has been given to the application of natural antioxidants in foods, because of their potential nutritional and therapeutic effects.

There is a continuous need for technological innovations on safe antioxidant systems. The best known and most effective primary antioxidant among natural substances are polyphenols. Primary antioxidants interfere with auto-oxidation by interrupting the chain

propagation mechanism. The auto-oxidation proceeds when the antioxidant has been destroyed completely.

Phenolic antioxidant commonly used in foods, are highly reactive, have two hydroxyl groups or one hydroxyl and one substituted hydroxyl group in *ortho* or *para* positions. These substances are effective at low concentrations; at high concentration some of them may accelerate the rate of auto-oxidation. They are most effective in foods that contain little natural antioxidants, while they are much less effective in vegetable oils that may contain amounts of naturally antioxidant. However, the naturally occurring antioxidants in oils as soybean oil are readily destroyed by heat during refining process.

Some sources of natural antioxidants are: algae, browning products, protein hydrolysate, plant (extracts), cocoa powder, oat flour, herbs, spices, fruits pulp and peel.

The peel and seeds of grapes are important sources of phenolic compounds, which are considered antioxidant substances. Phenolic compounds at concentrations as high as 718-1060 mg/100 g have been found in peel grapes (Molina-Quijada et al., 2010).

Gamez-Meza et al. (1999) evaluated the antioxidant activity in soybean oil of an extract from Thompson grape peel by Rancimat and Schaal methods. At all concentrations tested (0.1, 0.3, and 0.5% of total phenols), the grape peel extract exhibited appreciable activity, which exceeded the activity of BHA. The highest induction period (Rancimat test) was achieved with the extract at 0.5% of total phenols; this activity exceeds significantly ($p< 0.05$) the antioxidant activity of both synthetic antioxidants (Table 5).

In other study with soybean oil (Gámez-Meza et al., 2009), the antioxidant activity of grape peel extract (GPE) with those of rosemary extract (RE), and tocopherols mix (TM) known as covi-ox T-70 was compared. Thompson GPE at 0.3 and 0.5% (w/w) exhibited greater antioxidant activity than TM. Soybean oil containing GPE or RE at 0.5% (w/w) showed an OSI higher than 48 h at 110ºC. Also the synergistic effect of citric acid was evaluated. When citric acid was added, the RE exhibited a significantly greater antioxidant activity ($p<0.05$) than TM. A synergistic effect for TM at 0.02% (w/w) with citric acid at 1.0% (w/w) was observed. No synergistic effect of citric acid with Thompson grape peel extract was observed (Table 6). Banias et al. (1992) found an antagonistic effect when plant extracts (including RE) combined with citric acid were tested as antioxidants.

The results of studies suggest that it is important to pursue a characterization of the biologically active antioxidant compounds. Of the hundreds of substances that have been proposed to inhibit oxidation of oxidizable compounds, only a few can be used in products for human consumption. Antioxidants required to be approved for its food use by specialized agencies.

Treatment	Induction time[a] (110°C)
Soybean oil (control)	6.24 ± 0.20^{a}
Soybean oil + extract 0.1% TP	15.20 ± 0.56^{b}
Soybean oil + extract 0.3% TP	29.10 ± 1.84^{c}
Soybean oil + extract 0.5% TP	$> 48.00^{d}$
Soybean oil + BHA, 0.02% w/w	8.56 ± 0.37^{e}
Soybean oil + TBHQ, 0.02% w/w	21.70 ± 0.28^{f}

TP, total phenol; BHA, butyllatedhydroxyanisole; TBHQ, tertiarybutylhydroquinone.
[a]Values are mean + SD (n = 2). Mean with different superscript letters (a-f) are signicantly different ($p< 0.05$).

Table 5.Antioxidant activity (Rancimat test) of thompson grape peel extract and synthetic antioxidant.

Antioxidant %	Citric acid (%)					
	0.05		1.0		2.0	
	OSI (h)	SE	OSI (h)	SE	OSI (h)	SE
GPE						
0.00	7.50 (0.15)^{a}	-	8.24 (1.01)^{abc}	-	9.61 (0.48)^{bc}	-
0.02	7.70 (0.72)^{a}	0.45	7.87 (0.51)^{ab}	0.44	8.67 (0.66)^{bc}	0.45
0.10	10.24 (1.16)^{bc}	0.54	11.65 (0.75)^{d}	0.51	11.90 (0.40)^{d}	0.57
0.30	21.95 (0.95)^{g}	0.78	19.40 (0.30)^{f}	0.76	25.90 (3.10)^{h}	0.85
0.50	>48	-	>48	-	>48	-
TM						
0.02	8.67 (0.14)^{b}	0.56	17.00 (2.13)^{f}	1.04	17.25 (1.85)^{f}	0.97
0.10	7.87 (0.24)^{a}	0.48	13.60 (0.30)^{e}	0.79	17.50 (1.70)^{f}	0.94
0.30	8.76 (0.20)^{b}	0.50	13.65 (0.45)^{e}	0.75	14.60 (0.70)^{e}	0.75
0.50	9.66 (0.28)^{bc}	0.53	10.00 (0.90)^{c}	0.53	11.45 (0.76)^{d}	0.57
RE						
0.02	18.23 (1.89)^{fg}	0.86	19.60 (1.23)^{f}	0.89	29.00 (1.42)^{h}	1.24
0.10	27.00 (0.89)^{h}	0.81	34.65 (1.65)^{i}	1.04	42.50 (1.30)^{k}	1.20
0.30	37.80 (0.20)^{j}	0.96	37.87 (0.52)^{j}	0.94	43.80 (0.28)^{k}	1.06
0.50	>48	-	>48	-	>48	-

SE: values >1 define a synergistic effect between the implicated antioxidants, whereas values <1correspond to an antagonism.
Values represent the means (SD) (n = 3). Equal letters indicate equal means.
* Control oil (no antioxidants added) with an OSI = 6.63 (0.20) h at 110°C and 20 L/h air flow.
OSI, oxidative stability index; *SE*, synergistic effect; GPE, grape peel extract; –, not determined; TM, tocopherols mix; RE, rosemary extract.

Table 6. Synergistic effect of citric acid with thompson grape peel extract (GPE), tocopherols mix (TM), and rosemary extract (RE) on the oxidative stability index (OSI, in h Rancimat) in refined soybean oil*.

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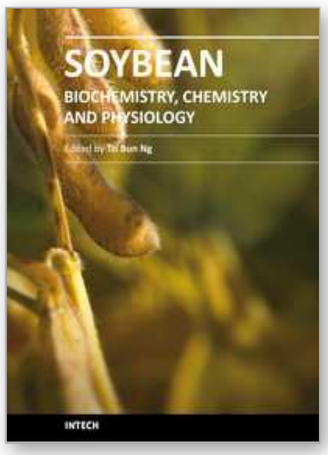

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