Characterization of natural edible oils regarding their quality and safety related constituents

MASTER THESIS

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HOANG QUOC TUAN

Abstract

This thesis provides data on some quality and safety related parameters in two species of corn oils (waxy- and conventional corn) and grape seed oils (red and white grape seeds). To date only limited information about characteristics, quality and safety related constituents of these oils, especially waxy corn oil and grape seed oils has been published. Therefore, it is of great interest to study the principal chemical constituents and quality- or safety-related parameters of these oils in order to explore potential benefits as well as hazards.

In this study, simultaneous distillation/solvent extraction-gas chromatography- mass spectrometry (SDE-GC-MS) was applied to study volatile compounds. The fatty acid composition was analyzed by gas chromatography. Phytosterols and tocopherols were analyzed by GC-MS and HPLC, respectively. The oxidative stability was investigated by the Rancimat method and the antioxidant activity was determined by β-carotene bleaching method. In addition, peroxide values, p-anisidine values and total phenolics of these oils were determined.

The major compounds, which were identified by SDE-GCMS, were aldehydes hydrocarbons and fatty acid esters. Linoleic acid ethyl ester was the most abundant component in corn oils. Red grape oil contained large amounts of fatty acid ethyl esters with practically none found in white grape oil.

Fatty acid composition was found to be dominated by linoleic acid, followed by oleic acid and palmitic acid in all oil samples.

Phytosterols were dominated by β-sitosterol. The predominant sterols of normal corn oil were higher than of waxy corn oil and the concentration of the three highest sterols found in red grape oil were considerably higher than in white grape seed oil.

Tocopherols were dominated by γ-tocopherol followed by α-tocopherol in corn oils.

The p-anisidine value of waxy oil was lower than in normal oil. However, the peroxide value was higher than of normal oil.

Red grape seed oil had the lower total phenolic content whereas its antioxidant activity was a bit higher than white grape seed oils that had a higher total phenolic content. Waxy corn oil had much higher total phenolic content than normal corn oil although its antioxidant activity was lower than was waxy corn oil.

Keywords: Red grape seed oils; White grape seed oils; Waxy corn; Corn oil; Flavour; Phytosterols; Tocopherols; Fatty acids; Quality;

List of Abbreviations

RGS	Red grape seed
WGS	White grape seed
W	Waxy
N	Normal
FA	Fatty acid
FFA	Free Fatty Acid
SMSDE	Simultaneous micro stream distillation/solvent extraction
GC-MS	Gas chromatography- mass spectrometry
HPLC	High performance liquid chromatography
PV	peroxide value
AV	p-anisidine value
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
BSTFA	Bis(trimethylsilyl)-trifluoroacetamide
TMCS	Trimethylchlorosilane
PS	Phytosterols
DPPH	2,2-diphenyl-1-picryhydrazyl
ABTS	2,2'-azinobis(3-ethylbenzenthiazoline-sulphonic acid)
IS	Internal Standard
NP	Normal phase
RP	Reserved phase
FID	Flame ionization detector
SPE	Solid phase extraction
SPME	Solid phase microextraction
OA	Oleic Acid
LA	Linoleic Acid
ALA	Alpha-Linolenic Acid
LDL	Low-density lipoprotein
SI	β-Sitosterol
CA	Campesterol
ST	Stigmasterol
AV	5-Avenasterol

I. Introduction

I.1 Key components of edible oils and their functions

I.1.1 Fatty acids

Edible oils differ in their composition of fatty acids, which are classified according to their degree of saturation. Fatty acids are saturated or unsaturated with one double bond (monounsaturated) or more than one double bond (polyunsaturated). The major unsaturated fatty acids are oleic acid (OA), linoleic acid (LA) and alpha-linolenic acid (ALA). The average levels of five major fatty acids in some edible plant oils are shown in table 1

Table 1. Fatty acid composition from different plant oils separated by capillary gas chromatography [1]

FAME[a]	Corn	Cotton	Grape	Olive	Peanut	Soy	Palm
14 : 0	0.2	0.8	0.0	0.0	0.0	0.0	0.9
16 : 0	13.0	27.3	7.0	10.2	12.5	11.6	43.7
16 : 1	0.0	0.8	0.1	0.7	0.0	0.3	0.1
17 : 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18 : 0	2.5	2.0	3.0	2.5	2.5	4.2	4.5
18 : 1	30.5	18.3	22.1	78.1	37.9	21.6	39.8
18 : 2	52.1	50.5	67.2	7.1	41.3	53.7	10.5
18 : 3	1.0	0.0	0.5	0.6	0.3	7.5	0.3
20 : 0	0.5	0.3	0.1	0.5	0.5	0.8	0.2
20 : 1	0.2	0.0	0.0	0.3	0.7	0.3	0.0
22 : 0	0.0	0.0	0.0	0.0	2.5	0.0	0.0
22 : 1	0.0	0.0	0.0	0.0	1.0	0.0	0.0
24 : 0	0.0	0.0	0.0	0.0	0.8	0.0	0.0

Edible oils are the main source of essential fatty acid for the body. Essential fatty acids are those fatty acids, that have integral functions in the body and cannot be synthesized endogenously and thus have to be supplied by the diet. Dietary fatty acids do also exert influence on plasma lipoproteins, which are correlated with cardiovascular health. LA and ALA are considered essential fatty acids, and are representing the omega-6 and omega-3 group, respectively. OA (an omega-9 fatty acid) is non essential because the body can synthesize it via delta-9 desaturation of stearic acid.

Essential fatty acids affect the function of the cardiovascular, reproductive, immune and nervous system [3-7]. In most animal organisms, LA and ALA can be transformed to their higher unsaturated derivatives, arachidonic acid from LA, eicosapentaenoic acid and docosahexaenoic acids (DHA) from ALA, by a series of desaturation and elongation reactions [8].

I.1.2 Lipid oxidation and its products

Unsaturated fatty acids tend to oxidize especially in the presence of radicals, singlet oxygen, metal catalysts or lipoxygenase enzymes. Many products of fatty acid oxidation exert aroma properties. Flavour compounds formed in this process may have positive or negative (off-flavour) characteristics. The volatiles formed from chemical oxidation of lipids are responsible for the off-flavor, referred to as oxidative rancidity. In contrast, enzymatic oxidation and β-oxidation of oils are considered responsible for the characteristic of the oil [9]. However, flavor compounds in edible oils not only are formed via lipoxygenase and beta oxidation pathway, but also from amino acids such as valine and leucine, which can be converted to volatile compounds, including methyl-branched alkyl and acyl compounds of esters, and into methyl-branched alcohols, which have the potential to change the sensory perception in edible oils such as olive oils [9].

Autoxidation or free radical oxidation

The classical oxidation route depends on the presence of free radicals which interact with intact olefinic chains of fatty acids by forming fatty acid radicals. The three stages of auto-oxidation are: initiation, propagation and termination. The initiation can occur by the action of external energy sources such as heat, light or high energy radiation, or by chemical initiation involving metal ions or metallo-proteins such as hemin. This process is described in some summary reaction below.

Initiation:

$$RH + O_2 \xrightarrow{\text{catalyst}} R\cdot + \cdot OOH$$

$$RH \xrightarrow{\text{catalyst}} R\cdot + \cdot H$$

Propagation:

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

$$RO_2\cdot + RH \rightarrow RO_2H + R\cdot$$

Termination:

$$R\cdot + R\cdot \rightarrow R\text{–}R$$

$$RO_2\cdot + R\cdot \rightarrow RO_2H$$

Photo-oxidation

Photo-oxidation is an alternative route to the free radical mechanism. It occurs when light and certain photosensitizer molecules are present. Photo-oxidation involves the formation of peroxides in a direct reaction of singlet oxygen with unsaturated lipids. The singlet oxygen ($^1O_2$) emerges during a r eaction of sensitizers (chlorophyll, haemoglobin, myoglobin, erythrosine, riboflavin and heavy metal ions) with atmospheric oxygen. Photosensitization can also occur *in vivo*. Photooxydation by singlet oxygen is 1,500 times faster with methyl linoleate than auto-oxidation and, as formerly stated, it reacts directly with double bonds by addition at either end of the double bond, producing an allylic peroxide in which the double bond has been shifted in the trans configuration. Two mechanisms have been proposed for photo-oxidation.

Type 1

$$\text{Sensitiser} + X + h\nu \rightarrow [\text{Intermediate I}]$$

$$[\text{Intermediate I}] + {}^3O_2 \rightarrow \text{Product} + {}^1\text{Sensitiser}$$

$${}^1\text{Sen} + h\nu \rightarrow {}^1\text{Sen}^{*\,1} \rightarrow {}^3\text{Sen}^*$$

$${}^3\text{Sen}^* + X\ (\text{acceptor}) \rightarrow [\text{Intermediate I}]$$

$$[\text{Intermediate I}] + {}^3O_2 \rightarrow XO_2 + {}^1\text{Sen}$$

Type 2

$$\text{Sensitiser} + {}^3O_2 + h\nu \rightarrow [\text{Intermediate II}]$$

$$[\text{Intermediate II}] + X \rightarrow \text{Product} + \text{Sensitiser}$$

$$\text{Sensitiser} + h\nu \rightarrow {}^1\text{Sensitiser}$$

$${}^1\text{Sensitiser} \rightarrow {}^3\text{Sensitiser}$$

$${}^3\text{Sensitiser} + {}^3O_2 \rightarrow \text{Sensitiser} + {}^1O_2$$

Secondary oxidation products are formed from hydroperoxides. Lipid hydroperoxides are very unstable compounds and break down in several steps, yielding a wide variety of decomposition products. Each hydroperoxide produces a set of initial breakdown products that are typical of the specific hydroperoxide and depend on the position of the peroxide group in the parental molecule. Peroxides first decompose to an alkoxy free radical which is broken down, mainly by cleavage on either side of the carbon atom bearing the oxygen atom.

Lipoxygenase (LOX) route

LOX-catalyzed oxidation differs from the free radical reaction by the formation of hydroperoxides at a certain position of the chain. Although the basic stoichiometry of LOX is the same as for autoxidation, LOX is very specific about the substrate and how the substrate is oxidized. For example, LA it is oxidized at positions 9 and 13 by LOX isolated from most natural sources. LOX prefers free fatty acids as substrates and the region-specificity and stereo-specificity of the reaction are illustrated in the figure 1.

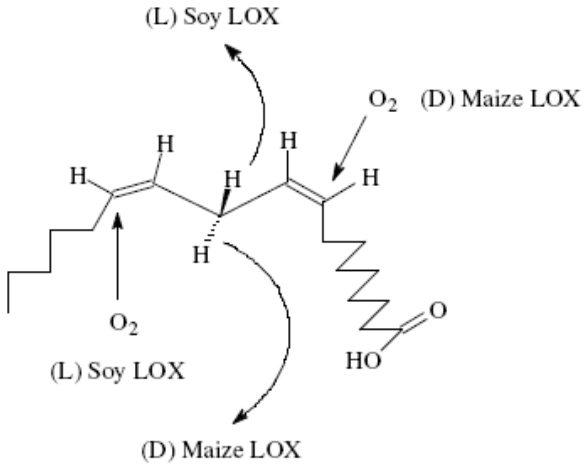

Fig.1. Steroespecific oxygenation of linoleic acid by lipoxygenase.

The lipoxygenase pathway in plants is initiated by the release of enzymes when vegetable tissues are disrupted. The reaction pathway involves a series of enzymes that oxidise (lipoxygenase) and cleave (hydroperoxide lyase) polyunsaturated fatty acids to yield mainly aldehydes. These are subsequently reduced to alcohols (by alcohol dehydrogenase) and esterified to produce esters or lactons [9].

β-Oxidation

β -oxidation can be involved in flavor formation, as for example in fruit such as pear, where decadienoate esters produced from β-oxidation of LA are flavour key compounds. [10].

I.1.3 Phytosterols

Phytosterols are present in all plant oils. Phytosterols regulate the fluidity and permeability of membranes and play an important role in adaptation of membranes to temperature. Cholesterol is the main sterol in animal cells but is only present in small amounts in plant cells [11-17].

Like cholesterol, phytosterols are made up of a tetracyclic cyclopenta (α) phenanthrene ring and flexible side-chain at C17. They are mainly 28-29 carbon atom steroid alcohols. Phytosterols differ from cholesterol by additional methyl or ethyl group in the side chain or be double bonds in the side chain (Figure 2).

In edible plant oils, a lot of different types of phytosterols have been reported. However, the most abundant in most oil are β-sitosterol (24-α-ethylcholesterol), campesterol (24-α-methylcholesterol) and stigmasterol (Δ^{22}, 24-α-ethylcholesterol). In nature, sterols can be found as free sterols or as four types of conjugates, in which the 3β-hydroxyl group is esterified to a fatty acid or a hydroxycinnamic acid, or glycosylated with a hexose (usually glucose) or a 6-fatty acyl hexose. Glycosides are the most common form found in cereals. Among vegetable oils, corn and rapeseed contain the highest amounts of phytosterols [18].

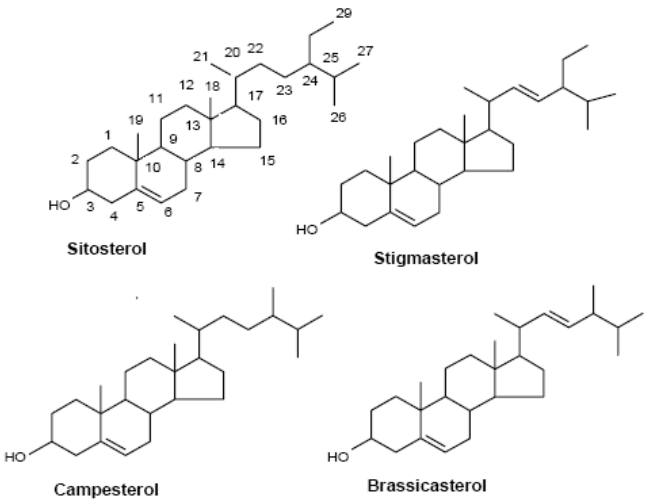

Figure 2. Chemical structure of some plant sterols (beta-sitosterol, campesterol, stigmasterol and brassicasterol)

The potential of phytosterol in decreasing serum low-density lipoprotein (LDL) cholesterol levels and thus in protecting against cardiovascular diseases, has led to the development of functional foods enriched with plant sterols [19-21]. At present, several functional food product types such as spreadable fats, yoghurts and milk, with free phytosterols or phytosteryl fatty acid esters or phytostanyl fatty acid esters added at high levels, are available in the market, especially in several European countries [22-24].

Several physiological functions have been described for phytosterols: S terols in soybean have been shown to inhibit the increases of plasma and liver cholesterol and

also reduce the severity of atherosclerotic lesions [25]. Beta- Sitosterol has been used as a supplement and as a drug for lowering serum cholesterol levels in hypercholesterolemic individuals. From this application, several studies have been made on the effect upon blood lipids of 4,4-dimethyl plant sterols or stanols, either in their free or esterified form. These compounds reduce total cholesterol and LDL-cholesterol levels through a reduction in cholesterol absorption. Properly solubilized free sterols and esterified sterols possess similar cholesterol-lowering activity. Phytosterols have also shown the following activities in animals: anti-cancer properties (with a beneficial effect upon the inhibition of colon cancer development), and anti-atherosclerotic, anti-inflammatory and anti-oxidative effects [26-29].

The beneficial effect of phytosterols on serum LDL-cholesterol levels has led to many food-related studies of these compounds. Therefore, today this increased attention on phytosterol research prompted the development of method for accurate measurement of these compounds in food.

I.1.4 Tocopherols and tocotrienols

Tocopherols are recognized as the principal natural antioxidants in edible oils [30-32]. In some plants and plant oils, considerable amounts of tocotrienols are found beside the tocopherols (table 2).

Table 2. Tocopherol and tocotrienol content (μg/g oil) of some edible oils[33-38]

Tocopherol/ tocotrienol	Palm oilc	Olive oil	Rapeseed oil	Corn oil	Sunflower oil
α-T	377	96	180	222	671
β-T	1	6	-	1	23
γ-T	4	12	340	570	4
δ-T	-	-	-	23	-
α-T3	52	-	-	54	-
β-T3	2	-	-	11	-
γ-T3	132	-	-	62	-

α-T, β-T, γ-T, δ-T (α, β, γ, δ-tocopherol); α-T3, β-T3, γ-T3 (α, β, γ-tocotrienol), respectively.

Both tocopherols and tocotrienols are further divided into individual compounds that are designated by the Greek letter prefixes α, β, γ, δ depending on the number and

position of methyl substitution on the chromanol ring. Tocopherols and tocotrienols are derivatives of 2-methyl-6-chromanol with a side chain of three terpene units. Tocopherols have the terpenoid side chain in saturated form, while the side chain in tocotrienols is unsaturated.

The main interest in vitamin E is its natural antioxidant capacity. It is considered to be one of the most efficient biological antioxidants for breaking free radical chain reactions. Thus, it protects cytoplasmic membranes from oxidation and guards low-density lipoproteins from dangerous lipid peroxidation processes. The antioxidant activities of tocopherols and tocotrienols are due to their abilities to donate their phenolic hydrogen to lipid free radicals and thereby retard the autocatalytic lipid peroxidation [39]. In general, α-tocopherol shows better antioxidant activity than γ-tocopherol in fats and oils, but at higher concentration γ-tocopherol is more active as antioxidant. For tocotrienols, in oils and fats, γ-tocotrienol was found to be a better antioxidant than α-tocotrienol, and tocotrienols were found have a higher antioxidant properties than their corresponding tocopherols [39]. In the field of cancer chemotherapy, as an example, tocotrienols display better anti-tumour activity than α-tocopherol. Tocotrienols reduce plasma cholesterol levels, as well as those of other lipids and non-lipids related to risk factors for cardiovascular diseases [40]. Tocopherols, especially in living cells, are acting together with other antioxidants such as ascorbic acid [41, 42].

Usually, the authentication of vegetable oils is conducted by quantification of chemical components present in edible oils and some authentication studies are based on tocopherol determination [43].

I.1.5 Carotenoids

Carotenoids are tetraterpenoids which show light absorption in the visual range at around 450 nm which makes them appear in a yellow to deep red colour. Carotenoids have a good antioxidant activity by scavenging free-radicals [44], They also seem to be valuable for cancer prevention. Epidemiological studies have shown that people with high β-carotene intake and high plasma levels of β-carotene have a significantly reduced risk of lung cancer [45]. However, there are some studies that report that, among smokers, carotenoid supplements can cause an increased risk for cancer. Although these studies are somewhat questioned and were intensively discussed in the last decade [46, 47]. Beta-carotene, α-carotene and β-cryptoxantin in edible oils are

also called pro-vitamin A, due to ability cleavage of the molecule at C 15-15' to form vitamin A [48].

I.1.6 Squalene

Squalene is a naturally occurring triterpenoid hydrocarbon found in numerous edible vegetable oil, especially in pumpkin (89mg/100g) and quinoa (58mg/100g) seed [49] and in fish liver oil. After intake, squalene is usually stored in the skin tissues and plays a number of important roles. Squalene is the last metabolite preceding sterol ring formation in the biosynthesis cholesterol pathway. It is also a potential oxidation inhibitor as it can protect cells against free radicals, strengthen the body's immune system and decrease the risk of various cancers [50].

I.2 Corn oil

Corn oil is extracted from corn germ (seed) by expelling and/or solvent extraction. The germ seeds are isolated during wet milling — a process designed to isolate starch from corn kernels. Corn oil, thus, is a by-product of the starch industry.

Corn oil is marketed as healthy oil, low in saturated fatty acids, rich in linoleic acid, with a very low ALA, and a high oxidative stability. With growing concern over the omega-6/omega-3 ratio, the low level of ALA acid seems to be a d isadvantage, especially with regards to marketing. Typically the oil contains C16:0 (10.9%), C18:0 (2.0%), C18:1n9 (25.4%), C18:2n6 (59.6%), and C18:3n3 (1.2%) [51].

Corn oil contains 1.3 to 2.3% of unsaponifiable material, including free and esterified sterols, tocopherols, and some squalene (~ 0.2%). Total sterols are mainly composed of β-sitosterol (55 to 67% of total sterol), campesterol (19 to 24%), Δ5-avenasterol (4 to 8%), stigmasterol (4 to 8%), and other minor sterol [52-54]. γ- Tocopherol is the most abundant tocopherol, followed by α-tocopherol and δ-tocopherol. High levels of carotenoids have been also reported in corn kernels, with primary (74–86%) being localized in the endosperm, 2–4% in the germ, and 1% in the bran. The most abundant carotenoids in corn kernels are lutein and zeaxanthin [55].

The important properties of corn oil include its pleasing flavor, its high levels of polyunsaturated (essential) fatty acids, and its low levels of saturated fatty acids and contain trace only of ALA. ALA oxidizes more than twice fast as LA and 20 times fast than OA [56]. Furthermore, ALA tends to polymerize, especially at high temperature. The low content of ALA is one of the reasons for a good stability during

frying of corn oil. Some studies have demonstrated that, compared to canola and soybean oils, corn oil produced the lowest levels of oxidation products and retained the highest levels of tocopherols, during five days at continuous frying temperatures [57, 58]. Another study on oxidative stability revealed that corn oil hybrids with higher levels of saturated fatty acids were more stable than traditional corn oils [55].

The antioxidant properties of tocopherols in corn oil may be involved in combating atherosclerosis by preventing the oxidation of low-density lipoproteins. Another recent study showed that the particular ratio of individual tocopherols in corn oil (a high ratio of γ- tocopherol/α-tocopherol) may achieve better protection against DNA damage than α-tocopherol alone [59]. Others have demonstrated beneficial effects of corn oil on blood pressure, platelet aggregation and diabetes [58].

I.3 Grape seed oils

Wine grapes are among the oldest cultivated plants in the Middle East/ European culture. Also today, they are of worldwide interest for nutritional purposes including raw and dried consummation, wine production, but also extracts of their peels and seeds are used in pharmaceutical applications related to their content of polyphenolic substances such resveratrol, phytosterol, tocopherol. Production of grapes generally is situated in moderate-warm climate zones such as Mediterranean or American west coast

Grape skin and seeds provide beneficial substances for lowering incidence of atherosclerosis and coronary heart diseases such as polyphenols [60].

Grapes seed oil is composed of approximately 90% poly- and monounsaturated fatty acids, which are responsible for its value as nutritive edible oil, particularly of linoleic acid (58–75%) followed by oleic acid (10-25%), palmitic (8-10%) and stearic (4-5%) [61]. Unrefined oils contain bioactive compounds including tocopherols and tocotrienols (5–52 mg/100 g) and numerous phenolic components, consisting of low and high molecular plant phenolics that may contribute to beneficial effects of vegetable oils. However phenolic compounds, maily proanthocyanidins, are not recovered with the oil after pressing and remain mainly in the aqueous phase due to their polar nature [62].

It is claimed that high-quality grape seed oil is characterized by light flavor with fruity touches, high smoke point (216^0C), high digestibility, and a s light increase in viscosity when used for batch frying [63].

Extraction methods usually used for seed oils are solvent and supercritical CO_2 extraction [64]. Extraction by physical means using a hydraulic or a screw press is also possible. Most of the grape oil on the market is obtained from solvent extraction and treated by refining methods. Thereby, the many beneficial effects, including the pleasant taste, are lost. Cold pressed grape oil, on the other hand, has a very fine, but fragile aroma, which can easily deteriorate and change into a mouldy and unpleasant taste. Since grape oil can be regarded as specialty oil, there is still very little information on the properties of the grape seed oil obtained by different extraction methods.

I.4 Measuring the quality of edible oils

I.4.1 Peroxide value (PV)

Peroxide value (PV) is one of the methods for determination of primary lipid oxidation products. The PV is expressed as milliequivalents oxygen per kg of fat/oil [65]. The number of peroxides present in edible fats and oils is an index of their primary oxidative level. The peroxide value test used is an index of its status of preservation. In fact, the lower the peroxide value, the better the fat or oil quality and its status of preservation. However, this method, although simple, does have disadvantages in that it tells nothing about the history of the oil – peroxides are increased at the beginning of oxidation progress but then will be decreased– especially if the lipid has seen high processing temperatures. Indeed the PV of oil may be significantly reduced by heating in the absence of oxygen. Chemical reactions involved in PV determination are given below:

$$ROOH + 2H^+ + 2KI \rightarrow I_2 + ROH + H_2O + 2K^+$$

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

I.4.2 p-anisidine value (p-AV)

The p-anisidine value (p-AV) estimates the amount of α or β-unsaturated aldehydes (mainly 2-alkenals and 2,4 dienals), which are secondary oxidation products in fats and oils. The aldehydes react with p-anisidine to form a chromogen that is measured spectrophotometrically [66]. An example of the reaction of p-anisidine with aldehydes is shown in Fig.3

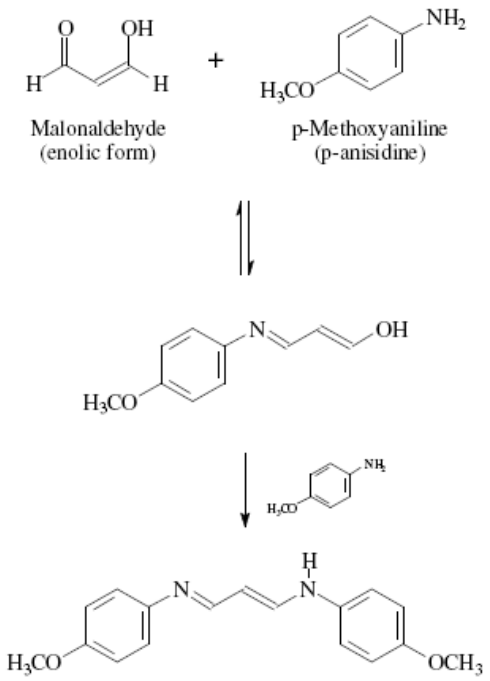

Fig. 3. Possibly reaction between p-anisidine reagent and maloaldehyde.

I.4.3 Rancimat method

The Rancimat test is a simple, quick and efficient way to screen the effectiveness of antioxidants used in liquid fats and oils. The method is an accelerated oxidation test in which the oil or fat to be tested is kept at elevated temperatures whilst exposing the sample to air accelerating the oxidation process of the oil. This results in autoxidation in a few hours, instead of weeks or months. Metabolites produced from the oxidation of the oil in the reaction vessel are driven off into a measuring vessel which measures changes in conductivity. At a cer tain point, at which there is a sudden change in conductivity caused by the formation of volatile polar compounds, the passed time is defined as the induction period, which is a measure for oxidative stability.

I.4.4 β-carotene bleaching method **[67]**

This method is based on the coupled oxidation of β-carotene and a fatty acid such as linoleic acid, methyl linoleate or methyl linolenate [68]. It is base on measuring the beaching of β-carotene resulting from oxidation by degradation product of linoleic acid (or methyl linoleate). The method, modified by Taga and his coworkers, uses a solution of β-carotene in chloroform showing an absorbance at 470 nm between 0.6 and 0.9 [69]. It is mostly used for evaluating the antioxidative power of phenolic extracts.

I.4.5 Radical-scavenging methods

The main mechanism of antioxidants in food is the cleavage of radicals. Several methods have been developed [70], in which the antioxidant activity of substances is assessed indirectly by measuring the scavenging of synthetic radicals in polar organic solvents, e.g. methanol, at room temperature. Synthetic radicals, which are commonly used for these assays are 2,2- diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzthiazoline-sulphonic acid) (ABTS) radicals. In the DPPH test, the decrease of absorbance at 515 nm of the scavenging of DPPH radicals is measured, which occurs due to reduction by the antioxidant (AH) or reaction with a radical species ($R^{\cdot}$).

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{–}H + A^{\cdot}$$

$$DPPH^{\cdot} + R^{\cdot} \rightarrow DPPH\text{–}R$$

Fast reaction of DPPH radicals occurs with some phenols e.g. alpha-tocopherol, but slow secondary reactions may cause a progressive decrease in absorbance, so that the steady state may not be reached for several hours. Most papers, in which the DPPH method has been used, report the scavenging after 15 or 30 min reaction time. The data is commonly reported as EC50, which is the concentration of antioxidant required for 50 % scavenging of DPPH radicals in the specified time period. The ABTS radical cation is more reactive than the DPPH radical, and reaction of the ABTS radical cation with an antioxidant is taken as complete within 1 min. The method of generation of the radical cation has changed several times since the method was first described. The most recent method describes the use of potassium persulphate to oxidize ABTS to the radical cation. The radical scavenging activity assessed by the ABTS method has been expressed as the TEAC (trolox equivalent antioxidant capacity) value in most papers employing this method. ABTS and DPPH methods may be useful for screening antioxidants, but antioxidant effectiveness in foods must always be studied by other methods because their activity in foods is dependent on a variety of factors including polarity, solubility, and metal-chelating activity [70].

I.5 Analysis of compounds, composition in edible oils

I.5.1 Determination of total phenolics content

Several methods have been developed for determination of total phenolics content [71]

I.5.1.1 Folin-Denis Assay

The Folin-Denis assay is the most widely used procedure for quantification of total phenolics in plant materials and beverages. Reduction of phospho-molybdicphosphotungstic acid reagent to a blue-colored complex in an alkaline solution occurs in the presence of phenolic compounds [72]. Swain and his coworker [73] modified this method for routine analysis of a large number of samples. The Folin-Denis assay is also used for determination of total phenolics using the AOAC official method [74].

I.5.1.2 Folin-Ciocalteu method

The Folin-Ciocalteu method is often used to determine the total content phenolics in food. However, Folin-Ciocalteu reagent is not specific and detects all phenolic groups found in extracts including those found in the extractable proteins. Another disadvantage of this method is the interference of reducing substances such as ascorbic acid with the determinations. The absorbance is often measured at 765 nm, however at 725 nm have been used, and the content of phenolics is expressed as gallic acid or catechin equivalents.

I.5.1.3 Prussian Blue Assay

Price and Butler introduced the Prussian blue method in 1977 for determination of phenolics from sorghum grain, which was later reevaluated and modified by Deshpande and Cheryan [75] for analyzing total phenol in dry beans. The principle of the method is the reduction of Fe^{3+} to Fe^{2+} by polyphenolic compounds and formation of a ferricyanide-ferrous ion complex, also called Prussian blue. The ability of polyphenolic compounds to reduce the ferric ion depends on their hydroxylation pattern and the degree of polymerization.

I.5.2 Tocopherols analysis

Many techniques are used in the analysis of tocopherols and tocotrienols. In the 1970s, the Association of Official Analytical Chemists' (AOAC) International Official Methods of Analysis presented several conventional analysis methods for α-tocopherol and a tocopheryl acetate [76]. These methods are based on colorimetric or

polarimetric analysis and thin-layer chromatography techniques. The problem with these methods is that many interfering compounds are not efficiently eliminated and have an impact upon the reliability of results. Gas chromatography (GC) methods were developed to determine tocopherols and tocotrienols in the late 1980s. The analytical sensitivity is significantly improved with the GC method compared with the colorimetric and polarimetric methods. However, because the boiling points of tocopherols and tocotrienols are high and relatively close together, GC separation of tocopherols and tocotrienols is difficult to achieve. To decrease their boiling points and avoid decomposition of tocopherols and tocotrienols at the high analysis temperature, a d erivatization procedure is usually carried out during sample preparation. Usually, tocopherol are derivatized to trimethylsilyl (TMS) ethers or to acetyl esters [77].

Currently, high-performance liquid chromatography (HPLC) methods are most widely used in the analysis of tocopherols and tocotrienols. Several chormatographics methods are available in the literature, and attempts to separate tocopherols and tocotrienols have been performed on both normal and reversed phase columns [78]. Although reversed phase columns are generally known to have the advantages of better stability and longer durability than normal phase columns, the latter are capable in separating β and γ isomers of tocopherols and tocotrienols. Moreover, they provide the possibility of operating with organic solvents, allowing a high solubility of lipids and can often skip the saponification step [77].

The most generally used mobile phases employ a b inary solvent system based on hexane together with a variety of organic modifiers including diethyl ether, diisopropyl ether, tert.-butyl methyl ether , methanol, isopropanol, 1,4- dioxane, or acetic acid ethyl ester. The use of ethers includes a risk of peroxide formation and the use of alcohols is accompanied by difficulties in achieving accurate mobile phase proportions since relatively very small volumes of these alcohols are needed because of their very high polarities. Compared to other ethers with similar polarity, tert.-butyl methyl ether was reported to show a lower risk of peroxide build-up [79]. Fluorescence detectors are generally used for the determination of vitamin E, because of their higher sensitivity and specificity compared with ultraviolet and evaporative light scattering detectors [79].

I.5.3 Phytosterol analysis

The determination of phytosterols in plant material is usually performed by capillary gas chromatography (GC), with flame ionization detection (FID) or mass spectrometry (MS) to confirm peak identity, although HPLC can also be used. However, it is GC of sterol TMS ether (or acetate derivatives) coupled with electron impact or chemical ionization MS that provides the most effective resolution, identification and quantisation [80].

Typically, the analysis of individual sterols includes the extraction of lipids, saponification or acid hydrolysis and saponification to liberate sterols, extraction of unsaponifiable matter and separation/partial purification of sterols, the formation of sterols derivatives, and their separation by capillary GC.

I.5.3.1. Sample preparation

One of the most important and critical steps in the analysis of sterol is sample preparation. Oxidation shoud be minimized by blowing nitrogen, or by the addition of anti-oxidants such as pyrogallol [81]. A reliable sample preparation method has been developed for the GC measurement of the total contents (including free, esterified and glycosylated forms) of individual sterols in foods [25]. However, oils can be directly saponified and do not require additional clean-up steps.

Solvent extraction

Phytosterols in seed oils or food can be isolated by solvent extraction with chloroform–methanol [82], chloroform–methanol–water [83], hexane [25], methylene chloride [84] or acetone [85], followed by alkaline hydrolysis and chromatographic purification to obtain enriched total sterols.

The solvent system chloroform–methanol–water [83] has been applied by Thompson and Merola [86] to determine cholesterol in multicomponent foods as an alternative method to the AOAC official [87], and has been reported to be capable of determining major non-glycosidic plant sterols. A modification of this method has been applied to the determination of plant sterols in research diets [88]. A solid phase extraction (SPE) method using neutral alumina cartridges has been applied for the extraction of free and esterified sterols from oils and fats [25].

Lipids from vegetables can also be extracted by supercritical fluid extraction (SFE) with supercritical CO_2, after which sterols can be enriched and isolated following saponification or supercritical fluid fractionation (SFF) together with additional sample clean up using different chromatographic techniques [89].

To isolate the non-glyceridic components (hydrocarbons, tocopherols, sterols and sterol esters) from the slightly more polar triacylglycerol matrix, solid phase extraction SPE (silicagel) has been used [18]. Prior to SPE, the free hydroxyl group of sterols and tocopherols were silylated to reduce their polarity. The eluted fraction was then analyzed by GC. As compared to solvent extraction, SPE is an environmentally more friendly technique which provides a more convenient way for sample extraction, with a reduced loss of sterol analytes.

Saponification

Some saponification methods have been described strong sodium hydroxide in ethanol saponification (alkaline saponification) with heating at 70^{o}C for 90 min or at 80^{0}C for 30 m in; strong sodium hydroxide saponification with BF_3/methanol methylation, the sample is boiled for 4 min; mild saponification using potassium hydroxide in methanol at room temperature for overnight [90]. After saponification the mixture should be diluted with water prior to liquid-liquid partitioning of unsaponifiable lipids into a solvent. The portions of alkaline alcohol, water, and solvent should be carefully controlled to avoid losses of phytosterol in the aqueous phase [90]. Solvents used to extract phytosterols include hexane, heptane, cyclohexane, chloroform, isopropanol, ethyl ether [25]. A comparison of different solvent systems, involving hexane, cyclohexane, and hexane-diethyl ether was done by Toivo and his coworkers. They found no statistical differences among them but they preferred cyclohexane than others in their method. One advantage of using cyclohexane instead of diethyl ether to extract nonsaponified lipids is that it dissolves much less water than diethyl ether which makes further steps in the analysis simpler [91].

I.5.3.2 Determination

Gas chromatography

Capillary gas chromatography (GC) has been the technique of choice for the analysis of sterols and related compounds. Capillary columns offer shorter analysis times and better chromatogram resolution and high thermal stability compared to packed columns [25]. With high-temperature capillary GC columns (e.g. phenylmethylsilicone) sterol, sample assays can be achieved with high detection sensitivity and component resolution. However, glass fused-silica capillary columns and packed columns can also be used to analyze sterols [92].

In a typical analysis, GC is interfaced with FID to monitor analytes in the column effluents or to MS for structural identification and quantisation by single-ion monitoring (SIM) or multiple-ion monitoring (MIM) [92].

Although the separation of sterols and stanols as such without derivatization is possible [93], the resolution of a sterol and its corresponding stanol is not as good as that of their trimethylsilyl (TMS) derivatives. Thus, sterols and stanols are conventionally transformed into derivatives that yield improved peak shape, resolution and sensitivity, and a higher stability for the thermally labile unsaturated sterols [92]. Sterols are commonly analyzed as their trimethylsilyl (TMS) or acetate derivatives. The former are more suitable for the GC–MS characterization and quantitation of sterols.

Derivatizing agents usually employed are: N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) in anhydrous pyridine and bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS) (1:1, v/v) added to a dried sample [93].

As in any chromatographic practice, the GC peaks of sterols are represented by their retention times. They can be express in absolute time or relative to a reference standard or an internal standard (IS) to eliminate analytical fluctuations of instrument operation conditions as well as other experimental variables.

Internal standard most commonly used in sterol determinations have been betulin, 5α-cholestane, 5β-cholestan-3α-ol (epicoprostanol) and 5α-cholestan-3β-ol [94-96]. An IS should be similar to the analytes, and cholestane is a sterol hydrocarbon lacking the position 3 hydroxyl group which is typical of sterols, while betulin has two hydroxyl groups and its structure and chemical properties differ even more from those of sterols. Thus, cholestane and betulin are not the best choices [25]. Cholestanol and sitostanol are not always applicable as IS, because they may occur in many fats.

Liquid chromatography

Compared to GC, HPLC offers the advantage of operating under milder column temperatures, and depending on the detector, non-destructive detection conditions. It therefore seems suitable for the analysis of thermally unstable compounds. However, the high lipophilicity of sterols can make sample processing and chromatography difficult [25].

Normal-phase (NP) and reversed-phase (RP) HPLC techniques have been widely used for the analysis of lipid classes and individual sterols in various sample matrices. RP-HPLC uses less volatile polar organic solvents in water, and offers ready equilibration in a bonded silica stationary phase with the mobile phase solvents, compared to NP-HPLC. In general, RP-HPLC has been more frequently and more widely used than NP-HPLC for the separation of individual sterols [25]. Sterol detection has been carried out by UV (200–210 nm), photodiode array detection (DAD), refractive index (RI) detection, evaporative light scattering detection (ELSD) and mass spectrometry (MS) [92].

I.5.4 Fatty acid analysis

Derivatization of fatty acids prior to GC analysis is necessary to liberate the FA from the glycerol molecular increase the volatility of the substances, to improve separation, and to reduce tailing. Normally, for analyzing fatty acids in most food matrixes, base-catalyzed or acid-catalyzed transesterification methods can be used.

I.5.4.1 Transesterification

Base-catalysed transesterification

For base-catalyzed transmethylation, $NaOCH_3$, NaOH, and KOH can be used. In all alkali methods, free fatty acids are not esterified. Morover, utmost care must be taken to exclude water from the reaction medium to prevent hydrolysis of lipids. Sodium methoxide in anhydrous methanol, prepared simply by dissolving metallic sodium in dry methanol, is the most popular reagent, but potassium methoxide or hydroxide have also been used as catalysts [97].

Acid-catalysed esterification and transesterification

FAs are transesterified in anhydrous methanol in the presence of an electrophilic catalyst such as HCl, H_2SO_4, or BF_3. Several procedures for the preparation of anhydrous methanol–HCl exist [98]. In this method, contrary to base-catalyzed methods, free fatty acids are also esterified, which is described in two equations below.

$$RCOOR' + CH_3OH \underset{}{\overset{H^+}{\rightleftharpoons}} RCOOCH_3 + R'OH$$

$$RCOOH + CH_3OH \underset{}{\overset{H^+}{\rightleftharpoons}} RCOOCH_3 + H_2O$$

If water is present, it may prevent the reaction going to completion. However, the influence of water in acidic transesterification methods is not as strong as in alkali

reactions [97]. In acidic reactions, the methylation of FFAs just takes some minutes. However, transesterification of TG to methyl esters also need considerably longer reaction times than FFAs. [97].

I.5.4.2 GC analysis

HPLC has also been used for analyzing fatty acids. However, GC is the best choice to identify and quantify FAs, since FAs are lacking good spectral properties [98]. Flame ionization detection (FID) is commonly used for FA analysis by GC. However, other kinds of detectors such as photo ionization detector, electrolytic conductivity detector , FT-IR,… have also been used, but only for special purposes [98]. The carrier gases may be nitrogen, helium, or hydrogen. Hydrogen is preferable for faster analysis, better separation of substances, and general usefulness with packed- and capillary column, but it not suitable for detection MS [99]. Almost all organic material, including fatty acids, can be detected by FID, except formic acid, which does not respond in the FID. So, the nitrogen-, phosphorous-sensitive detector (NDP) is useful in the detection of low concentration of formic acid and other fatty acid, including very long chain FAs [98]. Another kind of detector, which is a very powerful analysis of FAs is mass spectrometry detector (MS) [100]. It is widely used today for diagnostic fragmentation of saturated and unsaturated fatty acids, analysis of branching positions in fatty acids and for identification of substituted FA [101].

Basically two types of columns are suitable for separation of complex FA mixture: Wax colums (PEG phase-polyethyleglyco) and Polysiloxane columns with different amounts of cyanopropyl and phenylgroups as polar modifiers.

The technique most often used for injecting FAMEs into the GC is the classical split injection mode, in which the sample is introduced into a hot injector chamber. The advantage of split mode is that samples are applied in narrow band, thereby avoiding peak broadening. Typical split vent ratios used in FAME analysis range between 1:10 and 1:200. The major disadvantage of the classical split injection technique in FAME analysis is discrimination between high-boiling and low-boiling compounds, which can be minimize by the correct injection technical [102]. Splitless injection is used when the sample amount is too low to waste analyses through the split exits.

I.5.5 Flavour analysis

I.5.5.1 Sample preparation

Sample preparation for the subsequent chromatographic analysis of complex matrices still remains a difficult step that is not only time consuming, but may also alter the composition of the sample to be analyzed. Thus, many methods have been developed for isolating volatile compounds from edible oils, but several difficulties may be observed when analyzing volatile compounds from oils, because all these compounds are at least partially fat-soluble and, hence, the efficiency of the isolation procedure can be significantly reduced. So far, the use of purge and trap techniques and headspace sampling, thermal desorption-gas chromatographic analysis as well as simultaneous purging and solvent extraction has been proposed [103, 104]. In summary, the different sampling techniques offer a number of individual advantages but also suffer from specific limitations.

Headspace analysis

The advantages of headspace sampling for recovery of volatile compounds associated with aroma have long been recognized [105]. The original headspace procedure involves static recovery in which sample is equilibrated in a sealed container at a controlled temperature and the headspace sample is withdrawn via a septum. Applications of static headspace sampling are limited by a number of factors including low sensitivity. The dynamic procedure (termed purge and trap) involves passing an inert gas through the sample and collecting the stripped volatile constituents in a trap. The equilibrium between the food and head-space is constantly removed resulting in improved sensitivity [104]. Many studies suggested that head-space analysis accounts for the release of volatiles in the food and in edible oil samples which is more significant than total volatile analysis for the correlation of chemical analysis with sensory judgement. It allows more rapid analyses and analysis of volatiles without sample destruction. Extracts measured by head-space sampling contain fewer compounds than by solvent extraction or distillation methods.

Solid phase extraction (SPE)

SPE is used both in the isolation and clean-up of aroma extracts. Various problems, when first introduced, were associated with sorption of volatiles on the cartridge walls giving low recoveries, carryover and high blank values, large differences in properties between nominally equivalent sorbents from different manufacturers and large batch-to-batch variation, leading to poor reproducibility. However, many of these limitations

have been overcome and there is now a comprehensive range of phases available to allow selection of a suitable sorbent to retain the aroma compounds while allowing elution of interfering materials or vice versa. While head-space sampling is is more commonly use, SPE is better for recovery of semi-volatile aroma compounds [106].

Solid phase microextraction (SPME)

SPME is a solvent free technique for extracting analysts from a variety of matrices by partitioning them from a liquid or gaseous sample into an immobilized stationary phase. It uses a very simple setup and requires no additional instrumentation other than a conventional GC with a traditional injection port. The coated SPME fibber is immersed directly into an aqueous sample or into the headspace above a liquid or solid sample. Equilibrium is reached faster in headspace SPME than in immersion SPME as there is no liquid to impede diffusion of the analyst onto the coating. It has been shown to be a very sensitive method for headspace analysis and has been recommended for the quantitative analysis of flavour and fragrance compounds [107]. Headspace SPME has also been tested and has compared favourably to the commonly used purge and trap type analysis. The selection of an appropriate fibber for extraction can control the selectivity of the analysis because one may choose the stationary phase that best suits specific target analysts. By using headspace SPME, one can reduce matrix effects and interferences present in the liquid sample. The use of SPME in flavour analysis of volatile components can reduce the limitations associated with current methodologies [108].

Simultaneous distillation-solvent extraction (SDE)

SDE has received much attention in the last few years as successive modifications of the original design resulted in a micro version which allows operation with small amounts of extraction solvents without requiring a subsequent concentration of the extract, and hence, losses of volatile compounds can be reduced. Solvents, lighter or heavier than water, can be used in SDE, depending on the model of equipment (Figure 4)

The main advantage of SDE over direct solvent extraction is the absence of contact between the matrix and the organic solvent during the extraction procedure. This allows sample of various compositions, including fat, to be analyzed. Generally, compounds that are not soluble in water are well extracted. The major drawback of such a SDE is that very soluble compounds generally stay in the water phase and are not efficiently extracted. Another drawback is the possible formation of thermal artifacts [109].

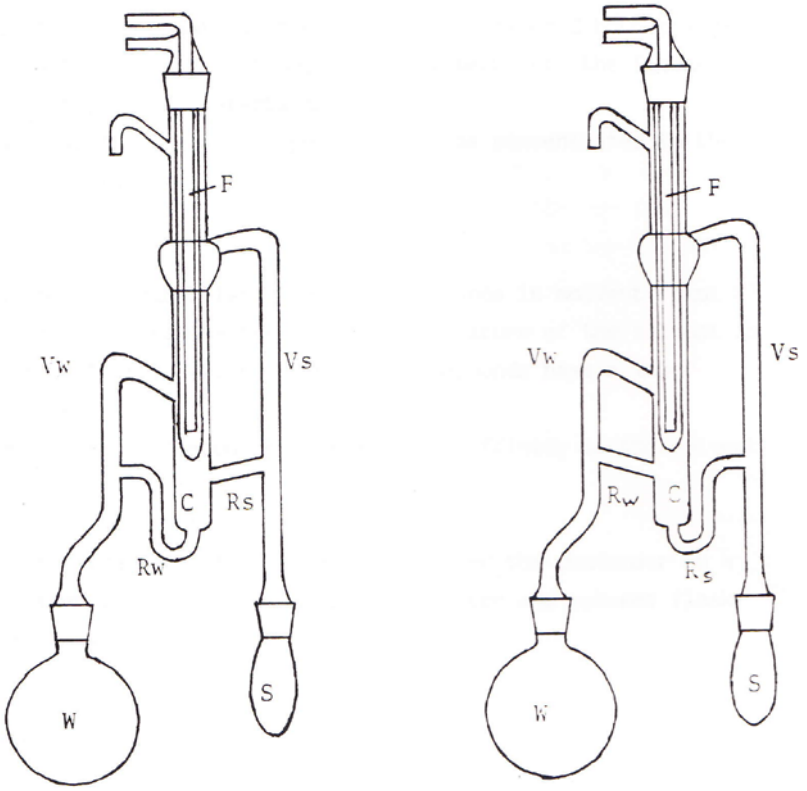

Figure 4.

Model A

Model B

C : separation chamber
F : cold finger
S : flask for solvent
W : flask for water

Vw : vapour tube for water
Vs : vapour tube for solvent
Rw : return tube for water
Rs : return tube for solvent

2. Gas chromatographic analysis

Some of the more common methods used for analyzing flavour compounds in food are gas chromatography (GC), gas chromatograpy- mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O) or gas chromatography mass spectrometry-olfactometry (GC-MS-O) [10].

In the past, flavour have typically been separated on capillary columns, mainly silicone-type Wall Coated Open Tubular (WCOT) and alumina- based Porous Layer Open Tubular (PLOT) columns for highly volatile compounds. Today, there are columns on the market, which are specially designed for flavour analysis according to EPA methods, e.g. VOCOL, RTX-VMS, RTX-VGC, RTX-VRX and DB-VRX [110].On the other hand unpolar WCOT columns with 100% poly dimethysiloxane are still standard for separating flavour compounds. For analyzing complex flavour

mixtures, two-dimensional GC has been developed. Two-dimensional GC involves collecting part of a GC run and re-chromatographing it on a different chromatographic phase. These systems typically permit the collection of a selected part of several GC runs, which improves on sensitivity. GC-O is used for investigation of aroma involves differentiation between odor-active or non-odor-active compounds. In GC-O systems, the nose is used as a GC detector. The GC system may be set up such that the column effluent is split so that a portion of the effluent goes to a sniffing port and the remainder goes to a GC detector (FID or an MS). Consequently, GC–O provides not only an instrumental, but also a sensorial analysis.

II. Objectives

The overall aim of this study was to characterize the main constituents of waxy and normal corn oils, red grape seed and white grape seed oils and to collect data on their quality related components.

The main objectives of the present work were to:

- Study some oxidation parameters such as peroxide value (PV), p-anisidine value (p-AV) and induction time in waxy corn and normal corn oil.
- Determination of total phenolics and antioxidant activity by β-carotene bleaching method in grape seed oils and corn oils.
- Study fatty acid composition in waxy and normal corn oils, and red grape and white grape seed oils.
- Study phytosterol and tocopherol composition of corn oils and grape seed oils.
- Study flavour compounds in waxy and normal corn oils, and red grape and white grape seed oils.

III. Materials and Methods

III.1 Materials

The corn oil samples were provided by an Austrian producer. Grape seed oils were provided by a local producer in Gumpoldskirchen/Lower Austria. All oils were produced by cool-press extraction.

III.2 Methods

III.2.1 Determination of the peroxide value (PV)

III.2.1.1 Materials

- Pipet-0.5 ml
- Erlenmeyer flasks-with glass stoppers, 250 ml
- Burette-50 ml
- Timer
- Acetic acid-chloroform solution (1.5:1 v/v)
- Potassium iodide (KI) solution-saturated (30 g KI in 20 ml of water)
- Sodium thiosulfate solution, 0,01N
- Starch indicator solution, prepared by making a paste with 1g of starch and a small amount of cold distilled water. Add, while stirring, to 100 ml of boiling water and boil for a few seconds [111]

III.2.1.2 Method

According to the original produce of AOCS Official method Cd 8-53 (reapproved 1997) [111], weigh 5.00 ± 0.05 g of sample into a 250 ml Erlenmeyer flask with glass stopper and add 30 ml of the acetic acid-chloroform. Swirl to dissolve the sample. Add 1 ml of saturated KI solution using a suitable volumetric pipette.

Allow the solution to stand with occasional shaking for 1 min, and then put in the dark for 5 min, and then add 75 ml of distilled water and 2 ml of starch solution. Titrate with 0.01N sodium thiosulfate, adding it gradually and with constant agitation. Continue the titration until the yellow iodine color has almost disappeared. Write the volume of standardized sodium thiosulfate solution was use for the test. The test is carried out in duplicate.

III.2.2 Determination of the p-Anisidine value (AV)

III.2.1.1 Materials

- Ten ml test tubes
- Twenty-five ml graduated flasks

- Pipette 1 ml
- Spectrophotometer suitable for observation at 350 nm
- Cuvettes 1 cm
- Iso-octane
- Glacial acetic acid (Sigma-Aldrich)
- p-Anisidine, 2.5 g/l solution in glacial acetic acid

III.2.1.2 Method

- Test solution A [112]: Weigh 0.5 g of the sample, to the nearest 0.001 g, into a volumetric flask (25ml). Add iso-octane to the volume. Measure the absorbance (A_b) of the solution at 350 nm in a cuvette with spectrophometer, using the reference cell fill with reference solution.
- Test solution B: Pipette exactly 5 ml of fat solution (test solution A) into a test tube, and then add exactly 1 ml of the p-anisidine reagent. After exactly 10 minutes measure the absorbance (A_s) of the solution in the test tube at 350 nm, using the reference cell filled with reference solution
- Reference solution: Pipette exactly 5 ml of iso-octane into a test tube, and then add exactly 1 ml of the p-anisidine reagent.

  The test is carried out in duplicate.
- Expression of result:

  The p-anisidine value is given by the formula:

$$p-A.V = \frac{25(1.2A_s - A_b)}{m}$$

  Where

  A_s is the absorbance of the fat solution after reaction with the p-anisidine reagent

  A_b is the absorbance of the solution of the fat

  m is the mass, in gram, of the test portion

III.2.3 Determination of the oxidation stability

III.2.3.1 Materials

- Rancimat equipment
- Distilled water
- Balance
- Acetone, using wash electrode

III.2.3.1 Method

Four gram samples are kept at a c onstant temperature of 120°C with an airflow passing through at the rate of 20 l/h. The airflow is passed through the measuring cell filled with oil– the volatile oxidation products are stripped into the distilled water, containing the conductivity cell. There the conductivity is determined continuously and recorded automatically. During the oxidation process volatile acids are formed. At the end of the ageing period the conductivity increases rapidly. The period up to this point is called 'induction period'. The test is carried out in duplicate.

III.2.4 Determination of total phenolics

III.2.4.1 Materials

- Methanol
- Folin-Ciocalteau (FC) reagent (Sigma -Aldrich)
- Sodium carbonate solution, 37% in water
- 100-ml volumetric flask
- Spectrophotometer set to 725 nm, with 1cm, 2 ml plastic cuvettes (HATICHI U2900)
- Gallic acid calibration standards (Sigma -Aldrich)
  0.1 g gallic acid in 100 ml methanol; Dilute 0.5; 1; 1.25; 1.50; 1.75; 2.25 ml to 5 ml with methanol to create standards with 100, 200, 250, 300, 350 and 400, mg/liter concentrations, respectively.

III.2.4.2 Method

Extraction of phenols was carried out according to the method presented by Parry J et al [113] with was changed a little bit.

- One gram oil, to the nearest 0.001 g, was added to a tube test 10 ml. And then adding 5 ml solvent (60:40 methanol/water) and 5 ml hexane for extraction of oils and defatted.
- Followed by vortex for 4 minutes and centrifugation for 10 minutes at 3700 RPM min^{-1} at room temperature. The extraction was carry out three times for each oil
- All methanolic extracts were combined and, after adding 10 ml 1-propanol, concentrated to dryness. Before measurement of total phenols, the dry matter was dissolved in 1ml methanol.

The total phenol content was analyzed according to the Folin-Ciocalteau reagent method was described by T. Gutfinger et al [114].

- Place 200 µl samples, a gallic acid calibration standard, or blank (deionized or distilled water) in a 10 ml tube test.
- Add 4.2 ml water, followed by 0.5 ml FC reagent. Swirl to mix and incubate 30 min.
- Add 1 ml sodium carbonate solution, mixed and allow to stand for 120 min.
- Transfer 2 ml to a cuvettes 1 cm and measure its absorbance at 725 nm in a spectrophotometer.

The TP content was expressed as gallic acid equivalents (GAE) in milligrams per gram of oil. The test is carried out in duplicate.

III.2.5 Determination of antioxidant activity by β-carotene bleaching method

III.2.5.1 Material

- β-carotene (Fluka Co.)
- Methyl Linoleate (Larodan)
- Tween 80
- n-hexane
- Methanol
- Chloroform
- Distilled water
- Rotary vacuum evaporator
- Oxygenated water which was prepared by bursting air directly into distilled water.
- Spectrophotometer set to 765 nm, with 1-cm, 2-ml plastic cuvettes (HITACHI U2900)

III.2.6.2 Methods

- *Extraction of phenols* [115]: Oils (1 g) dissolved in 4 ml n-hexane were extracted with 4 ml MeOH/H_2O (60:40 v/v). The mixtures were vortexed for 4 min and centrifuged at 3700 rpm for 10 min. After removal of the hexane layer, the lower layer, containing the phenolic substances,t was used for antioxidant activity analysis.
- The antioxidant activity was analyzed according to the method described by Dongmei Yang ME et al [116] with some minor modifications: Two

milligrams of β-carotene were dissolved in 10 ml chloroform and 3 ml of the β-carotene solution was mixed with 200 µl methyl linolenate and 400 µl tween-80 emulsifier in a dark round-bottom flask. Chloroform was then removed in a r otary vacuum evaporator. Oxygenated water (100 ml) was added to the flask and mixed well. The sample (200 µl) was mixed with 2 ml of the emulsion which was prepared as explained above. The control contained 200 µl methanol instead of the sample and was prepared accordingly. These test tubes were placed in a w ater bath of 50°C for 240 minutes and the absorbance value for each test tube was read at 470 nm at intervals of 30 minutes until the absorbance between close measurement did not change, the absorbance was recorded, $A_{A\,(T2)}$ and $A_{C(T2)}$. The control was also measured at 0 min, $A_{C(0)}$.

Antioxidant activity coefficient (AAC) was calculated with the following equation:

$$AAC = \frac{A_{A(T2)} - A_{C(T2)}}{A_{C(0)} - A_{C(T2)}} x1000$$

Where

$A_{A(T2)}$ is the absorbance of antioxidant at final time

$A_{C(T2)}$ is the absorbance of the control at final time

$A_{C(0)}$ is the absorbance of the control at initial time (t = 0 min)

- All samples were run in duplicate.

III. 2.6 Determination of tocopherols

III.2.6.1 Materials

- HPLC system including: a florescence detector with the excitation wavelength set at 295 nm and emission wavelength at 340 nm; column 5 µm particle size, L x ID 25 cm x 4.6 cm, matrix: silicagel, spherical particle platform, matrix active group: aminopropyl phase, pore size: 120 A_0, flow rate 1.6 ml/min.
- HPLC mobile phase, acetic acid ethyl ester in n-hexane (30/70 v/v; HPLC grade)
- Volumetric flask, 25 ml
- Solutions of tocopherol standards [31]

III.2.6.2 Method

Weigh accurately about 2 g of the oil into a 25 ml volumetric flask. Add a quantity of hexane, swirling to dissolve the sample and make up to volume with the same solvent. It is important that the test solution are protected from light prior to analysis, and analyzed on the day of preparation [112]. After that, injected 20 µl of the test solution was on to the column and record the areas of the tocopherol peaks. The test is carried out in duplicate. The tocopherol concentration of the sample is calculated from regression data of four points of calibrations of standard.

III. 2.7 Fatty acid analysis

III.2.7.1 Materials

- n-hexane
- Methanolic KOH 2 M
- Fifteen ml glass tubes with Teflon lined screw caps
- Sodium sulfate (anhydrous)
- Internal standard: heptandecanoic acid methyl ester
- Gas chromatograph system (Fisons 8000) with the following features:
  - Carrier gas: H_2
  - Split injection (temperature 240°C; Spit ratio 1/50)
  - Detector temperature 240^0C
  - Capillary column: CP-Sil88 (Varian Co)

III.2.7.2 Method

- Preparation of fatty acid methyl esters: Weigh exactly 10 mg of lipids into a 10 ml test tube and add internal standard. The mix is dissolved in 2 ml hexane followed by the addition of 2 ml of 2 M methanolic KOH. The tube is vortexed for 2 min at room temperature. The upper layer is taken and Sodium sulfate anhydrous is added, then the aliquot of the hexane layer is collected for GC analysis
- Gas chromatographic parameter: Analysis of fatty acid methyl ester was carried out on a s eries gas chromatograph with a C P-Sil capillary column (100m_0.25 mm I.D 0.2 mM film thickness). The injection volume was 1 ml and temperature was 240^0C. The column initial temperature 50^0C was kept for

3 min and then it was increased to 150^0C with 10^0C/min after which it was increased 2.5^0C/min until it achieved a temperature of 220^0C and then held at 220^0C for 20 min. The total time was 60 min. Following analysis, chromatogram peaks were assigned on the basis of comparison with reference supelco 37 component standards and quantification is carried out by peak area normalization.

- The analyse is carried out in duplicate.

III.2.8 Phytosterols analysis

III.2.8.1 Materials

- Postassium hydroxide (KOH) 40%
- Butylated hydroxyanisole (*BHA*), 1.58 mg/ml ethanol
- Cyclohexane, n-heptane
- Distilled water
- Silylation derivatization reagents: BSTFA + TMCS, 99:1 (SUPELCO Co.)
- Pyridine, 99+% (ALDRICH Co.)
- Internal standard A: 5α-cholestane: 1 mg/ml chloroform (SIGMA Co.)
- Internal standard B: Cholesterol: 1mg/ml chloroform (SIGMA Co.)
- Centrifuge
- Oven
- Vacuum evaporator (BUCHI ROTAVAPOR)
- Heating bath
- GC/MS system (GC equipped with a GC 8000 series and MS with MD 800 of FISIONS INSTRUMENT)

III.2.8.2 Methods

- ***Sample preparation***: *Saponification*: Phytosterols were extracted according to the method reported by Panfili. et al. [117] with some modifications as follows: Exactly 0.1 g of oil sample was weighed into a tube test. The oils were saponified in a screw-capped tube with 8 ml BHA solution, 1 ml KOH. The tube was flushed with nitrogen. The tube was placed in a 80^0C water bath for 15 min and mixed at 1, 2, 4 min. After alkaline treatment the tubes were cooled in an ice bath, and 15 ml cyclohexane and 10 ml H_2O were added. And Then the tube are centrifuged at 1100 rpm for 7 min and the upper layer was

collected and evaporated in a vacuum evaporator at 70 mbar and 40^0C to dryness. To the residue, 200 µl of each internal standard was added and solvent was removed by nitrogen.

Silylation derivatization: To the sample, 100 µl of pyridine and 100 µl BSTFA + TMCS reagents were added and then heated in an oven at 60^0C for 30 min. After that, the tubes were flushed by nitrogen to dryness and 300 µl of n-heptane were added.

GC-MS of phytosterols: Phytosterols in the saponified samples were quantitatively analyzed using GC-MS. The mass detector was set to scan form m/z 44 to m/z 641; source temperature and GC interface temperature were set to 220^0C and 300^0C, respectively. A HP-5 capillary column (length 30 m, ID 0.25 mm, Agilent technologies INC) was used. The oven temperature was programmed as follows: 100^0C (2 min) to 270^0C (1 min) at 20^0C/min; to 290^0C (1 min) at 1.0^0C/min. Split injection (split rate 1:50), with the injector temperature set to 270^0C was used and He was employed as carrier gas at a flow rate of 1ml/min.

III.2.9 Flavour analysis

III.2.9.1 Materials

- Heptanoic acid methyl ester standard (SIGMA Co.)
- 1-Decanol standard (SIGMA Co.)
- n-pentane
- Silicone oils
- Micro steam distillation/ extraction (SDE) apparatus
- GC-MS system (GC equipped with a TRACE GC ULTRA, Thermo electro corporation and MS with DSQ II, Thermo scientific)

III.2.9.2 Method

- ***Extraction [118]***: An accurately weighed sample of about 5 g grape seed oil or corn oil and 50 ml of water were placed in a 250 ml round bottom flask (A) and homogenized in ultrasound equipment for 30 minutes. After that, the flask (A) was connected to the micro steam distillation/solvent extraction (SD/SE) apparatus shown in Fig. 1 Approximately 1ml of pentane containing an internal standard (1% in pentane) was placed in flask "B" and before starting

the procedure water, 2.5 ml, and pentane, 3 ml, were introduced into bowl (C). This is sufficient solvent to fill the return arms "D"and "E"and to establish a demixing equilibrium between the two solvent layers in "C". The vapour channels "F"and "G"were wrapped with insulating tape to minimize condensation of the solvents during recirculation. Chilled water was circulated through the cold finger condenser. To commence operation an oil bath (silicone oils) on a hot plate/stirrer, was set at 140^0C, was used to heat flask "A" to a vigorous reflux and provides sufficient reflected heat to maintain a gentle reflux of the pentane in flask "B". The extraction was continued for 2 h with the steam generated in flask "A" returning to the flask after condensation via arm "E" and the pentane to flask "B" via arm "D". After the required time the source of heat was removed and the solvents allowed cooling until their recirculation stopped (around 20 minutes). The pentane extract in flask "B" and bowl "C" were then combined after disconnecting the apparatus from flask "A".

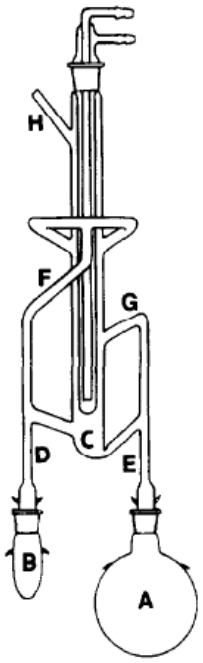

Fig. 1. Apparatus used for simultaneous micro steam distillation/solvent extraction of cinnamon samples. A = Boiling flask; B = receiver for extracting solvent (lighter then water); C = bowl for phase separation; D, E, F, and G are solvent or vapor transfer arms; and H = entry port for additional solvent or alternative sample introduction position for continuous liquid–liquid extraction.

- ***GC-MS of flavour concentrates:*** For GC-MS a TRACE GC connected to a DSQ II (Thermo scientific) quadrupole mass spectrometer was used. The injection temperature was 250^0C, split flow flow was 70 ml/min, split time was 0.25 min and surge pressure was 250 kP a. A HP-5 capillary column (length 30 m, ID 0.25 mm, Agilent Technologies Inc.) was used. The column temperature was programmed as follows: an initial temperature of 40^0C was held for 2 min, the ramped to 200^0C at 5^0C/min, and then held at the final temperature for 10 min. The mass detector set to scan form m/z 10 to m/z 350;

source temperature and GC interface temperature were set to 200^0C and 250^0C, respectively.

IV. Results and Discussion

IV.1 Stability and oxidation of Corn oils

The physicochemical properties of the tested corn oils are presented in Table 1. The Peroxide Value (PV) of waxy corn oil (6.33 meq/kg) was higher than of normal corn oil (2.07 meq/kg). Both of the peroxide value where is within the range of 0-10 mEq/kg stipulated for freshly prepared oil [119]. The higher peroxide value of the waxy corn oil indicated a more susceptibility to oxidation than the normal corn oil, considering that storage conditions were equal for both oils. However, a low value may represent either the beginning of oxidation or advanced oxidation. Thus, the formation of secondary oxidation products determined by the p-anisidine value (AV) is also presented in Table 1 and shows that the AV of waxy oil is lower than in normal oil. Another useful indicator of measuring the onset of progressive deterioration in oil and provides information regarding progression of the formation of primary and secondary oxidation products, is total oxidation value, the so-called Totox value, calculated from twice the peroxide value plus the p-anisidine value [120]. And TOTOX value of waxy corn oil (20.27) was found higher than in normal corn oil (13.60).

Table 2: General characteristics of corn oils

Analytical determination	**Waxy oil***	**Normal oil***
Peroxide Value (meq/kg)	6.33	2.07
p-Anisidine Value	7.60	9.46
Total oxidation value	20.27	13.60
Induction time (h)	6.15	6.97

* *Mean values of duplicate measurement*

Stability values of corn oil at 120 degree Celsius obtained by the Rancimat method expressed as Induction period (IP) are showed in Table 2. The IP of waxy corn oil and of normal corn oil were 6.15 ± 0.25 and 6.97 ± 0.07, respectively. The results showed that the IP of normal corn oil similar range in waxy corn oil. Literature values for other plant oils such as soybean oil (5.62 ± 0.09h [121]), palm olein (10.44 ± 0.19, [121]), rapeseed oil (5.62 ± 0.09h [121]) and sunflower oil (1.3h [122]) revealed that the IP of the corn oils studied was in the range of these oils with regards to their degree of unsaturation.

IV.2 Tocopherol of corn oils, total phenolics and antioxidant activity of corn oil and grape seed oils

As a further important quality-related parameter of seed oils, the content and composition of tocopherols, including tocotrienols was determined. These data are shown in Table 2. Tocopherols are particularly important functional constituent of the unsaponifiable fraction of vegetable oils. These compounds display antioxidant properties and are active as vitamin E, which make them particularly essential for human nutrition. The main tocopherols of corn oil in the two samples, waxy corn and normal corn, were γ-tocopherol and α-tocopherol (Tab.3) The total tocopherols content of corn oil showed a high amount of tocopherol in both of waxy and normal corn oil, compared to other edible plant oils such as sesame oil (647 mg/kg), cotton seed oil (778 mg/kg), sunflower oil (976 mg/kg) [123], soybean oil (316 mg/kg), peanut oil (164 mg/kg) [124].

Table 3. Table tocopherol of Corn oil

Compound name	**Waxy oil** (mg/kg oil)	**Normal oil** (mg/kg oil)	**Waxy oil** (IU)	**Normal oil** (IU)
α-tocopherol	243.8	131.3	363.3	195.6
γ-tocopherol	1248.0	1138.5	187.2	170.7

* *Mean values of duplicate measurement*

Tocotrienols (α, β, γ, δ -tocotrienol), β-tocopherol and δ-tocopherol were also reported in corn oil, as in the studies of Goffman et al [125] and Firestone et al [126], but they were not found in this experiment.

IV.3 Total phenolics and antioxidant activity of corn oil and grape seed oils

Phenolic compounds are important plant constituents, because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides in to radicals. The Folin-Ciocalteu method is a rapid and widely-used assay, to investigate the total phenolics content. Total phenolic content of corn oil and grape seed are demonstrated in Table 4. The amount of total phenolics ranged from 81.4 mg/kg to 198.5 mg/kg. Total phenolics content of normal corn oil (132 mg/kg) was significantly lower than waxy corn oil (198.5 mg/kg) ($p < 0.05$) and total phenolics content of white grape oil (132 mg/kg) was significantly higher than red grape oils (87.6 mg/kg). The total phenolic of corn oil and grapes seed oil is shown in

table 4. With grape seed oils, normally total phenolic in red grape oils is higher than of white grape oils. However, the result in Table 4 showed that it is opposite and it was also presented in study of Bail [128] with two kind of grape seed oils origin from Styria, Austria. May be explained that total phenolic are depended on grape variety.

Table 4 Total phenolics and antioxidant activity of Corn oil and Grape oil

	Total phenolics*	AAC*
Red grape oil	87.6	209.5
White grape oil	132.0	159.0
Normal corn oil	81.4	584.2
Waxy corn oil	198.5	373.3

Total phenolics: mg Gallic acid equivalent/kg
AAC: Antioxidant activity coefficient
** Mean values of duplicate measurement*

The β-carotene-linoleic acid method was used to evaluate the antioxidant activity of corn oils and grape seed oils and the results are presented in Table 3. The antioxidant activity coefficient (AAC) of corn oils and grape oils are ranged from 159 to 584. From The AAC was found to be 159.0 and 209.5 for white grape seed oil and red grape seed oil, respectively, while it was found to be 373.34and 584.2 for waxy corn oil and normal corn oil, respectively. The findings do not show any relationship between antioxidant activity and total phenolic contents (Table 4). For example, red grape seed oil had the lower total phenolic content whereas its antioxidant activity was a bit higher than white grape seed oils that had a higher total phenolic content. Waxy corn oil had much higher total phenolic content than normal corn oil although its antioxidant activity was lower than was waxy corn oil. The findings of this study indicate that antioxidant activity of corn- and grape seed oils are not primarily depending on the total phenolic content but also on concentration, other anti-oxidants such as tocopherol, carotenoids, and possibly also phytosterols or squalene. Squalene and phytosterols may have an antioxidant potential [129-131]. Therefore, normal corn oil and red grape oil (see Table 6), which have high squalene and phytosterols content may have higher antioxidant acitivity than waxy corn oil and white grape seed oil, respectively. This supposition is supported by studies of Dongmei et al, 2007 [116] and Vinson et al, 2001 [132].

IV.4 Fatty acid composition

Corn oil and grape oil are known to be some of the richest sources of linoleic acid. This property is shared with oils such as soybean, peanut, safflower and several others [55]. In this study, after alkaline hydrolysis and conversion of fatty acids to fatty acid methyl esters (FAMEs), the fatty acid composition of both corn oils (waxy and normal corn oil) and that of grape seed oils (red grape seed oil and white grape seed oil) were then analyzed by GC. The fatty acid composition of these oils is shown in Table 5.

Table 5 Fatty acid compounds of Grape seed oils and Corn oils

Compound name	Red grape oil (% of FA related to the fat content)*	White grape oil (% of FA related to the fat content)*	Normal corn oil (% of FA related to the fat content)*	Waxy corn oil (% of FA related to the fat content)*
C14:0	0.8	0.9	nd	nd
C16:0	7.5	6.4	11.0	11.6
C18:0	3.6	3.3	1.7	1.7
C18:1n9c	15.6	12.9	27.2	26.1
C18:1n7	0.7	0.7	0.5	0.5
C18:2n6c	72.0	74.7	58.9	58.3
C20:0	0.1	0.1	0.4	0.3
C20:1	0.1	0.1	0.2	0.2
C18:3n3	0.3	0.3	0.8	0.9

** Mean values of duplicate measurements ; nd: Not detected*
The range between duplicate measurements (%) relative to the mean was:
< 5% for fatty acids > 10% and
< 8 % for fatty acids < 10%

Linoleic acid was the most abundant fatty acid in all samples; its concentration was 58.3%, 58.9% in waxy corn oil and normal corn oil, respectively and 72.0%, 74.7% in red grape seed oil and white grape seed oil, respectively. Oleic acid was the next most abundant fatty acid; accounting for 12.9%, 15.6% in white grape and red grape seed oil, respectively and 26.14%, 27.24% in waxy and normal corn oil, respectively. Palmitic acid was next highest with 6.4%, 7.5%, 11.0%, 11.6% in white grape seed oil, red grape seed oil, normal and waxy corn oil, respectively, followed by stearic acid (1.6%-3.6%), iso-oleic, arachidic acid, gadoleic acid and alpha-linoleic acid.

The concentration of fatty acids, which were found in the corn oil samples did not differ from each other to a g reat extent. On the other hand, there were clear differences among the main fatty acids in grape oils. White grape seed oil had higher

Linoleic acid, whereas its oleic acid, palmitic acid, stearic acid were a bit lower than of red grape seed oil.

In summary, the result confirmed that both of corn oils and grape oils would be valuable natural oils from a nutritional standpoint regarding their fatty acid profiles. Epidemiological studies, animal experiments and clinical investigation have shown that diets high in unsaturated fatty acids from plant origin improve plasma lipid status, plasma antioxidant activity and also may reduce risk for cardiovascular disease [6].

IV.5 Phytosterol and squalene compostion

Phytosterols and squalene are of a g reat interest regarding their role as potential antioxidants and their involvement in anti-inflamatorv, antipyretic, immunomondulatory and antineoplastic mechanisms [26, 27, 29, 90, 130]. They are key components of the unsaponifiable matter of vegetable oils and fats. The analysis of the sterols provides rich information about the quality and the identity of the oils. The values for sterols and squalene of both corn and grape oils are given in Table 5. Squalene is a k ey intermediate in the biosynthetic pathway of sterols. It is also regarded as one of the compounds responsible for the beneficial effects against certain type of disease [133]. Antioxidant activity of squalene has been demonstrated with polyunsaturated fatty acids [134] and was found to be comparably active tophenols and tocopherols. Squalene content of waxy corn oil was slightly higher than of normal corn oil, while squalene was much higher in red grape oil than in white grape oil. To compare with other seed oils studied by Carlo I.G. Tuberoso et al [135], squalene concentration of both of corn oil and of grape oil is the same range of rapeseed oil and sunflower oil, but is lower than of peanut oils (1276 mg/kg), pumpkin seed oil (3529 mg/kg) and olive oils (5990 mg/kg).

Table 6 Phytosterol and squalene in corn oils and grape oils

Compound name	Normal corn oil (mg/kg)*	Waxy corn oil (mg/kg)*	Red grape seed oil (mg/kg)*	White grape seed oil (mg/kg)*
Squalene	423	473	490	133
Campesterol	1662	1474	526	407
Cholestanol	106	103	nd	nd
Stigmasterol	685	605	702	601
β-Sitosterol	5280	4707	5180	3465
C[a]	302	264	nd	nd
Δ5-Avenasterol	451	431	nd	nd
C[b]	57	58	nd	nd
Cycloartenol	102	120	nd	nd
Δ7-Avenasterol	29	35	nd	nd
C[c]	55	61	nd	nd

**Mean values of duplicate measurement*
[1]C[a]: 25(RS)-3a-hydroxy-5b-cholestane-26 oate
C[b]: 4a-methylergosta-7,24,(28)-dien-3b-ol
C[c]: 4b,5a- 4-4 dimethylcholesta-8-24-dien-3-ol
nd: Not detected

The most predominant sterol of all oil samples was β-sitosterol (SI), followed by campesterol (CA), stigmasterol (ST) and 5-avenasterol (AV) (not found in grape seed oils), with lesser amounts of some other phytosterols (not found in grape seed oil). Among the different phytosterols that were found, β-sitosterol had been most intensively investigated with respect to its physiological effect on health [136]. The predominant sterols (SI, CA, ST, AV) of normal corn oil were higher than of waxy corn oil and the concentration of the three highest sterols found in red grape oil were considerably different from white grape seed oil (see Table 6). The sterol composition in grape seed oils disagrees with data reported by other researcher in this study just found only three main sterols involve in SI, CA and ST, but in their study more compounds were found such as Δ7-Avenasterol, Δ5-Avenasterol, sitostanol, and cholesterol. However, total sterol content was much higher in this study and comparable to that found by Jose Emilio Pardo et al (3110 mg/kg) [137].

A remarkable high amount of 5-Avenasterol was found in corn oils (451, 431 mg/kg in normal corn oil and waxy corn oil, respectively). This compound is known to act as an antioxidant and as anti-polymerization agent in frying oil [138]. Gordon et al [138] hypothesized that lipid free radicals react quickly with the unhindered allylic groups in the unsaturated side chain of sterols to form stable tertiary free radicals that are retarding further reaction thereby delaying auto-oxidation. Sterol without unsaturated

side chain, such as sitosterol and campesterol, were found to have either neutral or slightly pro-oxidant activity. The study of Paniagua-Pérez et al that by means of the DPPH assay, the beta-sitosterol compound was able to trap free radicals *in vitro* when its concentration was high enough in the sample (250 μg/ml) [139]. Furthermore, other reports have show a significant scavenging capacity of beta-sitosterol when it was examined in plant extracts [140, 141].

IV.6 Flavour compounds of corn oils and grape seed oils

The flavour compounds of corn oils and grape seed oils were isolated by simultaneous mircro stream distillation/solvent extraction (SDE) and subsequently analyzed by GC-MS in order to identify flavor profiles. The chromatograms shown in Figs. 3, 4, 5 and 6, illustrate the GC profiles of the SDE red grape seed oil, white grape seed oil, normal corn oil and waxy corn oil. Identified flavor compounds with their relative peak area are summarized in Tables 7 and 8, respectively. A large number of substances consisting mainly of short chain fatty acid, alcohols, esters, hydrocarbons, as well as flavour active aldehydes and ketones were found.

Table 7: Flavour compounds of Grape seed oils

Compound name	(Rate of Asp/Asd)*100 (RED grape oil)	(Rate of Asp/Asd)*100 (WHITE grape oil)	% Area (Red grape oil)	% Area (white grape oil)
Octane	8.41	7.92	4.49	25.10
O-xylene	0.87		0.50	
2 Vinylbicydo(2.1.1)hex-2-ene		0.41		1.31
1-Butanol, 3-methyl-, acetate;	2.85	0.26	1.65	2.64
1-Butanol, 2-methyl-, 1-acetate	0.38		0.22	0.82
Hexanoic acid, methyl ester;	0.34	0.4	0.20	1.26
2 Heptenal (Z)	0.62	0.36	0.36	1.13
Acetaldehyde ethyl amyl acetal	0.09		0.05	
Benzen.1.2.3.trimethyl		0.45		1.42
Hexanoic acid, ethyl ester;	**14.91**	0.69	8.65	2.21
Acetic acid, hexyl ester	0.25	0.96	0.14	3.06
N-dodecane	0.23	0.19	0.13	0.59
Heptanoic acid	0.75	0.76	0.43	2.42
Heptanoic acid, ethyl ester	0.35	0.23	0.20	0.73
n-Nonaldehyde	0.37	0.42	0.21	1.31
Succinic acid, diethyl ester	2,32	0.59	1.34	1.88
Octanoic acid ethyl ester	**46.09**		26.73	
1-Hexadecanol		0.15		0.49

Table 7 (cont): Flavour compounds of Grape seed oils

Compound name	(Rate of Asp/Asd)*100 (RED grape oil)	(Rate of Asp/Asd)*100 (WHITE grape oil)	% Area (Red grape oil)	% Area (white grape oil)
Isoamyl caproate	0.41		0.24	
Acetic acid, phenyl-, ethyl ester;	1.10	0.55	0.64	1.74
p-ethylguaiacol	0.34		0.20	
2,4 Decadienal	0.36		0.21	
n-Tridecane		0.29		0.91
2-4 Decadienal	0.81		0.47	
p-menth-3 ene 2 isopropenyl-l-vinyl-(1S,2R)-(-)-S	0.42	0.17	0.24	0.55
Copaene	0.62	0.34	0.36	1.07
n-Decanoic acid	0.26		0.15	
Decanoic acid, ethyl ester	**31.31**	0.18	18.16	0.58
n-Tetradecane		0.41		1.31
Caryophyllene	2.53	1.01	1.47	3.20
Octanoic acid, isopentyl ester	1.15		0.66	
1,4,7 Cylcloundecatriene 1.5.9.9 tetramethyl -Z.Z.Z	2,35	0.97	1.36	3.07
1,6 Cylcloundecatriene 1 methyl 5 methylene- 8-(methyl ethyl)-(S-(E,E))	**3.40**	1.70	1.97	5.38
Unknown	0.72		0.42	
Cadina-1(10).4 diene	**3.59**	1.82	2.08	5.79
Lauric acid, ethyl ester	0.49	0.07	2.84	0.24
Lauric acid, isopentyl ester	0.62		0.36	
Heptadecane		0.09		0.28
Myristic acid, ethyl ester	0.69	0.08	0.40	0.26
1.2 Benzenedicarboxylic acid, dibutyl ester		0.17		0.52
Palmitic acid, ethyl ester	**11.53**	2.43	6.68	7.69
Labd-1.4- ene 8-13-epoxy-(13R)	0.67	0.05	0.39	0.15
9,12 octadecadienoic acid, ethyl ester	**21.10**	4,41	12.23	13.96
(E)-9-octadecadienoic acid, ethyl ester	**4.12**	1.68	2.39	5.32
Heptadecanoic acid.1 5 methyl-, ethyl ester	0.79	0.15	0.46	4.49

** Mean values of duplicate measurement*

-N/A : Not Available

-Asp : Area of sample

-Asd: Area of standard

Table 8: Flavor compounds of corn oils

Compound name	(Rate of Asp/Asd)*100 (waxy corn oil)	(Rate of Asp/Asd)*100 (Normal corn oil)	% Area (Waxy corn oil)	% Area (Normal corn oil)
Octane	104.12	119.04	30.97	40.54
2,4 Dimethylheptane	1.99	2.13	0.6	0.73
O-xylen	3.19	2,41	0.94	0.82
2.6-Dimethyl-3-heptanone	1		0.31	
2-Heptenal (Z)	1.61	1.37	0.47	0.47
2-n-pentylfuran	5.21	3.99	1.55	1.36
Decane		1.56		0.53
Acetaldehyde.Phenyl	2.84	3.49	0.81	1.2
4.7 Dimethyl undecane	1.45	2.04	0.42	0.69
Pentanoic acid-2-hydroxyl-4methyl-ethyl-ester	1.27	0.94	0.38	0.32
Undecane	1.1	1.88	0.33	0.64
Nonanal	0.36	2.62	0.1	0.88
2 isopropyl-5 methylhex-2-enal	5.72		1.71	
n-Dodecane	3.55	3.66	1.06	1.25
2-Decenal (E)	1.52	1.23	0.45	0.42
2.4 Dodecadienal	12.49	1.49	3.71	0.51
Dodecane-4-methyl		1.78		0.61
2.4 Decadienal	43.09	17.56	12.82	6.01
Tetradecane	4.93	4.81	1.45	1.64
5-methyl-2phenyl-2hexenal	14.79	10.31	4.36	3.5
n-Hexadecanoic acid	53.34	39.08	15.88	13.28
Hexadecane acid.ethyl ester	13.56	13.46	4.02	4.56
Heneicosane	2.6	2.46	0.76	0.84
9.12 Octadecadienoic acid.methyl ester	8.11	4.98	2.35	1.71
9. Octadecenoic acid.methyl ester	3.17	2.53	0.91	0.86
9.12 Octadecadienoic acid.ethyl ester	31.82	33.11	9.47	11.25
9. Octadecenoic acid ethyl ester	13.97	15.8	5.36	4.76

** Mean values of duplicate measurement*

** N/A : Not Available*

** Asp : Area of sample*

** Asd: Area of standard*

Totally, forty-four compounds were identified in both of grapes seed oil samples. Some of compounds were only found in red grape seed oil, those were O-xylene, acetaldehyde ethyl amyl acetal, isoamyl caproate, p-ethylguaiacol, n-decanoic acid, octanoic acid isopentyl ester, lauric acid, isoentyl ester, 2,4 decadienal and 2-4 decadienal, whereas some compounds such as 1-hexadecanol, n-tridecane, n-tetradecane, heptandecane not detected in red grape seed oil. Among these identified compounds of grape seed oils (Table 6), the dominating compounds were octanoic

acid ethyl ester (26.73 % related to the total level of flavour compounds in RGS); decanoic acid, ethyl ester (18.6% in RGS); 9,12 octadecadienoic acid, ethyl ester (12.23, 13.96 in RGS, WGS, respectively); hexanoic acid, ethyl ester (8.65% in RGS); palmitic acid, ethyl ester (6.68% in RGS and 7.69% in WGS); octane (25.10, in WGS). The concentration of those compounds in red grape seed oils are higher than of white grape oil when compared the result was calculated by rate of the area of sample and area of standard (Table 7).

In corn oils, totally twenty-five compounds were isolated by SDE. Those compounds were found to be dominated by octane (40.54%, 30.97% in normal and waxy corn oil, respectively) followed by n-hexadecanoic acid (15.88% in waxy corn oil and 13.28% in normal corn oil) and 2,4decadienal (12.82%, 6.01% in waxy and normal corn oil, respectively). 9,12-octadecadienoic acid-ethyl ester (11.25% in normal corn and 9.47% in waxy corn oil) and 9-octadecenoic acid-ethyl ester (5.62%, 4.76% in normal corn and waxy corn oil, respectively) were the next highest in corn oil.

Flavour esters like ethyl octanoate (octanoic acid ethyl ester) are well known for their pleasant, fruity, floral odor (wine–apricot note), as described in Fenaroli's handbook of flavour ingredients [142]. The aroma threshold values of octanoate range from 5 to 92 ppb, and taste characteristics at 5 ppm are waxy, fatty, aldehydic, coconut-like, creamy and dairy-like [142]. Ethyl decanoate (decanoic acid-ethyl ester), the second highest ester was found in red grape seed oils, giving a fruity odor reminiscent of grape (cognac). It has been also reported to have an oily, brandy-like odor, and taste characteristics at 20 ppm are waxy, fruity, sweet apple and aroma threshold value range from 8 to 12 ppb. Ethyl hexanoate (hexanoic acid, ethyl ester) has a powerful, fruity odour with a pineapple–banana note. It has been also reported to have a winy odour with taste characteristics at 10 ppm described as fruity and waxy with a tropical nuance. Palmitic acid (n-hexadecanoic acid), was also identified at high concentration in both corn oil samples, is virtually odourless. This compound is not volatile but it is co-extracted with other flavour compounds in SMSDE method. It is also reported as having a slight characteristic odour and taste. Ethyl palmitate (hexadecanoic acid, ethyl ester), which has a high concentration in both corn oil and grape seed oils, has a mild, waxy sweet odour. It is nearly tasteless and has a creamy mouth feel, and its taste characteristics at 30 ppm are waxy, fruity, and creamy and fermented with a vanilla, balsamic nuance [142].

Aldehydes were found in samples such as 2-heptenal; nonanal; 2-decanal; *2E,4E*,2,4-decadienal; 2,4-dodecadienal *E,E*, have a direct relation with rancidity, and 2-heptanal was the main indicator of rancidity [143]. The volatile carbonyl compounds formed in edible oils depend on the unsaturation level of the oil. Unsaturated acids are the main precursors of the volatile compounds found in oxidized oils [144]. Especially, the content of linolenic acid is a relevant factor in increasing the off-flavour of the oil during storing and frying [145]. When corn oils and grape seed oils were compared their fatty acids content to other oils such as palm oil, olive oil, rapeseed oil and sunflower, their showed the higher rate of unsaturated fatty acids, especially linoleic acid. Therefore, it may be had a low flavour stability.

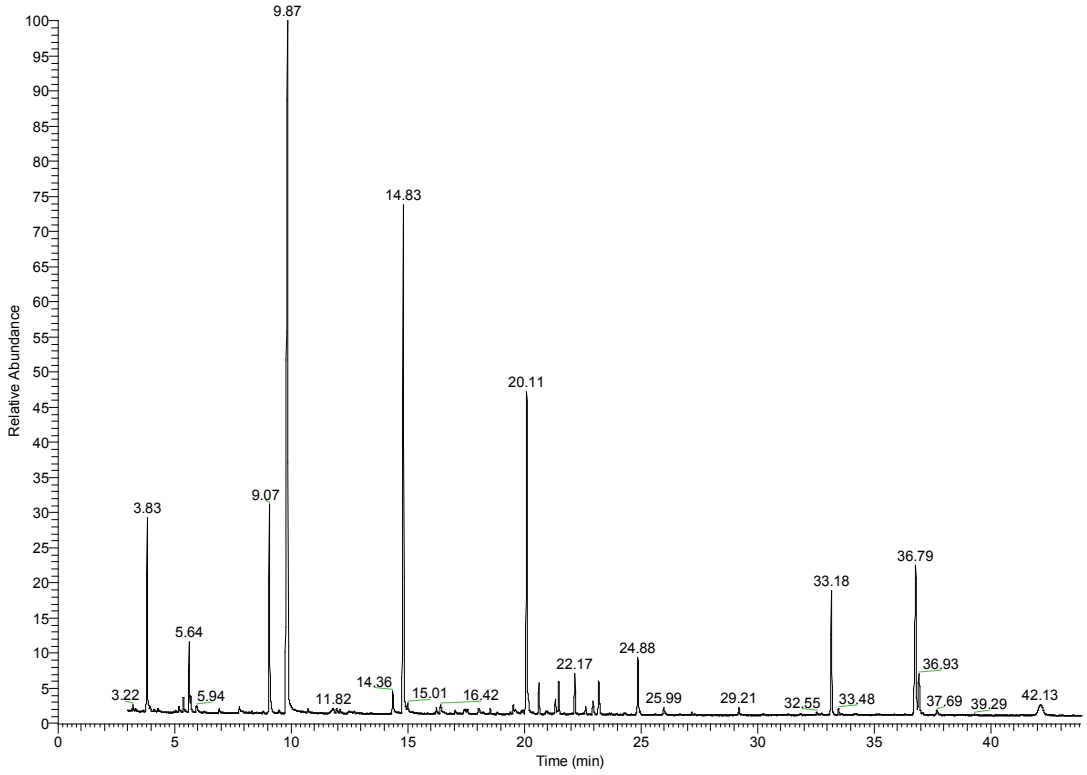

Fig 3 Chromatogram of red grape seed oil flavor compounds obtained with SDE extraction method

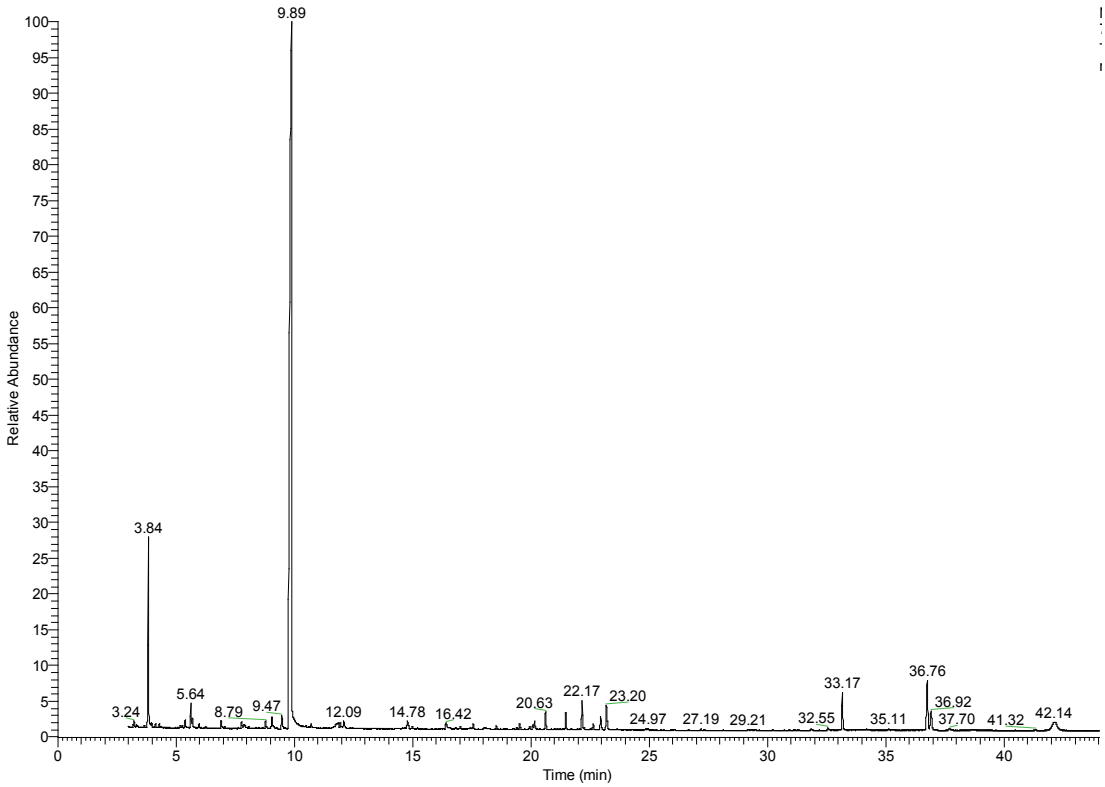

Fig 4 Chromatogram of white grape seed oil flavour compounds obtained with SDE extraction method

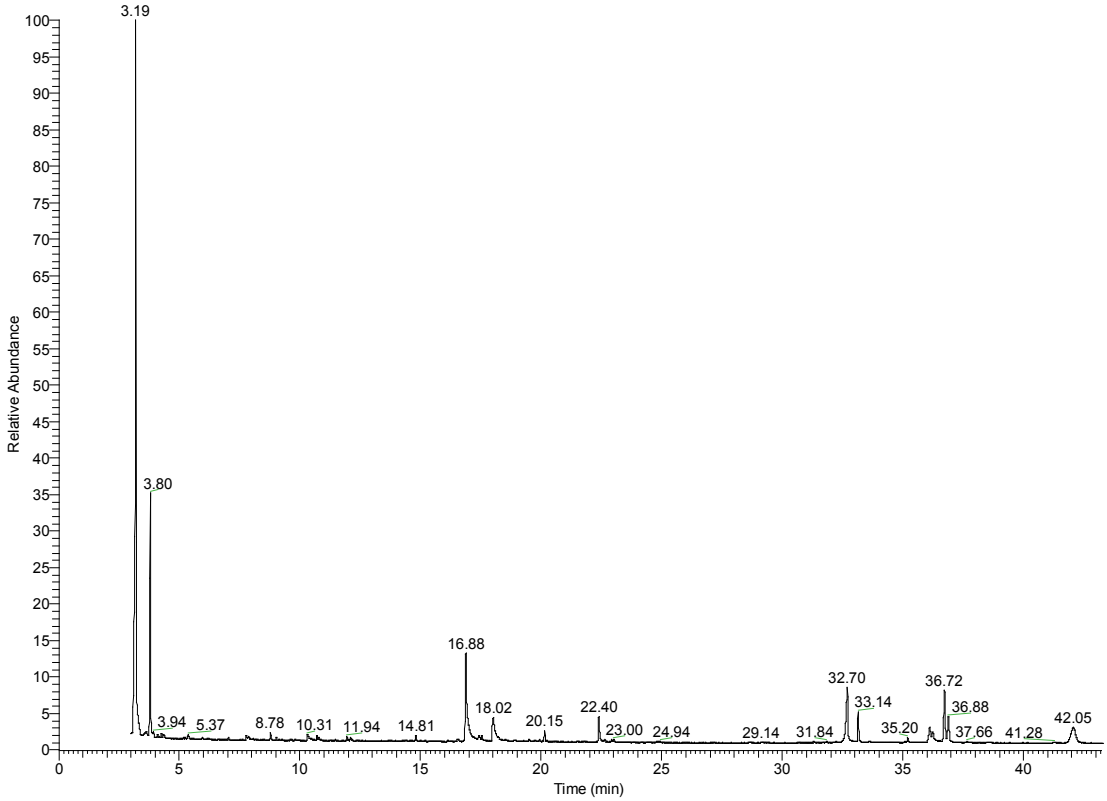

Fig 5 Chromatogram of normal corn oil flavour compounds obtained with SDE extraction method

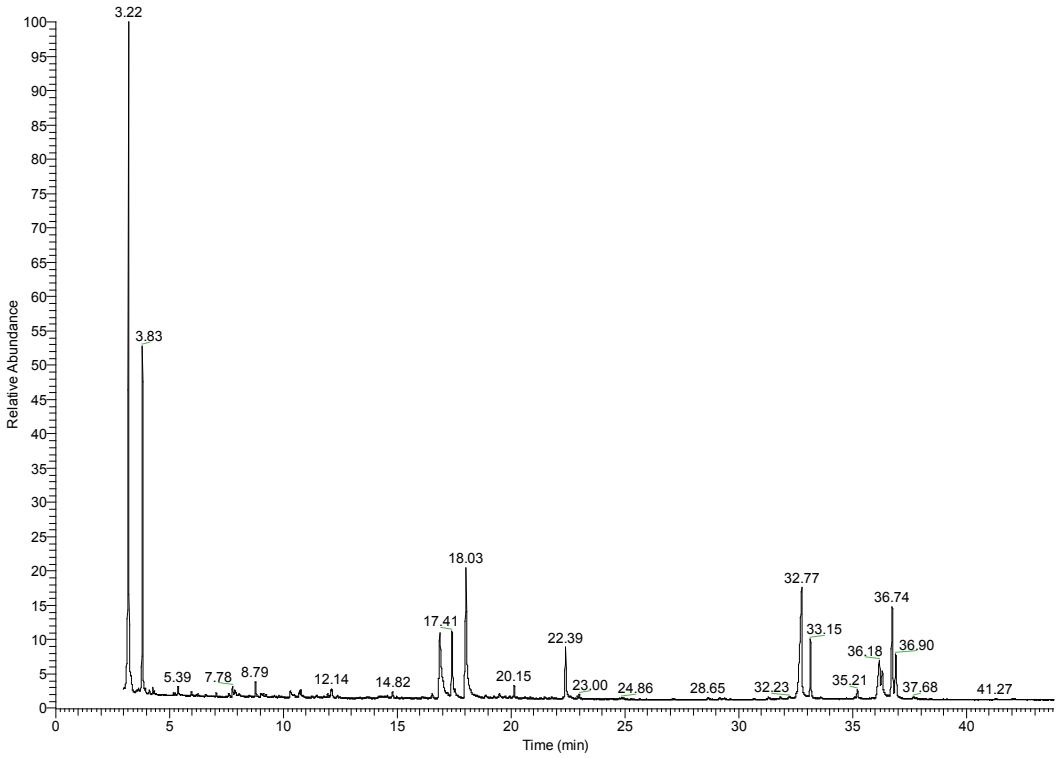

Fig 6 Chromatogram of waxy corn oil flavour compounds obtained with SDE extraction method

V. CONCLUSIONS

According to the analysis of quality parameters, total phenolics, antioxidant activity, tocopherols, phytosterols, flavour compounds of waxy corn oil, conventional corn oil, red grape seed oil and white grape seed oil, it can be conclude that both corn oils and grape seed oils contain a high number of phytosterols, and tocopherols There were considerable differences between the two respective groups of corn and grape oils with regards to their minor components. Regarding the phytosterols, normal corn oil had higher values than waxy corn oil, whereas total tocopherols in waxy corn oil were higher than in normal corn oil. In grape oils, total phytosterols were higher in red grape oil. Interestingly, white grape oil had higher total phenolics than red grape oil. However, red grape oil showed a higher anti antioxidant activity. Total phenolics were higher in waxy con oil than in normal corn oil, but the antioxidant activity was higher in normal corn oil, which was also reflected by the analysis of stability by the rancimat method.

It can be concluded that the analyzed oils differ in some chemical parameters, including minor components. Further investigation of these constituents, especially the phenolic fraction, would be a valuable contribution to explore possible nutritional benefits of these oils.

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