Corn and Coconut Oil

Antioxidant Properties, Uses and Health Benefits

Constantin Apetrei
Editor

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PREFACE

This book presents the properties, uses and health benefits of corn and coconut oils, which are important edible oils in different countries around the world. Corn and coconut oils are found in the market in two forms: virgin (crude, raw) or refined. Virgin coconut oil is a relative newcomer in the oil market and could be valuable oil, comparable with virgin olive oil. Furthermore, corn oil is a beneficial natural food product with excellent nutritional properties and health benefits.

In this book, we provide important data about the chemical composition, production, uses, and benefices of corn and coconut oils. The book comprises ten chapters and an index including numerous technical or scientific terms founds in all the chapters. The book is organized in the following way:

Chapter 1 deals with the description of the chemical composition of corn oil, highlighting the structure and properties of biologic active compounds present in this natural food product.

Chapter 2 describes the biosynthesis, analysis and antioxidant properties of tocopherols, tocotrienols and carotenoids from corn oil.

Chapter 3 presents technological and practical aspects related to supercritical fluid extraction of corn germ oil, a method that permits the efficient extraction of the oil and the preservation of biologic active compounds.

Chapter 4 describes different aspects related to uses and applications of the corn oil in different fields, such as foods, biodiesel production, etc.

Chapter 5 deals with the role of the corn oil in the aging process, presenting the long-term beneficial effects of corn oil in animals.

Chapter 6 present the coconut oil production methods highlighting the differences in quality of the oil produced in different manners.

Chapter 7 describes the most important methods involved in quality analyses and authentication of coconut oil.

Chapter 8 presents the antimicrobial activity of fatty acid derivates from corn and coconut oils against a large spectrum of Gram-positive and Gram-negative bacteria, yeasts, fungi, viruses and parasites, which cause spoilage or various infections in both animals and humans.

Chapter 9 describes the principal aspects related to the nutritional and health benefits of coconut oil.

The book closes with Chapter 10, which presents the beneficial effects of fatty acids from coconut oil on human metabolism and health.

I gratefully acknowledge the help of the distinguished specialists who contributed to this book. I consider myself lucky to have had the opportunity to collaborate with colleagues from universities from India, Spain, Sri Lanka, Turkey, the United States, and Romania, who have elaborated the chapters based on their research experience. Finally, I want to thank Nova Publishers for the opportunity offered to contribute, assemble and edit this book.

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Chapter 1

CHEMICAL COMPOSITION OF CORN OIL

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ABSTRACT

Corn or maize (*Zea mays L.*) originates from both North and South America. It is one of the most important cereal crops cultivated all over the world next to rice (*Oryza sativa*) and wheat (*Triticum spp.*). Corn belongs to *Maydae* tribe that is included in the sub-family *Panicoideae*, *Gramineae* family. The main cultivators are The United States of America, China, Brazil, Mexico, Romania, Argentina and France. A large percent of the corn grains is processed and fed to animals. Corn is also used for human consumption, fresh or dried and grounded into flour, being an important ingredient in various dishes around the world. Only a small fraction of the corn is used for obtaining corn oil. Usually, oil is extracted from the wet milled germ using a heating process, followed by mechanical expelling and hexane extraction. The major components of the crude corn germ oil are triacylglycerols. The crude oil also contains other minor nonpolar and polar lipid components such as free fatty acids, phytosterol fatty acyl esters, free phytosterols, phytosterol ferulate esters, tocopherols, tocotrienols, phospholipids, pigments, volatile compounds, waxes etc. The major undesirable components of the crude corn oil (the free fatty acids, the pigments, the volatile compounds, the phospholipids, and the waxes) are removed by appropriate refining steps. Corn oil is alsoused in cooking and in different industrial processes, i.e., margarine fabrication and biodiesel production. Among vegetable oils, corn oil is important because of its special characteristics, properties, nutritional and health benefits.

Keywords: corn oil, triacylglycerol, tocopherol, phytosterol, carotenoid

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INTRODUCTION

Maize oil (corn oil) is obtained from maize germ (the embryos of *Zea mays L.*) (Codex Alimentarius, 1999). Unlike most other vegetable oils, corn oil (maize oil) is obtained from seeds (kernels) that only contain 3-5% oil. Obtaining oil directly from the kernels is technically possible, but 'corn kernel oil' would be costly to produce, because of the low levels of oil in the kernels. Corn germ is rich in oil (>30%), and is the source of all the commercial corn oil, which could more accurately be called *corn germ oil* (Gunstone, 2011).

Corn oil is obtained from the germ, which is recovered during starch production. The germ from the corn flour industry contains 20% oil. The quality of the corn oil depends on the pretreatment and the separation of the germ. Traditionally, corn oil is obtained from the wet milled germ using a heating process, followed by mechanical press and hexane extraction. Extrusion is employed as a means of germ preparation for solvent extraction, producing a crude corn oil of high quality and high yield (Williams et al., 1997). Other scientists have demonstrated that corn germ can be effectively extracted by supercritical fluid extraction (King & List, 1996).

Crude corn oil contains principally triglycerides and minor nonpolar and polar lipid components such as free fatty acids, phytosterol fatty acyl esters, free phytosterols, phytosterol ferulate esters, tocols, tocopherols, tocotrienols; glycolipids, phospholipids, pigments, volatile compounds, waxes etc. (Gunstone, 2011).

Corn oil consists of about 95% triacylglycerols with 1.5% phospholipids, 1.7% free fatty acids, and 1.2% sterols (Fennema, 1996).

Free fatty acids, pigments, volatile compounds, phospholipids, and waxes are the major undesirable components of the crude corn oil and are removed by appropriate refining processes (Nagaraj, 2009).

In corn oil refining processes, the removing of the free fatty acids takes place by alkali refining, which involves adding base and subsequently neutralizing and separating the free fatty acid soaps. Otherwise, free fatty acids can be removed by physical refining, which involves treating the oil at high temperature and vacuuming, in order to volatilize the free fatty acids. The removing of the phospholipids is carried out by water degumming. If the phospholipids are not adequately removed, they result in a corn oil that will form dark colors and off-flavors when heated (Nagaraj, 2009).

The corn oil deodorization involves treatment at high temperature and vacuuming and the removing of the undesirable odors and flavor components (O'Brien, 2008).Pigments are usually removed by treating the oil with acid-activated bleaching clay. Another refining step that ensures the physical stability of the oils at low temperature is dewaxing or winterization. This involves cooling the oil to 5-10°C, and removing the precipitates by filtration (Antoniassi et al., 1998.

GENERAL COMPOSITION AND PHYSICAL PROPERTIES OF CORN OIL

Corn oil is primarily consists of triacylglycerols (> 95%) and secondarily free fatty acids, mono- and diacylglycerols, and several lipid compounds such as hydrocarbons, sterols, aliphatic alcohols, tocopherols, and pigments (Gunstone, 2011).

Table 1. Physical and chemical characteristics of corn oil (Maize)

Property	Values
Relative density (20ºC/water at 20ºC)	0.917-0.925
Refractive index (at 25°C)	1.470–1.474
Refractive index (at 40°C)	1.465-1.468
Saponification value (mg KOH/g oil)	187-195
Unsaponifiable matter ($g \times kg^{-1}$)	10-28
Iodine value (Wijs method)	103-135
Viscosity (cP)	30.80 at 40°C 18.15 at 60°C
Dielectric constant	3.954 at 26°C
Surface tension at 25°C (dyn/cm)	34.80
Thermal conductivity at 130°C ($J/s/cm^2/°C$)	4.2017×10^{-5}
Stability	6.2[a]
Melting point (°C)	−18 to −10
Ignition point (°C)	393
Flash point (°C)	321
Smoke point (°C)	230 to 238
Fire point (°C)	366 to 371
Cloud point (°C)	−14 to −11
Stable carbon isotope ratio	13.71 to 16.36

[a] Stability calculated as decimal fraction fatty acids multiplied by relative rate of reaction with oxygen of each fatty acid

Corn oil contains triglycerides plus phospholipids, glycolipids, phosphoinositides (phospholipids containing inositol), many isomers of sitosterol and stigmasterol (plant steroids), several tocopherols (vitamin E), waxes, unsaturated hydrocarbons such as squalene, carotenoids and chlorophyll compounds, as well as many products of decomposition, hydrolysis, oxidation, and polymerization of any of the natural constituents .

The commercial corn oil has been recognized as containing the highest levels of unsaponifiables (1.3–2.3%) of all the commercial vegetable oils (Strecker et al., 1996). The three main chemical components in the unsaponifiable fraction of corn oil are phytosterols, tocopherols and squalene.

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 linolenic acid.

The main physical and chemical characteristics of corn oil are presented in Table 1 (Woodbury et al., 1995a; Woodbury et al., 1995b; Woodbury et al., 1998).

FATTY ACID COMPOSITION OF CORN OIL

The fatty acid composition of oils is determined by the gas-liquid chromatography of the methyl esters of the fatty acids. Saponification (hydrolysis) followed by methylation is a

typical method for preparing the fatty acid methyl esters from glycerolipids and sterol esters (Sheppard & Iverson, 1975; Liu, 1994; Christie, 2003).

The fatty acids present in corn oil are palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3). Other fatty acids are found in small or trace amounts (Dubois et al., 2007).

The fatty acid compositional limits adopted in the most recent editions of Codex Alimentarius are presented in Table 4.

Table 2. Comparative composition of crude & refined corn oil (adapted from Blanchard et al., 1992; http://www.corn.org/)

	Typical Value (%)	
	Crude	**Refined**
Triglycerides	95.6	98.8
Free fatty acids	2.5	0.05
Phospholipids	1.5	0.0
Unsaponifiables		
Phytosterols	1.2	1.1
Tocopherols	0.12	0.08
Waxes	0.01	0.0
Color variable:	very dark to yellow	pale yellow
Odor and flavor	strong corn/feed	slight corn slight nutty/buttery

Table 3. Approximate composition of refined corn oil (adapted from Sayed & El Goud, 2010)

Amount in 100 g of oil	
Moisture	**None**
Protein (N×6.25)	None
Fats, total (g)	100.0
Triglycerides (g)	98.8
Polyunsaturated (g)	59.7
Monounsaturated (g)	26.0
Saturated (g)	13.1
Phytosterols (mg)	1000
Tocopherols (mg)	88
Carbohydrates (s)	None
Ash (g)	None

Table 4. Fatty acid composition of corn as determined by gas-liquid chromatography (expressed as percentage of the total fatty acids) (adapted from Codex Alimentarius)

Fatty Acid	Symbol	Percentage (%)
Lauric acid	C12:0	ND-0.3
Myristic acid	C14:0	ND-0.3
Palmitic acid	C16:0	8.6-16.5
Palmitoleic acid	C16:1	ND-0.5
Heptadecanoic acid	C17:0	ND-0.1
Heptadecenoic acid	C17:1	ND-0.1
Stearic acid	C18:0	ND-3.3
Oleic acid	C18:1	20.0-42.2
Linoleic acid	C18:2	34.0-65.6
Linolenic acid	C18:3	ND-2.0
Arachidic acid	C20:0	0.3-1.0
Eicosenoic acid	C20:1	0.2-0.6
Eicosadienoic acid	C20:2	ND-0.1
Behenic acid	C22:0	ND-0.5
Erucic acid	C22:1	ND-0.3
Lignoceric acid	C24:0	ND-0.5

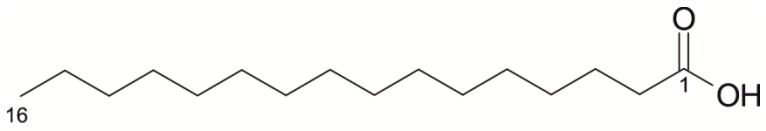
The chemical structures of the most important fatty acids in the corn oil are presented in the Figure 1.

Edible oils are often compared by examining their fatty acid profiles. Corn oil has a high content of the essential fatty acid linoleic acid (18:2). As is well known, an essential fatty is one that is required by humans but cannot be biosynthesized and must, therefore, be consumed from food (Das, 2006). Linoleic acid (also called *cis, cis*,-9, 12-octadecadienoic acid) is an ω-6 fatty acid. The numerous benefits of linoleic acid include cancer prevention, rheumatoid arthritis management, heart protection, etc. (Rainer & Heiss, 2004).

Another desirable characteristic of corn oil is that it contains low levels (<15%) of saturated fatty acids and very low levels of linolenic acid (18:3). It is well known that linolenic acid is especially susceptible to oxidation, which leads to rancidity. Another important free fatty acid present in corn oil is the oleic acid. The oleic acid ((9Z)-octadec-9-enoic acid) is a monounsaturated ω-9 fatty acid, associated with decreased low-density lipoprotein cholesterol, blood pressure reducing effects, decreased risk of breast cancer, etc. (Sales-Campos et al., 2013).

Besides the main fatty acids, corn oil contains up to 0.3% each of myristic and palmitoleic acid as well as 0.4% each of behenic and of lignoceric acid. The oils produced from the corn cultivated in the northern hemisphere contain 10-13% more linoleic acid than those in the southern hemisphere (Gunstone, 2011).

Furan fatty acids are part of the corn oil minor constituents. Two of these acids present the chemical structures exposed in Figure 2.



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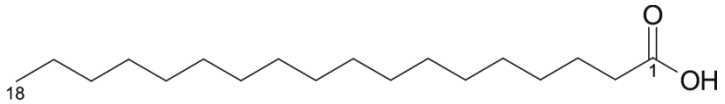

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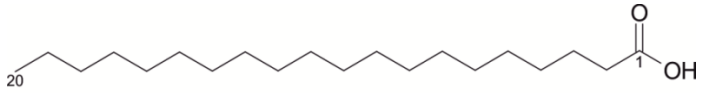

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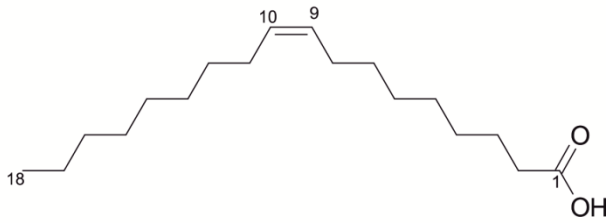

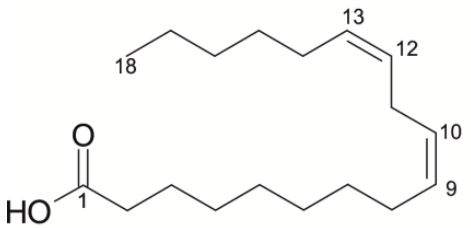

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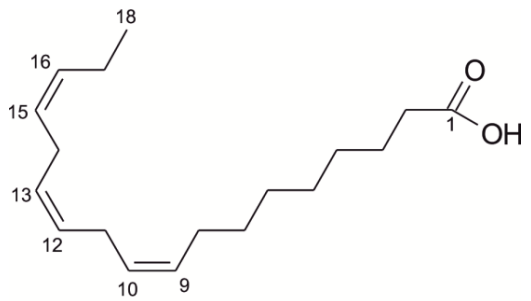


(f)

Figure 1. Chemical structures of a) palmitic acid (16:0); b) stearic acid (18:0); c) arachidic acid (20:0); d) oleic acid (18:1); e) linoleic acid (18:2); f) linolenic acid (18:3).

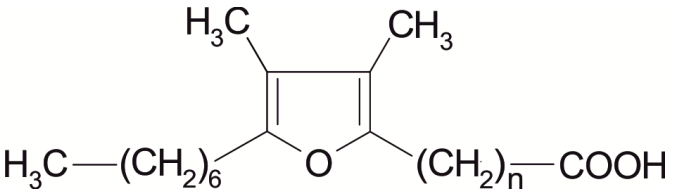


Figure 2. Chemical structures of furan fatty acids found in corn oil.

Table 5. Main triacylglycerols from corn oil and the number of double bonds per molecule

Triacylglycerol	Number of double bonds
LLO	5
LLL	6
LLP	4
OOL	4
PLO	3
PPL	2
OOP	2
LLS	4
LOS	3
OOO	3
PPO	1
PLS	2
LLLn	7
LnLO	6
OOS	2
POS	1
PLnL	5
PPP	0
OOLn	5
PLnO	4
PPS	0
SSL	2
LnLS	5
SSO	1
PPLn	3
SSP	0
SSS	0

Abbreviations: Ln, linolenic acid; L, linoleic acid; O, oleic acid; S, stearic acid; P, palmitic acid. Symbols such as LOS refer to all the possible triacylglycerols containing these three acids. (adapted from Strecker et al., 1990; Byrdwell et al., 2001).

The compound I (10, 13-epoxy-11, 12-dimethyloctadeca-10, 12-dienoic acid) is found in a concentration of 8-11 $mg \times kg^{-1}$ and the compound II (12, 15-epoxy-13, 14-dimethyleicosa-12, 14-dienoic acid) is found in a concentration of 9-13 $mg \times kg^{-1}$ (Belitz et al., 2009).

TRIACYLGLYCEROLS FROM CORN OIL

Triacylglycerols form the major part of corn oil, and they largely determine its main characteristics. The molecular structure of each individual triacylglycerol species has three basic characteristics: (a) the total carbon number; (b) the degree of unsaturation in each fatty acid; and (3) the position and configuration of the double bonds in each fatty acid (Buchgraber et al., 2004). Triacylglycerols are esters of the glycerol (a trihydroxyalcohol) with three molecules of one or more different fatty acids. Triacylglycerols are named in relation to the fatty acid components. For example, tristearin contains three molecules of stearic acid, and oleo-dipalmitin, one of oleic acid and two of palmitic acid. The types and relative proportions of triacylglycerols in oils vary with the species from which the oils have been obtained. The large number of triacylglycerols makes the analysis of the triacylglycerol composition a very challenging task. Reversed-phase HPLC techniques are currently used to quantitatively analyze the triacylglycerol molecules of plant and animal origin oils (Nikolova-Damyanova, 1997).

Reports on the triacylglycerol molecules from the refined corn oil have led to the identification of 19-27 individual molecules (Table 5), from which oleate-linoleate-linoleate and linoleate-linoleate-linoleate are the two most abundant molecules (Figure 3).

The fatty acids distribution in different triacylglycerols from corn oil shows that more than 65% of the triacylglycerols contain four or more double bonds and that more than 40% even contain five or more (Figure 4). Corn oil is considered a very stable oil.

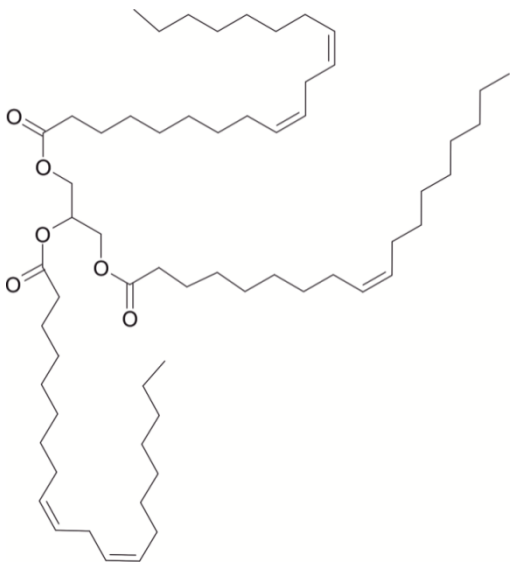


Figure 3. Chemical structures of 1-oleo -2-linoleo-3-linolein.

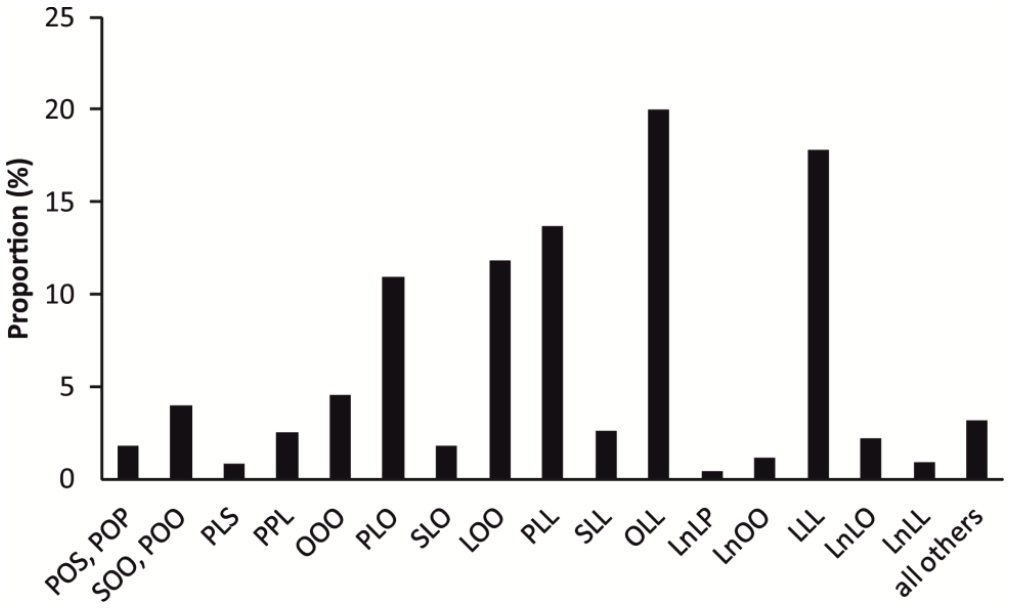


Figure 4. Triacylglycerols composition of corn oil (Strecker et al., 1990).

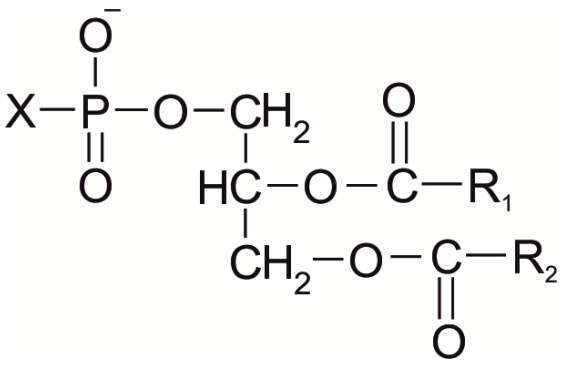
In Figure 3 presents the chemical structure of 1-oleo -2-linoleo-3-linolein, one of the most abundant triacylglycerol found in corn oil.

Monoacylglycerols and diacylglycerols are partial esters of glycerol and have one or two fatty-acid radicals, respectively. They are naturally associated with oils and fats. They can be produced by the reaction of fats and oils with glycerol or by the hydrolysis of the tryacylglicerols. Monoacylglycerol and diacylglycerol act as prooxidants, which increase oxidation at moderate temperature in the dark (Chaiyasit et al., 2007). Their surface-active properties that stabilize the emulsion are due to the hydrophilic properties of the glycerol-OH residue and to the hydrophobic properties of the fatty acid chains of the ester.

Monoglycerols and diacylglycerols should be removed from the oil during the oil-refining process, in order to improve the oxidative stability of the edible oils (Waraho, 2011). Monoglycerols and diacylglycerols are used as emulsifiers (Gulik-Krzywicki & Larsson, 1984; Byrdwell et al., 2001).

PHOSPHOLIPIDS

Crude corn oil contains lecithins, whose role is to stabilize the oil and to deposit it as energy reserve in the seeds. During the oil extraction, a part of these disperses in the oil; usually, they are separated and sold as special products (List, 1989).



Basic structure of phospholipids R_1, R_2 - fatty acid chains.

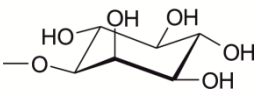
X	Name
$-OCH_2CH_2\overset{+}{N}(CH_3)_3$	Phosphatidylcholine (lecithin)
$-OCH_2CH_2\overset{+}{N}H_3$	Phosphatidylethanolamine (cephaline)
$-OCH_2CH(\overset{+}{N}H_3)COO^-$	Phosphatidylserine
$-OCH_2CHOHCH_2OH$	Phosphatidylglycerol
	Phosphatidylinositol

Figure 5. Chemical structure of the most important glycerophospholipids.

The term “lecithin” includes those phospholipids that are found in the oil after extraction. There are three main classes of compounds: phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinosite (Figure 5). The family of phospholipids includes a great variety of substances because of the very different fatty acid residues (R_1, R_2) and of the possibility of binding to two different positions, 1 or 2 (Shahidi, 1997).

In crude corn oil, phosphatides can be found at levels of 1-2%. The different varieties of crude oil had phospholipid-phosphorus values between 145 and 361 $mg \times kg^{-1}$. For oils at further stages of refining, the values dropped to 4.9 - 15.5 $mg \times kg^{-1}$. Degummed corn oils contained phosphatidylcholine (1.1 - 22 $mg \times kg^{-1}$ phosphorus), phosphatidylethanolamine (1.5 - 2 $mg \times kg^{-1}$ phosphorus), phosphatidylinositol (1.6 - 10 $mg \times kg^{-1}$ phosphorus) and phosphatidic acid (trace - 5 $mg \times kg^{-1}$ phosphorus). Further refined oils contained no phospholipids (Shahidi, 1997).

The level of phospholipids is important because these compounds carry out an antioxidant activity. The phospholipids may act as synergists (regenerating antioxidants such as tocopherols or phenolic compounds) or as metal scavengers (Pokorny & Korczak, 2001). At high levels, however, phospholipids may cause foaming or darkening during frying.

PHYTOSTEROLS FROM CORN OIL

Sterols are polycyclic alcohols derived from sterane (cyclopentanoperhydrophenantrene) as a base structure. One of the most important sterols is cholesterol, the first discovered sterol. Corn oils are cholesterol free, following the general agreement according to which all substances containing <50 ppm cholesterol are regarded as cholesterol free. Cholesterol, as a constituent of animal fat, is important as far as it can be linked to heart diseases (Dutta, 2003).

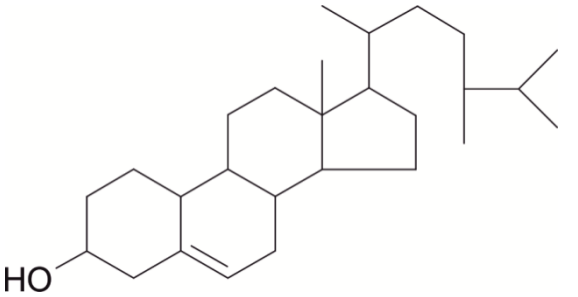
In general, vegetable oils have much higher concentrations of phytosterols than nonfatty vegetable foods. Among the commonly used commercial oils containing 0.77% phytosterols by weight (Ostlund et al., 2002), corn oil is one of the richest sources of phytosterol. Phytosterols are not absorbed by the human body (Weirauch & Gardner, 1987).

Corn germ oil contains two phytosterol lipid classes, free phytosterols and phytosterol fatty acid esters. Phytosterols have been recognized as one of the most important classes of phytonutrients (Ostlund et al., 2002). Most chemical identification processes of phytosterols in vegetable oils have been carried out by saponifying the oil and by measuring the resulting free phytosterols, usually trough GLC (Table 6).

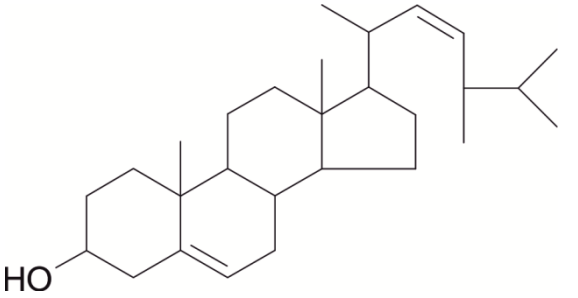
The chemical structures of sterols present in corn oil are presented in the Figure 6.

Table 6. Levels of sterols in crude corn oils as a percentage of total sterols (the values represent free and esterified phytosterols, measured after saponification)

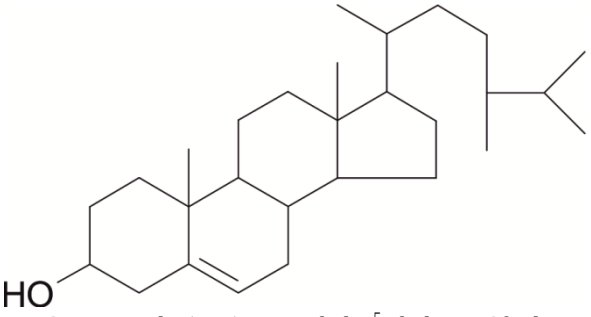
Sterol	Composition (%)
Cholesterol	0.2-0.6
Brassicasterol	ND-0.2
Campesterol	16.0-24.1
Stigmasterol	4.3-8.0
β–sitosterol	54.8-66.6
Δ^5-Avenasterol	1.5-8.2
Δ^7-Stigmastenol	0.2-4.2
Δ^7-Avenasterol	0.3-2.7
Others	ND-2.4
Stigmasta-8,22-dien-3β-ol	
5α-Stigmasta-7,22-dien-3β-ol	
$\Delta^{7,25}$-Stigmastadienol	
Δ^7-Campesterol	
Δ^7-Ergosterol	
$\Delta^{7,25}$-Stigmasterol	
Sitostanol	
Spinasterol	
24-Methylene cholesterol	
Total sterols (mg×kg^{-1})	7000-22100



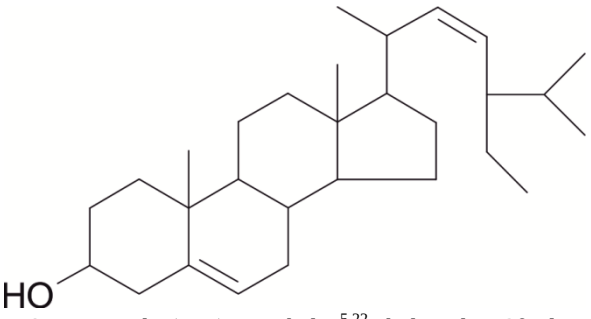
Cholesterol (Δ^5-Cholesten-3β-ol).



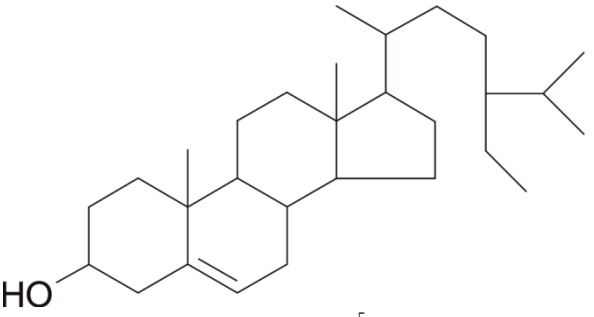
Brassicasterol - (24S)-24 Methyl-$\Delta^{5,22}$-cholestadien-3β-ol.



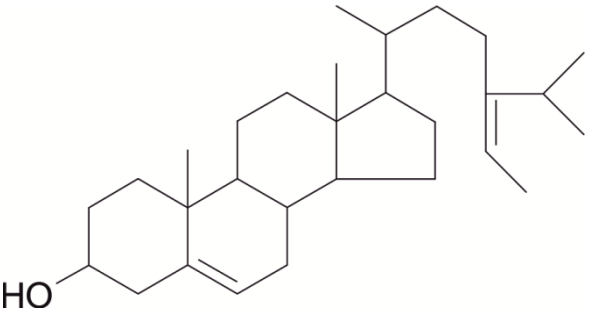
Campesterol - (24R)-24-Methyl-Δ^5-cholesten-3β-ol.



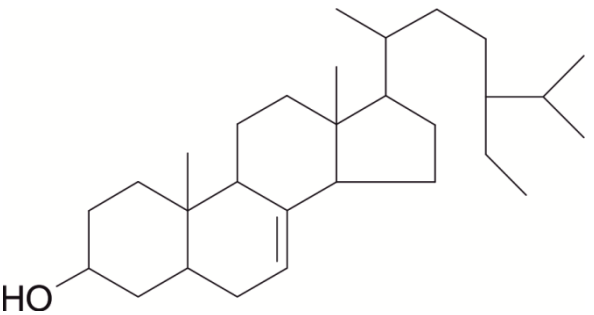
Stigmasterol - (24R)-24-Ethyl-$\Delta^{5,22}$-cholestadien-3β-ol.



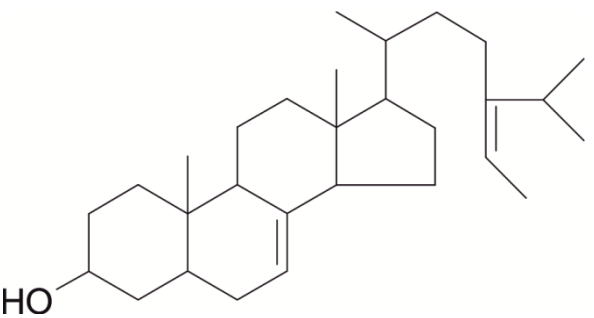
β-sitosterol - (24R)-24-Ethyl-Δ^5-cholesten-3β-ol.



Δ^5-Avenasterol - (24Z)-24-Ethylidene-Δ^5-cholesten-3β-ol.

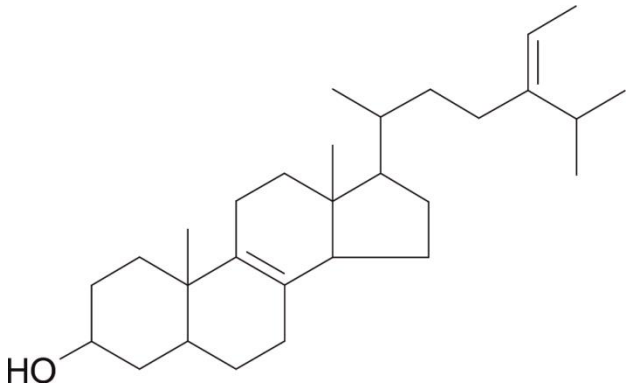


Δ^7-Stigmastenol - (24R,S)-24-Ethyl-Δ^7-cholesten-3β-ol.

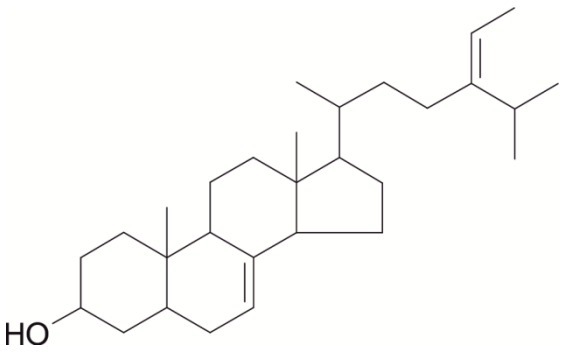


Δ^7-Avenasterol - (24Z)-24-Ethylidene-Δ^7-cholesten-3β-ol.

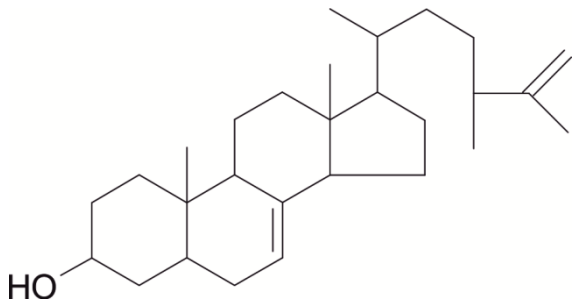
Figure 6. (Continued).



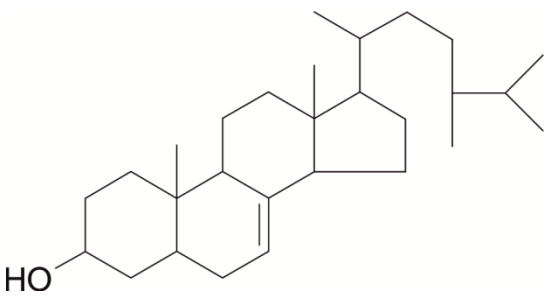
5α-Stigmasta-7,22-dien-3β-ol.



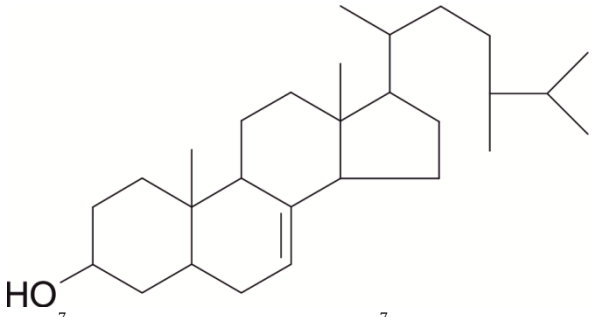
Stigmasta-8,22-dien-3β-ol.



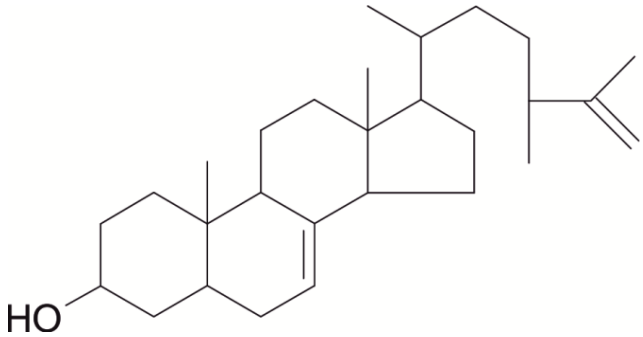
$\Delta^{7,25}$-Stigmastadienol - (24R,S)-24-Ethyl-$\Delta^{5,25}$-cholestadien-3β-ol.



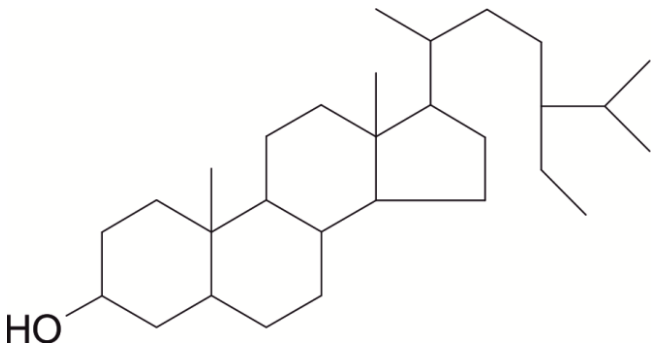
Δ^{7}-Campesterol - (24R)-24-Methyl-Δ^{7}-cholesten-3β-ol.



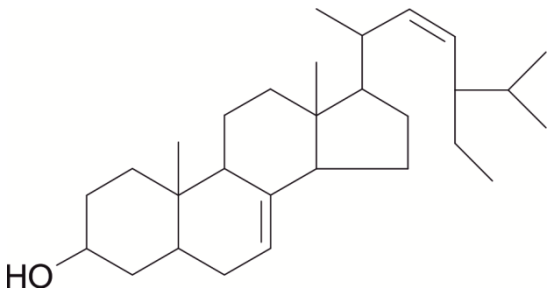
Δ^7-Ergosterol - (24S)-24-Methyl-Δ^7-cholesten-3β-ol.



$\Delta^{7,25}$-Stigmasterol - (24R,S)-24-Ethyl-$\Delta^{7,25}$-cholesten-3β-ol.

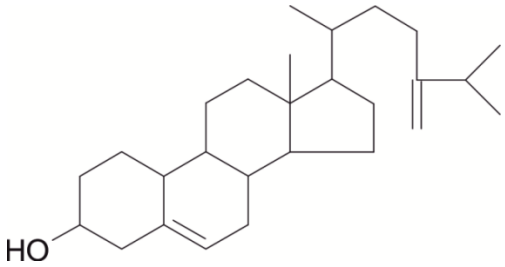


Sitostanol - (24R)-24-Ethyl-cholestan-3β-ol.



Spinasterol - (3β, 5α, 22E)-Stigmasta-7,22-dien-3-ol.

Figure 6. (Continued).



24-Methylene Cholesterol - 24- Methylene-Δ^5-cholesten-3β-ol.

Figure 6. Chemical structures of sterols from corn oil.

The examination of the total phytosterols in corn oil has revealed that the major phytosterol was β-sitosterol (Table 6). The major phytosterols in corn germ oil are β-sitosterol > campesterol > stigmasterol (Table 6). β-sitosterol is also found in the form of ferulate ester, displaying antioxidant properties.

TOCOPHEROLS AND TOCOTRIENOLS

Corn oil has long been recognized as a rich source of tocopherols, γ-tocopherol being the most abundant tocopherol, followed by α-tocopherol and δ-tocopherol (Table 7)(Codex Alimentarius, 1997; Fennema, 1996; Kamal-Eldin & Andersson, 1997).

Table 7. Levels of tocopherols in crude corn oils

Tocopherol	Composition (mg×kg^{-1})
α-Tocopherol	23-573
β-Tocopherol	ND-356
γ-Tocopherol	268-2468
δ-Tocopherol	23-75
Total, mg×kg^{-1}	**330-3720**

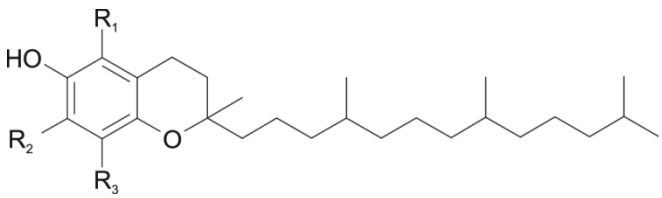


Figure 7. Chemical structure of tocopherols.

Tocopherol	R_1	R_2	R_3
α-Tocopherol	CH_3	CH_3	CH_3
β-Tocopherol	CH_3	H	CH_3
γ-Tocopherol	H	CH_3	CH_3
δ-Tocopherol	H	H	CH_3

Table 8. Levels of tocotrienols in crude corn oils

Tocotrienols	Composition ($mg \times kg^{-1}$)
α-Tocotrienol	ND-239
β-Tocotrienol	ND-52
γ-Tocotrienol	ND-450
δ-Tocotrienol	ND-20
Total, $mg \times kg^{-1}$	ND –709

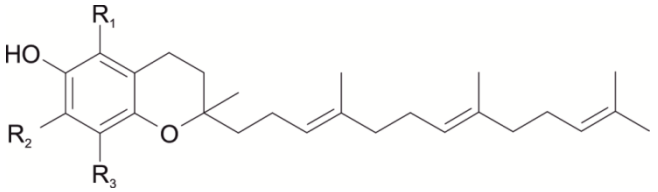


Figure 8. Chemical structures of tocotrienols.

Tocotrienol	R_1	R_2	R_3
α-Tocotrienol	CH_3	CH_3	CH_3
β-Tocotrienol	CH_3	H	CH_3
γ-Tocotrienol	H	CH_3	CH_3
δ-Tocotrienol	H	H	CH_3

High performance liquid chromatography (HPLC) is currently the preferred method for determining the tocols (Shahidi, 1997; Gimeno et al., 2000).

Tocopherols are the most important antioxidants present in corn oil. Among the tocopherols, α-tocopherol has received the most attention because of its vitamin E activity; however, other isomers also are known to display valuable antioxidant properties. Some studies suggest the fact that γ-tocopherol may be superior to α-tocopherol in preventing the oxidation of lowdensity lipoproteins and in delaying the thrombus formation. Tocopherols are used as antioxidants because they trap hydroperoxide intermediates both in vitro and in vivo, thus breaking the chain reaction. Tocopherols decrease corn oil oxidation under light by singlet oxygen ($^1O_2$) quenching (Burton & Ingold, 1981; Rizvi et al., 2014; Di Mascio et al., 1991).

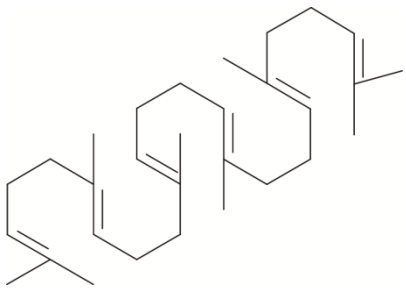


Figure 9. Chemical structure of squalene.

Significant levels of tocotrienols (the most abundant was γ-tocotrienol followed by α-tocotrienol) were found in corn oil (Dolde, 2009). Tocotrienols have antioxidant properties and in addition, it is currently believed that tocotrienols also possess cholesterol-lowering properties, which are related to their ability to inhibit the cholesterol biosynthesis (Palozza et al., 2006).

The refining process reduces tocopherol contents (Ergönül & Köseoğlu, 2014).

TERPENOIDS AND DERIVATIVES FROM CORN OIL

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is the last metabolite preceding sterol ring formation. Squalene consists of six isoprene-units and contains six (all-trans) double bonds. On the basis of GC examination it was reported that squalene was the major hydrocarbon in corn germ oil (Worthington & Hitchcock, 1984). Squalene is a compound beloging to terpenoid family.

Squalene is contained in corn oil in percentages between 0.016-0.042 and is a marker substance that proves the adulteration of the olive oil.

Squalene presence is regarded as partially responsible for the beneficial health effects of corn oil and its chemopreventive action against certain types of cancer (Rao et al., 1998).

Carotenoids. Carotenoids are terpenoids, the majority having a central skeleton consisting of 40 carbon atoms, formed of eight isoprenoid units. Carotenoids are widely found among living beings, plants and animals. Higher concentrations and greater varieties are found within the plant kingdom (Sajilata et al., 2008).

The amounts of carotenoids in the commercial corn oil are relatively low, partly due to their low concentrations in the germ and partly to their removal during bleaching (Moreau et al., 2007). The most abundant carotenoids in corn oil are lutein and zeaxanthin (Figures 10 and Figure 11).

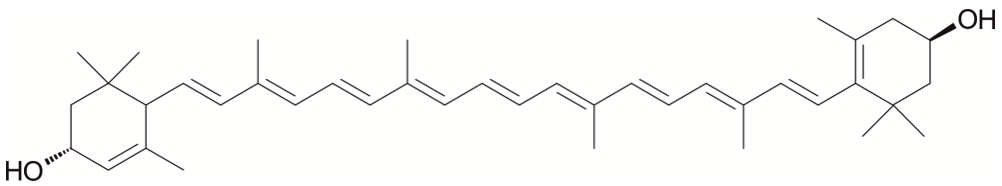


Figure 10. Chemical structure of lutein.

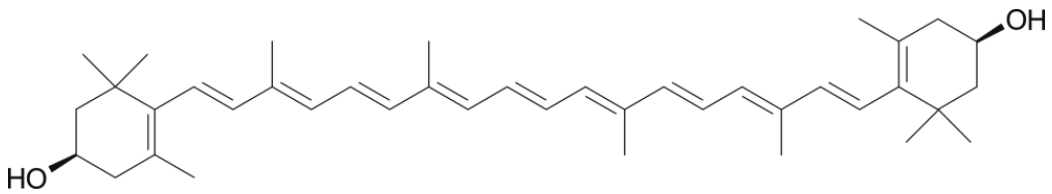


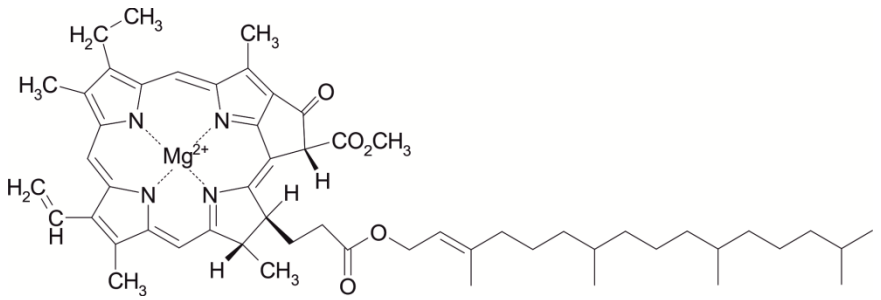
Figure 11. Chemical structure of zeaxanthin.

Lutein and zeaxanthin are yellow to red pigments founded broadly in vegetables, fruits, and cereals, including corn. Lutein and zeaxanthin are found in high quantities in the macula of the human eye (Sommerburg et al., 1998). Lutein is a yellow pigment which, in elevated concentrations, is orange-red. Lutein and zeaxanthin are detected and quantified by high-performance liquid chromatographic, using C18 column to increase the resolution of the peaks (Gilmore & Yamamoto, 1991).

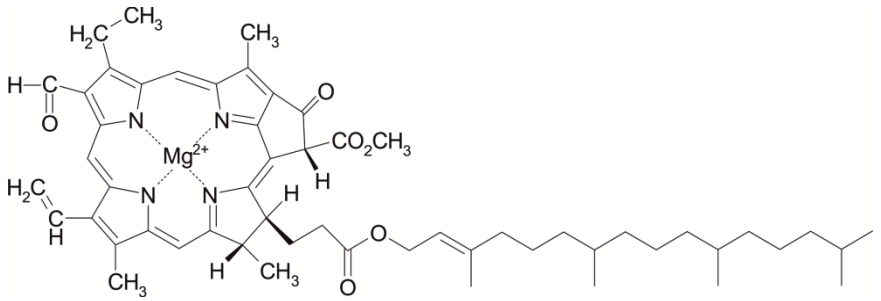
Lutein and zeaxanthin are important in nature because they absorb the light energy excess, avoiding damaging the plants from too much sunlight. In the human body, lutein and zeaxanthin play significant antioxidant roles. These pigments protect the body cell from the destructive effects of the free radicals. However, in certain conditions, carotenoids in vegetable oils and certain other food matrices may serve as pro-oxidants, especially at higher concentrations (Fiedor & Burda, 2014).

Lutein and zeaxanthin may reduce the risk of macular degeneration and cataracts. Besides the significant eye and vision benefits, lutein and zeaxanthin protect the body against atherosclerosis, the disease closely related to heart attacks (Sommerburg et al., 1998; Fiedor & Burda, 2014).

Chlorophylls and pheophytins. Chlorophyll pigments are widely spread in the natural environment, chlorophylls a and b being the most abundant. Chlorophylls are included in the porphyrin group. They have a basic common structure, the porphin, with four units of pyrrole linked at the alpha positions by methylene bridges in a planar aromatic system, which is highly stable and amenable to the formation of chelates with metal ions. For the chlorophylls, the metal ion that forms the complex is Mg^{2+} (Shahidi, 1997).



Chlorophyll a



Chlorophyll b

Figure 12. Chemical structures of chlorophylls.

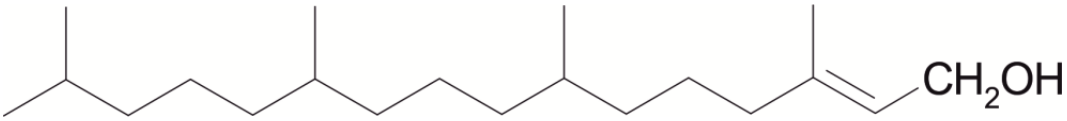


Figure 13. Chemical structure of phytol.

Chlorophyll and pheophytin contents have been determined in refined corn oils trough HPLC and fluorescence detection (Usuki et al., 1984). The total of the contents varied from 95 to 162 $\mu g \times kg^{-1}$ oil. The chemical structures of chlorophyll a and chlorophyll b are presented in Figure 12.

Pheophytin is a bluish-black waxy pigment that can be obtained from chlorophyll by replacing the magnesium with two hydrogen atoms by treatment with a weak acid (as oxalic acid). Chlorophylls are generally removed during oil processing through the bleaching process (Akoh & Min, 2008).

Chlorophylls and pheophytins act as sensitizers to produce $^1O_2$ in the presence of light and atmospheric $^3O_2$, and accelerate the oxidation of the oil (Choe & Min, 2006). Chlorophylls act as antioxidants in the dark probably by donating hydrogen to free radicals (Endo et al., 1985).

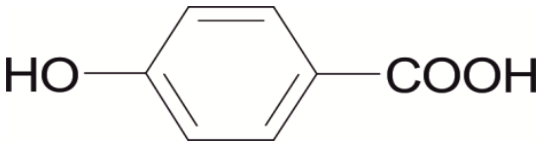
Phytol (3, 7, 11, 15-tetramethylhexadec-2-en-1-ol) is a diterpene, a member of the group of branched-chain unsaturated alcohols (Figure 13). Being the product of chlorophyll metabolism in plants, phytol is widely spread in nature. Phytol probably originates in corn oil from chlorophylls.

D Differetiang nonrefined and refined oils is an important task in food control. In chlorophyll only trans-phytol is to be found. The relative abundance of *cis*-phytol versus *trans*-phytol has been used as a marker in authenticating the nonrefined edible oils. The absence of cis-phytol in the nonrefined edible oils can be used as a marker for authentication (Vetter et al., 2012).

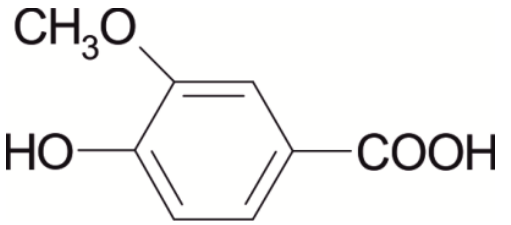
PHENOLIC COMPOUNDS FROM CORN OIL

Polar phenol fraction is a complex mixture of compounds with different chemical structures obtained from the oil by extraction with methanol-water. The methanol-water extract of corn oil mainly contains phenolic acids (Shahidi & Naczk, 2003).

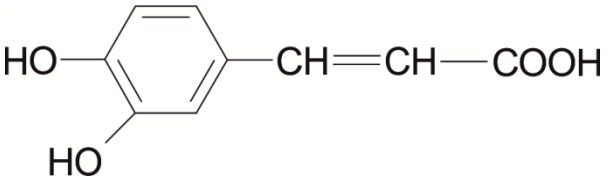
In corn oil, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric and ferulic acid have been detected; out of which the ferulic acid was the most abundant at 5.79 $mg \times kg^{-1}$, followed by the p-coumaric acid at 1.63 $mg \times kg^{-1}$ (Daigle et al., 1988; Shahidi, 1997). Figure 14 presents the chemical structures of the phenolic compounds from the corn oil.



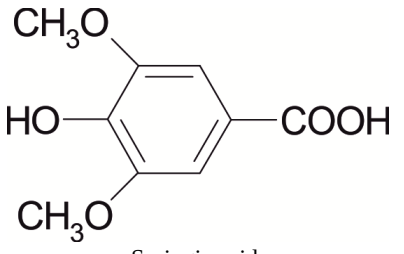
p-Hydroxybenzoic acid.



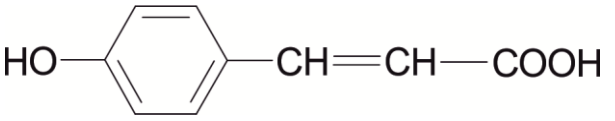
Vanillic acid.



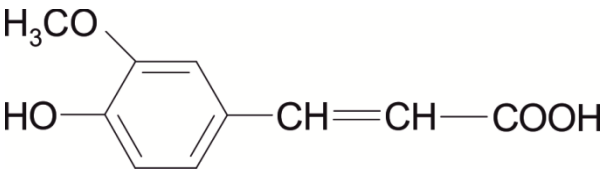
Caffeic acid.



Syringic acid.



p-Coumaric acid.



Ferulic acid.

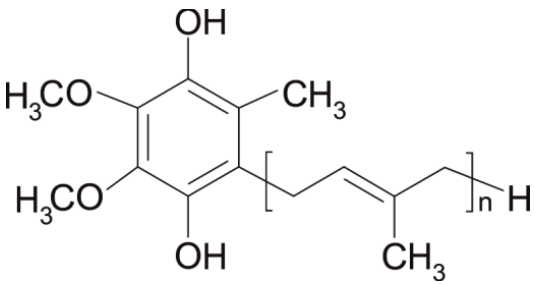
Figure 14. Chemical structures of the phenolic compounds from corn oil.

The spectrophotometric method commonly applied in determining the phenols in the water-methanol extract is based on the use of Folin-Ciocalteu reagent. Results are usually expressed as gallic acid equivalents (mg gallic acid/kg oil) (Rover & Brown, 2013).

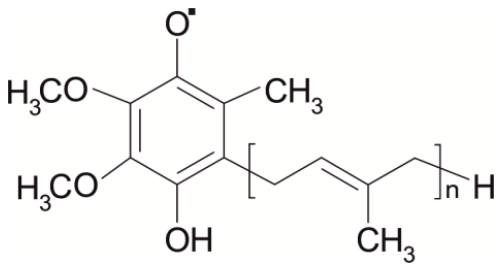
Sophisticated gas chromatographic and liquid chromatographic methods have been developed for the analysis of polar phenols. These methods are useful in elucidating the complex phenolic composition of the polar fraction of the oils, but they cannot be easily applied in the routine analysis because of its high overall cost (Snyder et al., 2011).

Corn oil phenols have been studied with respect to their potential to scavenge the free radicals, the peroxyl radicals, the superoxide radicals, etc. The free radical scavenging activity of the phenolic acids using model free radicals such as 1,7-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), or $ABTS^{\bullet+}$ (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt cation radical) is often measured. TEAC (Trolox equivalent antioxidant capacity), FRAP (Ferric-reducing antioxidant capacity) and TRAP (total-radical-trapping antioxidant parameter) of the corn oil are also used in order to obtain additional information, necessary to investigate the relation between the antioxidant intake and the oxidative stress related diseases (Stratil et al., 2008; Cetó et al., 2014; Apak et al., 2007).

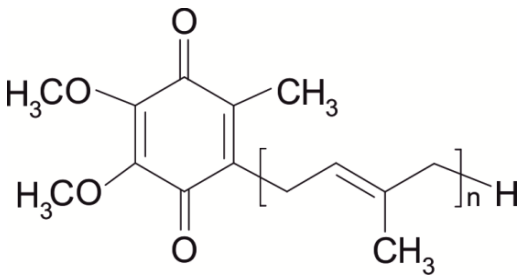
Phenolic compounds are related to the stability of the corn oil but also to its biological properties (Shahidi, 1997).



Ubiquinol.



Semiquinone radical.



Ubiquinone.

Figure 15. Chemical structure of ubiquinol, semiquinone radical and ubiquinone (n=9, 10).

Corn oil also contains ubiquinone (also known as co-enzyme Q), which in its turn, possess an anti-oxidant property. The presence of the oxidized and reduced forms of the Q (9) and Q (10) ubiquinones has been determined in the commercial corn oil (Cabrini et al., 2001). Very high concentrations of ubiquinones have been found in corn oils (Shahidi, 1997; Cabrini et al., 2001; Kokate et al., 2008; Guad et al., 2006).

Corn oil generally is considered to be fairly stable to oxidation. The quality of its flavor is very good. Corn oil is the only oil containing a significant amount of ubiquinone (200 $mg \times kg^{-1}$; a benzoquinone with a side chain of 6-10 prenyl units), an excellent antioxidant that helps protect oils against oxidative rancidity (Petillo & Hultin, 2008; Shahidi, 1997).

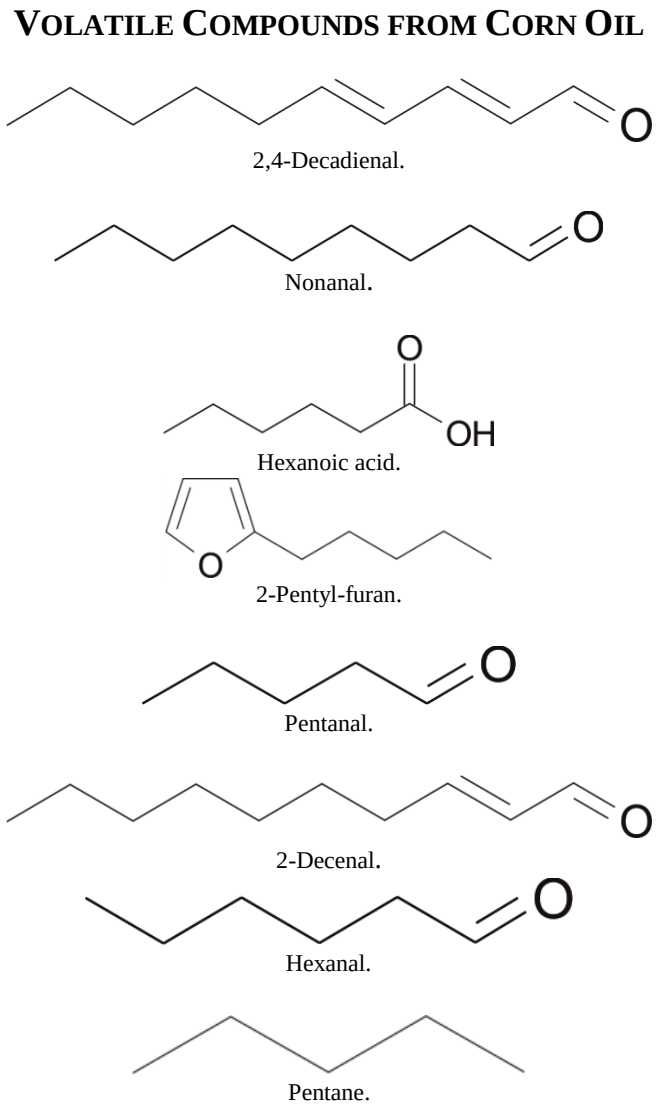


Figure 16. Chemical structure of volatile compounds from corn oil .

Several compounds belonging to different chemical classes have been identified and quantified in corn oil. Volatile compounds including 2,4-decadienal, nonanal, hexanoic acid, 2-pentyl-furan, pentanal, 2-decenal, hexanal, and pentane identified in corn oils from supercritical fluid-extracted oils have been analyzed with the help of the headspace gas chromatography (GC) (Figure 16) (Snyder & King, 1994).

2-Pentyl furan, which has been was identified in corn oil is a volatile compound obtained from the oxidation of the corn oil. The flavor threshold of this compound in oil, at room temperature, is 1 ppm. At concentrations of 1-10 ppm, it imparts, the oil a characteristic beany odor (Krishnamurthy et al., 1967; Steenson et al., 2002)

FUTURE TRENDS AND PERSPECTIVES

Corn oil's desirable properties include: a mild nutty flavor, high levels of unsaturated fatty acids, low levels of saturated fatty acids and very low levels of linolenic acid, high levels of desirable unsaponifiables (including phytosterols, tocopherols, phenolic compounds, etc.), and stability during frying. Nutritional properties and health benefits of the biological active compounds from corn oil have been proved by numerous studies published in literature. All these features make corn oil an excellent dietary choice that supports good overall health.

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Chapter 2

BIOSYNTHESIS, ANALYSIS AND ANTIOXIDANT PROPERTIES OF TOCOLS AND CAROTENOIDS FROM CORN OIL

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ABSTRACT

Corn oil is an edible oil extracted from the maize germ and has multiple applications. This chapter focuses on biosynthesis, analysis and antitoxidant activity of two classes of compounds, tocols and carotenoids, which are present in this vegetal oil. The demand to obtain corn oil with high levels of antioxidants should be considered. Tremendous work has been carried out in order to increase the level of these antioxidants by switching or modifying biochemical pathways. However, new approaches should be taken into consideration in order to increase their concentration in corn oil. A whole range of analysis or estimation methods of antioxidant activity are presented herein. The data suggest that tocopherols, as well as carotenoids, from corn oil are important antioxidant molecules. Moreover, development of more specific detection assays to distinguish their individual contribution to the total antioxidant activity should be taken into account.

Keywords: corn oil, antioxidant, tocol, carotenoid, biosynthesis

INTRODUCTION

Corn oil, the second most produced oil in the United States, gains a well-deserved status in our nutrition. Nowadays its production is ranked in the top ten worldwide. Maize oil can be found in foodstuffs or used in chemical industry and less commonly in pharmaceutical industry. Corn oil is characterized by elevated levels of unsaturated fatty acids and

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antioxidants. Particularly, tocols and carotenoids, biochemical compounds widely spread in plants, are valuable fat-soluble antioxidants for mammals (Ball, 2004; Kamal-Eldin & Appelqvist, 1996). In corn, these compounds are localized in the endosperm (tocopherols, tocotrienols, carotenoids), in the pericarp and germ (only tocopherols), and in the aleurone layer (carotenoids) (Balz & Schulte, 1992; Grams et al., 1970; Ndolo & Beta, 2013).

Corn oil, like soybean oil, presents a significant content (100-2000 mg/kg oil) of total tocopherol (Cerretani et al., 2010; Gliszczyńska-Świgłoet al., 2007; Grusak, 1999; Ndolo & Beta, 2013). However, the estimated tocopherol content in high-oil maize lines was higher than that in normal lines (Zhou et al., 2009). Furthermore, corn oil displays elevated levels of γ-tocopherol instead of its isomers (Eitenmiller & Lee, 2004; Lerma-Garcia, 2007; Shahidi, 2000). For this reason the use of corn oil induces an increase of γ-tocopherol in human serum (Lemcke-Norojärvi et al., 2001) and reduces cancer risk by minimizing DNA damage (Elmadfa & Park, 1999). γ-tocotrienol, an unsaturated variant of vitamin E, predominates in corn (Panfili et al., 2003). Conversely, moderate levels of α-tocotrienol were detected in maize germ oil (Speek et al., 2006).

By contrast, the concentration of β-carotene in corn oil is quite small (0.6 - 2 ppm) (Bootsma, 2012; E, Dauqan et al., 2011; Moreau et al., 2007). Among carotenoids, lutein and zeaxanthin are most abundant in corn kernels or corn products (de Oliveira & Rodriguez-Amaya, 2007; Gunstone, 2011) (more than 4 ppm). The analysis of six commercial corn hybrids reveals moderate levels of β-cryptoxanthin and β-carotene (1-3 ppm) (Kljak & Grbeša, 2015).

The reaction of homogentisic acid with phytyl-diphosphate (or geranyl geranyl-diphosphate) constitutes an important metabolic step for the biosynthesis of tocopherols (or tocotrienols). Conversely, autocondensation of geranyl geranyl-diphosphate leads to important precursors required for carotenoids biosynthesis. All these metabolic steps are assisted by specific enzymes. The attempts to modify or manipulate genes encoding these enzymes have revealed that these metabolic pathways can be targeted to achieving the desired metabolic product. The elevated levels of antioxidants could bring multiple benefits to consumers' life quality.

Initially, simple methods such as ultraviolet–visible (UV-Vis), fluorescence or infrared (IR) spectroscopy were proposed in order to estimate the total amount of antioxidants in various food samples. However, coupled methods such as gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography with ultraviolet-visible (HPLC-UV-Vis) or nuclear magnetic resonance detector (HPLC-NMR) and liquid chromatography-mass spectrometry (LC-MS) are employed more to quantify individual tocols or carotens from various corn-based food samples. In this chapter all these methods of analysis will be briefly described.

The last part of this chapter is focused on the antioxidant activity of tocols and carotenoids. Their antioxidant potency seems to be correlated with such factors as:

- chemical composition of oil;
- antioxidant conformation whithin membrane;
- physical parameters (temperature, intensity of light);
- type of system used (a heterogeneous system is prefered).

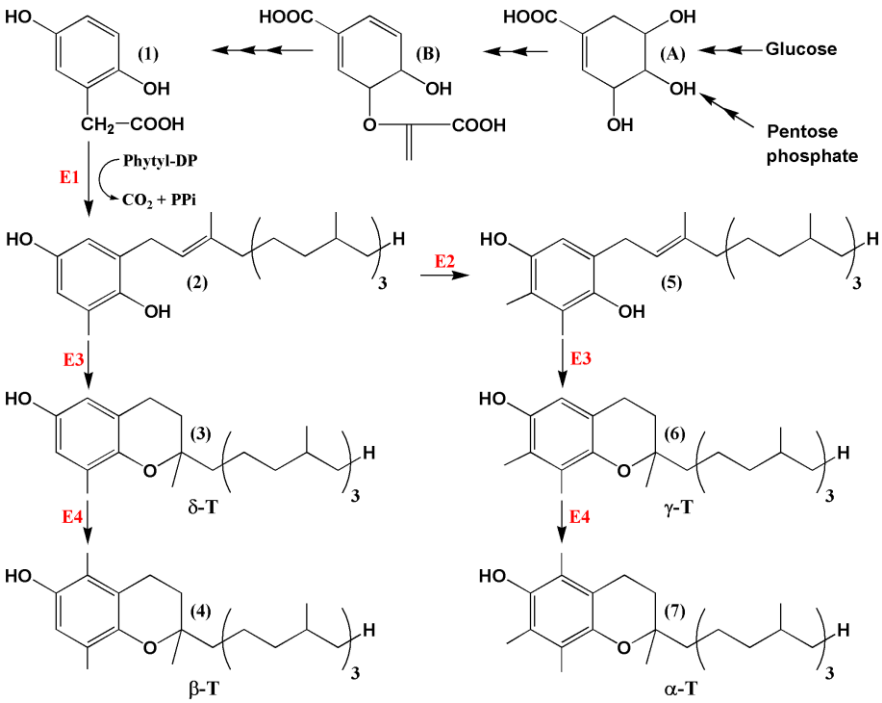

Figure 1. Tocopherol biosynthesis in plants. Enzyme abbreviation: E1-homogentisate phytyltransferase; E2-2-methyl-6-phytylplastoquinol methyltransferase; E3-tocopherol cyclase; E4-γ-tocopherol methyltransferase. γ-tocopherol (γ-T) is the major component (>70%) found in corn seeds oil.

Various methods for determining the antioxidant capacity are referred here. These assays are based on antioxidant capacity to scavange various radicals, reduce certain ions or compete with other compounds. However, this activity can be indirectely measured by monitorizing various compouds derived from radical-induced processes.

BIOSYNTHESIS

Biosynthetic Pathway of Tocols

In plants, the biosynthesis of tocols head group begins in the cytosol with the homogentisic acid (1), a product resulted from the shikimic acid pathway. Seven main steps are distinguished in the homogentisic acid biosynthesis. Shikimate (A) biosynthesis is based on intermediates from two pathways (glycolysis and pentose phosphate). A key intermediate, the chorismate (B), can be further used to obtain aromatic amino acids, vitamins and antibiotics. Moreover, during the homogentisic acid biosynthesis, tyrosine and p-hydroxyphenyl pyruvate were identified (Garcia et al., 1997). Conversely, the hydrophobic tail (phytyl) biosynthesis occurs in plastids (Ischebeck et al., 2006). Polyprenyltransferase (E1) catalyzes the coupling reaction of the homogentisic acid with the phytyl-diphosphate (phytyl-DP). The resulted compound, 2-methyl-6-phytylbenzoquinol (2) is and intermediate in δ- and β-tocopherol biosynthesis (Soll & Schultz, 1980). Similarly, the same enzyme (E1)

assists the initial reaction of homogentisic acid with geranyl geranyl-diphosphate, an essential step that precedes tocotrienols formation. On the other hand, 2-methyl-6-phytylbenzoquinol (2) can be further methylated. A methyltransferase (E2) assists its convertion to another intermediate, 2,3-dimethyl-6-phytylbenzoquinol (5) (Shintani et al., 2002). These two intermediates (2 and 5) are required during the cyclisation step catalyzed by tocopherolcyclase (E3). In parallel, two chromane derivatives are formed, namely δ- and γ-tocopherol (δ-T and γ-T) (Hunter & Cahoon, 2007). The last-mentioned compounds can be readily methylated using a γ-T-methyltransferase (E4) (Shintani & DellaPenna, 1998). Thus, two final tocols, β- and α-tocopherol (β-T and α-T) may also appear in this pathway.

Regarding tocotrienols biosynthesis (Figure 2), at first, geranyl geranyl transferase (E1') catalyzes the reaction of geranyl geranyl-diphosphate with homogentisic acid. The condensation product, 2-methyl-6-geranylgeranyl-1,4-benzoquinone (MGGBQ) is converted using an identical set of enzymes (E2, E3 and E4) to δ- and γ-tocotrienols (δ-T3 and γ-T3) respectively β- and α-tocotrienols (β-T3 and α-T3) (Abbasi, 2007; Cahoon et al., 2003; Herbers, 2003; Porfirova et al., 2002; Soll & Schultz, 1979).

There are several factors that influence tocols biosynthesis. The tocopherol level could be correlated with precursor (phytol, Phytyl-PPor, GGPP, HGA) concentration (Soll et al., 1980; Whistance & Threlfall, 1970). Many target genes encode enzymes such as γ-methyltransferase (d'Harlingue & Camara, 1985; Shintani & DellaPenna, 1998), GG reductase (Keller et al., 1998), GGPP synthase, HRP dioxygease (Falk et al., 2003; Tsegaye et al., 2002), prenyl transferase (Dada et al., 1968), HGGT-homogentisic acid transferase (Cahoon et al., 2003), cyclase (Porfirova et al., 2002), phytyltransferase (Schledz et al., 2001), homogentisate prenyltransferases (Dörmann, 2003; Sadre et al., 2006), phytyl kinase (DellaPenna & Last, 2006; Valentin et al., 2006). Among these enzymes, γ-tocopherol methyltransferase was overexpressed in *Arabidopsis sp.* inducing a higher α-T level in plant oils.

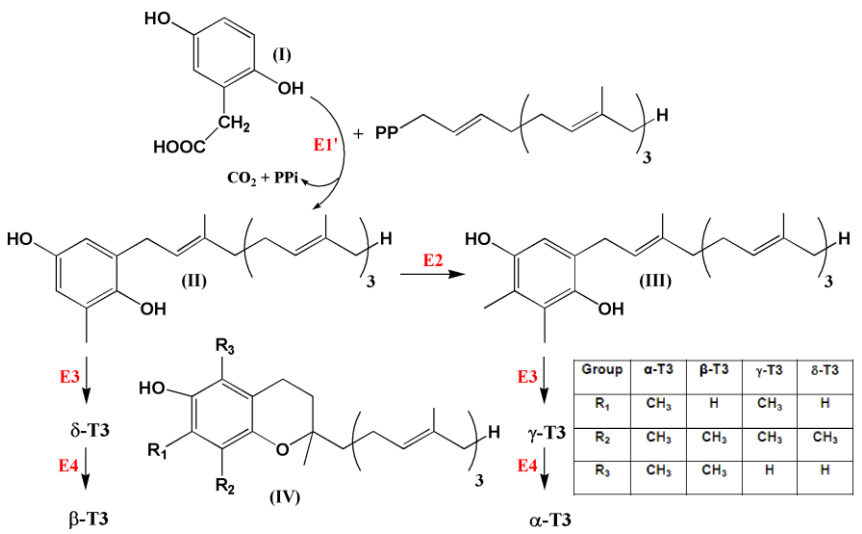

Figure 2. Tocopherol biosynthesis in plants. Enzyme abbreviation: E1'- geranyl geranyl transferase; E2-2-methyl-6-phytylplastoquinol methyltransferase; E3-tocopherol cyclase; E4-γ-tocopherol methyltransferase (Adapted from Kamal-Eldin et al., 1997).

Instead, tocochromanol levels are impaired in sucrose export defective 1 maize mutant (Provencher et al., 2001). Conversely, regulation and function of plants tocochromanols were demonstrated by simultaneous transgenic expression and genetic engineering (Hoa et al., 2003; Karunanandaa et al., 2005; Mène-Saffrané & DellaPenna, 2010). ROS (Reactive oxygen species) and phytohormones (salicylic, abscisic and jasmonic acids) (Falk et al., 2002; Sandorf & Holländer-Czytko, 2002) also regulate tocopherol biosynthesis. Abscisic acid was found to control genes expression of p-hydroxyphenylpyruvate dioxygenase respectively, the tocopherol amount (Munné-Bosch, 2007). Tocopherol level increases under stress conditions and can be correlated with jasmonic acid (Sandorf & Holländer-Czytko, 2002) or salyciclic acid concentration (Munné-Bosch & Falk, 2004) or lack of water (Ali et al., 2010). In this respect, the tocopherol is a signal molecule within ROS-antioxidant network (Shao et al., 2008).

Biosynthesis of Carotenoids

Tocols and carotenoids biosynthesis pathways have a key intermediate, geranyl geranyl-pyrophosphate (GGPP). The second pathway starts with the condensation of two GGPP molecules and is assisted by phytoene synthase (E1") (Shumskaya et al., 2012). This is a rate limiting step which dictates the carotenoid level in maize tissues. In the first instance, a long hydrocarbonated compound, 15-cis-phytoene (P1) possessing 9 double (C=C) bonds, is formed. Coupled desaturation-isomerization steps are necessary to generate all-trans lycopene. In the first sequence, 9,15-cis-phytoene and 9,15,9'-tricis-ζ-carotene are formed. These reactions are catalyzed by phytoene desaturase (E2") (Li et al., 1996). The last intermediate is further isomerized to 9,9'-di-cis-ζ-carotene (C1) by ζ-carotene isomerase (E3") (Chen et al., 2010). In the next step, ζ-carotene desaturase (E4") assists sequential conversion C1 to all-trans-lycopene (C2) via 7,9,7',9'-tetra-cis-neurosporene (Matthews et al., 2003). Lycopene cyclization constitutes a branching point in maize carotenoid biosynthesis. This reaction is catalyzed by lycopene cyclases (ε and β or E5" and E6") (Cunningham et al., 1996). The resulted compounds, δ- and γ-carotene (C3 and C4) possess an ionone moiety at the edge of the molecule. A similar strategy is used for α- and β-carotene (C5 and C6) biosynthesis. At this stage lycopene β-cyclase (E6") introduces the second ionone ring. Furthermore, mono and dihydroxylated carotens are resulted by grafting of one or two hydroxyl groups on ionone ring. Thus, lutein is obtained from α-carotene via a mono-hydroxylated intermediate, α-cryptoxanthin or zeinoxanthin. An enzyme, ε-carotene hydroxylase (E7"), assists the previous biochemical reaction (Tian et al., 2004). Analogously, zeaxanthin results from β-carotene via a mono-hydroxylated intermediate, β-cryptoxanthin (Figure 3).

Approaches in plants engineering to increase carotenoids concentrations have been reviewed (Hirschberg, 1999). Overexpression of 1-deoxy-5-D-xylulose-5-phosphate synthase (DXS) and phytoene synthase (PSY) genes was correlated with elevated levels of carotenoids (Aluru et al., 2008; Burkhardt et al., 1997; Ducreux et al., 2005; Lindgrenet et al., 2003; Shewmaker et al., 1999). Combinatorial nuclear transformation was successfully used to generate maize transgenic plants with high carotenoids levels (Zhu et al., 2008).

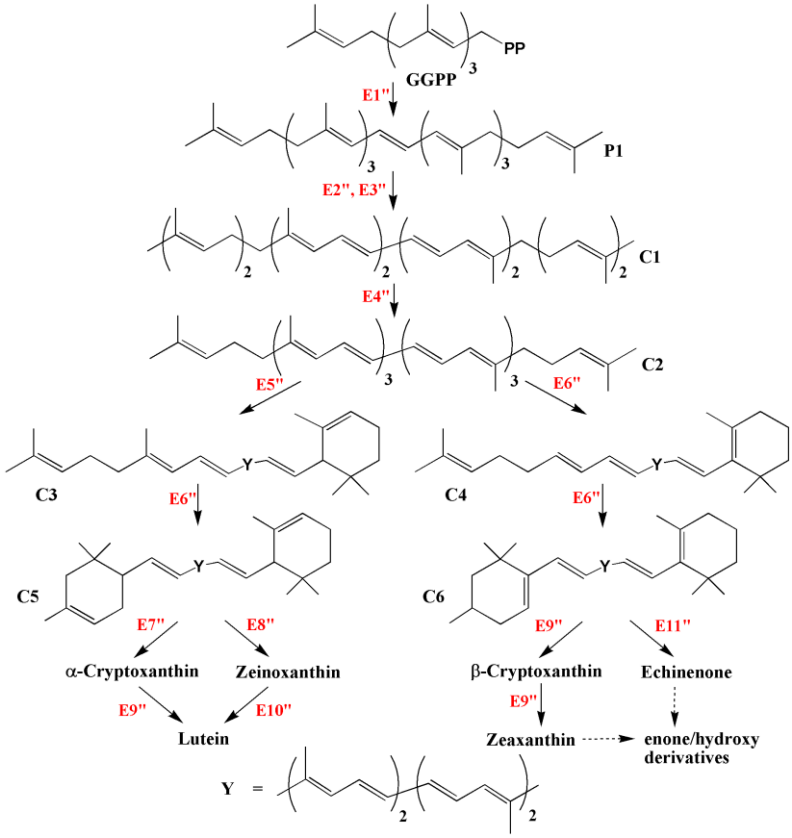

Figure 3. Carotenoid biosynthesis in plants. Enzyme abbreviation: E1"- phytoene synthase; E2"- phytoene desaturase; E3"- ζ-carotene isomerase; E4"- ζ-carotene desaturase; E5"- lycopene ε-cyclase; E6"- lycopene β-cyclase; E7"-ε-carotene hydroxylase; E8"- β-ring hydroxylase; E9"-β-carotene hydroxylase; E10"-ε-ring hydroxylase; E11"- β-carotene ketolase.

ANALYSIS METHODS OF TOCOLS AND CAROTENOIDS

Analysis of Tocols

Separation methods are most widely used for tocopherols and tocotrienols analysis from vegetable oils. Thin-layer chromatography (TLC) technique was earlier used to separate and estimate tocols and carotenoids (Jáky, 1967; Mishra & Singh, 2010) from vegetal samples. Two-dimensional TLC was successfully used for tocopherol and tocotrienol separation in several vegetable oils (Whittle & Pennock, 1967). The separated compounds could be developed using Emmerie-Engel reaction (Emmerie & Engel, 2010).

Derivative UV-Vis spectroscopy allows the estimation of individual or total tocopherol levels from their mixtures (Bukovits & Alberto, 1987). The UV spectra of different oils dissolved in n-hexan reveal characteristic signals of tocols (Figure 4). The concentration of tocopherol decreases when corn or soybean oil is heated to 50°C (Gonçalves et al., 2014). Tocopherols concentration was also estimated by chemiluminescence (Belyakov et al., 2004).

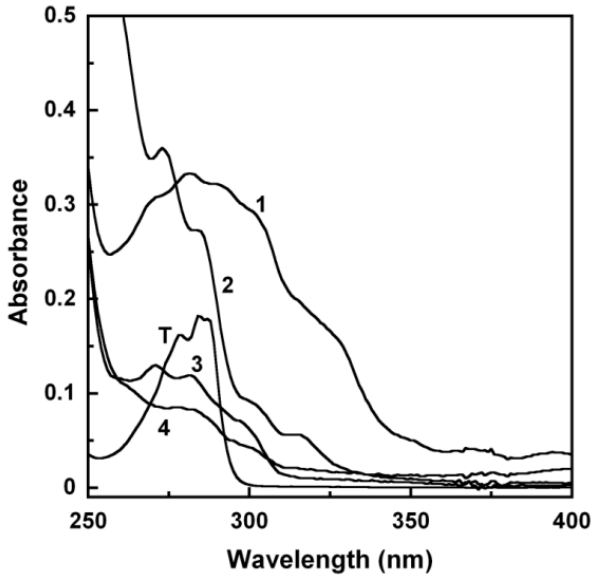

Figure 4. UV spectra of corn oil (1), linseed oil (2); sunflower oil (3) and olive oil (4) in n-hexane. 100 mg oil was dissolved in 500 µl n-hexan; the samples were diluted 1:20 in n-hexane with one exception - linseed oil sample was diluted 1:50. The spectrum of α-tocopheryl acetate (T; Biopharm pill; dilution of 1:8000) was recorded in hexane.

Isomers of tocopherols in edible oils were quantified using electrochemical methods: polarography (Smith et al., 1941), and differential pulse voltammetry (Galeano et al., 2004; Robledo et al., 2013). A couple of spectrophotometric assays were used to quantify tocopherols: copper(II)-neocuproin system (Tütem et al., 1997). GC-MS analysis reveals the instability at heating of γ-tocopherol from corn oil. This isoform is converted to α-tocopherol and later degraded when temperature is increased (Sim et al., 2014).

Quantification of tocopherols in edible oils was successfully achieved by HPLC with UV or fluorescent detection forty years ago (Abe et al., 1975). The typical UV absorption band of tocopherol has a characteristic peak at around 280-290 nm. This band attributed to vitamin E variant(s) is distinguished in various oils (Figure 4). The emission spectra of various oils are also illustrated in Figure 5. Thus, the corn oil is distinguished by higher emission intensity.

A lot of methods for tocols analysis by HPLC were reported in the literature (Bele et al., 2013; Cerretani et al., 2010; Ergönül & Köseo, 2013; Gimeno et al., 2000; Górnaś, 2015; Knecht et al., 2015; Ortíz et al., 2006; San Andrés et al., 2011; Taylor & Barnes, 1981; Thompson & Hatina, 1979). Tocopherol analysis by gas liquid chromatography was also reported (De Greyt et al., 1998). Tocopherols from rape oilseed were quantified by gas chromatography with flame ionization detector (Hussain et al., 2013). However, the sample preparation or detection procedures may substantially differ. Oils saponification was commonly used before HPLC separation (Ryynänen et al., 2004; Shammugasamy et al., 2013). Simultaneous analysis of both carotenoids and tocopherols was carried out by solid-liquid extraction followed by HPLC (Valdivielso et al., 2015). Similarly, RP-HPLC was used for simultaneous determination of α-tocopherol and β-carotene (Gimeno et al., 2000). Some analysts, including tochopherols, were analyzed by liquid chromatography coupled to mass spectrometry (Zarrouk et al., 2009).

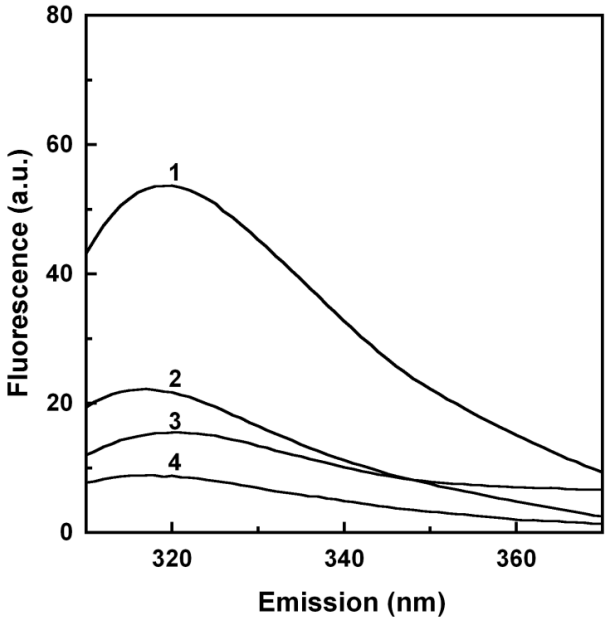

Figure 5. Emission spectra of corn (1), sunflower (2); linseed (3) and olive (4) oil in n-hexane (the excitation wavelength, λex = 295 nm). 100 mg oil was dissolved in 500 μl n-hexan; the samples were diluted 1:50.

Non-aqueous capillary electrophoresis with fluorimetric detection was also successfully used for the determination of tocopherols in vegetable oils (Galeano-Díaz et al., 2012). Infrared (IR) spectroscopy was also employed for tocols quantification (Ahmed et al., 2005; Zahir et al., 2014). In order to complete this overview, various methods for quantitative analysis of tocopherols were compared (Koswig & Mörsel, 1990).

Analysis of Carotenoids

The relative content of carotenoids in maize samples was estimated by TLC after extraction-saponification-extraction steps (Mishra & Singh, 2010). TLC separation of ethanolic extracts components followed by UV detection or iodine staining was used to estimate carotenoids content in plants (Natividad & Rafael, 2014). Recently, carotenoids concentration was determined by densitometry after their separation on high performance TLC (Rodić et al., 2012)

Total carotenoid content of corn oil could be assessed at 450 nm (Wolf, 1968). In order to strengthen this information several visible absorption spectra of some edible oils are displayed in Figure 6. Near-infrared reflectance spectroscopy (NIRS) technique was used to estimate carotenoids content in maize (Brenna & Berardo, 2004).

Various organic solvent mixtures have been used to extract carotenoids from maize. A recent study reveals that these compounds are easily extracted using a methanol (MeOH)-less polar solvent mixture. Acetone-water (5:1) was also used for initial extraction of carotenes from ground maize. The filtrate was further used and mixed with petroleum ether. The

concentrated sample was analyzed on a C30 YMC column (Howe & Tanumihardjo, 2006). A similar approach was used to extract carotenoids from cereal grain products using 75% acetone and hexane. The separation was performed on a reverse-phase HPLC column (C18 Vydac) (Luterotti et al., 2013). Corn xanthophylls were easily extracted using an 80% ethanolic alkaline solution and further separated by HPLC (C30 column). The elution profile was monitored at 450 and 445 nm (Moroset al., 2002). Rehydration of dry maize was recommended prior to MeOH:THF (1:1) extraction that precedes carotenoids separation on C18 or C30 column (Kimura et al., 2007). A hexane:ethyl acetate (9:1) extraction mixture was also used after saponification and the resulted carotenoids mixture was analyzed by HPLC on a Spherisorb ODS2 column (Scott & Eldridge, 2005). Boiling of maize samples (at 100 ºC for 30 minutes) proved to be a better choice to increase the concentration of extracted carotenoids (Muzhingi et al., 2008). Solid phase extraction (on de-activated alumina column) was successfully used for non- and monohydroxylated carotenes separation from maize kernel prior to a RP-HPLC separation (Hulshof et al., 2007). A suitable extraction mixture (MeOH:ethyl acetate or MeOH:THF 1:1) has turned out to be a better choice prior to carotenoids analysis by UHPLC-Vis (Rivera & Canela, 2012). More than 11 carotenoids were resolved by UHPLC-UV (Ultra high pressure liquid chromatographic system coupled with a UV detector) (Maurer et al., 2014). However, beside high levels of lutein and zeaxanthin, β-apo-8'-carotenal was also detected.

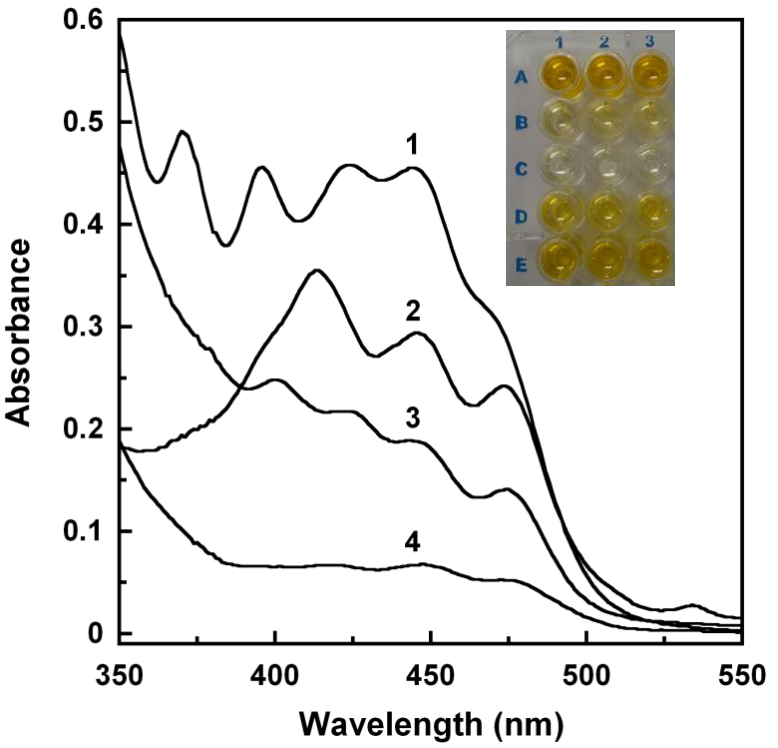

Figure 6. Spectra of n-hexane extracts of: corn (1), olive (2); linseed (3) and sunflower (4) oils (1,2 and 3- 100 mg oil / 900 µl n-hexan; 4-250 mg oil / 750 µl n-hexan). Inset: A-corn oil; B-sunflower oil (cold pressed); C-refined sunflower oil; D-linseed oil; E-olive oil.

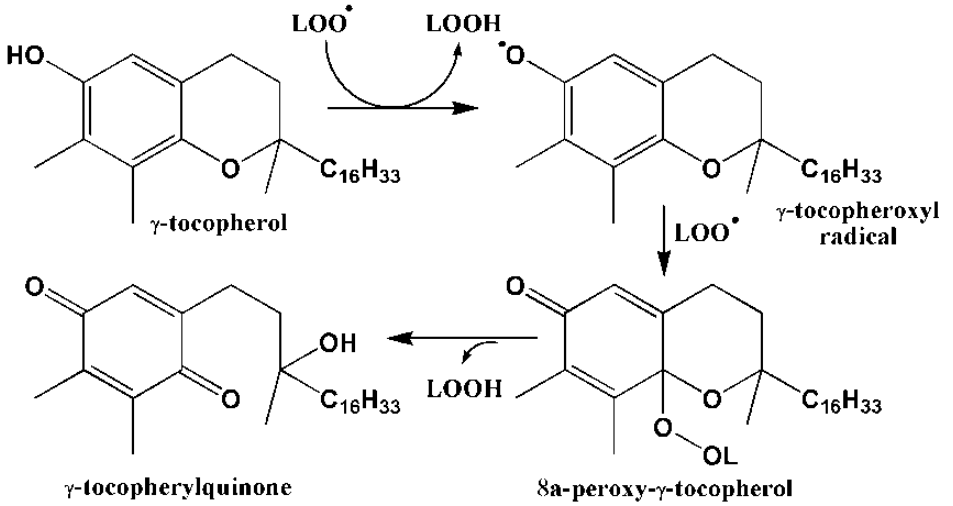

Figure 7. Reaction of γ-tocopherol with lipid peroxyl radicals (LOO•).

Since the carotenoids display similar absorption spectra it is recommended to identify these compounds after HPLC separation using a mass spectrometer or a NMR detector. Structural and geometrical isomers or epoxides were distinguished by this technique (Aman et al., 2005; Matsubara et al., 2012). An accelerated solvent extraction step in hexane-based solvent mixtures was successfully used prior to LC-ESI MS analysis of lutein and β-carotene from orange carrot (Saha et al., 2015).

ANTIOXIDANT PROPERTIES OF TOCOLS AND CAROTENOIDS

General Aspects

Corn oil contains natural antioxidants such as tocols, carotenoids and polyphenols. These compounds prevent protein oxidation, lipid peroxidation (Figure 7) and reactive oxygen species formation.

In this section, we focus on the antioxidant activity of lipophilic compounds such as tocols and carotenoids.

During the refining process the amount of antioxidants is reduced substantially. However, tocopherols are antioxidants which have a much higher stability compared with other antioxidants which are components of refined oils. The tocols manifest their antioxidant activity via their chromanol phenolic moiety that easily transfers hydrogen to a lipid-free radical (Kamal-Eldin & Appelqvist, 1996). The compounds obtained during these reactions could be reduced back to tocopherol by glutathione and vitamin C.

Earlier studies failed to establish correlations between the content of vitamin E and the formation of peroxide in corn and soybean oil (Chow & Draper, 1974). Additionally, it should be noted that the antioxidant capacity of α-tocopherol is greater than that of β-carotene in nonpolar environment (Tsuchihashi et al., 1995).

While the antioxidant-prooxidant switch seems to be dictated by α-tocopherol concentration (Huang et al., 2005), γ-tocopherol constitutes the main determinant of the total antioxidant capacity and this parameter is affected by other chemical components of the oil (Castelo-Branco & Torres, 2012). A recent study reveals that supplementation of four different vegetable oils with β and γ-tocopherol induces a slight increase of corn oil antioxidant activity (Hamdo et al., 2014).

Tocotrienols are distinguished by a higher antioxidant level than tocopherols. For example, α-tocotrienol is distinguished by superior antioxidant potency to that of α-tocopherol, having a pronounced disordering effect on membrane bilayer (Serbinova et al., 1991; Suzuki et al., 1993).

Electrochemical and Electron paramagnetic resonance (EPR) studies have been focused on neutral carotenoid radicals. These species are derived from carotenoid radical cations deprotonation (Focsan et al., 2015). At lower radical concentration this behaves like a scavenger for reactive species (single oxygen, hydroxyl or peroxyl radicals) (Foot, 1976). Although the antioxidant activity of carotenoids is higher than that of α-tocopherol, a modest contribution (less than 5%, due to modest concentration levels of carotenoids in oil) to the total activity is expected (Müller et al., 2011).

Compared to other tocopherols, α-tocopherol manifests a higher quenching ability for molecular oxygen, radical scavenging reactivity and biological potency (Fryer, 1992; Zingg & Azzi, 2004). Conversely, γ–tocopherol has a greater ability to scavenge nitrogen dioxide and peroxynitrite than the aforementioned isomer (Christen et al., 1997). It should be stressed here that the elevated levels of reactive oxygen species may be linked to several pathological states (Dreher et al., 1999; Jurkiewicz et al., 1995; Rahimi et al., 2005). Therefore, vitamin E was used as prophylactic and therapeutic agent for cancer, cardiovascular, chronic or autoimmune diseases (Borek, 2004; Hercberg et al., 1998; Ratnam et al., 2006; Suantawee et al., 2013). Moreover, γ–tocopherol specifically inhibits cyclooxygenase activity in macrophages and epithelial cells (Jiang et al., 2000).

The antioxidant activity is affected by the antioxidant structure (Lien et al., 1999), temperature (Réblová, 2006), light, relative humidity (Kimet al., 2015), oil physical state and presence of other compounds. Anyway, lipophilic antioxidants such as α–tocopherol were more effective in a heterogeneous system (Frankel et al., 1994; Losada-Barreiro et al., 2013a; Schwarz et al., 2000). In corn oil, tocopherol emulsion is localized toward oil-interfacial regions and for this reason it manifests a good efficiency in the inhibition of lipid oxidation (Losada-Barreiro et al., 2013b).

Measuring Antioxidant Activity of Oils

Trolox equivalent antioxidant capacity (TEAC) spectrophotometric assay was used to estimate antioxidant capacity (Miller et al., 1993; Re et al., 1999) of edible vegetable oils. This method relies on the capacity of antioxidants to scavenge a non-biological radical, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate) (ABTS) (Figure 8).

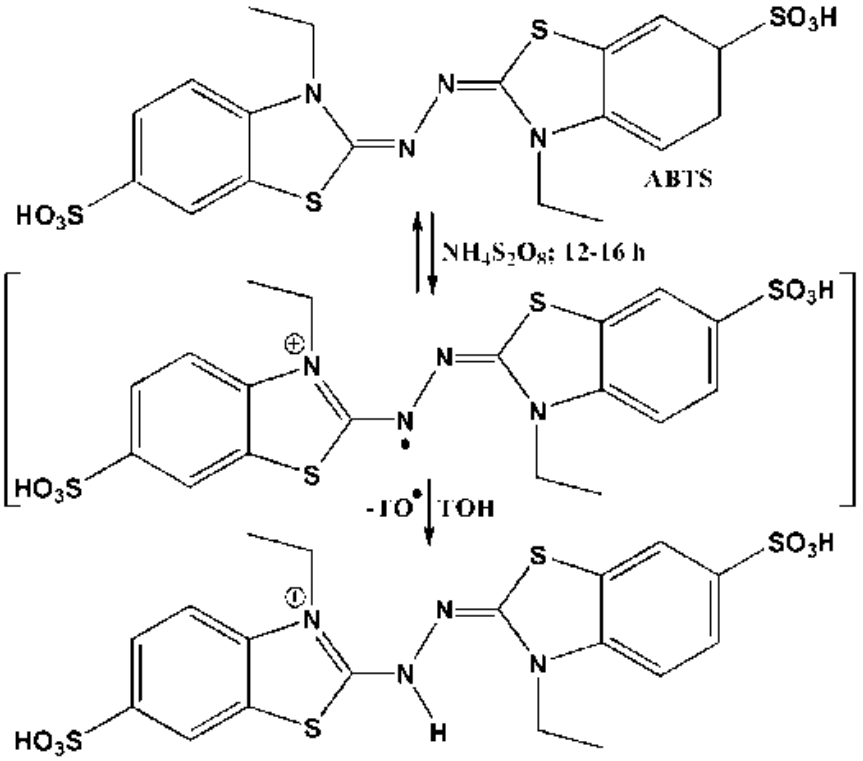

Figure 8. Chemical reaction steps that lie behind tocols antioxidant capacity. In the first instance a non-biological radical is generated. In the second reaction this radical species were counteracted by –OH phenolic moiety of tocols.

Two ferric-ion spectrophotometric methods were used to estimate the antioxidant capacity of the methanolic extract of corn oil (Szydłowska-Czerniak et al., 2008). One of these spectroscopic methods is exemplified in Figure 9.

A spectrophotometric method based on reduction of Mo(VI) to Mo(V) has been developed for the quantitative determination of the total antioxidant capacity of hexane extracts of corn and soybean samples (Prieto et al., 1999). The antioxidant activity of methanolic corncobs extracts was measured based on their ability to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (Sultana et al., 2007). This assay is based on radical disappearance according with sample antioxidant capacity (Figure 10).

Corn oil displays a modest DPPH radical scavenging activity when compared with olive or Perilla oils (Lee et al., 2015). However, the antioxidant activity of phenols from edible oils was estimated after a column extraction step (Siger et al., 2008).

Oxygen radical absorbance capacity (ORAC) and total trapping antioxidant parameter (TRAP) are two assays based on competition between an antioxidant and a substrate for peroxyl radicals (Cao & Prior, 1998; Cabrini et al., 2001).

Malondialdehyde (MDA) is a secondary carbonyl compound that arises from lipid peroxidation pathway. Gas chromatographic analysis of pentafluorophenyl-hydrazine derivatized carbonyl compounds was successfully used to assess antioxidant activity of vegetable oils (Stashenko et al., 1997).

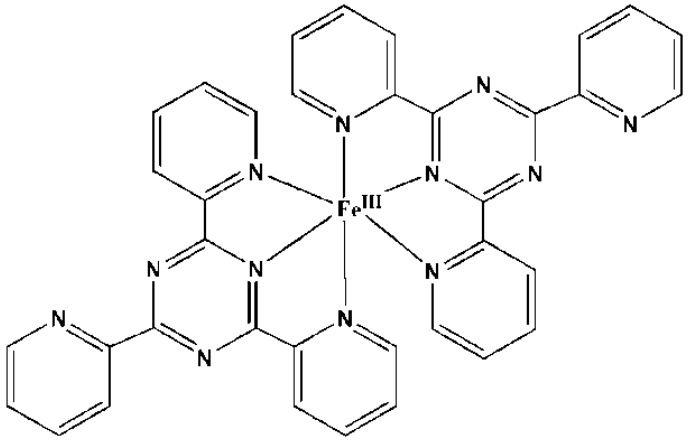

Figure 9. Reduction of ferric complexes by antioxidant (AOH) species.

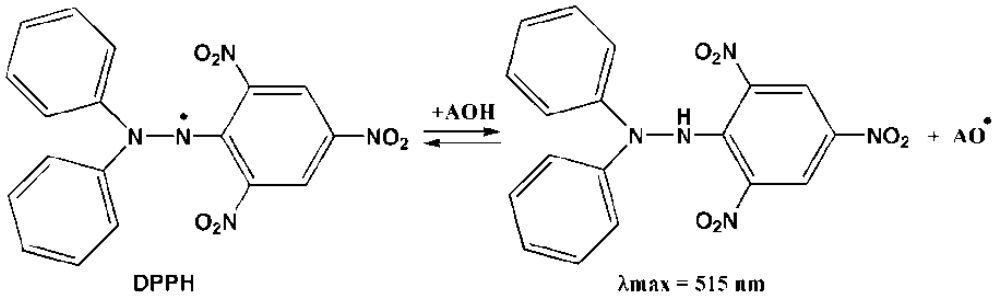

Figure 10. Capacity of antioxidants (AOH) to scavenge an organic radical.

CONCLUSION

In this chapter, a literature overview on corn oil antioxidants was performed. Corn oil displays a significant amount of tocols and low levels of carotenoids. Their biosynthesis routes have been well-understood. More attention should be paid to the metabolic engineering of antioxidants in corn. A significant increase in the concentration of one of these antioxidants will result in a higher nutritional value of corn oil. Such oils are rich in antioxidants and therefore are recommended as adjuvants in various therapies. An overview of the methods of tocols or carotenoid analysis has been one of main goals of this chapter. There were presented both simple and sophisticated methods of analysis and detection of these compounds.

The antioxidants have different structural features and hence they manifest distinct antioxidant properties. Most of the antioxidant assays presented are rather complicated and cannot distinguish the contribution of each antioxidant to the whole corn oil antioxidant power. Future studies should aim to design new more efficient methods to quantify their

specific antioxidant capacity. This will allow us to create an overall picture of the way antioxidants trapped in this complex corn oil matrix action.

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Chapter 3

SUPERCRITICAL FLUID EXTRACTION OF CORN GERM OIL

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ABSTRACT

In this chapter an environmental friendly extraction process of corn germ oil based on the use of supercritical CO_2 (SC-CO_2) is presented. The effect of important operating parameters in supercritical fluid extraction (SFE) processes such as pressure, temperature and flow rate on the extraction kinetics and the quality of the extracted oil is discussed. As for many SC-CO_2 extractions of vegetable oils, extraction curves of corn germ oil present an initially linear part with a slope close to the oil solubility value in CO_2. Then, a second section of the extraction curve is determined by the diffusional resistance in the solid matrix. Characterization of supercritical crude corn oil is presented by showing some properties reported in the literature such as physical parameters, fatty acid composition, neutral lipids, content of tocopherols, acid index, peroxide value, antioxidant capacity and the oxidative stability.

The quality of supercritical corn oil is also compared with the oil obtained by using conventional extraction process.

Keywords: supercritical fluid extraction, corn germ oil

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NOMENCLATURE

a_s	specific area between the regions of intact and broken cells (m^{-1})
C_1, C_2	fitting parameters
e	extraction yield, (kg extract×kg insoluble solid^{-1})
E	extract (kg)
k_s	solid-phase mass transfer coefficient (s^{-1})
n	number of experimental data
N_m	charge of insoluble solid (kg)
O.F.	objective function
Q	solvent flow rate ($kg \times h^{-1}$)
q	relative amount of the passed solvent (kg solvent×kg insoluble solid^{-1})
q_c	relative amount of the passed solvent when all the solute in broken cells has been extracted (kg solvent×kg insoluble solid^{-1})
r	grinding efficiency (fraction of broken cells)
t	extraction time (h)
x_u	concentration in the untreated solid (kg solute×kg solid insoluble^{-1})
y_s	solubility (kg solute×kg solvent^{-1})

INTRODUCTION

Corn (*Zea mays*) is mainly used for starch, sugar syrups and oil production. In the corn wet milling process, the corn kernel is separated into 3 main parts: the endosperm (gluten and starch), the outer skin, called the bran or hull, and the germ (around 8% of raw corn), which is the most valuable part of the kernel because of its high concentration of linoleic acid and its bland taste (Dijkstra et al., 2007). As depicted in Figure 1, after a cleaning process, corn undergoes a steeping stage that conditions the grain for subsequent germ separation. Corn germ, after removal of the starch adhered along the separation process from the whole corn, is dried to approximately a 4% moisture content to improve shelf life. The dry milling process does not start with a steeping step; instead, the corn kernels are directly ground and the germ separated. Whatever is the case, corn oil production should start with the dried germ. Extracting the kernel is limited to high-oil corn with an oil content of at least 8%. Corn oil concentrates in the germ with a content lying between 40% and 52% (Dijkstra, 2013).

Most of the corn germ oil is traditionally obtained from the germ using a conditioning process followed by mechanical expelling (prepress) and, in some cases, ending up with solvent extraction of the expeller cake. The conditioning process is diverse and several procedures as milling, cracking, flaking, heating, etc. have been proposed (Dijkstra, 2013). The corn germ meal obtained after pressing still contains 6-10% of residual oil while it contains 1-3% after solvent extraction (AWARENET, 2004). Extrusion has also been employed to prepare the germ for solvent extraction producing a crude corn oil of high quality and high yield (Gunstone, 2002). The aim of the extraction method is to optimize the oil yield while maintaining the oil quality (Matthäus, 2012).

After extraction, corn oil usually undergoes a refining process that comprises removal of gums, free fatty acids, adsorbable compounds, waxes and malodorous compounds (Dijkstra

and Segers, 2007). In some cases, specialty oil producers manufacture unrefined, 100% expeller pressed corn oil, with lower yield, thus more expensive and smaller market share.

Corn germ oil is mainly used in food and cosmetic applications and corn germ meal for animal feed production.

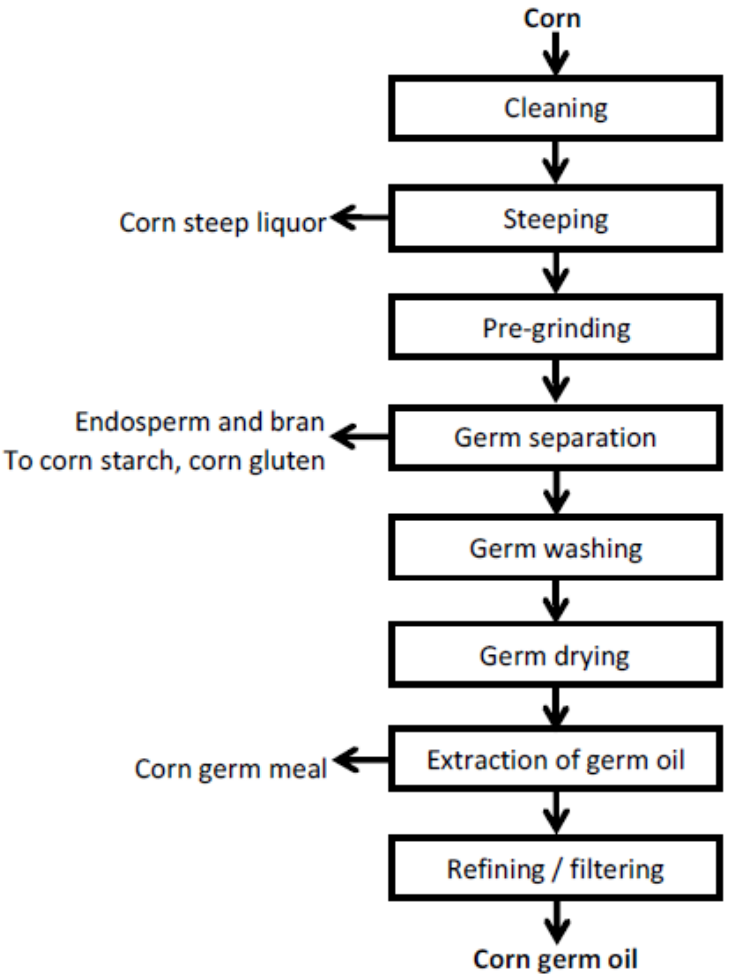


Figure 1. Flow diagram of the wet milling process for obtaining corn germ oil.

In the last decades, some more environment friendly procedures have been suggested for corn germ oil production, among them, the use of enzymes to enhance the oil recovery and/or extraction of oil by using supercritical fluid extraction (SFE) (Matthäus, 2012). This chapter is focused on the SFE process of corn germ oil, paying special attention to the description of the process and the influence of process parameters on the quality of the resulting oil.

SUPERCRITICAL FLUID EXTRACTION OF CORN GERM OIL

A supercritical fluid (SCF) is any fluid at a temperature and pressure above its critical point, where the liquid and vapor phases merge in one only phase because their densities

become equal. The physical properties of a supercritical fluid are thus intermediate to those of the respective liquid and gas, with good transport properties (viscosities and diffusivities) like gases, and high solvent power, like liquids. In addition, small changes in pressure or temperature result in large changes in density, allowing many properties of a SCF to be "tuned."

Specifically CO_2 shows mild critical conditions (Tc = 304.15 K and Pc = 7.38 MPa); therefore, although operation at high pressure is usually costly, pressures above 7.38 MPa can be cost effective. Analogously, temperatures above 31°C can be mild enough for processing thermo-sensible products. Carbon dioxide, together with water, are the only solvents that are not considered as solvents requiring process revaluation by the US FDA. In the cases where water cannot be used because solutes are non-polar, carbon dioxide can be used as a non-polar solvent. Carbon dioxide is non-toxic, not flammable, naturally abundant and inexpensive. In addition, at the critical point, surface tension approaches zero and there is no formation of a liquid-vapor meniscus, reasons for which supercritical fluids can easily penetrate into solid matrices and can be separated from the processed products by simple depressurization resulting products free from processing residues.

The number of processing plants that employ supercritical carbon dioxide (SC-CO_2) technologies, operating worldwide, is growing steadily. Most of the current plants are related to food processing, yet other type of processes are using these technologies. Some examples are fluoropolymer synthesis by DuPont, hydrogenation by Thomas Swan, coatings by Union Carbide or polyurethane processing by Crain Industries (Beckman, 2012).

In the food industry, the most widely commercialized SCF technology is SC-CO_2 extraction. SC-CO_2 is able to selectively solve caffeine from coffee, certain acids from hops, oil from seeds, various components from spices, etc. This ability has been fundamental to design different extraction processes that are today widely commercialized. The attractive properties of SC-CO_2 as solvent are currently being exploited in processes such as coffee decaffeination (Hag AG, Evonik, Kraft General Foods, etc.) or hops fractionation (NATECO2, Carlton, United Breweries, Hops Extraction Corp. of America, etc.) displacing the conventional solvents.

Also companies designing SCF processes and equipment are steadily growing. Companies such as NATEX (Natex, 2015) or Uhde High Pressure Technologies (Uhde, 2015) have designed supercritical fluid industrial plants for extraction of spices, herbs, hops, nutraceuticals or edible oil as well as for cork purification or rice treatment among other.

SFE of corn germ oil, although not yet at an industrial scale, has been studied by several authors, including some recent patents related to SFE of corn germ oil and SF fractionation of the extracted oil to obtain a refined corn oil (DeLine et al., 2009, DeLine et al., 2013, Marentis, 2013).

One of the first studies about SFE of corn germ oil was performed by the Agricultural Research Service of the United States Department of Agriculture (Peoria, Illinois). List et al., (List et al., 1984, List and Friedrich, 1989) and Christianson et al., (Christianson et al., 1984) compared the quality of crude oils obtained from dry and wet milled corn germ using SFE at 50-90°C and 55-83 MPa and conventional extraction methods. They concluded that the oil obtained by SC-CO_2 extraction exhibited lower refining loss and lighter color.

List et al., (List et al., 1984) concluded that, in general, the quality of crude oil obtained by SFE was unaffected by extraction conditions. The levels of tocopherols present in SC-CO_2 extracted corn oil were similar to those obtained by conventional methods (List et al., 1984,

List and Friedrich, 1989). However the phospholipids were almost absent in the SC-CO_2 oils, which is an advantage from a processing point of view.

Wilp and Eggers (Wilp and Eggers, 1991) proposed a SFE process followed by a fractionation separation step to improve the oil quality, since low polar triglycerides could be separated from high polar compounds such as water and free fatty acids.

Vigh et al., (Vigh et al., 1993) reported some extraction curves of wet corn germ oil with SC-CO_2 in an interval of pressure (27.0-33.0 MPa) and temperature (42-78°C) for corn germ with a particle size < 0.8 mm or 0.8-1.4 mm. Based on a second order orthogonal design, Vigh et al., (Vigh et al., 1993) concluded that, for the smaller particle size range, application of low pressures and high temperatures should be avoided. Ronyai et al., (Rónyai et al., 1998) studied the SC-CO_2 extraction of wet-milled corn germ oil with cosolvents (ethyl alcohol: from 0% to 10% by weight in CO2) at constant pressure and temperature (30 MPa and 42°C). The extraction time was reduced due to the higher solubility of corn oil the higher the ethanol concentration, although the amount of phospholipids in the oil was larger, since they are soluble in ethanol. Ronyai et al., (Rónyai et al., 1998) found that the emulsifying, foaming and absorption properties of the defatted meal and its protein isolates were better when using ethyl alcohol as cosolvent. Recently, Rebolleda et al., (Rebolleda et al., 2012) studied the SC-CO_2 extraction kinetics of oil from milled corn germ at different pressures (20.0-53.0 MPa), temperatures (35–86°C) and solvent flow rates (4-9 kg CO_2/h) concluding that the extraction process may be controlled by the solubility of the oil in SC-CO2 in the first stages of the extraction and that the external mass transfer resistance was not important. They also studied the oil quality concluding that the extraction temperature affects the oil quality, yielding oils with higher tocopherol content, and therefore higher antioxidant activity and lower oxidation level, when the extraction temperature increased. In addition, the oil they obtained by SC-CO2 extraction had lower values of acidity than oils obtained by conventional extraction methods. Rebolleda et al., (Rebolleda et al., 2012) also studied on-line fractionation of the oil extracted in two separators installed in series and separated the co-extracted water improving the oil stability against oxidation. In addition to oil, SFE of corn germ results in interesting defatted corn germ flour, due to its low fat content and lower peroxidase activity compared to hexane extracted corn germ flour, SFE flour having larger storage stability and better flavor, which are important economic factors. The bound lipid content of hexane extracted meal was nearly four times that of the SC-CO_2 extracted flour. Regarding the peroxidase activity, it is normally difficult to inactivate the enzyme by the normal toasting process, but it has been found that a sevenfold reduction in peroxidase activity can be achieved under SCF extraction (Christianson et al., 1984, Friedrich and Pryde, 1984, Christianson and Friedrich, 1985). Another important consideration is that deterioration of proteins and amino acids do not take place under the conditions usually employed in SFE in food, and a good balance of essential amino acids has been found compared to the published FAO/ALTO standards for highly nutritious proteins (Favati et al., 1996). Figure 2 provides a visual comparison of the corn germ meal obtained after SC-CO_2 extraction at the University of Burgos and corn germ bagasse obtained after mechanical expelling. In this chapter, the influence of different parameters on the extraction yield and oil quality obtained when using SC-CO_2 extraction as a process to obtain corn germ oil is presented by showing different results reported in the literature.

SUPERCRITICAL FLUID EXTRACTION EQUIPMENT AND PROCEDURE

The elements of a supercritical fluid extraction plant have to be designed taking into account the safety factors needed to operate safely under high pressure.

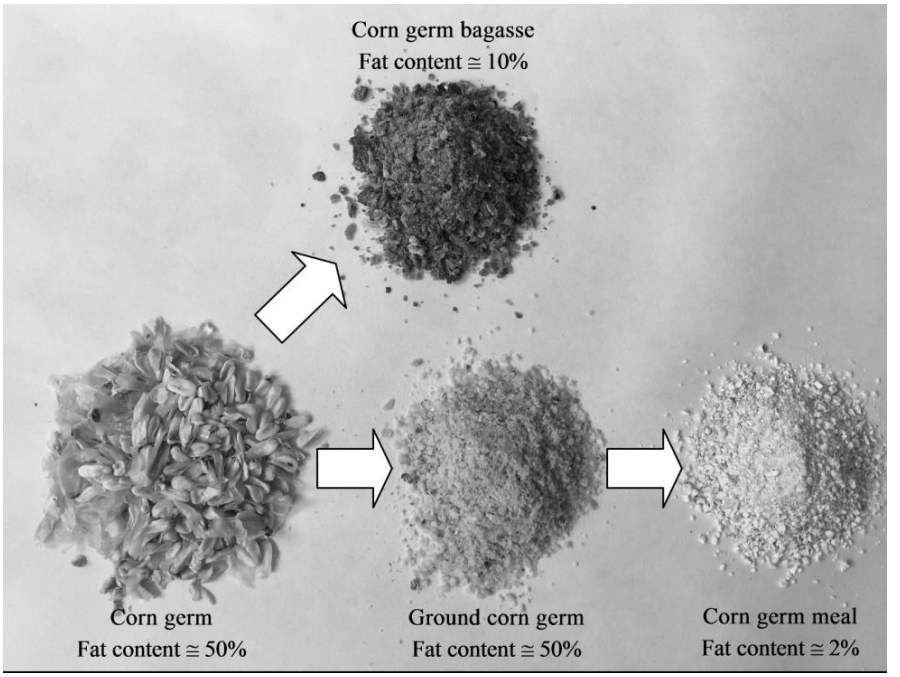


Figure 2. Samples of corn germ, ground corn germ, and corn germ meal obtained after SC-CO_2 extraction at the University of Burgos. Corn germ bagasse obtained after mechanical expelling is also included for comparison.

The most important elements to be assembled in a SFE plant are pump or compressor, extractor, one or more consecutive separators, heating and cooling systems, closures, tubing, valves, safety elements such as rupture disks and safety valves, and the instrumentation needed for measurement and control of the process parameters. As an example, the P&I diagram of the semi-pilot SFE plant of the University of Burgos is presented in Figure 3. This plant uses a pump for pressurizing liquid CO_2 after being cooled to conditions away from saturation. The maximum specifications of the SFE plant are: T = 200ºC, p = 65.0 MPa and solvent flow, F = 20 kg/h.

In order to illustrate the operation of a SFE plant, the procedure followed by Rebolleda et al., (Rebolleda et al., 2012) is presented. In a SFE experiment, a certain amount of corn germ is placed in the extractor (350 g). At a laboratory scale, the material to be extracted is sometimes mixed with inert fillings in order to avoid bed compaction; however bed compaction is usually avoided at industrial scale by using special designs or subtract pretreatments. The extractor was later pressurized with CO_2 up to the extraction pressure. Then, the solvent was circulated at the desired extraction pressure and temperature, with a certain solvent flow, F, and during a specific time, t. The solvent was continuously recycled

to the extractor after removing the solute in the separator. Solvent recycling is always used at an industrial scale.

Moisture content of the raw material is an important factor in SC-CO_2 extraction of natural products (Brunner, 1994) although many authors have not found a strong effect when moisture is lower than 20% (Rubio-Rodríguez et al., 2008). Christianson et al., (Christianson et al., 1984) reported that tempering the germ to 8% moisture prior to flaking did not influence extraction solubility or recovery. For instance, in the experiments carried out by Rebolleda et al., (Rebolleda et al., 2012) the corn germ was milled in a coffee grinder to a particle size ranging from 0.5 mm to 1 mm and had an average moisture content of 6.0 ± 0.4% and 46 ± 3% average fat content.

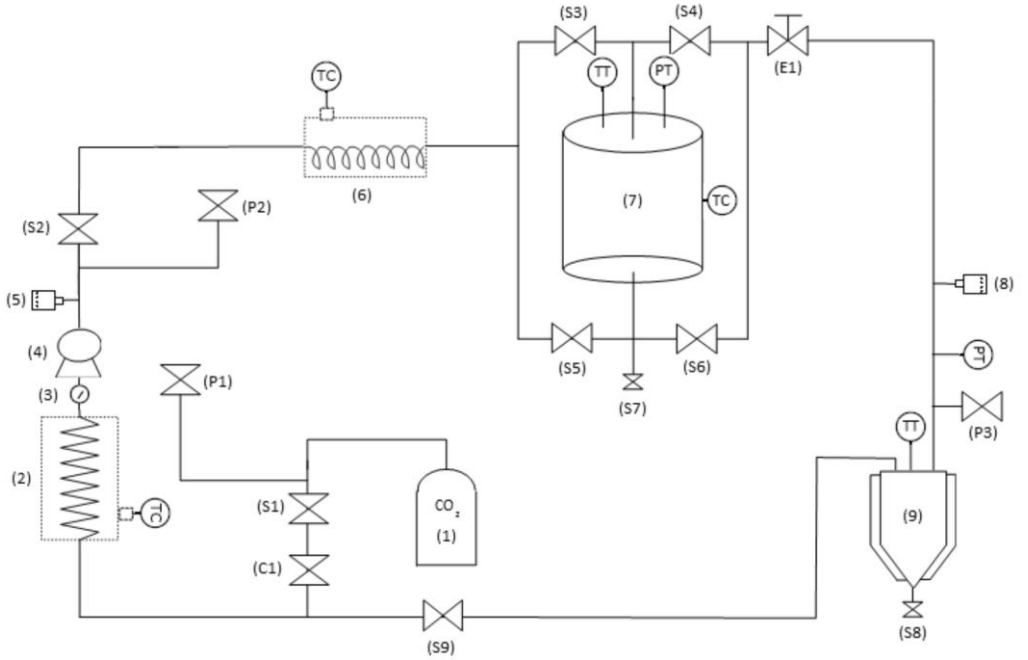


Figure 3. Flow-sheet diagram of the supercritical fluid extraction semi-pilot plant at the University of Burgos. CO_2 storage (1), Purge valves (P1, P2, P3), Shutoff valves (S1, S2, …, S8), Check valve (C1), Cooling bath (2), Temperature controllers (TC), Total mass flow meter (3), Pump (4), Rupture disks (5, 8), Heating bath (6), Extractor (7), Temperature transducers (TT), Pressure transducers (PT), Expansion valve (E1), Separator (9).

ANALYTICAL METHODS FOR CHARACTERIZATION OF CORN GERM OIL

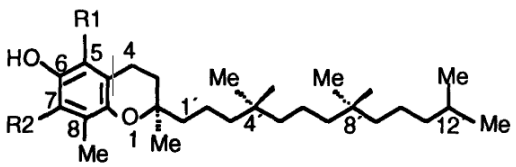
Lipid analysis comprises a vast range of methods, approaches and analyses that serve different purposes. A large amount of analytical methods have been developed and published by learned societies, and national or international bodies that issue standards. For source materials, meals and oils and fats alone, the AOCS (http://onlinemethods.aocs.org) has published more than 250 different Official Methods. A compilation of the most important

parameters and methods used for oil analyses is provided by Dijkstra et al., (Dijkstra et al., 2007).

Different parameters can be employed to characterize the extracted corn germ oil such as physical parameters (e.g.: refractive index, density, color) and chemical parameters, including composition in terms of fatty acids profile by gas chromatography (AOAC, 1995) and neutral lipids, that can be determined, among other analytical methods, by liquid chromatography (Schaefer et al., 2003).

Other important quality parameters are related to the antioxidant capacity of the extracted oil. Total antioxidant capacity can be evaluated through assays such as ABTS (Rebolleda et al., 2014), DPPH (Brand-Williams et al., 1995) or FRAP (Rebolleda et al., 2013) among others. Quantifying the main compounds that provide the antioxidant capacity is a different way of inferring such parameter for a substrate; Tocopherols for instance are important antioxidants that are found in many vegetable oils. Tocopherols have four isomers (α-, β-, γ-, δ-) differing in the number and position of methyl groups on the chromanol ring (Table 1). Tocopherols are well recognized for their effective inhibition of lipid oxidation in foods and biological systems. The antioxidant activity of tocopherols is mainly due to their ability to donate their phenolic hydrogens to lipid free radicals (Kamal-Eldin and Appelqvist, 1996). In the literature (Rebolleda et al., 2012) tocopherols have been successfully determined by HPLC-DAD after isolation by solid phase extraction (SPE). Individual compounds of α-, β-, γ- and δ-tocopherols can be identified and quantified using a calibration curve of the corresponding standard compound (Figure 4).

Table 1. Structure of the tocopherols



α-tocopherol	$R_1 = CH_3$ $R_2 = CH_3$
β-tocopherol	$R_1 = CH_3$ $R_2 = H$
γ-tocopherol	$R_1 = H$ $R_2 = CH_3$
δ- tocopherol	$R_1 = H$ $R_2 = H$

Kamal-Eldin and Appelqvist, 1996.

Other parameters that are usually determined to assess the oil stability and quality are the acid index (AI) and the peroxide value (Ca 5a-40 and Cd 8-53 AOCS Methods). The oxidative stability is also an important parameter in the characterization of fats and oils that can be determined among other methods by using the Rancimat test.

Further details for most of these analytic procedures can be found in the work published by Rebolleda et al., (Rebolleda et al., 2012).

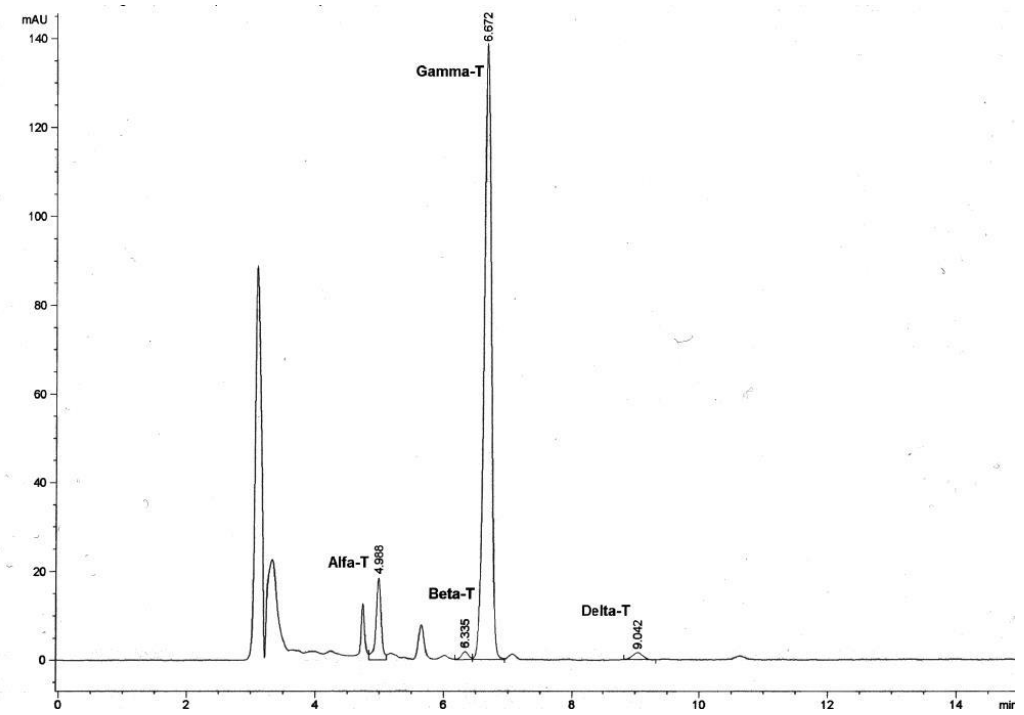


Figure 4. HPLC-DAD chromatogram of tocopherols in the SC-CO_2 extracted corn oil.

INFLUENCE OF PROCESS PARAMETERS ON THE EXTRACTION YIELD

The most important parameters that influence the supercritical fluid extraction process of solid substrates are extraction pressure and temperature, which determine solvent density, solvent ratio and solid substrate pretreatmente and moisture (Brunner, 1994).

By determining the influence of such parameters on the extraction kinetics, the mechanism controlling the extraction process can be elucidated. SFE can be governed by one or all of the following resistances to the process: internal mass transfer, equilibrium solute solubility and external mass transfer. The optimization of the process requires the knowledge of the stage controlling the process under different process parameters.

In order to illustrate the influence of the different process parameters on the extraction yield, recent results obtained by Rebolleda et al., (Rebolleda et al., 2012) under different extraction conditions (see Table 2) are presented in this chapter and compared with other results previously reported in the literature.

Similar to other vegetable oils, extraction curves present an initial section controlled by equilibrium constraints and therefore characterized by a linear relationship between the mass of oil extracted and the quantity of CO_2 employed. The characteristics of this curve depend on the pretreatment of the oilseeds which accelerates the release of the oil bound to the cells. In the second section of the curve, mass transfer is determined by diffusional resistance in the solid matrix (Eggers, 1996).

Table 2. Process parameters used in the experiments of SFE of corn germ oil with SC-CO_2

Run	P (MPa)	T (ºC)	Solvent flow rate, (kg CO_2/h)
R1	45 ± 2	40 ± 2	8.5 ± 0.8
R2	45 ± 2	63 ± 2	8.8 ± 0.8
R3	46 ± 2	79 ± 2	9.5 ± 0.9
R4	45 ± 2	85 ± 3	8.0 ± 0.9
R5	30 ± 2	80 ± 2	10.0 ± 0.7
R6	21 ± 1	39 ± 1	9.0 ± 0.7
R7	53 ± 2	80 ± 2	6.0 ± 0.5
R8	44 ± 2	78 ± 2	3.9 ± 0.5

Effect of extraction temperature. In SFE, an increase of the extraction temperature, although implying a decrease of the fluid density, could be also responsible for an increase in the extraction yield due to an increase of the solute vapour pressure.

As an example, Figure 5 shows the effect of the extraction temperature on the extraction yield reported by Rebolleda et al., (Rebolleda et al., 2012) from 40°C to 85°C (Runs 1-4) at 45.0 MPa and an average SC-CO_2 flow rate around 9 kg/h. It can be observed that the higher the temperature the higher the extraction rate. Therefore, this behavior may indicate that, at this pressure, the increase of oil vapor-pressure with temperature is more important than the decrease in SC-CO_2 density.

As it is well established in the literature (Salgın et al., 2006), the increase of seed oil solubility with temperature can be significant when the process is performed at pressures higher than 40 MPa, pressure at which a crossover behavior is usually observed in seed oils (Salgın et al., 2006).

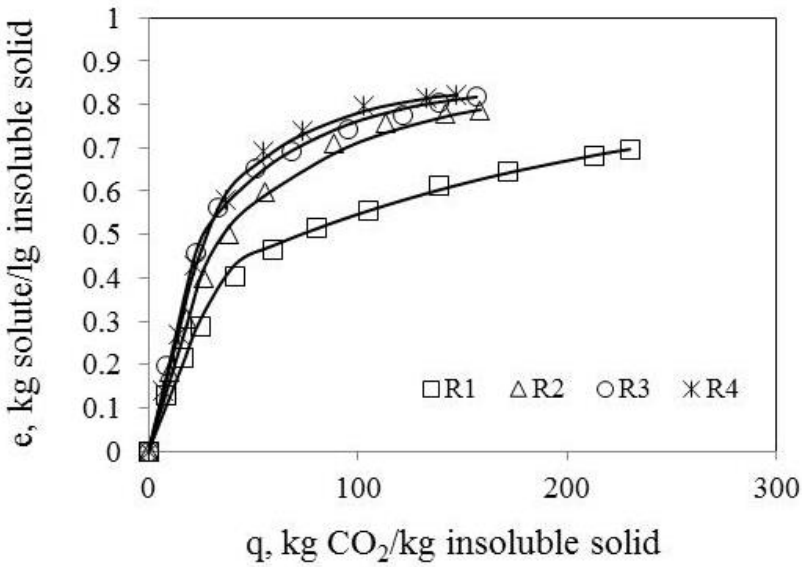


Figure 5. Influence of extraction temperature on corn germ oil extraction yield (□ 40ºC; △ 63ºC; ○ 79ºC; ∗ 85ºC), at around 45.0 MPa and 9 kg CO_2/h.. Solid lines correspond to the model of Sovová (Sovová, 2005).

Effect of extraction pressure. The CO_2 pressure is probably one of the main parameters that affect the extraction process. At a given temperature, an increase of the extraction pressure means an increase in the CO_2 density and consequently a higher solvent capacity. To illustrate the effect of extraction pressure, Figures 6a and 6b present the effect of pressure from 21 MPa to 53 MPa at two different operating temperatures of 40ºC and 80ºC, reported by Rebolleda et al., (Rebolleda et al., 2012) (Runs 1, 6 and 3, 5 and 7). In both cases, the extraction curves indicate that, at constant temperature, the higher the pressure the higher the extraction rate. This is mainly due to the higher density of SC-CO_2, and therefore, to its higher solvent power.

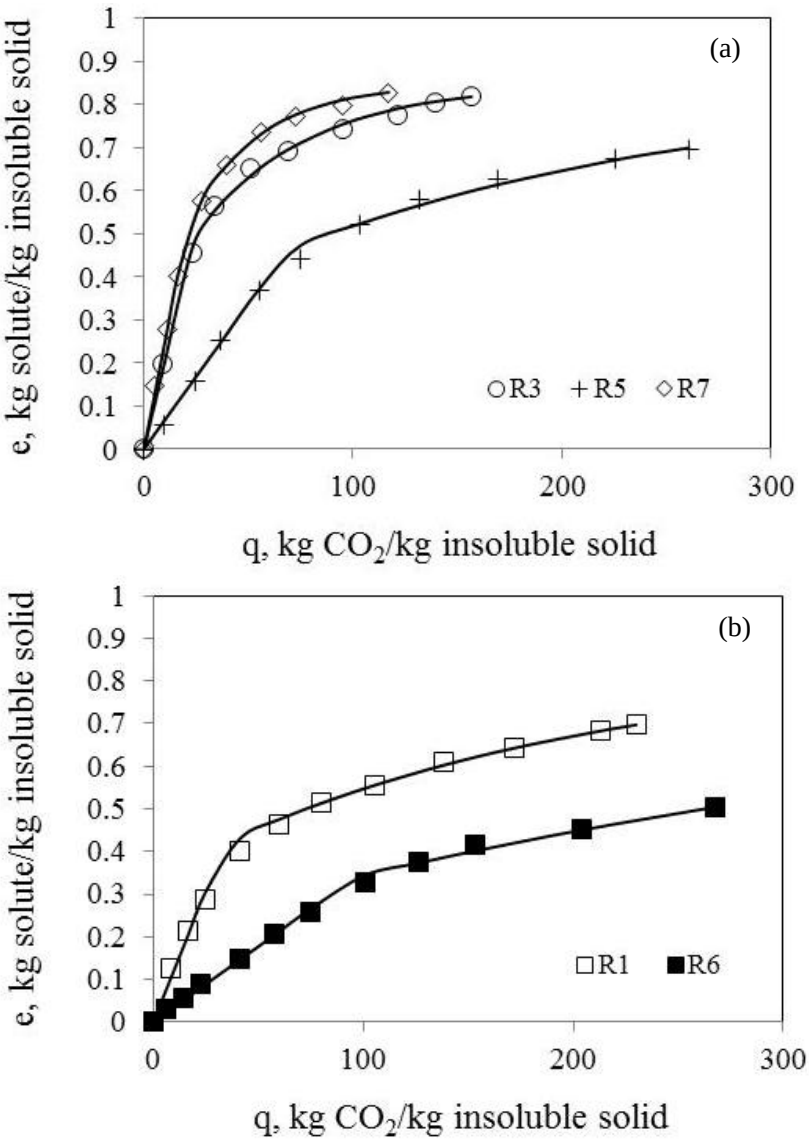


Figure 6. Influence of extraction pressure on corn germ oil extraction yield at constant extraction temperature of (a) 40ºC (+ 30 MPa; ○ 45 MPa; ◇ 53 MPa) (b) 80ºC (■ 21 MPa; □ 45 MPa). SC-CO_2 flow was around 9 kg CO_2/h in all cases. Solid lines correspond to the model of Sovová (Sovová, 2005).

In any case, Figure 2b indicates that the extraction at the lowest pressure (Run 6, 21 MPa) is very slow, which may be due to the low solubility of seed oil in SC-CO_2. An increase of oil solubility when extraction pressure is increased has been also reported for dry-milled corn germ (Christianson et al., 1984) and other seed oils (Friedrich and Pryde, 1984). As it will be explained later in this chapter, the first part of the extraction can be considered to be controlled by this thermodynamic parameter.

Effect of solvent flow rate. The effect of solvent flow rate has been studied by Rebolleda et al., (Rebolleda et al., 2012) and their results are presented in Figure 7 at a constant pressure and temperature. Extraction curves, expressed in dependence on the solvent-to-feed ratio, are not significantly affected by SC-CO_2 flow rate. This behavior supports the fact that solubility, not external mass transfer, is controlling the SC-CO_2 extraction process of corn germ oil.

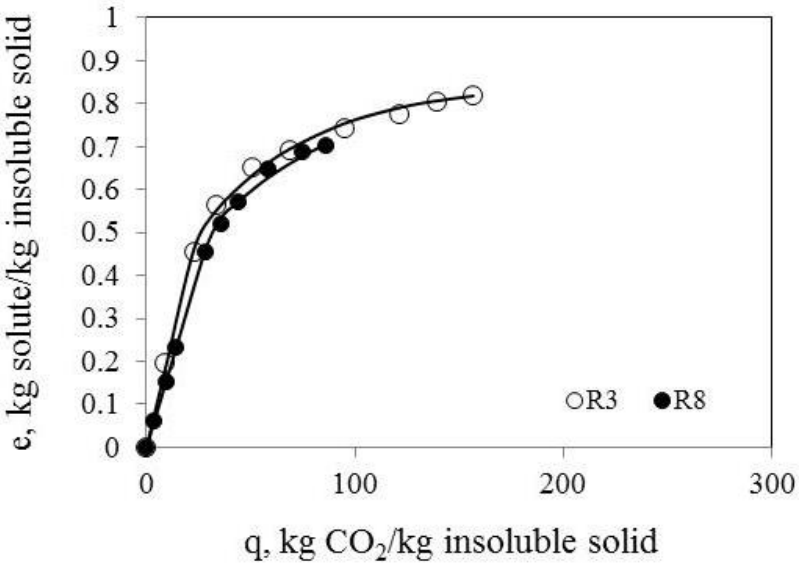


Figure 7. Influence of solvent flow rate on corn germ oil extraction yield at around 45 MPa and 79ºC: ● 4 kg CO_2/h; ○ 9.5 kg CO_2/h. Solid lines correspond to the model of Sovová (Sovová, 2005).

MODELLING OF THE SUPERCRITICAL FLUID EXTRACTION OF SOLIDS

Mathematical modeling of processes is a powerful tool to easily describe and optimize such processes and for scaling them up. However, modelling of extraction of natural materials is a difficult task since they have irregular structure and complicated geometry that change during the extraction process (Eggers, 1996).

For the specific process of supercritical fluid extraction (SFE) from solid substances, several models, from relatively simple theoretical models to more rigorous ones, have been developed (Brunner, 1994, Oliveira et al., 2011, Huang et al., 2012, Rai et al., 2014). When SFE is specifically applied to obtain extracts from plant materials, it should be considered that the solutes of interest are usually inside cell structures.

Plant materials are thus usually pretreated to break the cell structures and facilitate the contact of the solvent and the solute, thus increasing the extraction kinetics. Taking this into account, Sovová (Sovová, 1994) introduced the concept of broken and intact cells (BIC) to mathematically describe the extraction process from natural materials.

This concept has been since then extensively applied for modeling of SFE processes of natural substances in terms of the overall extraction curves (Huang et al., 2012). A BIC type mathematical model developed later by Sovová (Sovová, 2005) was the one chosen by Rebolleda et al., (Rebolleda et al., 2012) for modeling the experimental extraction curves previously presented This type of model assumes that the solute is regarded as a single pseudo compound. This simplification may lead to some errors since several components are generally involved in the extraction of seed oil (Reverchon and Marrone, 2001), but still keep models very useful for process description and scaling up. In this model (Sovová, 2005), the extraction yield is expressed as:

$$e = \frac{E}{N_m} \tag{1}$$

where E is the amount of extract (kg) and N_m the charge of insoluble solid (kg) in the extractor. The dimensionless amount of solvent consumed is obtained by:

$$q = \frac{Q\,t}{N_m} \tag{2}$$

where Q is the solvent flow rate (kg/h) and t the extraction time (h). Based on this model, the extraction curves consist of two parts. During the first one, the easily accessible solute from broken cells is transferred directly to the fluid phase, obtaining an initial part with a slope close to the solubility of oil in CO_2, while in the second one, the solute from intact cells diffuses first to broken cells and then to the fluid phase (Sovová, 2005).

Keeping this in mind, Rebolleda et al., (Rebolleda et al., 2012) calculated the initial slopes of different extraction curves of corn germ oil and compared them with data of solubility of oil in CO_2. To estimate the solubility of corn germ oil in SC-CO_2 these authors followed the general equation proposed by del Valle et al., (del Valle et al., 2012) to predict the solubility of many vegetable oils in high-pressure CO_2:

$$c_{sat}\left(g\cdot kg^{-1}\right) = 8.07\left(\frac{\rho}{910}\right)^{\left[9.59-8.45\left(\frac{\rho}{910}-1\right)-23.0\left(\frac{\rho}{910}-1\right)^2\right]} \exp\left\{-4182\left[1-259\left(\frac{1}{T}-\frac{1}{313}\right)\right]\left(\frac{1}{T}-\frac{1}{313}\right)\right\} \tag{3}$$

The reason for this behaviour is that the fatty acid composition of each oil is slightly different (Eggers, 1996). Del Valle et al., (del Valle et al., 2012) stated that Equation (3) can be applied to systems pure oil + high-pressure CO_2 as well as to oil containing vegetable substrates, since the initial stages of the extraction process is typically solubility-controlled. Del Valle et al., (del Valle et al., 2012) indicated that data obtained at a process temperature

different from 40ºC (T = 313.15 K) must be divided by a temperature-correction term (TCT) in order to compare the initial slopes of the extraction curves:

$$TCT = \exp\left\{-4182\left[1-259\left(\frac{1}{T}-\frac{1}{313}\right)\right]\left(\frac{1}{T}-\frac{1}{313}\right)\right\} \quad (4)$$

The corrected (at 40ºC) initial slope values calculated by Rebolleda et al., (Rebolleda et al., 2012) have been plotted in Figure 8 as a function of CO_2 density together with the prediction of the oil solubility from the General Model proposed by del Valle et al., (del Valle et al., 2012) and the error limit of this model. It can be observed that the values of the slope of the first part of the extraction are within the error limits for solubility of vegetable oils in CO_2.

In other studies of SFE of corn germ oil using ethanol as co-solvent (Rónyai et al., 1998) a linear increase at the beginning of the process has been also observed.

The value obtained in this study (Rónyai et al., 1998) has been also plotted in Figure 8. In this Figure, the solubility values of corn germ oil at 40ºC obtained by Soares et al., (Soares et al., 2007) have been also included.

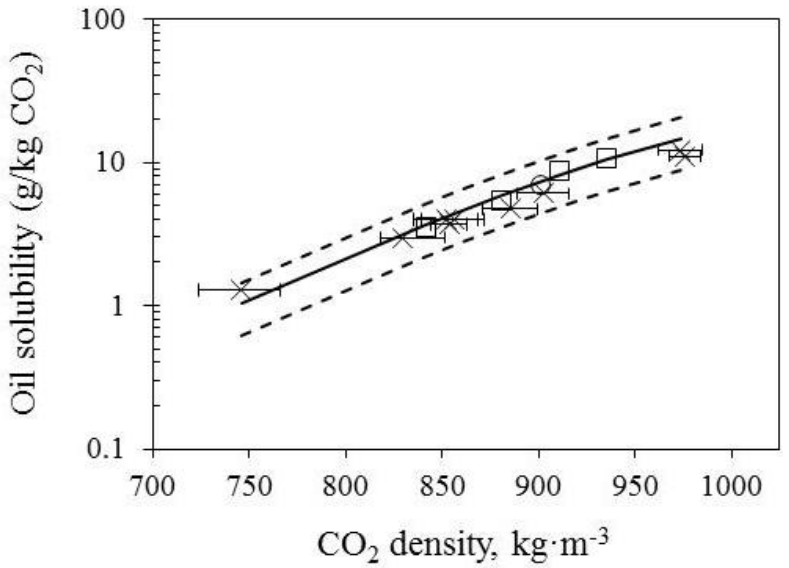


Figure 8. Corrected (at 40ºC) experimental solubility values of corn germ oil as function of pure CO2 density.(×) experimental initial slope values of the extraction curves obtained by Rebolleda et al., (Rebolleda et al., 2012); (—) prediction of del Valle et al., (del Valle et al., 2012) General Model; (- - -) error limits of the General Model; (□) solubility data from Soares et al., (Soares et al., 2007); (○) slope datum from Rónyai et al., (Rónyai et al., 1998).

Since the slope of the first part of the extraction curves can be considered of the same order as the oil solubility data, the model proposed by Sovová (Sovová, 2005) can be used to evaluate the first and second part of the extraction curves by using the following equations:

$$e = q\, y_s \text{, for } 0 \le q \le q_c \tag{5}$$

$$e = x_u\left[1 - C_1 \exp\left(-C_2 q\right)\right] \text{, for } q > q_c \tag{6}$$

C_1 and C_2 are model parameters, y_s is the experimental solubility datum (slope of the extraction curve), q_c the crossing point and x_u is the solute concentration in the untreated solid that can be obtained from the oil content in the corn germ.

The values of such constants obtained from the experimental extraction curves obtained by Rebolleda et al., (Rebolleda et al., 2012) are presented in Table 3. Model calculations have been represented along with the experimental data points in Figures 5-7 and good agreement can be observed between experimental data and model correlation. Following the model proposed by Sovová (Sovová, 2005), important parameters can be estimated from constants C_1, C_2 and co-ordinate q_c at the crossing point such as the volumetric fraction of broken cells in the particles, called grinding efficiency, r, and the solid-phase mass transfer coefficient, $k_s a_s$ (Table 3):

$$r = 1 - C_1 \exp\left(-C_2 q_c / 2\right) \tag{7}$$

$$k_s a_s = \left(1 - r\right)\left(1 - \varepsilon\right)\dot{Q} C_2 / N_m \tag{8}$$

In equation (8) solvent flow rate is expressed in $kg \cdot s^{-1}$. The estimated grinding efficiency was similar in all the extractions runs and it can be concluded that the volumetric fraction of broken cells in the corn germ particles is nearly 0.5. The crossing point, q_c, was found to increase with a decrease in the solubility value, specially marked at low operating pressure.

Table 3. Model parameters C_1, C_2, q_c, estimated grinding efficiency r and solid-phase mass transfer coefficient, $k_s a_s$

Experiment	C_1	C_2	q_c	r	$k_s a_s$
R1	0.6049	0.0054	25	0.43	$1.9 \cdot 10^{-5}$
R2	0.6728	0.0144	26	0.44	$5.0 \cdot 10^{-5}$
R3	0.6546	0.0188	23	0.47	$6.6 \cdot 10^{-5}$
R4	0.6586	0.0214	22	0.48	$6.2 \cdot 10^{-5}$
R5	0.6419	0.0050	55	0.44	$2.0 \cdot 10^{-5}$
R6	0.7478	0.0023	75	0.32	$1.0 \cdot 10^{-5}$
R7	0.7234	0.0297	17	0.44	$7.1 \cdot 10^{-5}$
R8	0.6509	0.0158	28	0.48	$2.3 \cdot 10^{-5}$

Obtained by Rebolleda et al., (Rebolleda et al., 2012).

QUALITY AND STABILITY OF CORN GERM OIL

Rebolleda et al., (Rebolleda et al., 2012) evaluated the quality of corn germ oil in two samples extracted with SC-CO_2 at a constant pressure near 45.0 MPa and two different temperatures of 56 and 84ºC (runs 9-10, Table 4).

The physical parameters evaluated (refraction index, density and color) for these authors showed no difference between the oils extracted at different temperatures. The average values they reported were a refraction index of 1.472 ± 0.001 at 25ºC, a density of 0.92 ± 0.01 kg/L at 20ºC and a color expressed as 100.07, -0.007, -0.012 corresponding to lightness (L*), redness (a*) and yellowness (b*), respectively.

Table 5 presents the fatty acid composition in different SFE extracts analyzed by Rebolleda et al., (Rebolleda et al., 2012) for the experiments presented in Table 4. It can be observed that the extraction temperature does not influence the fatty acid profile. Corn germ oil only contains 15% of saturated fatty acids, while it is very rich in polyunsaturated fatty acids (56%). A total of 8 fatty acids were identified by Rebolleda et al., (Rebolleda et al., 2012), being linoleic acid (more than 50%) the most abundant fatty acid, followed by oleic acid (28%) and palmitic acid (13%). Similar fatty acid profile of corn germ oil has been reported by other authors when using SFE (Vigh et al., 1993) and for crude germ oil obtained by pressing, followed in some cases by solvent extraction (Johnson, 2004).

Table 4. Experimental conditions in the SFE of corn germ oil with SC-CO_2 to evaluate the quality of corn germ oil

Run	P (MPa)	T (ºC)	Solvent flow rate, (kg CO_2/h)
R9	45 ± 2	56 ± 2	10.0 ± 0.9
R10	43 ± 2	84 ± 2	5.0 ± 0.5

One of the most important parameters to assess the oil quality is its acidity. Oil acidity depends on several factors such as oil composition, extraction procedure and raw material freshness. The acidity is related to the presence of free fatty acids (FFA) mainly generated by hydrolysis reaction of triacylglycerides and other non-lipid acid compounds such as acetic acid, that can be generated during spoilage of the raw material.

For corn germ oil extracted by SC-CO_2, the acidity index (AI) reported by Rebolleda et al., (Rebolleda et al., 2012) was found to be of the same order at different extraction temperatures (1.6 ± 0.1% oleic acid at 84ºC and 1.3 ± 0.1% oleic acid at 56ºC), and close to the lowest value of AI described in the literature for crude corn germ oils obtained by conventional extraction (from 1.5 to 4.0% oleic acid) (Johnson, 2004).

The AI values obtained by Rebolleda et al., (Rebolleda et al., 2012) agree with the results reported by List et al., (List et al., 1984) and Christianson et al., (Christianson et al., 1984) who compared the free fatty acid content of corn oil processing by expeller with those oils resulting of the SFE of corn germ obtaining lower values of acidity in the latter. Fractionation experiments will be explained later in this chapter since oils with higher quality and less acidity can be obtained by this procedure (Rubio-Rodríguez et al., 2012).

Table 5. Fatty acid profile of corn germ oil extracted with SC-CO_2 and comparison with crude oil

Fatty acids (FA)	R9, 56ºC mg/g oil	R10, 84ºC mg/g oil	R9-R10% (g/100 g FA)	Crude oil% (g/100 g FA)*
C16:0	107 ± 1	106 ± 1	12.5 ± 0.3	11.1-12.8
C18:0	20 ± 1	19 ± 1	2.3 ± 0.1	1.4-2.2
C18:1 n-9	245 ± 1	234 ± 1	28.1 ± 0.4	22.6-36.1
C18:1 n-7	5 ± 1	5 ± 1	0.6 ± 0.1	-
C18:2 n-6	475 ± 2	457 ± 2	54.8 ± 0.8	49.0-61.9
C18:3 n-3	8 ± 1	8 ± 1	0.9 ± 0.1	0.4-1.6
C20:0	4 ± 1	3 ± 1	0.4 ± 0.1	0.0-0.2
C20:1 n-9	3 ± 1	3 ± 1	0.4 ± 0.1	-
SFA	131 ± 3	128 ± 3	15.2 ± 0.5	12.5-15.2
MSFA	253 ± 3	242 ± 3	29.0 ± 0.7	22.6-36.1
PUFA	483 ± 3	465 ± 3	55.7 ± 0.9	49.4-63.5
Total FA	867 ± 9	835 ± 9	100 ± 2	84.5-114.8

*Johnson, 2004.

SFA: saturated fatty acids; MSFA: monosaturated fatty acids; PUFA: polyunsaturated fatty acids.

The composition of corn germ oil extracted with SC-CO_2 by Rebolleda et al., (Rebolleda et al., 2012), in terms of neutral lipids, is listed in Table 6. Triacylglyceride, free fatty acid and sterol content was nearly the same for the two temperatures studied. List et al., (List et al., 1984) reported values slightly lower for the unsaponificable content of SC-CO_2 extracted corn oil, in the range of 1.2-1.3 wt%, being sterols the majority.

Corn germ oil is rich in linoleic acid (Table 5), a polyunsaturated fatty acid, and therefore can suffer oxidation during processing. Lipids oxidation involves three different stages, initiation, propagation and termination, which rate depends on the substrates and reaction conditions. A common parameter to evaluate the oil oxidation is the Peroxide Value (PV) which indicates the total level of hydroperoxides in oil and is related to the primary compounds in the oxidation. Hydroperoxides are very unstable and decompose to different secondary compounds, especially at high temperatures. Therefore to assess the oxidative stability of SC-CO_2 extracted corn germ oil, the measurement of secondary oxidation compounds such as the anisidine value should be also considered. Unfortunately in the revised literature no data of the anisidine value or similar parameters related to the oxidation secondary products have been found for SC-CO_2 extracted corn germ oil and no comparison can be done with conventional corn germ oil extraction process.

Table 6. Neutral lipids profile of corn germ oil extracted with SC-CO_2

	% wt. in oil	
Neutral lipids	R9, 56ºC	R10, 84ºC
Triacylglycerides (TAG)	95.1 ± 0.8	95.4 ± 0.4
Free fatty acids (FFA)	0.8 ± 0.2	1.0 ± 0.1
Sterols (St)	2.3 ± 0.4	2.2 ± 0.1
Others	1.7 ± 0.2	1.4 ± 0.2

Table 7. Peroxide value, antioxidant capacity (DPPH) and tocopherol content of SC-CO_2 extracted oils

	R9, 56ºC	R10, 84ºC
Peroxide Value (meq/kg oil)	25 ± 2	20 ± 1
DPPH (mmol BHT/kg oil)	22 ± 3	32 ± 3
Tocopherol content (ppm)	1082 ± 14	1397 ± 17
α-tocopherol (ppm)	71 ± 5	86 ± 2
β-tocopherol (ppm)	65 ± 1	64 ± 1
γ-tocopherol (ppm)	945 ± 8	1232 ± 11
δ-tocopherol (ppm)	nd	15 ± 3

nd: not detected.

Temperature is one of the factors that could affect the initiation stage and, therefore, the oxidation of the oil (Anna, 2002), although results reported by Rebolleda et al., (Rebolleda et al., 2012) presented a slight decrease in the peroxide content of SC corn germ oil obtained at the highest extraction temperature studied (see Table 7). In any case, it must be highlighted that in the work carried out by Rebolleda et al., (Rebolleda et al., 2012), considerable high values of the peroxide content have been obtained (Table 7), especially when comparing with other values reported in literature for SC-CO_2 extraction of dry, freshly milled corn germ, which rarely exceed 0.5 meq/kg (List et al., 1989).

According to the specifications for corn oil by the Committee on Food Chemicals Codes of the National Academy of Sciences/National Research Council PV should be not more than 10 meq·kg^{-1} (NRC, 2003). Tocopherols have been described as effective antioxidants present in vegetable oils. Therefore, its presence contributes favorably to the conservation and the quality of oils. The tocopherol content of the oil obtained by Rebolleda et al., (Rebolleda et al., 2012) (Table 7) is even higher than the values reported for hexane prepress extracted wet corn germ (1000 μg/g) (List et al., 1984). The tocopherol profile of SC-CO_2 extracted corn germ oil obtained by Rebolleda et al., (Rebolleda et al., 2012) is also listed in Table 7; they found that γ-tocopherol was the major one followed by α-tocopherol and β-tocopherol, along with a small amount of δ-tocopherol. In Table 7, it can also be seen that, when extraction temperature increases from 56ºC to 84ºC, the yield percentage of tocopherols extracted increase by almost 30%. The solvent power of carbon dioxide decreases with increasing temperature at constant pressure because of its decreasing density.

Therefore, the higher tocopherol content found at the highest temperature might be due to an increase of vapor pressure of the tocopherols with temperature. No solubility data of tocopherols in CO_2 were found in the literature for the pressure and temperature range presented in Table 4. However, Chrastil (Chrastil, 1982) reported solubility data of α-tocopherol in the pressure and temperature range from100 to 250 bar and from 40 to 80ºC, respectively. These data show that α-tocopherol solubility decreases with temperature at low operating pressure (lower than 120-130 bar) while increases with temperature at operating pressure higher than 130-135 bar (Figure 9). Although no solubility data for other tocopherol isomers have been found in the literature, similar solubility behavior can be expected. Therefore, at pressure higher than 130 bar an increase in solubility is expected with increasing temperature. The increase of the tocopherol content with temperature can explain the

reduction of the peroxide content and the increase of the antioxidant level of oils (see value of DPPH in Table 7) at the highest extraction temperature. However, different results have been also found in the literature about tocopherol content in SC-CO_2 extracted corn germ oil, e.g.: List et al., (List et al., 1984) obtained SC-CO_2 extracted corn germ oil with less amount of tocopherols when temperature was increased from 70 to 90ºC at a constant pressure of 82.7 MPa (ranged from 1.840 to 1.180 μg/g at 70ºC and 90ºC respectively), although these authors concluded that the reason for this drop was unclear.

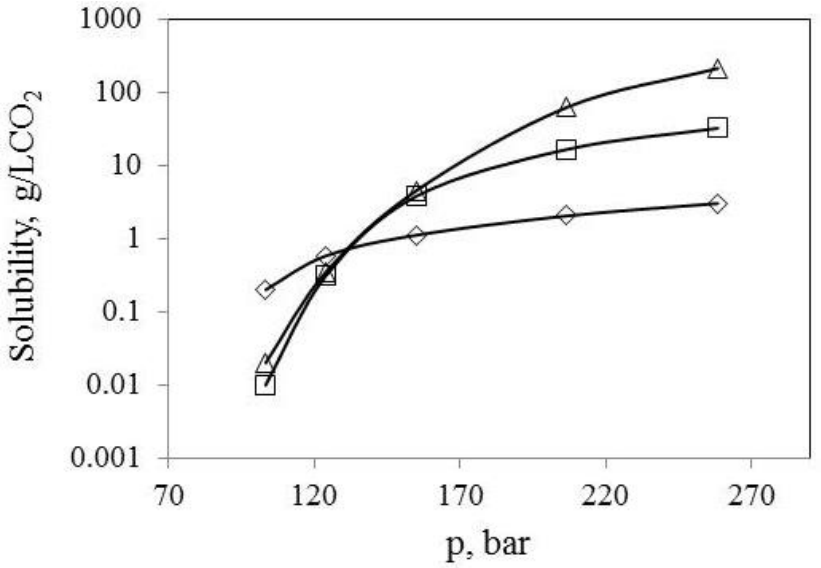


Figure 9. Solubility data of α-tocopherol in carbon dioxide reported by Chrastil (Chrastil, 1982). ◇ 40ºC, □ 60ºC, △ 80ºC.

List and Friedrich (List and Friedrich, 1989) and Rebolleda et al., (Rebolleda et al., 2012) found that SC-CO_2 extracted seed oils undergo rapid deterioration. Many factors can affect the oxidative stability of oils, including the different pre-treatments before oil extraction (corn steeping, germ separation, germ storage…), fatty acid composition, concentration and stability of antioxidants and the presence of prooxidant compounds (FFA, lipid peroxides, or prooxidant metals) (List and Friedrich, 1989, Winkler-Moser and Breyer, 2011).

List and Friedrich (List and Friedrich, 1989) suggested that the absence of phospholipids in the SC-CO_2 extracted corn germ oil could cause a decrease in the positive synergistic effects of tocopherols with phospholipids.

This synergic effect of phospholipids and tocopherols has been described in the literature as responsible for the oxidative stability in vegetable oils. In contrast, Calvo et al., (Calvo et al., 1994) suggested that oil instability may be related to the trace amounts of oxygen in the CO_2. In this case, during SC-CO_2 extraction of corn germ oil, oxidation would take place without mass transfer limitation since solvent and oil are in the same phase.

SUPERCRITICAL FLUID FRACTIONATION

Oils extracted by conventional solvents require refining processes to remove phospholipids, free fatty acids and oxidation products. Solubility of phospholipids and glycolipids in CO_2 is very low and therefore it is possible to eliminate the degumming step in the refining process, which is an advantage compared with the conventional process (Eggers, 1996). Another important advantage of SFE is that oil fractionation can be carried out under supercritical conditions since solubility of triacylglycerides in CO_2 below 16 MPa is fairly low, leading to save energy (Eggers, 1996). As an example, solubility data of trilinolein and linoleic acid, as the major fatty acid compound, have been represented in Figure 10 where higher values of solubility are observed for the FFA than for the corresponding triglyceride in SC-CO_2. Therefore, a simple process to obtain refined corn germ oil after SCCO_2 extraction can be proposed (Figure 11).

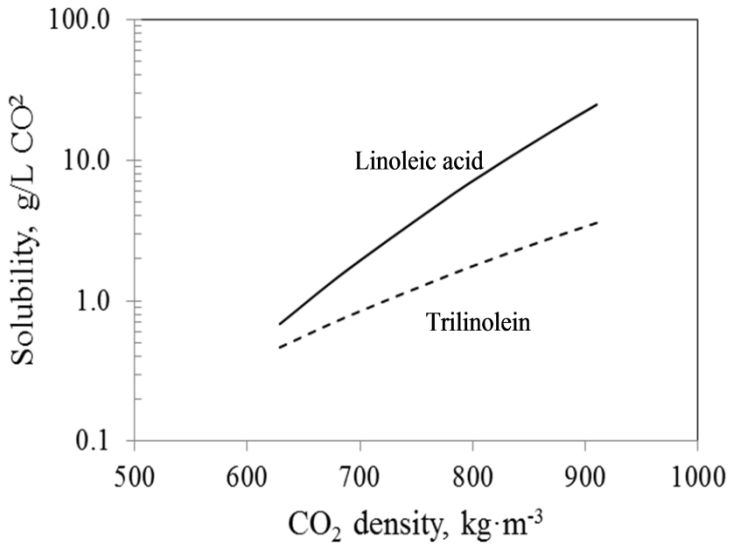


Figure 10. Solubility data of trilinolein (---- correlation of solubility data obtained by Chrastil (Chrastil, 1982)) and linoleic acid (Güçlü-Üstündağ and Temelli, 2000).

Rebolleda et al., (Rebolleda et al., 2012) reported results of an extraction-fractionation process in two separators installed in series, aiming to improve the oil quality. They maintained the first separator at 10.0 MPa and 40ºC. These authors evaluated the influence of extraction pressure and temperature on the tocopherol content of the oil and on their stability to oxidation in different experiments (runs 11-13), which conditions are here reported in Table 8.

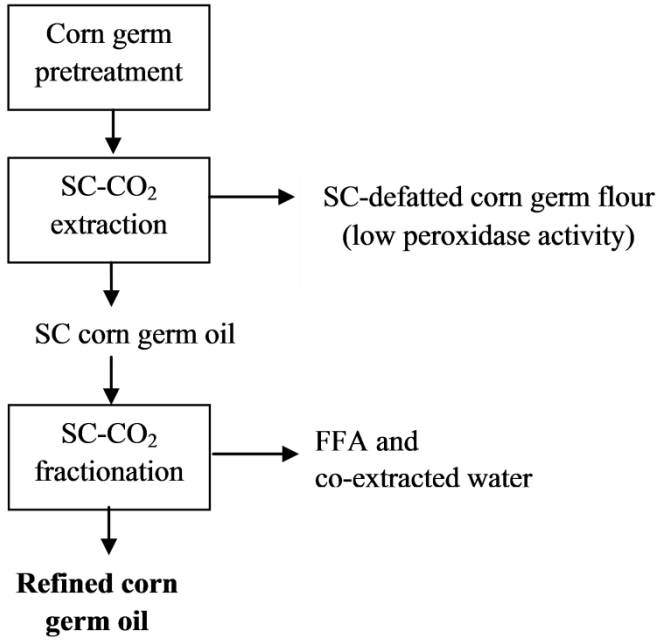


Figure 11. Obtaining refine corn germ oil by using supercritical CO_2 technology.

Table 8. Experimental conditions in the SFE of corn germ oil with SC-CO_2 to evaluate the effect of fractionation after extraction on oil quality (10 MPa and 40ºC in the first separator)

Run	P (MPa)	T (ºC)	Solvent flow rate, (kg CO_2/h)
R11	50 ± 2	35 ± 2	11.0 ± 0.7
R12	48 ± 2	86 ± 2	7.0 ± 0.9
R13	26 ± 2	85 ± 2	9.0 ± 0.9

These authors found that the fraction collected in the first separator was mostly oil whereas the fraction recovered in the second one was mostly an aqueous emulsion, what is probably due to the low solubility of triacylglycerides in CO_2 below 16 MPa (see Figure 10). The relative oxidative stability of the two fractions determined by the Rancimat test reported, show that the induction time in the oil fraction recovered in separator 1 (1.9 ± 0.3 h) was significantly higher than the induction time determined in the fraction recovered in separator 2 (0.5 ± 0.3 h). Water and free fatty acids, with a high tendency to oxidation, were mostly removed in separator 2, which agrees with the higher stability found in the oil fraction recovered in separator 1. In any case, the induction time of the oil fraction recovered in the first separator by Rebolleda et al., (Rebolleda et al., 2012) was lower than values reported by other authors (Winkler-Moser and Breyer, 2011) for hexane Soxhlet extracts of corn germ (3.91 ± 0.4 h). As previously explained, this could be because SC-CO_2 extracted oils suffer rapid deterioration. Another important result found by Rebolleda et al., (Rebolleda et al., 2012) is that total concentration of tocopherols in the oil fraction recovered in separator 1 is

almost double when temperature increased form 35ºC to 86ºC at a constant extraction pressure of around 49.0 MPa (see Figure 12).

This result agrees with the values of tocopherol content presented in Table 7 where the effect of extraction temperature on the oil quality was analyzed. However, different results are also found in the literature. Wilp and Eggers (Wilp and Eggers, 1991) found that an increase in extraction temperature from 50ºC to 80ºC at constant extraction pressure of 50 MPa results in a decrease in tocopherols concentration in the first separator of more than 30%. On the contrary, the effect of extraction pressure, at constant extraction temperature on tocopherol content has been found negligible in different studies.

Rebolleda et al., (Rebolleda et al., 2012), at constant temperature, around 85ºC, when the extraction pressure is increased from 26 MPa to 49 MPa, found that tocopherol concentration in the oil fraction recovered in the first separator was only slightly increased (less than by 10%). Similar results were obtained by Wilp and Eggers (Wilp and Eggers, 1991) for a extraction pressure range from 50 MPa to 32 MPa at a constant extraction temperature of 50ºC. Based on these results, it could be concluded that the effect of temperature on the extraction of tocopherols is more notable than the effect of pressure.

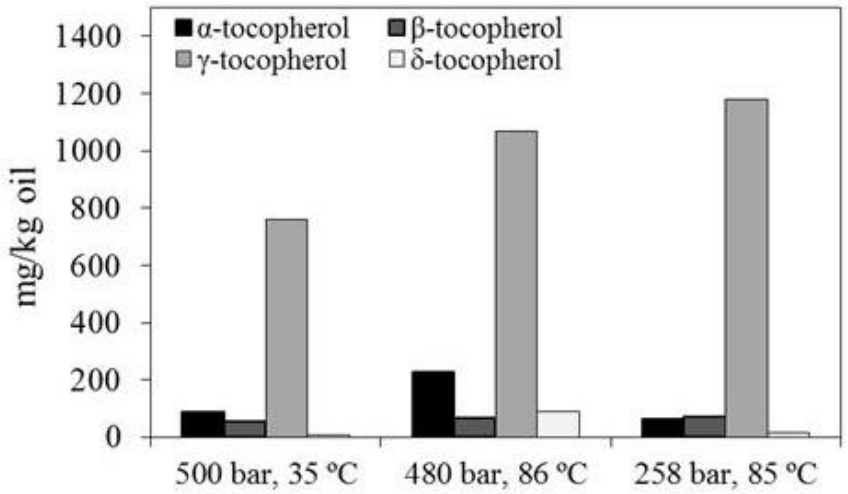


Figure 12. Tocopherol concentration of fractionated oils in the first separator (10.0 MPa and 40ºC) at different extraction temperatures (a) and pressures (b).

CONCLUSION

Supercritical carbon dioxide extraction has been presented as an alternative procedure to obtain oil of high quality from milled corn germ. Typical extraction curves of vegetables oils, including corn germ oil, present two different sections.

In the first stages of the extraction, the process may be controlled by the solubility of the oil in SC-CO_2 since the initial slope calculated for SC-CO_2 extracted corn germ oil is of the same order as the solubility of vegetable oils in CO_2. While, a second section of the extraction curve is determined by the diffusional resistance in the solid matrix.

SC-CO_2 extraction of corn germ permits to obtain oil with good quality with lower values of acidity than for oils obtained by conventional extraction. High tocopherol content and therefore high antioxidant activity has been reported in the literature for SC-CO_2 extracted corn germ oil. However, SC-CO_2 extracted seed oils undergo rapid deterioration. Nevertheless, fractionation of the extracted oil in two separators installed in series allows separating the co-extracted water and seems to improve the oil stability against oxidation.

It should also be highlighted that the defatted corn germ meal obtained after removing the oil with SC-CO_2 could be used in food formulation due to its good stability and protein quality.

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Chapter 4

USES AND APPLICATIONS OF THE CORN OIL

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ABSTRACT

Corn or maize oil is an important vegetable oil obtained from the germ part of the corn kernel. It is used in food, biofuel and some other important industrial applications for a long time. Corn oil use is increasing gradually as a result of its production as a co-product in the ethanol manufacturing. Its use in food and biofuel markets grows as its price reduced as a result of increasing production rates. Corn oil is used as a frying oil, salad oil, margarine and spread oil etc. in food markets. It is also used to produce biodiesel which is an alternative biofuel obtained from vegetable oils and animal fats. Biodiesel is compatible in terms of many fuel properties with petroleum diesel fuel. Corn oil has also some other important applications in chemical industry such as polymers, coatings and corrosion inhibitors etc. In this chapter, main uses and applications of corn oil will be elucidated in order to show its growing importance in related sectors.

Keywords: corn oil, biodiesel feedstocks, vegetable oil

INTRODUCTION

World corn production was about 38,065 million bushels in 2013-2014 marketing year. Corn is mainly used as an animal feed, a food component and an ethanol feedstock (Anonymous 2014). Corn oil is one of the co-products of the corn seeds. Its capacity depends on the corn production. Corn oil production and consumption data show that the largest corn oil producers and consumers are United States and China respectively. The recent increase in

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the ethanol production particularly in the United States results in an increase in corn oil production (Menon 2014).

Corn kernel contains about 4% oil mainly in its germ parts (Moreau 2005). Corn oil is produced as a co-product in industrial ethanol production processes. There are two methods of ethanol production from the corn seeds: Dry-grind and wet milling. In dry-grind process, which is the common one, corn grains are crushed by the high speed rotating hammer tips into the fine particles with 3-5 mm diameter. After fermentation and distillation steps, corn oil is obtained by extracting the oil from the thin stillage portion of the distiller's dried grains with solubles (DDGS) (Anonymous 2011).

In wet milling process, which is more capital and energy intensive, wet mills separate the corn kernel into its components such as starch, fiber, gluten and germ. After germ is removed from the kernel, corn oil is extracted from the germ (Bothast & Schlicer 2005). In both processes, corn oil containing intermediate products are first pressed and then corn oil is extracted with using hexane or 2-methylpentane (isohexane) as a solvent (Anonymous 2006). Some of the physical properties of corn oil are given in Table 1.

Table 1. Some chemical and physical properties of corn oil (Gunstone 2002).

Property	Value
Iodine value	127-133
Saponification number	187-193
Specific gravity 25°C/25°C	0.919
Viscosity, 40°C	30.80 cP
Surface tension, 25°C	34.80 dyn/cm
Thermal conductivity, 130°C	4.2017×10^{-5} J/s/cm^2/°C
Unsaponifiables	1-2%
Melting point	-11 to -8 C
Smoke point	230 to 238°C
Flash point	323 to 338°C
Cloud point	-14 to -11°C

Corn oil is used in industry mainly in two applications: As a food and biofuel feedstock. Some other notable uses are soap making, rustproofing, medicine and insecticide production. Some important uses of corn oil are explained in detail as following.

FOOD USES OF CORN OIL

Corn oil has different uses in food sector such as cooking or salad oil, margarine oil, blends of butter (40%) and corn oil margarine (60%), mayonnaise and emulsion type salad dressings as either consumer or institutional products. It is also used as an oil ingredient in many packaged and restaurant foods, including spaghetti sauce, potato chips and snack foods, French fries and breaded fried foods, baking mixes, frosting, whipped toppings, crumb coatings for meat and poultry, and baked goods. Corn oil is often used without blending with other oils except for packaged foods (Anonymous 2006).

Salad and Cooking Oils

Approximately half of the food use of corn oil is as salad/cooking oil. It is preferred as a salad/cooking oil because of its mild flavor, good nutritional properties, highly digestible structure and good oxidative stability (because of low linolenate levels). It is also a better source of essential fatty acids (Meurant 1983, Dupont 1990). Corn oil is used as 100% corn oil or in blends with other vegetable oils.

Deep fat frying is one of the most common cooking methods because fried foods have desirable flavor, color and crispy texture (Boskou et al., 2006). Food is immersed in hot oil at high temperatures (150-190°C) in the cooking container where heat and mass transfer of oil, food and air are occurred simultaneously. As a result of favorable conditions, chemical reactions such as oxidation, hydrolysis and polymerization take place during the deep frying. Many factors such as frying time, temperature, composition of food, type of fryer and antioxidant content, quality of frying oil are significant parameters on the quality criteria of fried food (Choe & Min 2007). It is reported that (Warner & Knowlton 1997) oxidation rate of the oil increased as the unsaturated fatty acid content of the oil increased. That is why corn oil with less unsaturated fatty acid is better frying oil than soybean and canola oils with more unsaturated fatty acids.

In 69% of the popular chain fast food restaurants in the United States, corn oil is used as frying oil in French fries while by 20% of the small businesses use it as fry oil. This is attributed that major users have large-scale cost effective corporate agreements with the suppliers; whereas minor users find it more expensive than other plant oils such as soybean oil (Jahren & Schubert 2009). In addition, fast food companies have changed potato frying applications from animal fat to non-hydrogenated corn oil because of the *trans* fatty acid concerns (Anonymous 2006).

Vegetable oils and fats incline to form polar compounds and polymerized triglycerides. Corn oil has one of the fastest rates of production of polar compounds and polymerized triglycerides among the common oils at 190°C temperature as a result of its higher polyunsaturated fatty acid content (Takeoka et al., 1997).

Margarines and Spreads

Margarine is mainly classified into two types: stick and tub margarines. Stick margarine is formulated to approximate the butter properties. It is a little bit softer than butter under the ordinary storage conditions and can melt in the mouth. Tub margarine contains higher unsaturated fatty acids and easily spreadable at lower temperatures (McGee 2004).

Corn oil use is expanded over the years especially after the invention of that corn oil has a favorable effect on serum cholesterol in 1950s. It became the highest consumed oil among the polyunsaturates of all important margarines (Anonymous 2006). *Trans* fatty acid formation during the hydrogenation process turned out to be a major concern in margarine production. Several methods were suggested in order to avoid *trans* fatty acid formation (List et al., 1995; Sundram et al., 1999). Corn oil can be interesterified with fully hydrogenated vegetable oil to produce *trans* free margarines (Anonymous 2006). The corn oil margarines and other food products containing corn oil have a wide variety of applications in food industry (Gunstone 2002).

Mayonnaise and Salad Dressings

Mayonnaise is defined as “an emulsion of oil droplets suspended in a base composed of egg yolk, lemon juice or vinegar, which provides both flavor and stabilizing particles and carbohydrates” (McGee 2004). Varieties of oils are used in mayonnaise and it can contain vegetable oil up to 80%. The vegetable oil used in mayonnaise purveys a medium for the rest of the ingredients. The pH of the mayonnaise is 3.6 to 4.0 because of the acetic acid. It contains 9-11% salt and 7-10% sugar.

Salad dressings have similar composition with mayonnaise except for finished product contains 30% vegetable oil. pH of the salad dressing is in the range of 3.2 to 3.9 as a result of acetic acid content. The aqueous phase contains 3.0 to 4.0% salt and 20 to 30% sugar (Jay 2000). Use of corn oil in mayonnaise improved sensory characteristics such as color, taste, texture and flavor. Corn oil use in the mayonnaise also provides good emulsion stability (Rasool 2013).

BIODIESEL FEEDSTOCK

Biodiesel Production

Biodiesel is an alternative diesel fuel obtained from vegetable oils and animal fats. The term of biodiesel is commonly referred for mono-alkyl esters obtained from vegetable oils and animal fats via transesterification even though it implies other lipid products having a potential to be used as a biofuel. Other plant oil based biofuels can be obtained via different methods such as direct use, blending, microemulsions and pyrolisis (Ma & Hanna 1999). Mono-alkyl esters and other liquid biomasses are used as a fuel usually after a blending step with petroleum diesel fuel in order to improve its operational characteristics such as viscosity and cold flow properties. Biodiesel, as a renewable diesel fuel, has mild exhaust emission characteristics and faster biodegradability along with a lubricating effect in diesel engines.

Fatty acid structure (composition and unsaturation degree) of vegetable oils used as feedstocks considerably affect the physical properties of biodiesel such as viscosity, cold flow properties, oxidation stability and exhaust emission profile (Knothe 2008). Even, there are many successful attempts to predict the biodiesel properties from the fatty acid composition of the plant oils. Fatty acid distribution of some common vegetable oils is given in Table 2. Higher saturated fatty acid based biodiesel has higher viscosity, cold flow temperatures, oxidation stability and calorific value while higher unsaturated fatty acid based biodiesel shows a combination of improved fuel properties as a whole (Knothe 2005; Ramos 2009; Kumar 2013).

Transesterification of vegetable oils into the methyl ester fatty acid mixture (alcholysis) is achieved at similar reaction conditions for almost all oil feedstocks. Biodiesel is produced with the reaction of a vegetable oil with an alcohol (mostly methanol) in the presence of a catalyst (a strong base such as NaOH or KOH) at about 50^{o}C temperature in a short time (shorter than 1 hour). The overall chemical reaction is presented in Scheme 1.

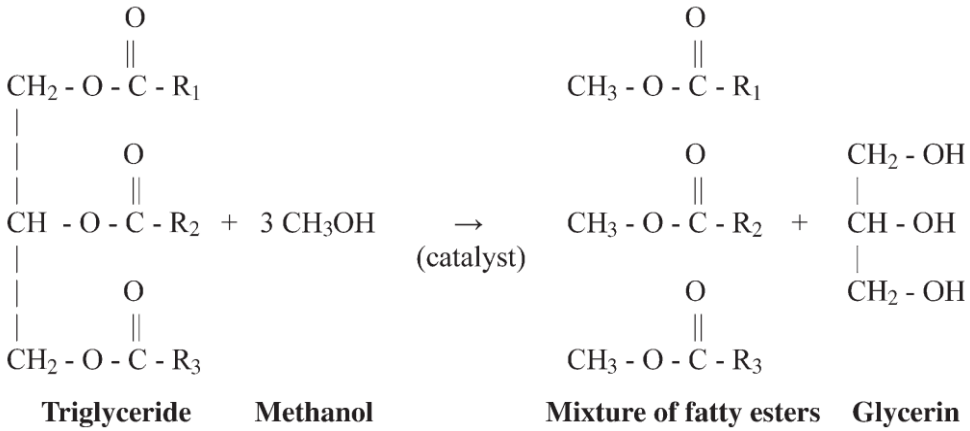
Vegetable oils with higher amount of free fatty acid and water are needed to first introduce a neutralization step in order to prevent saponification between the base catalyst

and free fatty acids. Another method is to use acidic catalyst such as sulfuric acid in the transesterification. When the free fatty acid content of the triglycerides is high, use of acid catalyst is the best way of transesterification to avoid undesired neutralization of the base catalyst (Ma et al., 1998). Figure 1 shows the process steps in the biodiesel production via basic transesterification. Biodiesel is used after blending with a higher percentage of petroleum based diesel fuel in practice. The percentage of biodiesel is designated by terms such as B5 or B20 which means 5% or 20% of the blend is biodiesel respectively.

Table 2. Fatty acid composition of some common plant oils[a] (Kirk-Othmer 1991)

C atoms: double bond	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
Canola oil	-	-	-	0.1	4.0	0.3	1.8	60.9	21.0	8.8	0.7	1.0	0.3	0.7	0.2
Castor oilb	-	-	-	-	2.0	-	1.0	7.0	3.0	-	-	-	-	-	-
Coconut oil	7.1	6.0	47.1	18.5	9.1	-	2.8	6.8	1.9	0.1	0.1	-	-	-	-
Corn oil	-	-	-	0.1	10.9	0.2	2.0	25.4	59.6	1.2	0.4	-	0.1	-	-
Cottonseed oil	-	-	0.1	0.7	21.6	0.6	2.6	18.6	54.4	0.7	0.3	-	0.2	-	-
Linseed oil	-	-	-	-	6.0	-	4.0	22.0	16.0	52.0	0.5	-	-	-	-
Olive oil	-	-	-	-	9.0	0.6	2.7	80.3	6.3	0.7	0.4	-	-	-	-
Palm oil	-	-	0.1	1.0	44.4	0.2	4.1	39.3	10.0	0.4	0.3	-	0.1	-	-
Peanut oil	-	-	-	0.1	11.1	0.2	2.4	46.7	32.0	-	0.1	0.1	-	-	-
Rapeseed oil	-	-	-	0.1	3.8	0.3	1.2	18.5	14.5	11.0	0.7	6.6	0.5	41.1	1.0
Safflower oil	-	-	-	0.1	6.8	0.1	2.3	12.0	77.7	0.4	0.3	0.1	0.2	-	-
Soybean oil	-	-	-	0.1	10.6	0.1	4.0	23.3	53.7	7.6	0.3	-	0.3	-	-
Sunflower oil	-	-	-	0.1	7.0	0.1	4.5	18.7	67.5	0.8	0.4	0.1	0.7	-	-

[a] Some oil compositions may not add to 100% due to the presence of minor fatty acids.
[b] Contains 87% OH-bearing ricinoleic acid (C18:1).



Scheme 1. Transesterification (methanolysis) of vegetable oils.

Enzymes can also be used as catalysts in transesterification. The enzymatic route can provide advantages such as elimination of organic solvents, chemical catalyst and waste water (Ranganathan 2008). On the other hand, it has several disadvantages such as high enzyme cost, low yield and longer reaction time.

Corn oil is used as a feedstock in biodiesel production via transesterification either as a raw vegetable oil or as waste frying oil. Advantages and specifications of biodiesel obtained from corn oil are examined in detail in the following subtopics.

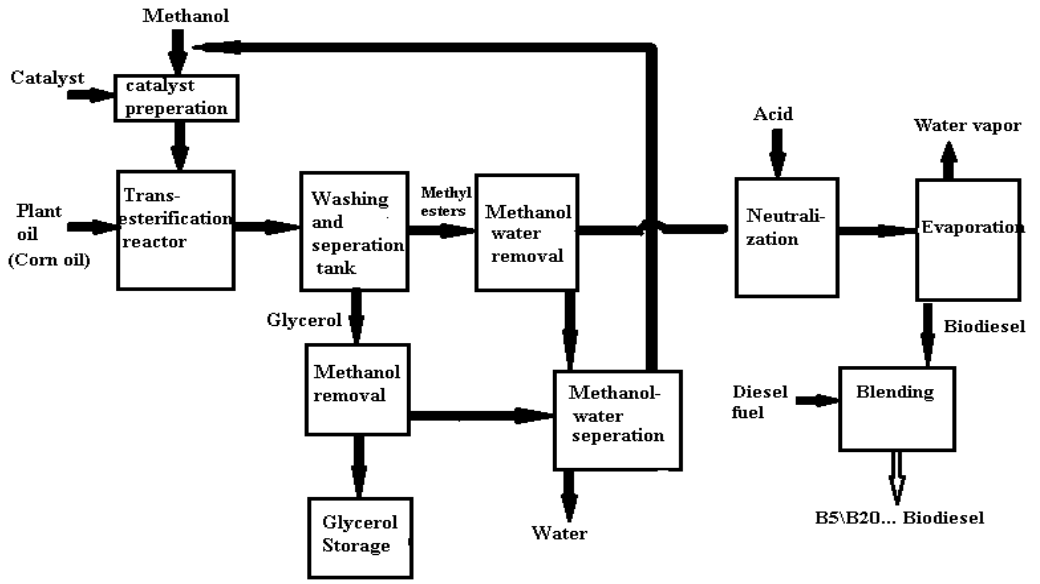


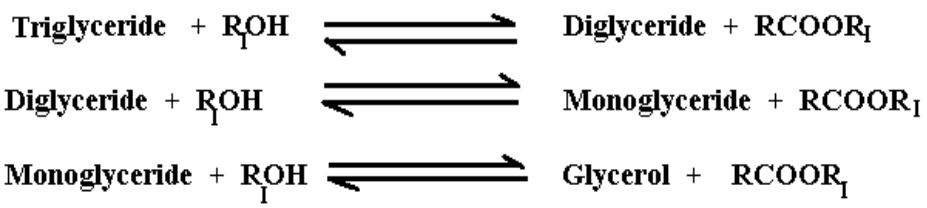
Figure 1. Process flow diagram for (corn oil based) biodiesel production.

Reliability of Corn Oil as a Biodiesel Feedstock

Corn oil has been ignored as a biodiesel feedstock formerly because of its higher cost and value as edible oil. However, developments in the ethanol production processes such as dry grinding have made way for higher amount of corn oil production. Corn oil is the fastest growing biodiesel feedstock in biodiesel production in the United States (Kotrba 2014). Ethanol producers began to utilize corn oil as a biodiesel feedstock onsite biodiesel plants in order to take advantage of lower operational and transportation costs. Increases in ethanol production made the corn oil a reliable feedstock in biodiesel production.

Transesterification Kinetics

Transesterification of triglycerides has a stepwise reversible reaction route in which diglycerides and monoglycerides are intermediates (Scheme 2):



Scheme 2. Stepwise transesterification of triglycerides.

An excess alcohol makes the forward reaction pseudo-first order whereas reverse reaction second order in the case of corn oil (Meher et al., 2006). When corn oil is transesterified in a pressurized batch reactor in the presence of sodium methoxide and methanol, higher conversion can be obtained. Kinetic constants of the stepwise reactions are increased in the direction of the progressing steps of the transesterification (Velazquez 2007).

Biodiesel production from corn oil can also be carried out in enzymatic catalysis. In a study using immobilized lipase enzyme (Novozym 435) as a catalyst, it was reported that 81.3% fatty acid methyl ester content was obtained at 15% enzyme load, 60^{o}C temperatures and 10 MPa pressure in 4 hours (Ciftci & Temelli 2013). Corn oil was also transesterified with methanol by injecting it into a supercritical carbon dioxide stream in the presence of immobilized lipase enzyme. Fatty acid methyl ester yield was observed as greater than 98% (Meher et al., 2006).

Cold Flow Temperatures

Behavior of biodiesel from corn oil at lower ambient temperatures is specified by cloud point, pour point and cold filter plugging point temperatures. Cloud point is the temperature by which liquid material begins cloudy since the saturated esters become crystallized. If the temperature decreases more, it will stop flowing. The lowest temperature it can keep flowing is called pour point. Cold filter plugging point gives a more practical measure in terms of cold flow properties of liquid fuels. It is defined as the highest temperature at which a given amount of sample cannot pass through a standard filter in a settled time (Knothe et al., 2005). Cold flow properties of biodiesel samples obtained from corn oil were seen acceptable in terms of common biodiesel standards (Rasimoglu & Temur 2014). In this study, the cloud point of the B100 corn oil based biodiesel was reported to be -4˚C while the pour point and cold filter plugging point to be -10˚C and -12, respectively.

Kinematic Viscosity

Kinematic viscosity of corn oil biodiesel is strongly affected by the structure of fatty acids. Fatty acid properties such as chain length, position, double bonds (double bond location, number, and nature) have effects on the kinematic viscosity of biodiesel. Biodiesel products obtained from shorter fatty acids and longer alcohol moieties have lower viscosity than those have longer fatty acids and shorter alcohol moieties (Knothe & Stidley 2005; Knothe & Stidley 2007). Allen et al. (1999) developed an empirical model to predict the

viscosity of 15 biodiesel samples including corn oil based one from the fatty acid contents of the parent oils.

Engine Performance and Emission Characteristics

Biodiesel obtained from corn oil has been reported as having improved engine performance (Kumar & Kumar 2013). Emission characteristics of biodiesel-diesel fuel mixtures enhance as biodiesel ratio in the mixture increases (Gopinath & Suresh 2015). On the other hand, fuel consumption increased by about 10% while maximum engine power slightly decreased in the case of corn oil biodiesel (Pullen and Saeed, 2014). Tesfa at al. (2013) pointed out that there is no significant difference between the engine performances of different vegetable oils.

A Valuable Co-product: Glycerol

As a result of transesterification process, a mixture of fatty acid methyl esters (biodiesel) and glycerol are obtained. These products are in different phases and can be separated by gravitational settling (Figure 2).

Glycerol recovery plays an important role in biodiesel production in terms of making whole process more profitable. The glycerol phase separated from the biodiesel contains about 50% glycerol together with excess methanol, catalyst and soap and has a little economic value. The soaps formed during transesterification are decomposed again into free fatty acids and salts by adding acid (Gerpen 2005). Methanol is evaporated after free fatty acids are precipitated and separated. The glycerin purity can reach to 85% as a result of these purification steps.

Figure 2. Corn oil biodiesel and glycerol separation (upper phase is biodiesel).

Alternative Production Routes

The quality specifications of biodiesel fuel obtained from corn oil is changeable as a result of its broader fatty acid distribution since corn oil has a wide range of saturated-unsaturated fatty acid contents from region to region as well as time to time (Lofland & Quasckenbush 1956; Ericson 1989; Gunstone 2011; Vesna et al., 2013).

Mendes (2011) produced biodiesel from corn oil with using ethanol as an alcohol reactant and NaOH as a catalyst. He pointed out that 0.4% NaOH in weight, 40˚C reaction temperature and 90 minutes transesterification time is enough to obtain a higher biodiesel yield. One step alkali transesterification is sufficient to obtain higher biodiesel yield when corn oil contains lower amount of free fatty acid (Patil & Deng 2009).

Thermal behavior of corn oil-based biodiesel obtained by using methanol and ethanol was investigated by (Dantas et al., 2010). They reported that corn oil was thermally stable up to 336˚C, methyl biodiesel up to 145˚C, and ethyl biodiesel up to 169˚C in nitrogen atmosphere.

Biodiesel production from corn oil can also be carried out via enzymatic route as well. When the Lipozyme TL IM-catalyzed transesterification was performed in the presence of absolute ethyl alcohol, 98.95% fatty acid ethyl ester conversion was obtained at 35^{o}C in 12 hours reaction period (Mata et al., 2012).

As a result, the corn oil biodiesel satisfies ASTM D6751 standard and it can firmly claimed that corn oil is a rational biodiesel feedstock (Alptekin et al. 2014; Rasimoglu & Temur 2014).

OTHER USES

Polymerization

Corn oil together with other vegetable oils is seen nowadays as important natural raw materials used in many chemical industries because of their availability, biodegradability, cheapness and harmless health effects. Vegetable oil based polymers and composites have a wide range of applications such as paints and coatings, adhesives and biomedicine (Ligadas et al., 2013). A mixture of vegetable oils including corn oil is polymerized with styrene and divinilbenzene in the presence of different fillers and fibers such as organic clays, glass, hemp, flax, wood flour, corn stover, wheat straw and sugarcane bagasse. These polymeric materials show improved mechanical and thermophysical performance (Frederick at al., 2004; Lu 2009; Samarth & Mahanwar 2014).

Paints-coatings and Corrosion Inhibition

Poly(ether amide urethane) resin produced from corn oil was used as a corrosion protective and environmental friendly coating material. The coatings have good scratch harness, flexibility, corrosion performance and temperature resistance up to 250°C (Alam & Alandis 2014). Corn oil can be used as a coating material for eggs to extent their shelf life.

Corn oil coating retains internal quality of eggs by 3 weeks longer than that of non-coated eggs with minimized weight loss (0.8%) at 25°C storage temperature (Ryu et al., 2011). Similar results can be observed with other vegetable oils.

Some surfactants based on corn oil and monoethanolamine exhibited better corrosion inhibition effect for the mild steel in 1% NaCl solution saturated with CO_2 at 323 K temperature (Ismailov 2013).

Insulating Applications

Petroleum based insulating liquids are generally used as a dielectric and coolant in electrical power applications. They are not only toxicant but also inflammable as a result of higher temperatures and required costly fire protection precautions. Al-Eshaikh and Qureshi (2012) offered the food grade corn oil to overcome these problems because it is non-toxic and has a higher fire point. Furthermore, corn oil has higher breakdown strength and less degradation than mineral oils.

CONCLUSION

As a result of increasing corn production in worldwide, corn oil is expected to gain more importance as a vegetable oil. In addition to this, corn oil consumption as a food source is increasing since it has several benefits as a valuable food component in terms of health effects. It has lower blood cholesterol and higher polyunsaturated fatty acid content. It has a potential to be one of the most reliable biodiesel feedstock in the near future. It can be also used in many other industrial applications such as medication, chemical and soap production. Finally, it is an important lipid product already having many important uses and applications. More researches on corn oil applications are needed to clarify its benefits extensively.

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Chapter 5

CORN OIL AND AGING: INSIGHTS FROM BASIC RESEARCH

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ABSTRACT

Corn oil, having one of the highest polyunsaturated (PUFA) levels after sunflower, safflower, walnut and wheat germ oil, is the second leading vegetable oil consumed in the United States. While corn oil is well known in reducing blood lipids in humans, there are very few studies on the long-term beneficial effects of corn oil in animals. In the present chapter, the beneficial effects of corn oil on survival rate, bodyweight, food intake and other physiological parameters were described in aging mice and rats fed with corn oil long time. The possible mechanisms of these corn oil effects were also discussed. Long-term corn oil intake reverses aging-increased blood lipids and circulating pro-inflammatory cytokines as well as aging-damaged rotarod performance test and liver function, and thus increases longevity of aging mice. These health benefits of corn oil may result from the combinations of the high levels of PUFA, vitamin E and plant sterols. Therefore, corn oil, even at a high energy percentage, is a favorable replacement of animal fats in the human diet if the total energy intake is controlled.

Keywords: corn oil, aging, mice, rats, mechanism

INTRODUCTION

High saturated fat diets are well associated with obesity prevalence and the increased risk of cardiovascular disease, diabetes and cancer. Reducing saturated fats from the diet is recommended to eliminate Western diet-induced health problems.

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The common alternatives of animal (saturated) fats for humans are plant oil, including soybean oil, peanut oil and corn oil because of the high percentage of unsaturated fat acids.

Corn oil, a co-product starch manufacture named corn wet milling process, is majorly (85%) from corn germ although germ is only about 11.9% dry weight of conrn kernel (Figure 1). However, corn oil was increasing demanded after it was reported in reducing human serum cholesterol in 1950s. Annual production of crude corn oil increased 5.75% each year from 1974 to 2002. In 2005, crude corn oil production in US was 2.48 billion pounds and ten years later increased to 4.45 billion pounds in 2015, and nearly all of it is refined into high-quality oil for the food industry and direct use by consumers (USDA, 2015; Association, 2006). Corn oil is now the second importance only to soybean oil in the United States (USDA, 2015; Corn Refiners Association, 2006).

Corn oil is composed mainly (99% of the refined or 96% of the crude oil) of acylglycerols (mono-, di- and primarily tri-), and has 59% polyunsaturated (PUFA), 24% monounsaturated (MUFA) and 13% saturated fatty acid (SFA). The PUFA to SFA ratio (P/S) is about 4.6 (Table 1).

Corn oil has one of the highest PUFA levels after sunflower, safflower, walnut and wheat germ oil (Landers and Rathmann, 1981) (Table 2). The primary PUFA is linoleic acid (C18:2n-6), with a small amount of linolenic acid (C18:3n-3) giving a n-6/n-3 ratio of 83.

Corn oil contains a significant amount of ubiquinone and high amounts of gamma-tocopherols (vitamin E). Corn oil contains a significant amount of ubiquinone and high amounts of gamma-tocopherols (vitamin E) (Table 1). These high contents of PUFA, phytosterols and vitamin E may contribute to the health benefits of corn oil consumption.

The beneficial effects of PUFA have been extensively investigated, however, there are very few studies investigating the effects on human health with long-term corn oil consumption, particularly on the older population.

This is very important because corn oil is the second leading vegetable oil consumed and produced 4.45 billion pounds in 2015 in the United States (USDA, 2015; USDA, 2014). Since US adults age 65 and older heavily consume this high fat oil, their population is rapidly increasing and is projected to reach 71 million by 2030. The objectives of the present study are to summarize the long-term health effect of high volume of corn oil consumption in aging animals and to understand the relevant mechanisms.

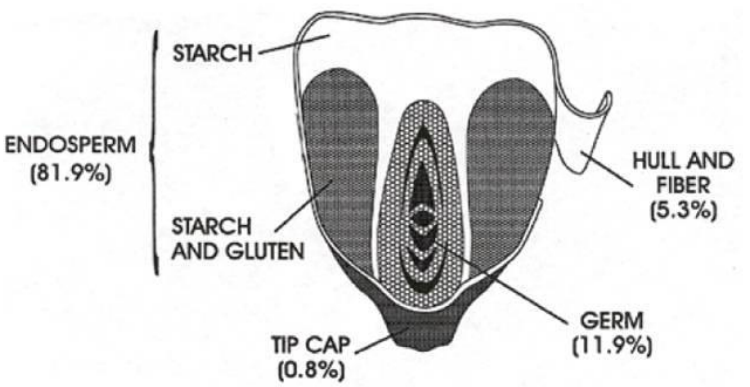


Figure 1. A kernel of corn.

Table 1. Nutrients values of refined corn oil (100 g)

Nutrient compostion	Values
Protein (g)	0
Fat, total (g)	100
Triglycerides (g)	98.8
Polyunsaturates, total (g)	59.7
Cis, Cis only (g)	58.7
Monounsaturates (g)	26.0
Saturates, total (g)	13.1
Unsaponifiable Matter (g)	1.2
Cholesterol (mg)	0
Phytosterols (mg)	1000
Tocopherols, total (mg)	88
Alpha-tocopherol (mg)	19
Gamma-tocopherol (mg)	67
Delta-tocopherol (mg)	3
Carbonhydrate (g)	0
Ash (g)	0
Sodium (mg)	0
Energy (Cal)	885

Table 2. Composition comparison of major vegetable oils

Type	Saturated fatty acids	Mono-unsaturated fatty acids	Polyunsaturated fatty acids			Oleic acid (ω-9)	Smoke point (^{o}C)
			Total poly	Linolenic acid (ω-3)	Linolenic acid (ω-6)		
Coconut	91.0	6.0	3.0	-	2.0	6.0	177
Corn	12.9	27.6	54.7	1.0	58.0	28.0	232
Cottonseed	25.9	17.8	51.9	1.0	54.0	19.0	216
Flaxseed	7.5	15.5	79.0	64.0	15.0	11.0	107
Peanut	16.9	46.2	32.0	-	32.0	48.0	225
Soybean	15.7	22.8	57.7	7.0	50.0	24.0	238
Sunflower	10.1	45.4	40.1	0.2	39.8	45.3	227

RESULTS OF ANIMAL STUDIES

Aging Mice

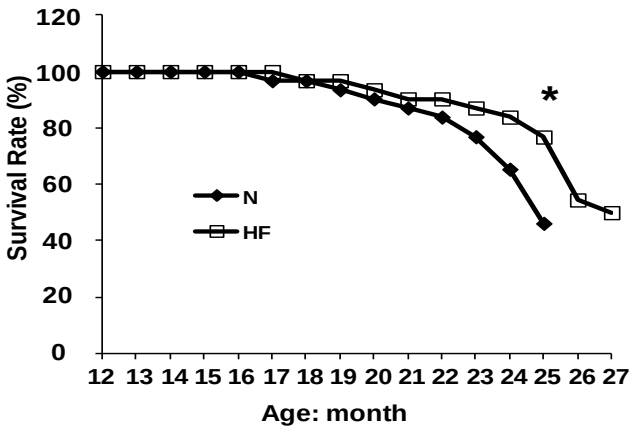
We recently conduct a study investigated whether dietary intake of phytochemical epicatechin in extending lifespan in mice, we unexpectedly found that high corn oil dietary intake improves health and longevity of aging mice (Si et al., 2014).

Twelve-month old male C57BL/6 mice purchased from the National Cancer Institute (Bethesda, MD) were randomly divided into two groups (n = 31) and given either a normal diet (N) or a high corn oil diet (HF).

Both diets are based on the AIN-93 produced by Dyets Inc. (Bethlehem, PA) with two exceptions 1) soybean oil was replaced with corn oil and 2) the percentage of corn oil in each diet. The normal diet has 10% fat derived calories (FDC) and the HF diet has 58% fat derived calories (FDC) (Reeves, et al., 1993). This dose of corn oil (58% FDC) was calculated based on previous studies using high fat diet (60% FDC, 6% from soybean oil and 54% from lard) (Sung et al., 2014). We also collected samples from young control mice (YC) to compare changes between YC and normal diet fed old mice (N).

Our results shown that at 25 months of age, 53.8% of mice had died in the N group, whereas the mortality rate was only 23.2% in the HF group ($P = 0.02$, Figure 2).

The median for the HF group was reached two months later at 27 months of age. Although the average body weight in the HF group was significantly higher than that in the N group, the food consumption in the HF group was significantly lower than that in the N group. This may be a result of the texture of the HF diet, which has the consistency of paste. Some days an oily liquid would form on the top of the rodent feeding jars, while the N diets were typical pellets. Interestingly, there was no significant difference in energy intake between these two groups. Physical activity and locomotor function continuously diminishing with aging (Yankner et al., 2008; Baur et al., 2006) and although corn oil increased longevity, it is important to know whether quality of life was maintained. We evaluated the ability to perform on a rotarod, a classic method of testing balance and motor coordination. While the time on the rod was significantly decreased as mice aged in the N group (decreased from 115 sec at month 14 to 80 sec at month 25), the HF group maintained their motor skills until they were 25 months of age. Histological examination of liver sections stained with haematoxylin and eosin revealed a loss of cellular integrity and the accumulation of large lipid droplets in the livers of the N group, but the HF group reduced the size of intracytoplasmic lipid vacuoles. Circulating lipid abnormalities are increasingly recognized as playing an important role in the aging process and age-related disorders such as diabetes and vascular dysfunction (Labinskyy et al., 2006). Compared to the YC group, serum triglyceride, total cholesterol and LDL-cholesterol were significantly increased in the N group; however, all these three serum lipids were reversed in the HF group.



*P < 0.05. (Si et al., 2014).

Figure 2. Suvival curve in normal diet (N) and high corn oil diet (HF) mice for 13-15 months. There initially was 31mice/group.

Consistent with observations of the pathological alterations in liver and remarkably shortened lifespan of mice in the N group, circulating levels of cytokines including IL-1β, IL-6, IFN-γ and MCP-1 were significantly elevated in the N group compared to those in the YC group. However, dietary consumption of corn oil significantly reduced these pro-inflammatory markers, indicating that corn oil may suppress chronic inflammation caused by aging. In addition, GSR activity in the livers of the N group was significantly decreased compared to that in the YC group, whereas the activity of this enzyme was reversed in the HF group.

Aging Rats

The National Toxicology Program (NTP) initiated studies to evaluate the role of several oils in altering cancer rates in male rats in early 1990s (Program, 1992). 7 weeks old male F344/N rats (50 rats/group) were administrated 2.5, 5, 10 mL corn oil/kg body weight or 10mL saline/kg body weight by gavage, 5 days a week, for 2 years.

The results shown that two-year survival was increased in male rates receiving corn oil (untreated control, 26/50; saline control, 32/50; 2.5mL/kg, 33/50; 5mL/kg, 38/50; 10mL/kg, 40/50). The major reason was the dose-related decreased incidence of mononuclear cell leukemia (untreated control, 27/50; 2.5mL/kg, 16/50; 5mL/kg, 11/50; 10mL/kg, 7/50).

Although the mean body weights of all dosed groups were at least 5% higher than those of the untreated and saline controls by week 48, the mean body weights of groups receiving 2.5mL/kg, or 5mL/kg corn oil/kg decreased during the final weeks of the study (after week 89) and were similar to those of the controls at the end of the study.

This study was further extended to explore whether sex and diets affect survival in rats (Rao and Haseman, 1993). Four to five weeks old male and female F344/N rats (50 rats/ group) fed either commercial diet or a NIH-07 diet were administrated with 5 mL corn oil/kg body weight or 10mL saline/kg body weight by gavage, 5 days a week, for 2 years. Corn oil intake with either commercial diets or NIH-07 diet significantly increased the body weight and survival but significantly lowered the incidence of leukemia compared with their corresponding diet control groups at 106 weeks (Table 3), however, these beneficial effects were not observed in female rats. Moreover, oil intake worked slightly better in reducing leukemia in NIH-07 diet (from 48.9% to 21%) than that in commercial diet (from 28.3% to 12.6%) in male rats although the control groups without corn oil in commercial diet had higher survival and lower leukemia than these in control group without corn oil in NIH-07 diet. Interestingly, the gross energy intake (per kilogram body weight) was essentially the same for male and female rats with or without corn oil intake but reduced food intake and increased PUFA in corn oil gavage groups comparing to the control groups (40 g/week vs. 32 g/week, 2.7 g/week vs. 1.1 g/week respectively), this is in line with our recently studies in mice.

MECHANISMS

Aging is well-known as an inevitable process that is physiologically characterized as a progressive, generalized systematic dysfunction of almost or all organs, giving rise to the escalated vulnerability to environmental challenges and resulting in increased risks of disease and death. Indeed, aging is associated with a greatly increased metabolic and oxidative stress, elevated chronic, low-grade inflammation, and accumulated DNA mutations as well as increased levels of its DNA damages (Heininger, 2000a; Heininger, 2000b; Frisard and Ravussin, 2006). It is established that calorie restriction delays age-associated organ disorders and increases longevity as well as improves inflammation and oxidative stress in a wide range of species, suggesting that targeting nutrient-sensing and energy metabolism pathways may be an effective approach to delay the aging process and age-related diseases.

Indeed, our recent study in mice and previous studies in rats provide evidence that long-term corn oil intake improved general health, decreased incidence of mononuclear cell leukemia and extended longevity. These health benefits of corn oil may be associated with reversing aging-increased blood lipids and pro-inflammatory makers as well as aging-damaged brain and liver functions. These results are very important for us to understand the health effects of corn oil consumption, the second largest vegetable oil consumption in the United States, although more studies, particular clinical studies are needed to evaluate the beneficial effects of long-term corn oil consumption.

Corn oil was first attended by its highly effective in lowering blood cholesterol, particularly LDL-cholesterol (Ahrens et al., 1957; Hill et al., 1979), one of the major risks of cardiovascular disease. Reducing blood LDL is recommended to lower cardiovascular disease as well as other aging-related chronic diseases (2002). Our results in mice are in line with these previous studies that corn oil lowers LDL-cholesterol, total cholesterol and triglyceride (Si et al., 2014).

This effect may be due to the high level of PUFA, which is supported by evidence that corn oil is more effective than olive oil in lowering LDL-cholesterol because corn oil has higher PUFA (58.7 g/100 g oil) than olive oil (8.4 g/100 g oil) (Howell et al., 1998; Dupont et al., 1990). Corn oil has a plant sterols content of 128 mg/1000kcal vs. 66 mg/1000kcal for olive oil, and these plant sterols can reduce cholesterol absorption from the gut which in turn lowers body pools and enhances synthesis rate through de-suppression of cellular hydroxy-methylglutaryl-CoA reductase activity (Howell et al., 1998; Maki, et al., 2015). This is supported by a recent human study that the hypocholesteremic effect of corn oil is connected with the high amount of phytosterols (Wagner et al., 2001).

Indeed, corn oil intake affected lipid metabolism genes including cholesterol 7α hydroxylase, hydroxyl-3-methylglutaryl-Coenzyme A reductase, fatty acid synthase and angiopoietin-like protein 4 at a circadian rhythm (decreased at hour 3, increased at hour 6 to 9, and decreased at hour 24) in rats (Takashima et al., 2006).

Table 3. Influence of corn oil on body weight, survival and leukemia in F344 rats

	Male				Female			
	Commercial diet		NIH-07 diet		Commercial diet		NIH-07 diet	
	Control	Corn oil	Control	Corn oil	Control	Corn oil	Control	Corn oil
Body weight, g	446±20	473±21*	478±18	504±19*	320±22	298±20*	348±15	330±16*
Suvival, %	74±7.4	82±5.1*	63±7.4	71±8.4*	79±7.0	78±7.3	71±8.2	73±8.6
Leukemia, %	28.3±10	23.6±8.2*	48.9±10.3	21.0±9.2*	18.7±6.2	18.7±10.3	25.2±8.1	22.7±7.6

*Significant ($p < 0.01$) compared with corresponding control.

Rao and Haseman, 1993.

These genes/proteins changes may lead to the decrease of food intake (15% in weight) (Takashima et al., 2006), which is similar with our results (Si et al., 2014). These results suggest that the animals can self-control their total calorie intake to a constant level by changing these genes expressions. A large body of evidence indicates that increased generation of reactive oxygen species (ROS) which are chemically reactive molecules with most of them containing oxygen and unpaired electrons is one of the major triggers of aging. There is a strong correlation between chronological age and the levels of ROS generation and oxidative damage of tissues.

ROS are primarily produced by mitochondria during energy production (about 2% of total oxygen consumption was funneled to ROS) (Chance et al., 1979). Extra amounts of ROS induces oxidation of fatty acids and proteins and causes oxidative damage of DNA that may lead to cellular senescence, functional alterations, and pathological conditions (Harman, 1972; Linnane et al., 1989). This extra amount of ROS is deactivated to water and oxygen by endogenous enzymes including superoxide dismutase (SOD), catalase or glutathione peroxidases (Chang et al., 2004).

Endogenous antioxidant glutathione (GSH) and exogenous antioxidants including vitamins C and E are also important ROS scavengers. Reducing ROS is proposed as a leading strategy to delay aging and related degenerative diseases. Corn oil is one of the highest natural sources of vitamin E (62.01 mg/100 g oil) and is just a little less than cottonseed oil (62.37 mg/100g oil). This high content of vitamin E may prevent aging-increased ROS and extend the lifespan of aging mice. This is supported by our results showing decreased GSR activity, a critical endogenous antioxidant enzyme, was reversed by corn oil intake (Si et al., 2014). Aging-induced ROS also contributes to low-grade chronic inflammation (Brod, 2000), a crucial player of the process of aging and age-related diseases in older adults. Indeed, chronic pro-inflammatory markers including IL-6, MCP-1 and TNF-α are consistently elevated with age in the absence of acute infection or other physiological stress (Ferrucci, et al., 2005). Consequently, the sustained increases of these pro-inflammatory molecules impair the function and integrity of various tissues and organs and thus accelerate aging and aging-related chronic diseases, although this increase is still in the sub-acute range (Chung, et al., 2006). Interestingly, calorie restriction significantly attenuates the increase of these pro-inflammatory markers while extending the lifespan (Chung et al., 2006; Zou et al., 2004), suggesting that anti-inflammatory agents may have the potential to extend a healthy lifespan. Results from our study shows that dietary intake of corn oil significantly reversed the increase of circulating pro-inflammatory markers including IL-1β, IL-6, IFN-γ and MCP-1 in aging mice and therefore increased longevity (Si et al., 2014).

A lower ratio of omega-6/omega-3 fatty acids (n-6/n-3) is recommended to reduce the risk of many highly prevalent chronic diseases in Western societies (ratio of (n-6/n-3 in Western diets is 15/1–16.7/1) (Simopoulos, 2002). Mammalian cells cannot convert omega-6 to omega-3 fatty acids because they lack the converting enzyme, omega-3 desaturase.

These two classes of essential fatty acids (EFAs) are not interconvertible, are metabolically and functionally distinct and have opposing physiological functions. Therefore, too much omega-6 may be detrimental for cells. Corn oil is not a good source for EFAs because the ratio of omega-6/omega-3 fatty acids from corn oil is 83, which is much higher than the recommended ratio (1/1 to 4/1) (Simopoulos, 2002).

However, the present study and other studies show that high corn oil intake improves health and longevity in mice and rats (10 mg/kg/d by oral gavage) (1994).

One explanation is that majority of omega-6 PUFA from corn oil is used for energy, and is not used to produce thrombi and atheromas, which are required for cardiovascular disease development. Moreover, high levels of vitamin E (majorly γ-tocopherol) (Elmadfa and Park, 1999) and plant sterols (0.77% by weight) (Ostlund, et al., 2002) may counter the bad effects of omega-6 PUFA of corn oil. For example, γ-tocopherol, the major form of vitamin E in the corn oil, and its metabolite have more anti-inflammatory properties than α-tocopherol, the predominant form of vitamin E in the tissues and most supplements (Jiang and Ames, 2003).

Taken together, if total calorie intake is kept in a normal range, long-term corn oil intake reverses aging-increased blood lipids and circulating pro-inflammatory cytokines as well as aging-damaged brain and liver function, and thus increases longevity of aging mice and rats.

CONCLUSION

These health benefits of corn oil may result from the combinations of the high levels of PUFA, vitamin E and plant sterols. Therefore, corn oil, even at a high energy percentage, is a favorable replacement of animal fats in the human diet if the total energy intake is controlled.

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Chapter 6

PRODUCTION METHODS AND COCONUT OIL QUALITY

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ABSTRACT

Among many edible oils, coconut oil can be considered a unique oil because of its unique short and medium chain saturated triglyceride composition. Coconut oil is extracted by dry and wet extraction methods. In dry extraction, coconut oil is extracted from dried coconut kernels by pressing or solvent extraction. In wet extraction, an aqueous emulsion of white coconut kernel (coconut milk) is either boiled or treated under cold conditions to separate coconut oil. The cold methods include freezing, enzymatic treatment or fermentation of coconut milk emulsion to break down proteins and separate oil. Copra oil is prepared by pressing dry coconut kernel known as copra. Virgin coconut oil is prepared by both wet and dry extraction methods. Coconut oil contains two fractions, fatty or lipid fraction and non-lipid fraction. The quality parameters such as acid value, peroxide value, anisidine value, iodine value and saponification value are originating from the lipid fraction. Among these quality parameters, iodine value and saponification value of coconut oil are not significantly affected by the method of extraction. Fatty acid composition of coconut oils extracted by any dry or wet methods remains reasonably similar. However, acid value, peroxide value and anisidine value vary with the extraction method of coconut oil. Non-lipid fraction also changes with the method of extraction. Health properties of coconut oil are associated with not only lipid fraction but also non-lipid fraction. As a result, health properties of coconut oil considerably vary with the method of extraction. Therefore, it is advisable to verify the origin of coconut oil when coconut oil is used for research purposes or for consumption.

Keywords: coconut oil, quality parameters, oil extraction, wet process, dry process, antioxidant properties

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INTRODUCTION

The average height of a coconut tree of ordinary tall coconut cultivars is about 12 meters. Coconuts become mature within 12-14 months. Coconuts can be harvested manually or by allowing the nuts to fall naturally. Manual harvesting can be done by hand picking after climbing the tree or by using a knife attached to a long bamboo pole. A coconut tree produces a single bunch of mature coconuts every month and the number of nuts harvested can be up to about 75 nuts a year. A mature fresh coconut with the husk weighs about 1.5 kg. The husk weighs about 0.5 to 0.8 kg and it can be removed by dehusking the coconut using a sharp iron spike fixed in the ground. About 1750 coconuts can be dehusked per eight-hour period by an experienced worker. Inside the husk there is a thick hard shell covering the coconut endosperm. Dehusked coconuts have to be cracked using a chopping knife or hatchet to open the coconut. This process is called shelling. During this shelling, coconuts are cracked into two equal halves. After opening the coconut by shelling, coconut kernel is still attached to the shell halves and can be further processed to obtain coconut oil. This coconut kernel, which weighs about 0.35 kg, is either dried to produce copra or grated to make desiccated coconut.

PROCESSING COCONUT KERNEL FOR OIL EXTRACTION

Coconuts have a solid as well as a liquid endosperm. Solid endosperm is the white coconut kernel which is used to extract coconut oil. The extraction process can be categorized based on the nature of coconut kernel and the methods used to obtain oil from coconut kernel. If dry coconut kernel is used for the extraction of coconut oil, the process is called dry process. Fresh coconut kernel is used in the wet extraction process. Here, a water emulsion of coconut kernel is prepared by mixing crushed or scraped coconut kernel with water. The resultant white-colored, milk-like emulsion, usually known as coconut milk is used for the extraction of coconut oil.

Dry Process

This process is used for the preparation of coconut oil in industrial scale for human consumption as cooking oil or as an ingredient for preparation of other foods. This oil is also used to prepare soap and other skin care products. The first step of the dry extraction of coconut oil is the drying of coconut kernel. Usually the fresh coconut kernel contains about 50% water, 34% oil, 7.3% carbohydrates, 3.5% proteins, 3.0% fiber and 2.2% ash (Banzon & Velasco, 1982). The water content in coconut kernels has to be reduced from 50% to about 6% during the drying process for the oil extraction by pressing. The dry coconut kernel or copra is prepared from mature coconut kernel. There are two types of copra, cup copra and ball copra, which reflect the shape of the copra. Ball copra is mainly used in making sweets or consumed directly. Ball copra is commonly prepared by mildly drying mature coconuts for 8-9 months. For this, it is not necessary to expose coconuts to direct sunlight. In this method, coconuts are stored in the shade. The coconut kernel inside the nut shrinks due to slow evaporation of moisture from the liquid endosperm of coconut and further drying of the

coconut kernel. During this process, the kernel separates from the shell as a ball. This detachment of copra ball from the coconut shell can be confirmed by shaking the nut to check for the rattling sound made by the detached kernel. At this stage, the husk can be removed and the shell can be broken to take copra out of the coconut shell as a ball. In the production of cup copra, coconuts are split-open into two halves and dried using various methods.

Sun Drying

Sun drying is the cheapest and probably the greenest way of drying copra. After removing the husk, coconuts are halved by breaking the thick shell of the coconut. When these coconut halves are dried for two days, the low moisture coconut kernels from the halves can be removed including the thin brown outer skin of the coconut kernel, known as testa. These separated coconut kernel halves are still not suitable for extraction of oil as they contain about 30% moisture. The moisture content has to be further reduced to 6% for the copra to be suitable for extraction of oil. Copra has to be dried in bright sunlight for 8 hours a day for at least 7-14 days to lower the moisture content down to 6%. Though this drying process takes a long time, this method is popular among small-scale copra producers, as it does not require any special instrument or additional expense for drying. However, the drying process can be easily disturbed by rain. In addition to getting copra wet during rain, the lack of sunlight delays the drying process.

As coconut kernel contains proteins and carbohydrates in addition to the moisture and oil, the kernel is prone to fungal attacks, which produce aflatoxins in fresh coconut kernel. Fresh coconut kernel is very susceptible to deterioration, which results in producing free fatty acids and rancidity due to fungal attacks and other reasons. To minimize fungal contaminations, the drying process should be started as soon as the nut is open. The drying process has to continue without any disruption due to rain or lack of sunlight in order to prepare good quality sundried copra. In some instances, coconut kernels or partially dried copra is cut in to small slices to accelerate the drying process. This slicing requires a lot of labor and as a result, the cost of production of copra increases. As fungal attacks occur on the surfaces that are exposed, slicing may promote deterioration of the kernel if proper sunlight is not provided. Therefore, it is safer to split the nuts into halves.

Usually the drying process takes about two weeks. With the evaporation of water, the oil to water ratio of the coconut kernel increases. It is easier to extract oil from copra, which has low moisture content compared to the extraction of coconut oil from fresh coconut kernel containing a lot of water. Properly drying copra to minimize water is important for the production of coconut oil by pressing copra. The water content of the coconut oil extracted using properly dried copra is negligible. Water content affects the quality of the coconut oil as the presence of water in coconut oil promotes the hydrolysis of triglycerides to give hydrolytic rancidity. In the production of desiccated coconut, the drying is done after removing the testa, which gives the light yellowish color to coconut oil. In the production of virgin coconut oil, the testa is removed and the white coconut kernel is used to produce the colorless oil.

Kiln Drying

Coconut kernel can be dried by heating the coconut halves in a special kiln or on an open stage as well. In this case, copra is smoked by a fire underneath. This process dries coconut kernel more quickly than the sun drying process. Kiln drying is important during the rainy season and this drying can also be combined with sun drying. The special platform used for this purpose can be set up using bamboo stems or some wood planks. This platform has many empty or open spaces. Coconut halves are spread on the platform and a fire is made using coconut shells on the floor under the platform. With slow burning of coconut shells, the coconut kernels on the platform are dried due to the contact of smoke generated by slow burning process underneath. The kiln where this platform drying takes place is covered by a brick structure. The temperature of the smoke generated in this drying process is about 40-50°C and the drying time is reduced to 4-5 days due to the hot smoke, which is in contact with the copra. Coconut oil produced by this copra is inferior in quality and brownish in color. Of the different parameters, the width of the drying chamber and the distance between the floor where the burning takes place and the drying platform are critical in the drying process (Rodrigo et al., 1996). Two types of fuels are used in traditional kilns, coconut shells and coconut shell charcoal. Better quality copra based on commercial grading system can be obtained by using charcoal as fuel.

Hot Air Drying

This drying process needs fuels other than coconut-based fuels. Therefore, this is a more expensive drying process. However, the quality of copra produced by this method is superior to kiln dried copra because of the more efficient drying without exposing copra to smoke. The drying time of copra by hot air drying is also shorter compared to other drying methods. Hot air can be generated within the kiln or can be pumped from an outside source.

Desiccated Coconut

Desiccated coconut is obtained from small particles of coconut kernel by drying ground or shredded coconut kernel after the removal of the brown testa. It is an important ingredient in confectioneries, puddings and many other food preparations. Husk is removed from fresh matured coconut. Then the hard shell is removed and coconut meat is separated. The testa is removed and the white coconut kernel is washed with clean water. Then it is grated and sulfur dioxide is passed to obtain a white color. The grated coconut kernel mass is blanched with live steam for about 20 minutes to bring down the microbiological counts. The blanched mass is then dried in a hot air drier at a temperature of 80-90°C for about 10 hours to bring down the moisture content below 3%. The dried coconut kernel is tested for moisture, free fatty acids and microbial counts. This desiccated coconut kernel contains 2-3% moisture, 65-68% fat and 30-32% other solids such as proteins and carbohydrates.

EXTRACTION OF COCONUT OIL

Pressing Copra

The main method of extraction of coconut oil from copra is by pressing copra using expellers. Copra is pressed in large expeller presses that generate heat and pressure. The resultant crude coconut oil is brown and turbid in appearance. This oil can be further purified by filtering and refining to remove free fatty acids (a breakdown product from the oil), any remaining moisture, any bad flavor or smell. Coconut oil made this way is the least expensive of all coconut oils, which is used in food preparations. Pressing is also used to produce virgin coconut oil in the dry extraction process. The quality of copra used in the extraction of virgin coconut oil is of high quality and free of fungal contaminations. Special driers can be used to prepare copra for this purpose and the brown portion of copra is not used to prepare virgin coconut oil by pressing copra. Here, the pressing can be considered as cold pressing, since the temperature is controlled.

Solvent Extraction

A solvent can be used in the extraction of coconut oil from copra. *n*-Hexane is considered to be the most efficient solvent for oil extraction as oils easily dissolve in hexane. It is the most suitable solvent also because of the low boiling point, which makes it easier to remove from the oil. It is also a relatively low cost solvent. However, its flammability, mild toxicity, explosiveness and environmental impacts are the concerns of industrial scale solvent extraction of coconut oil. The solvent extraction leaves low levels of solvent residue in the oil, which is safe but undesirable for food purposes. During the extraction, the oil in copra is leached out with the solvent and the insoluble copra meal is retained unaffected. The efficiency of extraction depends upon the temperature of the solvent, the ratio of the solvent to copra meal, size and the porosity of the copra particles and contact time with the solvent. Oil extraction by solvent extraction is more suitable for oil seeds containing relatively low amounts of oil. As copra contains about 70% oil, mechanical extraction by pressing is more efficient and economical. In addition to the full solvent extraction, prepress solvent extraction can also be used. In the mechanical extraction by pressing under moderate pressure, the oil content of copra can be reduced to nearly 15%. This remaining oil can be further extracted by solvent extraction. The resultant mixture of solvent extraction includes solvent, oil solution and extracted coconut meal. The solvent in the meal is removed by heating to boil off the volatile solvent and the solvent is recovered by condensation. The remaining solvent in the oil solution is removed by distillation. Traces of solvent left in the meal and the oil are removed by steam-stripping under reduced pressure. Various high temperature steps in the solvent extraction process may thermally degrade the oil to a very small extent and about 500-1000 ppm concentrations of solvent will also remain in the oil after purification.

Extraction of Coconut Oil by Supercritical Carbon Dioxide

Extraction of seed oils by supercritical carbon dioxide (SC-CO_2) can overcome the problem of solvent residues present in oils extracted by solvent extraction. Different compounds in edible oils may be incorporated into seed oils by changing pressure and temperature of carbon dioxide. However, the establishment of a supercritical extraction unit needs high investment. Thus, the technology is not in widespread use, though the quality of oil extracted by this method is claimed to be high. Information about the operational costs of this method of extraction based on systematic studies is not available for coconut oil and several other vegetable oils. The SC-CO_2 extraction of soybean oil has been studied and the equilibrium solubility data for different temperatures and pressures have been reported. The operating costs for SC-CO_2 and solvent extraction of soybean oil have been studied and compared (Reverchon & De Marco, 1994). Though the initial costs of the establishment of SC-CO_2 system is higher, the operational costs fell in the same range as conventional hexane extraction plants.

Triglycerides in seed oils are readily soluble in SC-CO_2 at 40°C and at pressures higher than 280 bars. The main parameters important for efficient oil extraction in this method are particle size, pressure and residence time. Small particles of about 1mm mean diameter or less and high pressures (300-500 bar) can strongly reduce the extraction time. After extraction, the SC-CO_2 tryglicerides solution is sent to a separator working at subcritical conditions. This operation reduces the solvent power of CO_2 and allows the recovery of oil. The complete elimination of gaseous CO_2 from oil is also accomplished in this step. The SC-CO_2 extraction of seed oils has been tested up to pilot scale for several seed oils.

Wet Process

In the wet process of extraction of coconut oil, fresh coconut kernel is scraped and pressed with water. Due to the presence of proteins in coconut kernels, an emulsion is made. Separation of oil from this milk-like emulsion is a difficult task. This separation of the oil from the emulsion can be done in various ways. As the solution is in emulsion form due to the presence of proteins, these proteins have to be broken down in order to separate oil. Prolonged heating is the traditional way of extracting coconut oil from a coconut oil emulsion. Modern techniques use centrifuges and pre-treatments including cold, heat, acids, salts, enzymes, electrolysis, shock waves, or some combination of them for the extraction of oil from the emulsion. Despite numerous variations and technologies, wet processing is less efficient in oil extraction than dry processing, resulting in a 10-15% lower yield, even after considering the losses due to spoilage and pests during dry processing. Wet processes also require investment of equipment and energy, incurring high capital and operating costs. High quality virgin coconut oil is prepared by some wet extraction methods where temperature can be maintained below 40-50°C. However, coconut oil prepared by any wet extraction method is sometimes called virgin coconut oil even though higher temperatures are involved in some wet extractions.

Wet Extraction by Boiling Coconut Milk

One traditional way of extracting coconut oil from the coconut milk emulsion is by prolonged heating of the emulsion. Heating breaks down and deposits proteins at the bottom of the container. When the heating is continued, water in the emulsion evaporates. Due to its high boiling temperature, coconut oil does not evaporate significantly during this process. Finally, the coconut oil can be separated by decanting from the residue containing proteins, carbohydrates and other substances. The resultant coconut oil gives a nice coconut aroma and the oil is free of water. This oil can be kept for a very long time without forming oxidation products that cause rancidity. However, due to caramelization and other reactions such as Maillard reaction, the coconut oil produced by this method has a light yellowish color. One disadvantage of this method is the high amount of energy needed and relatively longer period of time taken to evaporate water from the coconut milk emulsion. In addition, there are no machines designed to produce coconut oil in industrial scale using this method. Therefore, this method is limited to the preparation of coconut oil in small scale for household consumption.

Centrifugation Process

The coconut milk emulsion prepared by pressing fresh coconut kernels contains approximately 40% oil. In the production of high quality virgin coconut oil, the pressing should be done using a special machine of which both the pressing plate and the sleeve are cooled by chilled water. Using a centrifuge, the cream is then concentrated to yield a higher percentage of oil while the proteins and water-soluble substances are separated out. Coconut oil produced by this method has a very light coconut flavor and the texture of coconut oil is extremely mild and smooth. In some instances, coconut milk is chilled at 10°C for 10 h to solidify the lipids. Then the aqueous layer is discarded and the lipid block is allowed to stand at 30°C until it dissolves completely. Then the mixture is centrifuged and the oil layer is separated. The coconut oil produced by this centrifugation method is considered to be one of the highest quality coconut oils. These oils are expensive and usually labeled as extra virgin coconut oil.

Fermentation Process

Fermentation method is the least consistent of all the coconut oil production processes. Therefore, the quality of coconut oil produced by this method varies for different producers. The oil has to be further purified for food purposes. In the preparation of coconut oil by this method, fresh coconut kernel is first grated and then coconut milk or cream is pressed out from the white flesh. This milk is placed into vats or buckets and allowed to ferment at about 37°C. The enzymes and bacteria break the proteins in emulsion and separate the milk into different layers which include a top protein curd layer, a coconut oil layer underneath, another curd layer and a layer of water. The protein curd on the top can be removed and then the oil layer can be siphoned.

Enzyme Assisted Extraction

Enzyme assisted extraction of coconut oil is an eco-friendly process based on isolation of coconut oil and proteins in an emulsion. Copra meal prepared by cutting copra and passing through a 1 mm mesh or grated fresh coconut kernels is mixed with water and the mixture is treated with enzymes. The enzymes can be crude mixtures of protease, cellulase, hemicellulase and amylase enzymes. The presence of enzymes facilitates oil recovery by breaking cell walls and oil bodies. The oil recovery can vary from 80-98%. A single enzyme is not sufficient to break the insoluble cell wall materials to release oil (Rosenthal et al., 1996).

The polysaccharides of fresh mature coconut kernels are galactomannans (61%), mannans (26%) and cellulose (13%) (Balasubramanium, 1996). When an aqueous medium of ground desiccated coconut kernel was treated at 50°C with a mixture of commercially available enzymes including a hemicellulase preparation, a pectinase preparation, a cellulase preparation and an enzyme complex with multiple activities, 84% of the coconut oil present in coconut kernel was released (Chen & Diosady, 2003). The incubation followed by centrifugation gave a clear oil phase, an emulsion layer, an aqueous phase, and a solid phase containing the remaining coconut meal. Proteins of the solid residue were extracted by freeze drying the solid and treating with aqueous alkali, which dissolved 88% of the proteins in the solid phase. The acidification of the protein solution towards the isoelectric region precipitated 93.5% of dissolved proteins. Therefore, enzymatic extraction has an additional advantage because of the recovery of proteins.

QUALITY PARAMETERS OF COCONUT OILS

Various quality parameters have been proposed in order to establish the quality of coconut oil. Asian Pacific Coconut Community (APCC) and Codex Alimentarius have established the values of quality control parameters in physical, chemical and microbiological aspects of coconut oil. Quality parameters established by Codex Alimentarius for coconut oil are acid value or free fatty acid percentage (none), peroxide value (< 15 meq/kg oil), iodine value (6.3-10.6) and volatile matter percentage at 105°C (0.2%). APCC standards of quality parameters for virgin coconut oil are given in the Table 1.

Color

Colorless coconut oil is considered as commercially more acceptable good quality coconut oil. Due to this reason, refined, bleached and deodorized (RBD) coconut oil is prepared. One source of the light yellow or light brown color of coconut oil is coconut testa. When processing copra, some colored substances get incorporated into coconut oil. If copra without coconut testa is used for the dry extraction of coconut oil, the color of coconut oil will be colorless. In both dry and wet extraction of virgin coconut oil, this brown thin skin of coconut kernel is avoided. As a result, the extracted virgin coconut oil is colorless. Coconut oil can be extracted by heating coconut milk emulsion during wet extraction. In this case,

light yellow or light brown colored coconut oil is resulted and this color is due to caramelization of non-lipid substances such as sugars present in coconut milk or due to various oxidation reactions of the non-lipid substances. Even though the color of coconut oil is important as a commercial parameter, the color does not reflect the nutritional quality of coconut oil.

Table 1. APCC standards for virgin coconut oil

Parameter	Maximum value or range
Moisture (%)	0.1
Volatile matter, 120°C (%)	0.2
Free fatty acid (%)	0.2
Peroxide value, meq/kg	3
Density g/mL	0.915-0.920
Refractive index, 40°C	1.4480-1.4492
Insoluble impurities (%)	0.05
Saponification value, mg KOH/g oil	250-260
Iodine value	4.1-11
Unsaponifiable matter (%)	0.2-0.5
Total plate count	0.5
Color	Colorless

Adapted from APCC, 2009 (APCC, 2009).

Acid Value

Acid value of an oil is a measure of the amount of free acids present in the oil. The major component of oil is the triglyceride portion. These triglycerides produce free fatty acids upon hydrolysis. The hydrolysis can occur due to the action of lipase enzyme. The source of enzyme can be from the broken cells or tissues during the extraction process of coconut oil or from microorganisms. The acidity is an indicator of aging oil. The free acid formation due to hydrolysis of oil can accelerate due to poor extraction and storing conditions of coconut oil. Acid value is defined as the weight of potassium hydroxide (KOH) in mg needed for the neutralization of free fatty acids from 1 g of the oil. Even though the acid value unit, mg KOH/g oil is frequently used by chemists, the acidity is also expressed as a percentage by weight of total fat. Here, the number of moles of free acids found by the titration can be converted to mass by multiplying the number of moles by the molecular weight of fatty acids. As it is a fatty acid mixture, the molecular weight of the major fatty acid of the oil is usually considered for the calculation. In coconut oil, free fatty acid content is given as lauric acid equivalents while in olive oil it is given as oleic acid equivalents. When the acid value percentages are given as the major fatty acids in different oils, such acid values for different oils cannot be easily compared. Therefore, the acid value expressed as mg KOH/g oil is always easier for comparison purposes.

As triglycerides get hydrolyzed to form free acids, glycerol is also formed. Due to volatility of free short chain fatty acids, a light rancid smell may be observed. Free fatty acids and glycerol also change the taste of the oil or oil based food. Rancidity due to the free fatty

acids and glycerol formed by hydrolysis of triglycerides is called hydrolytic rancidity. This rancidity is milder than the rancidity resulting from the aldehydes and ketones due to oxidation of fatty acids. Therefore, hydrolytic rancidity arising mainly from free fatty acids is not seriously objectionable to the consumers as to cause serious economic disadvantageous.

Peroxide Value

Peroxides are primary oxidation products of edible oils formed due to free radical reactions and these peroxides further break in to shorter chain secondary oxidation products such as aldehydes and ketones that are volatile giving the smell of rancidity, which is known as oxidative rancidity. Therefore, the peroxide value may increase in the beginning and then secondary oxidation products appear later. Virgin coconut oil has a lower peroxide value compared to copra oil, which indicates the freshness of virgin coconut oil. Recommended maximum value for peroxides in coconut oil is 3 meq/kg. Copra oil, even after freshly collected from a mill has a peroxide value higher than that of virgin coconut oil prepared by dry or wet methods. This indicates that longer drying period of copra may also contribute to the formation of peroxides. Peroxide formation is also catalyzed by the presence of metal impurities. Pressing and grinding of copra and storing coconut oil in metal barrels may introduce dissolved metals such as iron in to coconut oil. This dissolving of metals is supported by the acidity of coconut oil if the acid value of coconut oil is high.

The traditional coconut oil, prepared by prolonged boiling of coconut milk also has low peroxide values. Surprisingly, peroxide value of this high temperature-processed coconut oil is lower and comparable with the peroxide value of virgin coconut oil prepared under cold conditions, indicating that peroxide formation may not be induced by the heating of coconut milk during this wet extraction. High content of saturated fatty acids of coconut oil gives a high oxidative stability to coconut oil. Therefore, peroxide formation is minimal in coconut oil prepared by any method of extraction compared to other polyunsaturated oils. Then coconut oil can be considered as the most stable and, therefore, most suitable oil for deep-frying.

Iodine Value

Iodine value reflects the degree of unsaturation of the fatty acids of oils. This parameter does not change significantly with the method of extraction if same quality coconut kernel is used for the extraction of coconut oil. However, low quality coconut oil for nonfood purposes can be obtained by pressing coconut pairings and this oil is called pairing oil. Such pairing oil may have slightly higher iodine values compared to other coconut oils. However, quality of pairing oil is low not because of the slightly different iodine value but due to higher peroxide value and higher acidity.

Saponification Value

Saponification value reflects the number of fatty acid units in a given weight of oil. If the fatty acids present in an oil are relatively small, the saponification value is relatively high as more units of fatty acids are present in a given weight that is going to be saponified by potassium hydroxide. This means that if the average molecular weight of the oil is low, the saponification value is high. Fatty acid compositions of all common edible oils are well known. Therefore, average molecular weight, which reflects the average chain length of fatty acids, can easily be calculated. When the average chain length of the fatty acids present in triglycerides of an oil is shorter, the saponification value becomes higher. Table 2 shows this relation between saponification value and average chain length of fatty acids of four common edible oils. When comparing reported saponification values for different oils or fats, it is important to check the units and the base used for saponification. The saponification values are given as milligrams of KOH per gram of oil, milligrams of NaOH per gram of oil, grams of KOH per gram of oil or grams of NaOH per gram of oil.

Table 2. Average chain length and saponification values for common edible oils

Oil	Average chain length	Saponification value mg KOH/g oil
Canola	17.92	185-195
Olive	17.74	184-196
Sunflower	17.86	185-198
Coconut	12.86	250-264

Table 3. Fatty acid composition (%) of common edible oils

Oil	C8	C10	C12	C14	C16	C18	C18:1	C18:2	C18:3
Canola	-	-	-	-	4	2	62	22	10
Olive	-	-	-	-	13	3	71	10	1
Sunflower	-	-	-	-	7	5	19	68	1
Coconut	8	7	48	18	9	2	6	2	-

Coconut oil has a unique fatty acid composition of short and medium chain fatty acids as indicated in Table 3. Due to the presence of this unique composition of shorter and medium chain fatty acids, coconut oil has a higher saponification value and this value in coconut oil is the highest among almost all other edible oils. Saponification value also does not change significantly with the method of extraction of coconut oil. However, slightly high saponification values may be observed for oils with other non-lipid substances, which may react with potassium hydroxide.

Anisidine Value

Peroxide value is used to analyze primary oxidation products. It is important to evaluate the oxidative status of oils by both primary oxidation products as well as secondary oxidation products. If an oil initially has a high peroxide value, keeping the oil in stock for a long time

in absence of oxygen, produces secondary oxidation products that determine the decrease of peroxide value but the increase of anisidine value. Over time, these peroxides get further oxidized to volatile compounds such as hexanal and other non-volatile aldehydes and ketones. Anisidine values reflect the amount of relatively non-volatile aldehydes and ketones.

Unsaponifiable Substances

Even if all the insoluble impurities are removed from coconut oil, the oil still has up to about 0.5% of unsaponifiable matter, which includes polyphenols, alcohols, terpenoids, and several other organic substances (Kirschner & Harris, 1961). Quality parameters of coconut oil are mainly and traditionally defined for the lipid fraction. However, many studies conducted in recent times show that unsaponifiable components are also important to evaluate the quality of oil.

COCONUT OIL QUALITY

Coconut oil is a stable oil due to its high saturated fatty acid content. Due to this thermal stability, coconut oil is considered probably the most suitable oil for frying purposes. The progress of lipid oxidation of coconut oil was assessed by measuring peroxide value, anisidine value and total oxidation value during a period of 12 months. The low peroxide value (0.24-0.49 meq/kg oil) signifies a high oxidative stability, while anisidine values were in the range 0.19-0.87. Fourier transform infrared (FTIR) spectroscopy was used to monitor the peak changes due to oxidation during storage. The results of this study suggest that coconut oil retains its good chemical properties during 12 months of storage (Moigradean et al., 2012). When the quality of coconut oil is evaluated, it is important to consider both nutritional quality and commercial quality. Acid value, peroxide value and anisidine value are commercially attractive parameters because these values are directly connected to the rancidity of oil. Iodine value and saponification value are important in checking the authenticity of an oil. The most crucial parameters important for the nutritional quality are the identity of fatty acids in the triglycerides of the oil and the non-lipid components.

Basic Quality Parameters and the Extraction Method

Quality of coconut oil is evaluated by basic parameters such as color, acid value, peroxide value, iodine number, saponification value and anisidine value, etc. These parameters change over a range of values not only because of the method of extraction but also because of cultivar and maturity of coconut. Most of the records in literature give the values for copra oil and virgin coconut oil. Copra oil refers to the coconut oil extracted by dry process by pressing sun dried or kiln-dried copra. Virgin coconut oil has been made either dry process or wet process. Careful observation of the reported data indicates that the quality parameters such as iodine value and saponification value do not change significantly in coconut oils extracted by any method. However, acid value and peroxide value can vary

significantly in oils extracted by different methods and also with the aging of oils. Wet extracted coconut oil by centrifugation or enzymatic degradation contains lower free fatty acid content and peroxide value. Coconut oil extracted by dry methods or copra oil has a relatively higher acidity and peroxide value. However, for the extraction of virgin coconut oil by dry method, high quality copra prepared by quick drying in better conditions is used. Therefore, acid values of such coconut oil are lower compared to normal commercial coconut oil.

Among drying methods of copra, hot air drying is used to produce high quality copra in a relatively shorter period of time compared to other drying methods. Studies have been conducted to assess the quality of copra and coconut oil prepared using copra, dried in laboratory scale hot air driers (Guarte et al., 1996). In a laboratory scale drier, at a constant air velocity of 0.5 m/s, the moisture reduction rate of copra (down to 7%) increases with the drying temperature from 40-90°C. At this temperature range, the quality of copra or coconut oil was not affected. At temperatures above 90°C browning of copra occur due to Maillard reaction. However, drying temperature up to 100°C does not affect the oil content, acid value, anisidine value or saponification value. Drying copra at 90°C in a hot air dryer can reduce the moisture down to 7% within one day without making any changes of color, smell or taste of copra. Drying under this condition does not affect the contents of fatty acids such as lauric and palmitic. Other fatty acids showed small differences with no definite trend in variation with the temperature. Coconut oil prepared by dry and wet methods have been compared for copra oil prepared by pressing dry copra in a small scale expeller and coconut oil prepared by prolonged boiling of an emulsion of coconut milk in the traditional method (Seneviratne & Dissanayake, 2005). The quality parameters are given in the Table 4 and fatty acid compositions are given in the Table 5.

Table 4. Quality parameters of traditional coconut oil and copra oil

Source	Acid value (mg of KOH/g)	Saponification value (mg of KOH/g)	Iodine value (g of I_2/100 g)	Peroxide value (absorbance at 500 nm)
Traditional oil	0.40 ± 0.02^a	274 ± 2^a	6.6 ± 0.2^a	0.034 ± 0.002^a
Copra oil	3.5 ± 0.9^b	241 ± 3^b	6.2 ± 0.3^a	0.62 ± 0.04^b

Adapted from Seneviratne & Dissanayake, 2005 (Seneviratne & Dissanayake, 2005). Each data point represents the mean of eight replicates ± standard deviation; Different superscript letters in a same column denote a significant difference ($p < 0.05$) by MINITAB 2-sample t test.

Table 5. Fatty acid compositions of traditional coconut oil and copra oil

	Fatty acid								
Source	6:0	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
Traditional oil	0.63 ± 0.04	9.2 ± 0.1	6.5 ± 0.1	49.6 ± 0.2	19.2 ± 0.1	7.2 ± 0.1	2.4 ± 0.1	4.5 ± 0.1	1.1 ± 0.1
Copra oil	0.53 ± 0.04	8.6 ± 0.1	6.4 ± 0.2	48.7 ± 0.2	18.9 ± 0.1	7.6 ± 0.2	2.4 ± 0.1	4.9 ± 0.2	1.3 ± 0.1

Adapted from Seneviratne & Dissanayake, 2005 (Seneviratne & Dissanayake, 2005). Each data point represents the mean of three replicates ± standard deviation.

According to this study, acid value, peroxide value and saponification value of the two types of oils are significantly different and the iodine values showed no significant difference. Fatty acid compositions of the two types of oils were not significantly different enough to cause any nutritional changes.

In wet processing, natural fermentation carried out by microbes present in the natural environment is well known for its traditional and industrial applications. In this process, the fermentation of coconut oil is usually carried out without applying any heat.

This natural fermentation process has contamination problems due to the presence of unwanted microorganisms and uncontrolled conditions. It leads to the production of poor quality coconut oil, usually yellow in color, with a rancid smell. Therefore, induced fermentation method, using certain species of probiotic microorganisms, can be used under semi-controlled conditions. Quality parameters for virgin coconut oil produced both by natural fermentation and induced fermentation under semi-controlled conditions have been studied and compared with the quality standards given by APCC (Neela & Prasad, 2012). In this experiment, coconut milk was allowed to ferment naturally in a laboratory scale experiment in a separating funnel or in a clean container and the mixture was allowed to stand for 48 hours at room temperature. During the production of virgin coconut oil by natural fermentation, contaminations by different microbes occurred, resulting in yellow-colored, spoiled, coconut oil. The fermentation was also carried out under controlled conditions. Here, coconut milk was sterilized and a pure culture of *L. plantarum* was used in fermentation. The yield of virgin coconut oil from the natural fermentation was 25.68 ± 0.96% while that of induced fermentation was 28.47 ± 1.07%. Physico-chemical parameters such as specific gravity, refractive index, insoluble impurities, free fatty acid content, peroxide value, iodine value, saponification value, unsaponifiable matter, and fatty acid compositions of virgin coconut oil produced by natural fermentation and induced fermentation were within the limits of APCC standards. However, the moisture content of virgin coconut oil by natural fermentation (0.52%) and induced fermentation (0.56%) is little higher than the APCC values (0.1- 0.5%). Total aerobic plate count of virgin coconut oil by natural fermentation was 31 colony forming units (CFU) per 0.1 mg while that of virgin coconut oil by induced fermentation was 49 CFU/0.1 mg. Both oils contained about 6 mg/100 g tocopherols. The impurities and free acids of the coconut oil prepared by fermentation process can be removed to some extent by diatomaceous filters and carbon filters. As the moisture content of this coconut oil is high, the oil has to be heated for several hours at 100°C to remove water. Due to the presence of moisture, free fatty acids and a high peroxide value, coconut oil produced by fermentation is of poor quality.

A detailed study has been conducted to differentiate virgin coconut oil, RBD coconut oil and copra oil (Dayrit et al., 2007). Virgin coconut oil prepared by centrifugation, expeller process, enzymatic treatment, fermentation with and without heat and settling methods were used for the study. According to this study, gas chromatography analysis done with virgin coconut oil, RBD coconut oil and copra oil indicates that these oils cannot be differentiated by their fatty acid composition due to similar fatty acid compositions. However, volatile organic matter, moisture content and peroxide values are different in these oils. Moisture contents of virgin coconut oils extracted under different conditions varied from 0.05 to 0.12%. RBD coconut oil had a lower moisture content ranging from 0.01 to 0.1%. Copra oil had a higher average moisture content at a range of 0.08 to 0.14%. Volatile organic matter contents of virgin coconut oils ranged from 0.00 to 0.08%. RBD coconut oil had no volatile

organic matter while copra oil had a level 1.77% of volatile organic matter by weight. APCC standards for free fatty acids expressed as lauric acid equivalents is 0.5%. Average free fatty acid contents of virgin coconut oil, RBD coconut oil and copra oil were 0.131%, 0.021% and 1.41% respectively. There were no significant differences in iodine values of the three types of oils tested. The average peroxide values were 0.56, 0.98 and 1.48 meq/kg for virgin coconut oil, RBD coconut oil and copra oil respectively and these values are well below the Codex Alimentarius peroxide value limit of 15 meq/kg for virgin oils. In the assessment of microbial contamination, it was observed that the CFU/mL for most of the virgin coconut oil and RDB coconut oil samples were below 10 CFU/mL, which is the APCC standard value. However, there were randomly distributed samples with higher CFU values. The microbial contamination depends on the quality of production including the quality of copra but not on the method of preparation of virgin coconut oil. Production quality of copra oil is usually poorer than that of virgin coconut oil. The microbial contamination of the samples of copra oil used in this study was not less than 10 CFU/mL but was only less than 250 CFU/mL range.

Enzymes in the Extraction of Coconut Oil

In the wet extraction of coconut oil from a coconut milk emulsion, the most difficult part is the separation of oil from the proteins and cell wall matter. The yield of coconut oil prepared by wet extractions is usually less unless modifications are done to the extraction method. In a wet extraction method, conditions for destabilization of coconut milk emulsion have been studied by employing different treatments such as thermal, pH, enzyme, chilling and combination of enzymes and chilling treatments (Raghavendra & Raghavarao, 2010). The coconut milk emulsion was treated with protease enzyme at 0.1% concentration and incubated at 25°C and 37°C for 2 h. Out of these treatment methods, the most effective method for destabilization of coconut milk emulsion was found to be combination treatments, which gave a high yield of 94.5% oil. The oil obtained by combination of treatments contained lower free fatty acids and peroxides and higher lauric acid content compared to commercial coconut oil.

In addition to the separation of coconut oil from a coconut milk emulsion, enzyme treatment can also be used to extract coconut oil from finely divided copra meal. Coconut oil extraction based on the enzymatic action of polygalacturonases, α-amylase and proteases on a diluted coconut paste has been tested (McGlone et al., 1986). After the reaction with enzymes, the mixture gave three phases upon centrifugation. The upper phase contained high quality coconut oil and the middle layer and the lower layer contained water and coconut meal respectively. This process gave a yield of 80%, which is a much higher yield compared to other traditional wet extraction methods. Enzyme assisted extractions have also been used to extract coconut oil from powdered copra. The crude commercial enzyme used in this study contained α-amylase, neutral protease, acid protease, cellulase/hemicellulase, and pectinase. The enzyme treatment in this process can be considered as a pretreatment of copra prior to the oil extraction. The enzyme was added 1% rate of the copra and allowed to stand for 30 min. After enzyme treatment, the meal was extracted by hot water and the emulsion was boiled to evaporate water. This enzyme pretreatment of copra prior to the extraction improved the yield of coconut oil by 50% compared to the same extraction procedure without enzyme

pretreatment. However, the quality of oil extracted by enzyme pretreatment method was similar to the oil extracted without enzyme pretreatment (Tano-Debrah & Ohta, 1997).

Supercritical Carbon Dioxide in the Extraction of Coconut Oil

Extraction of coconut oil by supercritical carbon dioxide (SC-CO_2) extraction has been reported by several authors. CO_2 is a nonpolar solvent at supercritical conditions and triglycerides in coconut oil are easily soluble in CO_2. In a study, coconut kernel has been dried by a microwave-assisted method and the resultant dried kernel material has been used to extract oil by supercritical extraction (Quitain et al., 2003). The sample of grated coconut kernel was dried using microwave-assisted low-temperature air-drying method. The microwave frequency used in the drier is 2.45 GHz and this is similar to the frequency of domestic microwave ovens. The typical operating temperature was set at 40°C. About 95% of oil recovery can be achieved within 20 h using this method while the cold press method of virgin coconut oil extraction gives a recovery of about 50%. Therefore, the supercritical extraction method seems to be more efficient than the cold press method. However, the economic aspects of the two methods have not been compared. In the supercritical extraction method, the drying method, whether it is microwave assisted, hot air or freeze drying have no effect on the yield of coconut oil. There is no significant difference in physical properties of super critically extracted oil compared to cold pressed oil. However, the vitamin E content of coconut oil extracted by SC-CO_2 extraction is not significant. Fatty acid compositions showed no significant difference in cold pressed virgin coconut oil, or supercritical oil or ordinary coconut oil prepared by pressing copra. However, phenolic contents of the super critically extracted coconut oil can be different. Due to the low polarity of CO_2 and the high polarity of phenolic compounds, solubility of phenolic substances in CO_2 is poor. Organic modifiers can be coupled with this extraction and the amount and the nature of organic modifier is important for incorporating phenolic substances into oil during this extraction (Palma et al., 2000).

Distinguishing between Virgin Coconut Oil and RBD Coconut Oil

Both virgin coconut oil and RBD coconut oil are colorless and they have low acid values and peroxide values. Therefore, it is difficult to distinguish between these two oils by simple experiments. Attempts have been made to distinguish between virgin coconut oil and RBD coconut oil using standard quality parameters such as free fatty acid content and peroxide value, ^{31}P NMR spectroscopy and head space solid phase microextraction gas chromatography coupled to mass spectroscopy (SPME/GCMS) (Dayrit et al., 2011). Virgin coconut oil has higher free fatty acids, moisture and volatile matter and lower peroxide value. ^{31}P NMR analysis has shown that virgin coconut oil and RBD coconut oil can be distinguished by total amount of diglycerides. Virgin coconut oil contained 1.55 w/w% and RBD coconut oil contained 4.10 w/w% diglycerides. Virgin coconut oils prepared by different methods such as expeller process, centrifugation process and fermentation with and without heat cannot be distinguished by standard quality parameters. However, SPME/GCMS analysis showed that virgin coconut oil produced by fermentation process with and without

heat can be distinguished from virgin coconut oil produced by expeller or centrifugation process based on higher levels of volatiles such as acetic acid and octanoic acid present in virgin coconut oil produced by fermentation process (Dayrit et al., 2011).

Coconut Hybrids and Coconut Oil Quality

Quality parameters of virgin coconut oil from different hybrids have been compared. For this study, virgin coconut oil was prepared by cold press method and fermentation method. The results showed that some hybrids produced significantly high lauric acid contents compared to their parents. However, there was no significant difference in the lauric acid content of virgin coconut oil extracted by cold pressing and fermentation. This study also compares the free fatty acid contents of virgin coconut oil prepared by cold press method and fermentation method. The results indicate that cold pressed coconut oil contains lower free fatty acids compared to coconut oil prepared by fermentation process. The lowest and highest acid values of the cold pressed coconut oils of the tested varieties were about 0.05 and 0.25 mg KOH/g oil respectively while the lowest and highest acid values for the coconut oils prepared by fermentation process for the tested cultivars were about 0.25 and 0.65 mg KOH/g oil respectively (Arlee et al., 2013).

Phenolic Substances and the Extraction Method

It is well-known that phenolic substances provide protection against the oxidation of unsaturated fatty acids in triglycerides. Phenolic substances are naturally present in seed oils and these phenolic substances improve the shelf life of oils. The naturally present phenolic substances of oils can be removed by passing oils through a column containing activated charcoal and silicic acid (Waraho et al., 2009). Such stripped oils are oxidatively less stable than unstripped oils. Phenolic substances can also be added to oils to improve the oxidative stability. Added phenolic compounds such as caffeic acid and *p*-coumaric acid in avocado and coconut oils improved the oxidative stability and nutritional quality. However, those phenolic acids facilitated the hydrolysis of triglycerides (Sun-Waterhouse et al., 2011). Adding the carotenoids and vitamin E fraction back to refined, bleached and deodorized palm oil reduced its atherogenicity (Wilson et al., 2005).

Total phenol contents of seed oils vary significantly with the extraction conditions. Solvent-extracted oils contain more phenolic substances than virgin oils (Gutfinger, 1981). The most striking difference between the traditional coconut oil extracted by prolonged heating of coconut milk and copra oil is in the phenol content. Total phenol content of traditional coconut oil was 618 ± 46 mg while that of copra oil was 91 ± 11 mg as gallic acid/kg oil (Seneviratne & Dissanayake, 2008). This clearly indicates that extraction methods have a remarkable impact on the total phenol content of coconut oil.

The effect of the use of cell-wall-degrading-enzyme preparations during the mechanical extraction process of virgin olive oil on the phenolic compounds and polysaccharides have been investigated. The use of the enzyme preparations increased the concentration of phenolic compounds in olive oil. Especially, the contents of secoiridoid derivatives such as the dialdehydic form of elenolic acid linked to 3, 4-dihydroxyphenylethanol (3, 4-DHPEA-EDA)

and an isomer of oleuropein aglycon (3, 4-DHPEA-EA), which have high antioxidant activities, increased significantly in the olive oil (Vierhuis et al., 2001).

According to a recent study, virgin coconut oil prepared by fermentation process contains higher amount of phenolic substances compared to virgin coconut oil prepared by other methods. Total phenol contents of virgin coconut oil prepared by fermentation process by leaving coconut milk standing for 12 h, chilling coconut milk and RBD coconut oils measured as gallic acid equivalents are about 250 μg/g, 180 μg/g and 120 μg/g respectively (Marina et al., 2009). The total phenol contents of coconut oil extracted by chilling coconut milk and traditional coconut oil prepared by boiling coconut milk emulsion have been compared (Seneviratne et al., 2009). In the cold extraction, coconut milk was chilled at 10°C for 10 h to solidify the lipids. Then the aqueous layer was discarded and the lipid block was allowed to stand at 30°C until it dissolved completely. Then the oil layer was separated by centrifugation. In the traditional extraction, coconut milk emulsion was heated to 100-120°C and the water in the emulsion was evaporated. Finally, the oil was decanted from the deposits at the bottom of the container. According to this study, the maximum total phenol content as gallic acid equivalents of cold pressed coconut oil was 66 mg/kg oil and that of traditional coconut oil was 449 mg/kg oil.

The higher phenolic antioxidant contents of coconut oil prepared at high temperatures can be easily explained by considering the polarities of phenolic substances and coconut oil. Coconut oil can be considered as a non-polar substance and oil dissolves in nonpolar solvents such as hexane. On the other hand phenolic substances are polar substances and, therefore, the solubility of polar phenolic substances in nonpolar coconut oil is low. More phenolic substances are incorporated into coconut oil when oil is extracted by wet extraction with prolonged boiling of coconut milk emulsion. High temperatures improve the solubility of phenolic substances in coconut oil. In the cold extraction methods, the phenolic substances are not properly incorporated into coconut oil due to the mild temperature conditions.

In the wet extraction methods, the phenol content of coconut oil also depends on the nature of the endosperm components used to make coconut milk emulsion (Seneviratne et al., 2009). White coconut kernel is a poor source of phenolic substances with only 61 mg/kg of phenolic substances. Phenolic contents of copra, copra meal (coconut cake or poonac, which is the residue after the extraction of coconut oil by pressing copra) and coconut testa are 405 mg/kg, 2156 mg/kg and 3946 mg/kg respectively (Seneviratne & Dissanayake, 2006). Therefore, when white kernel is used in the extraction of virgin coconut oil under mild temperature conditions, high quantities of phenolic substances are not incorporated into coconut oil as in the high temperature methods. Even when coconut oil is produced by boiling coconut milk, the phenolic content of coconut oil will be lower if only white coconut kernel is used for the preparation of coconut milk. Coconut oil produced using only the white coconut kernel by any method contains lower amount of phenolic substances. Coconut oil from the pairings which contain the rind or testa has slightly higher phenolic content, higher iodine value and higher free fatty acid content compared to the coconut oil extracted from white coconut kernel or meat. Though these differences do not affect the nutritional quality of coconut oil, the oil extracted from pairings is considered as low quality coconut oil (Zajew, 1956).

Identified Phenolic Substances

Phenolic substances present in coconut oil have been identified and quantified. HPLC with diode array detection and mass spectroscopic methods have been used for this purpose (Seneviratne et al., 2009). The identified phenolic substances present in coconut oil extracted by boiling coconut milk are given in Table 6. The quantities of phenolic substances are compared for copra oil and traditional coconut oil prepared by boiling coconut milk extracted from white coconut kernel are given in Table 7.

Table 6. Quantities of phenolic substances present in coconut oil prepared by boiling coconut milk

Phenolic compound	Quantity mg/kg oil
Gallic acid	20.2 ± 10.1
(-)-Epigallocatechin	26.7 ± 1.7
(+)-Catechin	81.7 ± 22.7
p-Hydroxybenzoic acid	4.8 ± 1.0
(+)-Epicatechin	1.4 ± 0.6
Caffeic acid	4.6 ± 1.5
Syringic acid	4.1 ± 0.9
Ferulic acid	22.1 ± 8.9

Adapted from Seneviratne et al., 2009 (Seneviratne et al., 2009). Each data point represents the mean of five replicates ± standard deviation.

Table 7. Comparison of phenolic substances present in copra oil and traditional coconut oil prepared by boiling coconut milk based on white coconut kernel

Compound	Quantity (mg/kg)	
	Copra oil	Traditional coconut oil
(+/-)-Catechin	0.87 ± 0.1	2.9 ± 0.4
Caffeic acid	0.13 ± 0.06	3.0 ± 1.1
p-Coumaric acid	0.34 ± 0.01	2.0 ± 0.2
Ferulic acid	0.31 ± 0.2	3.3 ± 1.2
Unidentified phenolic acids	11.2 ± 2.1	97.2 ± 5.7
Unidentified flavonoids	1.6 ± 0.3	10.8 ± 5.0

Adapted from Seneviratne & Dissanayake, 2008 (Seneviratne & Dissanayake, 2008). Each data point represents the mean of three replicates ± standard deviation.

Vitamin E Contents

Comparison of tocopherol contents of cold pressed and fermented coconut oils from different coconut cultivars indicate that the cold pressed method provides highest tocopherol content in coconut oil. According to this study, cold pressed coconut oil contains tocopherol contents ranging from 3.17 mg/100 g oil to 5.78 mg/100 g oil for different coconut cultivars. Coconut oil produced by fermentation process contained tocopherol contents ranging from

2.14 mg/100 g oil to 4.15 mg/100 g oil for different coconut cultivars. According to the same study, the total phenol contents of cold pressed coconut oils were slightly higher compared to fermented coconut oil for each cultivar tested. However, the differences are not very important nutritionally as the values are very close to each other (Arlee et al., 2013).

As a general statement, fatty acid composition of coconut oil is more or less same in all the coconut oils extracted by different methods. However, the unsaponifiable matter content varies significantly with the method of extraction. Vitamin E content in coconut oil is usually low and the quantities are negligible in coconut oils extracted by dry methods. However, virgin coconut oil extracted by cold wet methods contains higher amounts of vitamin E. Vitamin E and phytosterols in virgin coconut oil prepared by chilling coconut milk and copra oil have been compared and the results are given in Table 8 (Nevin & Rajamohan, 2009). Studies also show that not only vitamin E levels but also vitamin A levels are higher in virgin coconut oil compared to copra oil. When exposed to sunlight for several days, vitamin content of virgin coconut oil decreased sequentially, which confirms the loss of vitamins from copra when exposed to UV radiation from sunlight (Nevin & Rajamohan, 2006).

Table 8. Vitamin E and phytosterol contents of coconut oils

	Virgin coconut oil	Copra oil
Vitamin E (μg/100g)	30.87	12.76
Campesterol (ng/dL)	17.00	25.07
Stigmasterol (ng/dL)	63.13	57.05
β-Sitosterol (ng/dL)	73.03	57.00

Adapted from Nevin & Rajamohan, 2009 (Nevin & Rajamohan, 2009).

Antioxidant Capacities and Related Health Benefits

It is common opinion that saturated fat is bad for health and it increases cholesterol levels in blood. However, when considering health effects of saturated fat, it is also important to consider the nature of saturated fatty acids. In this case, chain lengths of fatty acids are important, as the metabolic pathways of fatty acids depend on the chain length of fatty acids. A study has been conducted with ^{14}C labeled synthetic triglycerides containing saturated fatty acids such as butyric acid (C 4:0), caprylic acid (C 10:0), lauric acid (C 12:0) and palmitic acid (C16:0). Feeding Sprague-Dawley rats with these synthetic triglycerides indicated that the excretion rate of $^{14}CO_2$ as the end product of fatty acid metabolism decreases with the chain length of fatty acids. This observation is probably due to, at least in part, differences in the route of transport of the fatty acids to the tissues following absorption from the gastrointestinal tract. The major fatty acid in coconut oil is lauric acid and percentage of the administered radioactivity recovered as $^{14}CO_2$ in the breath of rats after 48 hours was 69% compared to 50% in longer chain palmitic acid (Kirschner & Harris, 1961). These results indicate that shorter chain fatty acids oxidizes more easily instead of entering the fat deposits compared to longer chain fatty acids.

Antioxidant capacity of coconut oil varies with the phenolic substances present in coconut oil. As the contents of phenolic substances and other antioxidants depend on the method of extraction of coconut oil, antioxidant capacities of coconut oils also vary with the

extraction method. The antioxidant properties of virgin coconut oil produced through chilling and fermentation have been investigated and compared with those of RBD coconut oil. Virgin coconut oil showed better antioxidant capacity than RBD coconut oil. The virgin coconut oil produced through the fermentation method had the strongest scavenging effect on 1,1-diphenyl-2-picrylhydrazyl and the highest antioxidant activity based on the β-carotene-linoleate bleaching method. However, virgin coconut oil obtained through the chilling method had the highest reducing power (Marina et al., 2009). These antioxidant activities, which correlated with the phenolic contents were lower in RBD coconut oil. During the refining and bleaching steps natural phenolic antioxidants are removed and antioxidant capacities decrease accordingly.

In another study, rats were fed with diets containing virgin coconut oil prepared by chilling coconut milk and copra oil. After 45 days, antioxidant vitamin levels were higher in rats fed with virgin coconut oil. Low-density lipoprotein (LDL) isolated from the rats fed with virgin coconut oil was found to be more resistant to oxidation compared to LDL isolated from rats fed with copra oil or sunflower oil. Virgin coconut oil also showed beneficial effects on blood coagulation. The lower levels of fibrin and fibrinogen in virgin coconut oil fed rats compared to copra oil fed rats indicate that the blood clotting tendency decreased in virgin coconut oil fed rats (Nevin & Rajamohan, 2008).

Phenolic fractions of virgin coconut oil prepared by chilling coconut milk and copra oil have been compared for their potential to inhibit Cu^{2+} induced *in vitro* LDL oxidation (Nevin & Rajamohan, 2004). The results indicated that the phenolic fraction of virgin coconut oil prepared by chilling coconut milk has better potential to inhibit LDL oxidation compared to the phenolic fraction of copra oil as measured by carbonyl formation. Most of the studies on health effects of coconut oil, do not report the origin or method of extraction of coconut oil. As mentioned earlier, the fatty acid composition of coconut oil does not significantly vary with extraction method. However, due to the differences in non-lipid components, coconut oil prepared by chilling coconut milk has a better beneficial effect in lowering lipid components compared to copra oil. Lower levels of total cholesterol, triglycerides, phospholipids, LDL and VLDL cholesterol levels and higher high-density lipoprotein (HDL) cholesterol levels were observed in rats fed with virgin coconut oil compared to rats fed with copra oil (Nevin & Rajamohan, 2004).

The beneficial effects of virgin coconut oil extracted by wet extraction by chilling coconut milk and by boiling coconut milk have also been compared. The antioxidant activity of the phenolic fraction of coconut oil prepared by boiling coconut milk, tested by DPPH radical scavenging assay and deoxyribose degradation assay is at least two times higher than that of the phenolic fraction of coconut oil prepared by chilling coconut milk. Serum Trolox equivalent antioxidant activity studies of the rats fed with special diets containing traditional coconut oil prepared by boiling coconut milk and coconut oil prepared by chilling coconut milk indicate that traditional coconut oil improves the serum antioxidant status more pronouncedly compared to coconut oil prepared by chilling coconut milk (Seneviratne et al., 2009). These antioxidant activity studies indicate that most of the antioxidant substances of coconut oil are stable at high temperatures. The polyunsaturated oils have a lower thermal stability in the saponifiable fraction due to various oxidation processes of fatty acids possible at high temperatures. However, due to high saturated fat content of coconut oil and due to the thermal stability of phenolic substances in coconut oil, high temperature extraction of coconut oil appears to not cause any nutritional damage to coconut oil. A later study also further

verifies the findings on the properties of traditional and cold-extracted coconut oils. In this study reported in 2011, coconut oil was prepared from coconut milk emulsion by cold and hot extraction under similar conditions used by Seneviratne et al. (2009) (Siddalingaswamy et al., 2011). The results of this study indicated that coconut oil extracted by boiling coconut milk at high temperatures (393 K, or 120°C) reduced blood glucose, total cholesterol, triglycerides, LDL, and very low density lipoprotein. This oil also elevated activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione while it decreased lipid peroxidation in the liver more effectively than coconut oil extracted by freezing the coconut milk emulsion. The improved antioxidant properties are due to the increased amounts of phenolic and other antioxidants present in coconut oil extracted at high temperatures from coconut milk emulsion. The results of these studies further prove that antioxidants present in coconut oil are stable at high temperatures and their activities remain unaffected even at 120°C. According to this study, the total phenol contents of commercial copra oil, cold-extracted coconut oil and hot-extracted coconut oil are 64, 75, and 242 μg/g respectively. Some antioxidants may inactivate or destroy at high temperatures. For example, α-tocopherol was destroyed during the processing of raw corn and other selected foods (Wyatt, 1998). Hydroxytyrosol derivatives present in virgin olive oil were also destroyed due to thermal oxidation at high temperatures (Nissiotis & Margari, 2002). Even though high temperatures may destroy or inactivate certain phenolic compounds in coconut oil, the better incorporation of thermally stable phenolic substances during high temperature extractions may compensate for this loss.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a class of carcinogenic compounds produced due to incomplete combustion of organic compounds. At high temperatures, certain bonds in organic compounds are broken and small unstable fragments are formed due to a process called pyrolysis. These unstable fragments are mostly radicals, which recombine to give PAHs. The process, which occurs at high temperatures, is called pyrosynthesis. Due to their nonpolar nature, PAHs can easily dissolve in oils. Both light and heavy PAH levels have been tested in common edible oils such as coconut, groundnut, mustard, olive, palm, rice bran, safflower, sesame, soybean and sunflower. Among 296 samples tested from these oils, 88.5% of the samples contained total PAH levels above 40 μg/kg and range was 40.3 to 624 μg/kg indicating that PAHs are common contaminants of edible oils (Pandey et al., 2004). PAH contamination in seed oils can occur in various stages of oil preparation. One major source of contamination is the seed or kernel drying by direct fire. Refining process can remove PAH considerably. Levels of low molecular weight PAHs containing up to four aromatic rings can be reduced by deodorization. The main process of removing PAHs from edible oils is charcoal treatment.

In coconut oil, PAH contamination occurs during the drying of coconut kernel. Coconut oils prepared from kernels dried in the sun, in direct heating kilns and in indirect heating kilns have been tested for PAH levels (Wijeratne et al., 1996). According to this study, coconut kernels dehydrated inside the husk and shell by prolonged storage (ball copra) did not contain any PAH. Smoke dried coconut kernels contained in the order of 100 μg/kg PAHs. Unrefined

coconut oil contained 359 μg/kg of total PAHs and extremely low levels of PAHs (11 μg/kg) were detected in desiccated coconut, dried using an indirect drying method. The results also indicate that modifying the drying method by avoiding direct fire or smoke during copra preparation lowers the PAH levels in coconut oil.

Among PAHs, fluoranthene, pyrene, and benzo(a)anthracene are light PAHs while benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h) anthracene and benzo(g,h,i)perylene are considered as heavy PAHs, which are known to be carcinogenic. This study showed that 95% of PAHs in the tested coconut kernel products were light PAHs and heavy PAHs were only 5%. The PAH levels of soybean oil, mustard oil and coconut oil have been compared. For this purpose, PAHs in oils have been extracted by liquid-liquid extraction using acetonitrile–acetone 60:40 (v/v) (Hossain & Salehuddin, 2012). The study showed that soybean oil contained lowest PAH levels compared to mustard oil and coconut oil. As the study has been conducted for oil samples available in the market, the processing methods and conditions of oil preparation are not clear. However, results indicate that both coconut oil and soybean oil contain levels of PAHs that are below the accepted limits by the World Health Organization (WHO). Mustard oil has relatively high levels of PAH exceeding the WHO recommended maximum value for safety.

Various purification steps remove PAH from soybean oil. The main refining process includes degumming, neutralization, bleaching and deodorization. Because of the presence of PAHs in raw soybeans and solvents, PAH levels in solvent extracted soybean oils were high and reached 45.16μg/kg. Degumming can remove enzymes, microorganisms and other impurities to ensure the stability of the oil. The PAH content decreased to 12.00μg/kg with the removal of impurities in the degumming process because most of the light PAHs in extracted oils were removed. Deacidification is one of the important steps in the soybean oil refining process. Its main purpose is to remove free fatty acids in crude oils and to remove impurities such as pigments, phospholipids, hydrocarbons and mucilaginous materials. The deacidification of soybean oils is achieved by neutralization of free fatty acids with alkaline solution. The content of PAHs was further reduced after the deacidification process and the total amount of PAHs in neutralized soybean oils decreased to 6.64μg/kg. In the decolorization process, the content of light PAHs has a slight decrease showing that the bleaching clay has a little adsorption capacity for light PAHs. The purpose of the deodorization step is to remove unpleasant odors and remaining free fatty acids. It further reduced light PAHs and the total PAHs in the deodorized oil decreased to 1.88μg/kg. Degumming has the most prominent effect of decreasing the amount of light PAHs, followed by neutralization and deodorization (Yu et al., 2014).

Aflatoxin Levels

Many oil seeds such as groundnut, cottonseed, coconut, soybean and sunflower are susceptible to fungal attacks in the field or during storage. The natural mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are the main aflatoxins in seed oils. Humans can be exposed to aflatoxins by consumption of contaminated food, contributing to an increase in nutritional deficiencies, immunosuppression and hepatocellular carcinoma (Wagacha & Muthomi, 2008). Among the 18 different types of aflatoxins identified, the

major ones are, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, aflatoxin M1 and aflatoxin M2, which are produced by *Aspergillus flavus* and/or *Aspergillus parasiticus*.

The aflatoxin contamination in coconut oil occurs during the preparation of copra. Therefore, the quality of coconut oil depends on the quality of copra used in the extraction of coconut oil. According to a study conducted in Sri Lanka, the aflatoxin levels of coconut oil prepared by small-scale mills are higher than that of oils prepared in large-scale mills. The reason is, in small-scale mills low quality copra with fungal contaminations are used for the oil extraction while better-processed copra is used in large scale mills. According to this study, the tested coconut oils from the small-scale mills contained a mean value of 186 ppb of aflatoxin B1 in 115 samples while coconut oil from large-scale mills contained only about 50 ppb of aflatoxin B1.

In the dry extraction of coconut oil, copra is produced in various ways. During the rainy season, levels of aflatoxins in copra and coconut oil increases. Fungal growth occurs in copra when the moisture level of copra is above 6%. During any curing process, the moisture level in copra decreases to 6%. However, due to high humidity in rainy season, copra can absorb moisture from the atmosphere so that the moisture levels will be high enough for fungal growth. In addition, some mills store copra for some time before pressing for oil production. During this period, fungal contaminations occur (Samarajeewa & Arseculeratne, 1983).

CONCLUSION

In evaluating the quality of coconut oil, several quality parameters are considered. Parameters such as acid value, peroxide value, iodine number, saponification value, anisidine value and fatty acid composition in triglycerides are the parameters related to the lipid fraction of coconut oil. Among these quality parameters, acid value, peroxide value and anisidine value are important in quickly monitoring the commercial quality of coconut oil and these parameters vary significantly with the method of extraction. Variation of iodine value and saponification value with the extraction method of coconut oil is not significant even though iodine value varies with the quality of copra used for the extraction of coconut oil. Fatty acid composition of the triglycerides of coconut oil remains relatively unaffected by the method of extraction even though the content of some minor fatty acids vary by small percentages.

As the fatty acid composition of coconut oil is independent of the method of extraction, it can be assumed that any health effect purely connected to the fat content may not vary with the type of coconut oil. However, non-lipid components of coconut oil significantly vary with the method of extraction. As most of these minor compounds are polar in nature, their solubility in coconut oil is higher at high temperatures. Therefore, coconut oil prepared by boiling coconut milk contains higher amounts of phenolic antioxidants compared to other coconut oils. Virgin coconut oil produced under cold conditions can also retain thermally unstable non-lipid matter such as vitamins, some phenolic compounds and several other compounds such as sterols. Copra oil contains lower amount of important non-lipid compounds. Therefore, coconut oil prepared by wet extraction under cold or hot conditions seem to confer better beneficial health effects compared to copra oil.

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Chapter 7

QUALITY ANALYSES AND AUTHENTICATION OF COCONUT OIL

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ABSTRACT

Coconut oil contains mainly triacylglycerols and free fatty acids. Coconut oil is a very important source of medium chain fatty acids. Three important medium chain fatty acids exist in coconut oil: caprylic acid (C8:0), capric acid (C10:0) and lauric acid (C12:0), lauric acid being about 50% of the total fatty acids content. Medium chain fatty acids exhibit good properties in different metabolism pathways. The unsaponifiable matter consists principally of sterols, tocols, squalene, phenols, color compounds and odor compounds such as γ- and δ-lactones. Physico-chemical analysis can be used for quality detection and monitoring of coconut oils. The virgin coconut oils authentication is of central importance for consumers, food processors and food industries. Virgin coconut oil can be adulterated with other vegetable oils affecting the quality and physicochemical and organoleptic properties. Palm kernel oil, canola oils and others vegetable oils were used as adulterants in different studies. These oils were chosen because of their similarities with chemical composition of virgin coconut oil. The principal analytical techniques used for authentication of virgin coconut oil are refractometry, Fourier transform infrared spectroscopy, differential scanning calorimetry, electronic nose, fiber optical sensor and nuclear magnetic resonance spectroscopy. The analytical technique results combined with multivariate data analysis tools (principal component analysis, discriminant analysis, partial least square regressions, etc.) were able to detect adulteration down to 1% limit.

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INTRODUCTION

Coconut oil is obtained from copra, which is the dried kernel of coconuts. The coconut palm is the species *Cocos nucifera L.*, which grows well in the humid regions near to the equator. Usually, fresh coconut kernel contains (% weight) moisture (50%), oil (34%), ash (2.2%), fibre (3.0%), protein (3.5%) and carbohydrate (7.3%) (Hui 1996; APCC 2006; Gopala Krishna et al., 2010).

Virgin coconut oil is obtained from the mature kernel of the coconut by mechanical or physical means with or without the application of minimal heat (Dia et al., 2005). Coconut oil contains about 90% saturated fats. The oil contains predominantly triglycerides with 86.5% saturated fatty acids, 5.8% monounsaturated fatty acids and 1.8% polyunsaturated fatty acids (Gunstone 2011; Hui 1996; Marina et al., 2009).

Raw coconut oil (or virgin coconut oil) is colorless at or above 30°C and it is white when in its solid form, being an unctuous mass. Coconut oil melts at 25°Cand becomes solid below this temperature. The typical smell of coconut oil is that of coconuts (if not refined, bleached and deodorized). Coconut oil is insoluble in water at room temperature (APCC 2006; Gunstone 2011).

Refined coconut oil is the oil obtained from the dried, solid part of the endosperm of *Cocos nucifera L.*, then refined. It appears as a white or almost white, unctuous mass, insoluble in water, easily soluble in methylene chloride and in light petroleum and very slightly soluble in alcohol (Baldwin 1986; European Pharmacopoeia).

Coconut oil contains mainly triacylglycerols and free fatty acids. Typical fatty acid composition of coconut oil is 16:0 (9 wt%); 18:1 (6 wt%); 18:2 (2 wt%); 8:0 (8 wt%); 10:0 (7 wt%); 12:0 (48 wt%); 14:0 (18 wt%). As can be seen, coconut oil is rich in medium chain fatty acids and exhibits good digestibility (Che Man & Marina 2006; Marina et al., 2009a).Coconut oil contains among 0.5%-0.8% of unsaponifiable matter (Malaysian standard (MS2943 2007; Codex 2003). The term *unsaponifiable matter* refers to those substances present in oils that are not saponified by alkali hydroxides and are extractable into ether. This unsaponifiable matter consists principally of sterols, tocols, squalene, pheols, color compounds, carbohydrates and odor compounds (such as lactones). The characteristic odor and taste of coconut oil is principally due to γ- and δ-lactones, which are present in trace amounts (Young 1983).

METHODS FOR THE ANALYSIS OF MAIN CONSTITUENTS OF COCONUT OIL

Determination of Triacylglycerols

The analysis of vegetable oils by their triacylglycerol (TAG) content has assumed a great importance as far as quality control and possible origin determination (Graciani-Constante et al., 1997; Nollet & Toldra 2012; Aitzetmuller 1993).

The various TAG of oils, may be separated and quantified by high-performance liquid chromatography (HPLC), according to their carbon numbers (Moreda et al., 2003; Aitzetmüller et al., 1988). The values obtained can then serve to distinguish them from other oils or to interpret their properties. For example, the percentage of trilinolein (LLL) was adopted as criterion to detect the presence of seed oils in olive oils (EEC 2568/91). TAGs are effectively separated byHPLC on reversed-phase columns, containing silica with chemically bonded to octadecyl groups (RP-18) as stationary phase. Using acetonitrile as mobilephase, separation of TAGs occurs according to the chain length and degree of unsaturation of the fatty acids in the glycerol moiety (Schulte 1981). Other solvents were used as mobile phase. One of the most frequently employed is acetone indifferent proportions. In the standard official methods, acetone/acetonitrile (1 volume: 1 volume) is used as mobile phase (IUPAC 1987; EEC 2472/97).

Regarding the differences between oils, only oils that are rich in one fatty acid contain much monoacid triacylglycerol, for example, olive oil, linseed oil and sunflower oil containing triolein (OOO), trilinolein (LLL) and trilinolenin (LnLnLn) (Reske et al., 1997).

The triacylglycerol compositions of several oils are presented in the Table 1.

Table 1. Triacylglycerol composition of several oils (molecular species (wt%))

Coconut oil		Olive oil		Soybean oil	
Triacylglycerol	wt %	Triacylglycerol	wt %	Triacylglycerol	wt %
12, 12, 8	12	OOL	11	LnLL	7
12, 12, 10	6	OOO	43	LnLO	5
12, 12, 12	11	POP	3	LLL	15
12, 12, 14	11	POL	4	LLO	16
14, 12, 8	9	POO	22	LLS	13
		StOO	5	LOO	8
				LOS	12
				OOS	5

where: L-linoleic acid; Ln-linolenic acid; O-oleic acid; P-palmitic acid; S-saturate acids; St-stearic; 8-caprylic acid (8:0); 10-capric acid (10:0); 12-lauric acid (12:0); 14-myristic acid (14:0). (Laureles et al., 2002).

Determination of Fatty Acids

Fatty acids are usually analyzed by gas-liquid chromatography (GLC). This method is applicable to oils containing fatty acids with chain length in the range C14 to C24. GLC analysis of fatty acids is performed following their conversion to methyl ester derivatives. Columns with polar phases are used, for example polyethylene glycol stationary phase (Carbowax) (Jennings 1987).

When samples contain short-chain fatty acids, methods of derivatization have been proposed, for example benzylation and ter-butyldimethyl silylation (Monseur et al., 1981; Burger et al., 1990).

Analysis of free fatty acids can be carried out by Gas Chromatography: Flame Ionization Detection (GC-FID), this technique being an alternative method of analysis (Hajimahmoodi et

al., 2005; Regulation (EEC) N8 2568/ 91, annex X; Lerma García 2012). Furthermore, other analytical methods, such as High-Performance Liquid Chromatography (HPLC) (Shahidi 2005; Kotani et al., 2002), or ^{31}P-NMR (Dayrit et al., 2008) were also developed for this purpose.

The use of pre-column HPLC method for the analysis of fatty acid with 2-nitrophenylhydrazine hydrochloride was reported (Miwa & Yamamoto 1990). After alkaline hydrolysis of coconut oil free fatty acids are reacted with 2-nitrophenylhydrazine hydrochloride and then derivatized to corresponding fatty acid hydrazides. Each of the derivatives was separated on reversed-phase HPLC with isocratic elution and detected at VIS 400 nm (Miwa & Yamamoto 1996). In Table 2 is presented the typical fatty acid composition of coconut oil.

Table 2. Fatty acid composition of coconut oil as determined by gas liquid chromatography from coconut oil samples (expressed as percentage of total fatty acids)

Fatty acid	Fatty acid concentration (%wt)
C6:0	ND-0.7
C8:0	4.6-10.0
C10:0	5.0-8.0
C12:0	45.1-53.2
C14:0	16.8-21.0
C16:0	7.5-10.2
C18:0	2.0-4.0
C18:1	5.0-10.0
C18:2	1.0-2.5
C18:3	ND-0.2
C20:0	ND-0.2
C20:1	ND-0.2

Adapted from Codex 2003.

Determination of Sterols

The methods for the analysis of total sterols in coconut oil involve sample preparation steps such as saponification, extraction of the neutrals from the soap solution, pre-separation by preparative thin-layer chromatography, silylation of sterols and GC-FID analysis (ECCR N8 2568/1991, annex V; Ham et al., 2000).

The major disadvantage of GC is the requirement of thermally stable columns and the need of chemical derivatization prior to analysis. For this reason, alternative methods were described based on the use of liquid chromatography-mass spectrometry (HPLC-MS) (Santos et al., 2011; Itoh et al., 1973). In Table 3 are presented the levels of desmethylsterols in crude coconut oil samples.

Table 3. Levels of desmethylsterols in crude coconut oil samples as a percentage of total sterols

Sterols	Fatty acid concentration (%wt)
Cholesterol	ND-3.0
Brassicasterol	ND-0.3
Campesterol	6.0-11.2
Stigmasterol	11.4-15.6
Beta-sitosterol	32.6-50.7
Delta-5-avenasterol	20.0-40.7 1
Delta-7-stigmastenol	ND-3.0
Delta-7-avenasterol	ND-3.0
Others	ND-3.6
Total sterols ($mg \times kg^{-1}$)	400-1200

Adapted from (Firestone 2013).

In general, the desmethylsterols are useful markers to assess authenticity. Taking into account that beta-sitosterol is the most abundant sterol in the majority of oils, its value has only limited use for the authenticity assessment and differentiation of vegetable oils.

Determination of Tocopherols and Tocotrienols

Tocopherol itself is a term that constitutes eight different subtypes namely alpha-, beta-, gamma- and delta-tocopherol and alpha-, beta-, gamma-and delta-tocotrienols.

Tocopherols analysis is usually performed by using high performance liquid chromatography (HPLC) with fluorescence detection. Coconut oil samples are dissolved in n-hexane and n-hexane/tetrahydrofurane/2-propanol can be used as the mobile phase (Mansor et al., 2012; Nollet & Toldra 2012).

In Table 4 are presented the levels of tocopherols and tocotrienols in crude coconut oil samples.

Table 4. Levels of tocopherols and tocotrienols in crude coconut oils samples

Tocopherols and Tocotrienols	Concentration ($mg \times kg^{-1}$)
α-tocopherol	ND-17
β-tocopherol	ND-11
γ-tocopherol	ND-14
α-tocotrienol	ND-44
γ-tocotrienol	ND-1
Total ($mg \times kg^{-1}$)	ND-50

Adapted from Codex 2003.

Determination of Volatile Compounds

Volatile compounds of coconut oil were determined by GC-MS. Volatile compounds can be identified and quantified by analysis of the headspace using methods such as static head space, dynamic headspace (purge-and-trap), direct thermal desorption,and solid phase microextraction (SPME) techniques (Santos et al., 2011).

Different studies on coconut oil have identified hydrocarbons, alcohols, aldehydes, ketones and δ-lactones that have significant contributors to its aroma (Pai et al., 1970; Allen, 1965; Lin & Wilkins 1970).

The distinct coconut odor has been shown to be due to δ-octalactone (Padolina et al., 1987; Maarse 1991).

Other approach was proposed for the determination of volatile compounds, based on the use of gas sensor arrays with overlapping selectivities. The electronic nose consists of an array of gas sensors with different selectivity, a signal collecting unit and suitable pattern recognition software (Gardner and Bartlett 1999).

Different gas sensors are presently used for construction of electronic noses. They are based on different sensitive materials (i.e., metal oxides, conducting polymers etc.) and detection principles (resistive, piezoelectric, optical, electrochemical sensors, etc.) (Patel 2014).

Determination of Phenolic Compounds

Virgin coconut oil is a important source of phenolic compounds. Total phenolic contents of the virgin coconut oil are quantified by means of Folin-Ciocalteu method. For detection a quantification of of phenolic compounds several methods have been described. The most used technique has been HPLC, coupled to florescence detection (Seneviratne & Sudarshana Dissanayake 2008), diode array detector (Seneviratne et al., 2009), or UV-Vis detector (Santos et al., 2013).

CHEMICAL AND PHYSICAL METHODS OF ANALYSIS OF COCONUT OIL CHARACTERISTICS

The most important physico-chemical characteristics included in national and international standards and used for coconut oil quality control are as follows.In the case of virgin coconut oils the physico-chemical characteristics include the free fatty acids content, iodine value, saponification value, refractive index, specific gravity, unsaponifiable matter, moisture and impurities. All these characteristics are related to the authenticity of the coconut oil and the values are decreasing during refining. For refined coconut oils, the physico-chemical characteristics also usually include the color, peroxide value, slip melting point and solid fat content. All these characteristics are indications of quality and behavior during cooking or using as food (MMAF 2005).

Determination of the Free Fatty Acids content

The free fatty acids content (acid value or acidity index) is the number of mg of potassium hydroxide required to neutralize the free fatty acids in 1 g of the coconut oil.

The chemical reaction involved is:

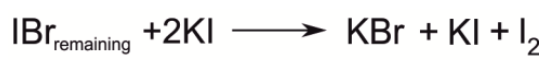
The acidity is an expression of the content (in %, weight/weight; percentage) of free fatty acids as content of dominant or chosen fatty acid. In the case of coconut oil the chosen fatty acid is lauric acid that has the molecular weight of 200 $g \times mol^{-1}$ (I.S. 1964)

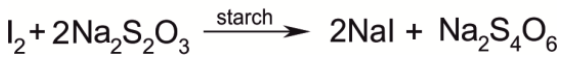
Iodine Value

The iodine value gives primarily a measure of unsaturation of a lipid being directly proportional to the degree of unsaturation (Yasuda 1931). Therefore, a higher iodine value indicates a greater number of C = C double bonds. The iodine value is expressed as the grams of iodine bonded per 100g of lipid (A.O.A.C. 2000c).

The methods for determining the iodine value of lipids are Hanus method and Wijs method. The lipid to be analyzed is weighed and dissolved in a suitable organic solvent, to which a known excess of iodine monobromide (or the monochloride) is added. Some of the IBr reacts with the double bonds in the unsaturated lipids, whereas the rest remains unreacted:

The amount of IBr that has reacted is determined by measuring the amount of IBr remaining after the reaction has gone to completion ($IBr_{reacted} = IBr_{excess} - IBr_{remaining}$). The amount of IBr remaining is determined by adding excess potassium iodide to the solution to release iodine and then titrating with a sodium thiosulfate ($Na_2S_2O_3$) solution in the presence of starch to determine the concentration of iodine released:





In the case of unhydrogenated solid oils, the iodine value is a very good measure of consistency and it is inversely proportional to the melting point of lipid. An increase in iodine value indicates high susceptibility of lipid to oxidative rancidity. In addition, there is a very good correlation between the iodine value, the sleep melting point of unhydrogenated fats of the same species.

The Codex standard (Table 8) specifies the iodine value range of coconut oil as 6.3-10.6, while the Malaysian Standard (Table 7) and give the iodine value range of coconut oil as 7.5-

10.5. The Codex (2003) standard is shown in Table 5 and the Malaysian standard for crude and refined coconut oil (MS 2007) is shown in Table 5.

Table 5. Typical iodine values for several oils

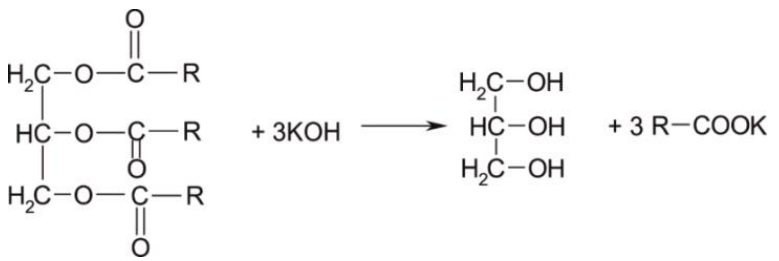
Oil	Grams of iodine absorbed per 100g of oil
Coconut oil	6.3-10.6
Palm kernel oil	14.1-21.0
Palm oil	50.0-55.0
Olive oil	75-94
Peanut oil	86-107
Cottonseed oil	100-123
Corn oil	103-135
Soybean oil	124-139
Safflower oil	136-148
Sunflowerseed oil	118-141
Rapeseed oil	94-120
Grapeseed oil	128-150

Adapted from Codex 2003.

Saponification Value

Saponification value is a measure of the molecular weights of the triacylglycerides in coconut oils. The triacylglycerides with high value of saponification value are considered to make better quality soaps than those with low saponification value. As definition, saponification value is the number of milligrams of potassium hydroxide required to saponify completely the fatty material present and also to neutralize the free fatty acids present in one gram of the coconut oil.

A scheme of the saponification process is presented below:



Triacylglycerol Glycerol Potassium salt of fatty acid

The saponification value is an indirect measure of the amount of free acids present in coconut oils (I.S.I. 1984d).

Ester value is another important parameter. It is defined as the number of milligrams of KOH required to combine with fatty acids present in the triacylglyceride form in 1 g of oils. It is a measure of amount of triacylglyceride present in a sample of oil, which is saponificable.

Refractive Index

The refractive index (*n*) of coconut oil is the ratio of the velocity of light in a vacuum to its velocity in the coconut oil. The refractive index is also defined as the ratio of the sine of the angle of incidence (i) to the sine of the angle of refraction (r):

$$n = \frac{\sin i}{\sin r}$$

It varies with the wavelength of the light used in its measurement and with the temperature. Therefore, it is necessary to specify these conditions when the values are reported. Refractive indices are usually stated in terms of sodium light of wavelength 589.3 nm (line D) at a temperature of 20 ± 0.5°C (n_D^{20}). The Abbe's refractometer is used for determination of refractive index with very good accuracy (Khanna & Gulati 1985; A.O.A.C. 2000b). In the case of coconut oil determination of refractive index is carried out at 40°C.

The measurement of the refractive index can be employed to establish the identity and authenticity of coconut oil.

Specific Gravity

The specific gravity (or relative density) of coconut oil is the ratio of the mass of the coconut oil in air at 30°C to that of an equal volume of water at the same temperature. Determination of relative density (d_{30}^{30}) is carried out using a hydrostatic balance (if it is necessary a precision with three decimal digits) or a pycnometer.

The specific gravity of coconut oils, containing only carbon, hydrogen and oxygen is less than one (A.O.A.C. 2000).

Unsaponifiable Matter

The term unsaponifiable matter is applied to the substances non-volatile at 100-105°C obtained by extraction with an organic solvent from the oil to be examined after it was saponified. The result is calculated as percent weight/weight (I.S.I. 1984e).

Saponification of the coconut oil is usually done with ethanolic potassium hydroxide solution. After that, extraction of the unsaponifiable matter is then carried out with petroleum ether for several times.

Unsaponifiable mater of coconut oil is a variable mixture of hydrocarbons, alcohols, aldehydes, ketones, pigments and fat-soluble vitamins which occur naturally or may be formed during processing or degradation of the oil (Moura Fe et al., 1975).

Moisture and Impurities

The determination of the water content is one of the most frequently used methods in laboratories for foods around the world. One method is the drying method using drying ovens

or infrared lamps at temperature 105°C (I.S.I. 1984). By this method the loss on drying is determined and not just the water content because the method is not specific.

The titrimetric determination of water by the Karl Fischer method depends on the reaction that takes place quantitatively between water and a reagent consisting of sulfur dioxide and iodine in anhydrous pyridine and methanol. The reaction is carried out in methanol.

The chemical reaction is:

$$H_2O + CH_3OH + I_2 + SO_2 + R_3N \longrightarrow [RNH]SO_4CH_3 + 2[RNH]I$$

In contrast to drying, this is a specific method. In the absence of side reactions only water will be determined (Pande 1974).

The term *insoluble impurities* refers to extraneous substances such as dirt, debris and fibers. They are defined as those substances which remain insoluble and can be filtered off after the oil is dissolved in a specific solvent such as petroleum.

Volatile Mater

The matter volatile at 105°C in oil sample is usually determined according to I.S.I. procedure (I.S.I. 1984). The test sample is heated at105°C until moisture and volatile matter were completely removed and the loss in mass was recorded. The result was expressed in percentage by mass.

Color

Coconut oil in its virgin form is clear in color. High heat exposure during deodorization process (230°C) normally will lead to polymerization and consequently the oil will turn yellow. Color of virgin coconut oil in this survey was in range of 0.1 Red and 0.5 Yellow max.

The liquid oil samples are placed in a Lovibond 5 ¼" cell and the color is determined at 30°C by achieving the best possible match with the standard color slides of red and yellow indices using a Lovibond tintometer.

The method determines the color of oils by comparison with Lovibond glasses of known color characteristics. The color of coconut oil is expressed as the sum total of the yellow and red slides used to match the color of the oil in a cell of the specified size in the Lovibond tintometer (I.S.I. 1984b).

The color of the oil calculated in terms of Lovibond units as follows:

Color reading = (a Y + 5 b R) or (a Y + 10 b R)

in (5 ¼" cell) where

a: sum total of the various yellow slides (Y) used;
b: sum total of the various red (R) slides used;

Y + 5R is the mode of expressing the color of light colored oils;
Y + 10 R is for the dark-colored oils.

Peroxide Value

Peroxide value is a measure of the amount of peroxidesformed in fats and oils through autoxidation and oxidation processes (Rohman et al., 2011). It is a measure of the degree of initialoxidation of fats and oils.

The peroxide value is determined iodometrically according to standard methods for the oils analysis and the results are expressed in meq O_2/kg oil (AOCS 1998).

The sample is treated in solution with a mixture of acetic acid and a suitable organic solvent and then with a solution of potassium iodide. The liberated iodine is titrated with a standard solution of sodium thiosulfate in presence of starch.

Peroxides and similar products which oxidize potassium iodide under the conditions of the test will contribute to the peroxide value. Peroxide values are expressed either in milliequivalents of O_2/kg oil.

The peroxide value is determined by measuring the iodine released from potassium iodide by peroxide, using sodium thiosulfate solution as the titrant. In acetic acid solution, the reaction scheme for is as follows:

$$R{-}H + O_2 \longrightarrow ROOH$$

$$KI + H_3C{-}COOH \longrightarrow HI + H_3C{-}COO^-K^+$$

$$ROOH + 2HI \longrightarrow ROH + H_2O + I_2$$

$$I_2 + 2Na_2S_2O_3 \xrightarrow{\text{starch}} 2NaI + Na_2S_4O_6$$

The reaction of peroxides of the structures R-O-O-R' follows an analogous pathway. Cyclic peroxides do not react quantitatively under these conditions.

Alternatively, the ion reaction is of more of general applicability:

$$O_2^{2-} + 2I^- + 4H^+ \longrightarrow I_2 + 2H_2O$$

$$I_2 + 2S_2O_3^{2-} \xrightarrow{\text{starch}} 2I^- + S_4O_6^{2-}$$

The Reichert Value

The Reichert value is the number of millilitres of 0.1N aqueous sodium hydroxide solution required to neutralize steam volatile water soluble fatty acids distilled from 5g of an

oil/fat under the given conditions. The Reichert value is a measure of water soluble steam volatile fatty acids mainly butyric and caproic acids present in oil or fat (I.S.I. 1984f).

The Polenske Value

The Polenske value is the number of mililiters of 0.1N aqueous alkali solution required to neutralize steam volatile water insoluble fatty acids distilled from 5g of the oil/fat under the prescribed conditions. It is a measure of the steam volatile and water insoluble fatty acids, chiefly caprylic, capric and lauric acids present in oil or fat (I.S.I. 1984f).

Melting Point

Coconut oil is predominantly mixture of triglycerides. Coconut oil does not exhibit either a definite or sharp melting point. For that reason the melting point does not mean the same characteristics that it does with pure crystalline substances.

Melting point is undoubtedly the most widely used measure of the consistency of oils and fats.

Fats pass through a stage of gradual softening before they become completely liquid. Therefore, the melting point depends by the specific conditions of the method by which it is determined. The test is empirical and has a large experimental error. There are several versions of the method. One method is open-tube capillary-slip method. In this method, the melting point is considered the temperature at which the oil or fat softens or becomes sufficiently fluid to slip or run as determined by the open-tube capillary-slip method (I.S.I. 1984c).

Neither Codex, or the Malaysian Standard specify a melting point for coconut oil but a literature survey it were found 24.2-25.0°C ranging values (Gunstone 2011; Firestone 2013).

Other Physical Characteristics

Other physical characteristics of coconut oil (Table 6) such as density, viscosity, heat capacity and heat of fusion, are important for theoretical considerations and for food engineering purposes.

For example, viscosity is an important property. Viscosity of the oil determines the diameter of pipes and the power needed for pumping or stirring of oils. Viscosity is also important for taste and mouth feel, for example, in the case of fried foods.

SPECIFIC CHARACTERISTICS OF COCONUT OIL

Coconut oil is commercially important oil. Coconut oil differs significantly from other fats and oils in that that it pass abruptly from a brittle solid to a liquid, within a narrow temperature range. Coconut oil is a hard brittle solid at ambient temperature (21.1°C), but it

melts sharply and completely below body temperature. The sharp melting characteristics of the coconut oils are derived from the similarity of the melting points of the triglycerides. Coconut oils leave a clean, cool, nongreasy sensation on the palate, which is difficult to match with other oils (Gopala Krishna et al., 2010).

More than 90% of the coconut oil fatty acids are saturated, which accounts for its excellent oxidative stability. It is the richest source of medium-chain triglycerides, which are composed of C-6, C-8 and C-10 fatty acids. Due to the high medium-chain triglycerides content, coconut oil is a major component of infant formulas and medical foods for people who cannot absorb longer chain fatty acids (Erickson 1990).

Table 6. Miscellaneous physical properties of coconut oil

Parameter	Value or range
Boiling point (°C) at 760mmHg	298.9
Boiling point (°C) at 1mmHg	130.2
Dielectric constant	ca. 3.1
Heat of combustion (cal×g^{-1})	9020
Heat of fusion (cal×g^{-1})	46.2
Heat of vaporization (cal×g^{-1})	69
Heat of vaporization (cal×g^{-1})	51
Kinematic viscosity (C. Stokes) (37.8°C)	29.79
Kinematic viscosity (C. Stokes) (98.9°C)	6.06
Refractive index (n_D at 40°C)	1.448-1.450
Relative density (40°/20°C)	0.908-0.921
Slip melting point (°C)	23.0-25.0
Specific gravity (20°/4°C)	0.9226
Specific gravity change/°C	7.13×10^{-4}
Specific heat (cal×g^{-1}) 66°C	0.510
Specific heat (cal×g^{-1}) 97°C	0.530
Vapor pressure (mmHg) 188°C	0.001
Vapor pressure (mmHg) 244°C	0.05

Data adapted from (Swern 1979; Codex 2003; Cocks & Van Rede 1966).

Table 7. Malaysian standard MS 239 (1987): requirements for coconut oil

Characteristics	Crude		Refined
	Grade 1	Grade 2	
Free fatty acids (as lauric acid) (%) max.	1.0	3.5	0.1
Moisture and insoluble impurities, (%) max.	0.50	0.50	0.10
Iodine value (Wijs), g I_2/100g of oil, range	7.5-10.5		
Color (5 ¼") Lovibond cell, max.	3R	4R	1.5R
Refractive index at 40°C, range	1.4480-1.4490		
Specific gravity at 30/30°C, range	0.915-0.920		
Saponification value (mg KOH×g^{-1}), range	248-264		
Unsaponifiable matter (%) max.	0.8		0.5

Adapted from Gunstone 2011).

Table 8. Codex (2001) draft standard for coconut oil

Chemical and physical characteristics	Values
Relative density 40/20°C	0.908-0.921
Refractive index (n_D 40°C)	1.448-1.450
Saponification values (mgKOH×g^{-1})	248-265
Iodine value (g I_2/100g of oil)	6.3-10.6
Unsaponifiable matter (g×kg^{-1})	≤ 15
Matter volatile at 105°C	0.2% m/m
Insoluble impurities	0.05% m/m
Soap content	0.005% m/m
Iron (Fe)	virgin oil 0.5 mg/kg
	refined oil 5.0 mg/kg
Copper (Cu)	virgin oil 0.1 mg/kg
	refined oil 0.4 mg/kg
Acid value	virgin oil 4.0 mgKOH/g oil
	refined oil 0.6 mgKOH/g oil
Peroxide value	virgin oil up to 15 me/kg
	refined oil up to 10 me/kg
Reichert value	6-8.5
Polenske value	13-18

Adapted from (O'Brien 2008; Gunstone 2011).

Table 9. Coconut oil composition and physicochemical properties

Characteristic	Range
Unsaponificable matter, %	0.1-0.8
Solidification point, °C	14-22
AOM stability, h	30-250
Oxidative stability index (110°C), h	8.5-85

Adapted from (Fritsch et al., 1971; Gunstone 2011).

The color of crude coconut oil varies from a light yellow to brownish yellow. The National Institute of Oilseed Products specification limit is 15.0 maximum Lovibond red color. Normal processing techniques will produce deodorized oils with very pale yellow colors. The odor and taste of coconut oil are largely due to the presence of small quantities of lactones (less than 150 ppm). Because coconut oil is low in unsaturated fatty acids, it has a high resistance to oxidation; however, coconut oil will hydrolyze two to ten time faster than normal oils to produce a disagreeable soapy flavor. Coconut oil hydrolysis proceeds slowly in the presence of free moisture alone, but rapidly when an enzyme lipase is present in the food product. Pastry dough and cake batters are examples of such products (O'Brien 2008).

The typical characteristics for the coconut oil are presented in Tables 7, 8 and 9.

Trade Specifications

The specifications and test values of oils provide a useful insight into their properties and can be used as guides for good quality and/or authenticity.

The quality of commercial coconut oil quality is based on trade specifications which give rounded values for a small number of basic characteristics. The specifications of the Malaysian Edible Oil Manufacturers' Association (MEOMA 2001) for crude and refined coconut oils are shown in Table 10 and those of a major EU refiner for both refined and hydrogenated CNO are shown in Table 11.

Table 10. MEOMA specifications for crude and refined coconut oil: for export market (MEOMA 2001)

Parameter	Crude coconut oil, Grade 1	Crude coconut oil, Grade 2	RBD coconut oil
	Values		
Free fatty acids (as lauric acid)	1.0% max.	3.5%max.	0.1% max.
Moisture and insoluble matter	0.5% max.	0.5% max.	0.1% max.
Iodine value (Wijs), g I_2/100g of oil	7.5-10.5	7.5-10.5	7.5-10.5
Color (5 ¼" Lovibond cell)	-	-	Red 1.5 max.

Adapted from (Gunstone 2011).

Table 11. Selling specification for RBD coconut oil and hydrogenated coconut oil

Characteristics	RBD* coconut oil	Hydrogenated coconut oil
Color (5 ¼") Lovibond cell, max.	1.2R-4.5Y	1.2R-4.5Y
Free fatty acids (as lauric acid), %max.	0.1	0.1
Peroxide value, milliequivalents O_2/kg oil max.	1.0	1.0
Iodine value (Wijs), g I_2/100g of oil	7-10	0-2
Saponification value, mgKOH/g oil	255-260	255-260
Slip melting point, °C	24-26	32-34

Adapted from Codex 2009, 2011, 2013.

* RBD - refined, bleached and deodorized coconut oil.

CLASSIFICATION OF COCONUT OILS

Different terms employed for commercial coconut oils (Fife 2004; Gopala Krishna et al., 2010; Kamariah et al., 2008). Some of them are presented in the following sentences.

100% Pure Coconut Oil

The term 100% pure coconut oils are describing two categories of oils: a) 100% pure-natural (only minor treatments were used for obtaining pure oil); b) 100% pure-refined (the purity was achieved by refining, bleaching and deodorizing or other treatments).

Virgin Coconut Oil

Virgin coconut oil is the clear oil extracted from fresh coconut milk by coconut wet-process, without refining. Oil is extracted only from fresh high quality coconuts and extraction and purification processes are through mechanical means only. The resulting coconut oil has a very low free-fatty acid content. Virgin coconut oil has very long shelf life, natural coconut aroma and flavor, light viscosity and non-oily character.

RBD Coconut Oil

RBD refers refined, bleached and deodorized coconut oil. The process for coconut oil obtaining consists in raw material preparation, extraction of oil and purification. Extracted crude oil is treated with alkali to remove free fatty acids. In the subsequent stage oil is treated with steam under vacuum to remove odors and flavors. In the last stage oil is filtered through carbon to decolorize yellow or dark colors. RBD coconut oil is edible oil and can be used in food and cosmetic applications.

AUTHENTICATION AND ADULTERATION STUDIES ON VIRGIN COCONUT OIL

The oils authentication, especially functional food oil such as virgin coconut oil and virgin olive oil is of crucial importance for consumers, food processors and food industries (Rossell et al., 1985; Fakhri & Qadir 2011). The adulteration often involved the replacement of high-cost ingredients with cheaper substitutes. Virgin coconut oil can be adulterated with other vegetable oils of lower commercial value or with similar composition (Fakhri & Qadir 2011).

Although the adulteration is carried out by economic reasons, the action can affect the quality of food, where virgin coconut oil is one of the food components (Flores et al., 2006).

Adulteration has been a problem in the oil trade for a long time. It is sometimes deliberate, sometimes accidental. Indeed, accidental contamination is hard to avoid in modern bulk handling installations. However, it is sometimes remarked that it is the expensive oil that usually gets contaminated with the cheaper one. Numerous tests were developed for the characterization of oils and fats in order to characterize and identify the oils. These include determination of iodine value, to give a measure of oil's unsaturation and of saponification value, which gives a measure of the average molecular weight of the constituent fatty acids (Nollet & Toldra 2012).

The knowledge of vegetable oil characteristics is important not only with regard to the commercial importance of establishing edible oil authenticity, but also with regard to the need to comply with foodstuff labeling legislation in many countries. Descriptive sensory analysis can be carried out to differentiate virgin coconut oil and refined, bleached and deodorized (RBD) coconut oil samples (Villarino et al., 2007).

Palm kernel and coconut oils both contain about 47% lauric acid. This gives the oils close similarities in physical and chemical properties. The oils are different and it is important to be able to distinguish between them (Fakhri & Qadir 2011). The fatty acid profile plays a key role to the physicochemical properties of the oil rather than evaluation of oils based on sensory evaluation especially visual appearance (Sodamade et al., 2013).

Palm kernel and coconut oils are somewhat alike in physical and chemical properties. Nevertheless the slight differences in their properties are real. It is frequently necessary to distinguish the oils, e.g., for labelling purposes, or decide on their proportions in a blend.

In one study, palm kernel and coconut oils samples were analyzed for triglyceride, fatty acid, sterol and tocopherol compositions. The melting points were also determined. Statistical analysis of the results has shown that a combination of values from the carbon number analysis differentiates palm kernel and coconut oils. Furthermore, carbon number analysis can be used to determine the proportion of each in a blend (Rossell et al., 1983).

Other study based on physicochemical properties has shown that coconut oil can be distinguished from similar oils such as palm kernel oil. Significant differences are observed for iodine value ($\leq$ 11 for coconut oil and $\leq$ 19 for palm kernel oil). Other parameters are related to content of oleic, stearic and linoleic acids, the sum of these values should not be greater than 11.5% for coconut oil and 22% for palm kernel oil. Furthermore, it is a clear difference between coconut oil and palm kernel oil regarding the content of fatty acids with 10 or fewer carbons. The content is higher in coconut oil in comparison with palm kernel oil (Hamilton 2012). Triglyceride analysis was also useful since palm kernel oil contains higher levels of C-46 to C-54 triglycerides than coconut oil (Ashurst & Dennis 2013).

Comparison of the sterols esters of coconut and palm kernel oil showed enough differences to form a basis for distinguishing between the two oils (Yildiz 2009; Gordon & Griffith 1992). Coconut oil had a lower β-sitosterol content and higher Δ^5-avenasterol content than palm kernel oil (Ashurst & Dennis 2013).

In a study including numerous samples of coconut oil from a variety of geographical origins have been analyzed for their overall fatty acid composition, triglyceride carbon number composition, tocol concentrations, sterol compositions and slip melting points. The use of triglyceride carbon number analysis has been useful for distinguish between coconut oil, palm kernel oil and their blends. The coconut oil samples contained only low levels of the known tocopherols and tocotrieneols in comparison with other oils. Sterol analysis was carried out by Downes (Downes 1982) in a related project on a selection of the same set of samples. The major sterols in coconut oil were β-sitosterol, Δ-avenasterol and stigmasterol. The relatively high, Δ-avenasterol level in coconut oil can be used to distinguish palm kernel from coconut oil.

Virgin coconut oil, a relative newcomer in the fats and oil market and could be valuable oil, comparable with virgin olive oil. For that reason, it is important to establish reliable purity criteria to assure its premium quality. Even physicochemical parameter values are useful for distinguish among oils, the obtaining and the analysis of these are very difficult,

time-consuming and require expensive materials and equipments as well as high qualified personal. Therefore, for practical applications in quality control several methods were developed.

Several analytical techniques were used for authentication of virgin coconut oil such as:

- Refractometry
- Fourier transform infrared (FTIR) spectroscopy
- Differential Scanning Calorimetry
- Electronic Nose Technology
- fiber optical sensor
- $^{-13}$C NMR spectroscopy and ^{31}P NMR spectroscopy

Refractometry

Refractive index plays an important role in different fields of chemistry, physics and biology. Knowledge of the refractive index of oil is of critical importance in applications of adulteration of oils and purity (Yunus et al., 2009).

Refractive index of oils increases with the increase in unsaturation and also chain length of fatty acids (Sivasankar 2002). Therefore, this parameter can be used for distinguish among oils. The coconut oil and sunflower oil were mixed with palm oil in different percentages. The refractive indices of oils and mixtures were determined by using Abbe's refractometer. The percentage of adulteration of these oils was calculated using refractive index values and the calibration plot. From this study, it was observed adulteration of the coconut oil starting with 30% percentage of adulterant oil in the mixture. This study reveals that the measurement of refractive index can be used as a quality control technique for finding the adulteration of oils but, the sensibility is reduced (Ariponnammal 2012).

In other study, refractive indexes of virgin coconut oil and virgin olive oil were measured and compared. The refractive index for virgin olive oil is higher than the refractive index for virgin coconut oil and this can certificate the purity of the oil (Yunus et al., 2009).

FTIR Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is well known in the analytical field for quantitative analysis of oils and fats (Rohman et al., 2009). FTIR is also becoming popular for authentication analysis.

FTIR spectra of coconut oils present a series of bands with different intensities and forms. Compared with other edible oils and fats, virgin coconut oil has characteristic FTIR spectra. The functional groups responsible for infrared absorption at each frequency in virgin coconut oil spectrum were assigned as follow. The major peak in the region 3,100-2,800 cm^{1} is due to C-H stretching mode. The stretching of > C = O is observable in the region 1,700-1,800 cm^{-1}, while wave number region 1,400-900 cm^{-1} is associated with C-O-C stretching and C-H bending (Rohman et al., 2009; Moigradean et al., 2012).

FTIR spectra of other vegetable oils present some particularities that can be useful in authentication analysis. For discrimination and classification studies discriminant analysis or partial least square (PLS) regression were performed (Manaf et al., 2007; Che Man & Rohman 2013).

In one study, attempts were made to adulterate virgin coconut oil with palm kernel olein. The study was conducted to evaluate the effectiveness of FTIR spectroscopy in detecting adulteration of virgin coconut oil. As adulterant was used palm kernel olein because of its similarity with virgin coconut oil in chemical composition (Manaf et al., 2007.

The spectra of virgin coconut oil and palm kernel oil show the typical characteristic absorption bands for common vegetable oils (Rohman et al., 2009; Moigradean et al., 2012). In the entire range of wave numbers, both virgin coconut oil and palm kernel olein spectra seems almost identical. This is related to lauric acid content of about 47-48% (Rossell et al., 1983). If a meticulous check is carried out some differences between spectra were observed. For instance a peak at 3,006 $cm^{-1}$is observed for in palm kernel olein spectrum that is not present in the spectrum of virgin coconut oil. The absorption peakat 3,006 cm^{-1} are due to the *cis* C = C-H stretching (Rohman et al., 2009; Moigradean et al., 2012). A higher content of unsaturated fatty acids contributes to an absorbance in the region of *cis* C = C-H stretching. This peakcan be used for distinguishing of palm kernel olein from virgin coconut oil.

The band absorbance increases in the range 3,000-3,010 cm^{-1} upon increase of the percentage of palm kernel oil in a blend.

FTIR spectroscopy was used in combination with partial least square (PLS) to differentiate and quantify these two oils. The calibration plot of PLS regression model was shown a good linearity between the actual value and FTIR predicted value of percentage of palm kernel olein in virgin coconut oil. The differences between the actual adulteration concentration and the calculated adulteration predicted from the model were very small, with a determination coefficient (R^2) of 0.9973 and root mean error of calibration of 0.0838.

Discriminant Analysis was carried out for pure virgin coconut oil and the blended samples and a classification into two groups, pure virgin coconut oil and adulterated oils was observed. The model demonstrated the classification of pure virgin coconut oil with addition of 1-50% of palm kernel olein using10 principal components. Therefore, detection of adulteration was possible down to 1%.

Other study was carried out to develop a method based on FTIR spectroscopy combined with chemometrics of multivariate calibrations (partial least square and principal component regression) as well as discriminant analysis for quantification and discrimination of canola oil in virgin coconut oil (Che Man & Rohman 2013).

FTIR spectra of virgin coconut oil revealed some differences to other vegetable oils including canola oil, especially in the regions around 3007 cm^{-1} and 1654 cm^{-1} and at wave number regions of 1120-1090 cm^{-1}. There was no band at frequencies of 3007 and 1655 cm^{-1} for virgin coconut oil and the otherwise was observed for canola oil. Furthermore, at frequency region of 1120-1090 cm^{-1}, virgin coconut oil showed one peak and canola oil exhibited two peaks. Therefore, these differences were used for detection and quantification of canola oil in virgin coconut oil.

Quantification of canola oil in virgin coconut oil was performed using multivariate calibrations of partial least square (PLS) and principal component regression (PCR). The relationship between actual and predicted values of canola oil in virgin coconut oil was

demonstrated that FTIR spectroscopy combined with PLS can be a promising method for quantification of canola oil in virgin coconut oil.

DA was used to determine the capability of the system to distinguish between pure and adulterated oil. The DA model classified 100% of all samples accurately according to its group therefore the detection limit of this system is 0.5% (volume/volume).

Other study regarding the authentication of virgin coconut oil adulterated with cheaper plant oils such as corn and sunflower oils. Methods involving Fourier transform mid infrared (FT-MIR) combined with chemometrics techniques (PLS and DA) were developed for quantification and classification of corn oil and sunflower oil in virgin coconut oil (Rohman & Che Man 2011). The classification of virgin coconut oil and that adulterated with corn oil was carried out using spectral regions at combined frequencies of 3028-2983, 2947-1887 and 1685-868 cm^{-1}, meanwhile frequencies at 3030-2980 and 1300-1000 cm^{-1} was exploited for classification of virgin coconut oil adulterated with sunflower oil.

The quality of developed PLS model was evaluated by cross-validation technique. The values of root mean square error of cross validation obtained are relatively low, 1.68% and 1.32% (volume/volume), respectively for corn oil and sunflower. Based on this result, the method developed has a good ability to estimate the percentage of corn oil and sunflower as oil adulterants in virgin coconut oil samples.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) provides unique thermal profiling for each oil and can be used to detect cheaper oils or lard adulteration in virgin coconut oil (Biliaderis 1983).

DSC was used to detect adulteration in virgin coconut oil (Marina et al., 2009b). Soybean, sunflower and palm kernel oil were used as adulterants. Each oil was considered representative oil from linolenic, oleic-linoleic and lauric acid group. The heating curves of sunflower and soybean adulterated samples showed that adulteration peak appeared at the lower temperature region starting at 10% adulteration level. For the data analysis, regression analyses using stepwise multiple linear regression (SMLR) was used. SMLR model was able to predict the percent adulterant with a determination coefficient (R^2) of 0.9390 for sunflower oil and 0.9490 for soybean oil. In the case of palm kernel oil, which belongs to the same group as virgin coconut oil (lauric acid), no adulteration peak was observed. For the palm kernel oil, a good relationship between the main exothermic peak height of palm kernel oil and percentage of adulteration was established.

Other work showed the ability of DSC to detect changes in the cooling and heating curves of virgin coconut oil when it is adulterated with lard (Mansor et al., 2012). Lard can be used as an adulterant in virgin coconut oil. The similar physical characteristic of virgin coconut oil to lard makes lard a possible adulterant in virgin coconut oil.

The exothermic and endothermic peaks were changed for adulterated samples comparing with those observed for pure samples. Thus, the DSC curves showed slight changes such as the size increase or reduction of exothermic peaks and the smoothing effect of shoulder peak in the endothermic peak as the percentage of lard adulteration increased.

Two independent DSC parameters were chooses and by means of multiple linear regression analysis prediction of lard percentage adulteration in virgin coconut oil with a

determination coefficient (R^2) of 0.9582. Therefore, these parameters can be used as a good measurement index in detecting lard adulteration in virgin coconut oil.

In conclusion, DSC is a simple and chemical free method in the study of adulteration in oils.

Electronic Nose

An electronic nose can be useful for analyzing volatile compounds. It works in a way analogous to the way a human nose functions and does not require previous separation of individual volatile compounds. The system provides a pattern of volatile compounds present in the samples. For data analysis chemometrics methods are necessary.

The potential use of electronic nose in detecting adulteration in virgin coconut oil was studied by Marina et al., 2010. An electronic nose (zNose^{TM}) was applied to the detection of adulteration of virgin coconut oil. The system is based on a surface acoustic wave sensor. Virgin coconut oil was mixed with refined, bleached and deodorized palm kernel olein at a level of adulteration from 1 to 20% (weight/weight).

Principal component analysis (PCA) was used to differentiate between pure and adulterated samples. The PCA provided good separation of samples with 91% of the variation accounted for the PC1 and the PC2. Pure samples formed separated cluster from all adulterated samples.

Excellent results were obtained for the differentiation between pure and adulterated samples down to the 1% detection limit.

The adulterant concentration was quantified using PLS analysis. The correlation between the signals measured with the electronic nose and adulteration percentages of samples was established. The coefficient of determination (R^2) for the model was 0.91, indicating that the model fit the data well.

This study has demonstrated the application of electronic nose as a tool to detect adulteration of virgin coconut oil.

Fiber Optical Sensor

The research field in optical fiber grating technology has opened a new platform for sensor applications (Othonos & Kalli 1999). This technique can be used to discriminate different oils as well as to detect adulteration.

A fiber optic sensing system for the detection of adulteration of coconut oil by paraffin oil was demonstrated (Libish et al., 2011). Paraffin oil is extremely hazardous to human health as and up to 20% of paraffin oil can be mixed with coconut oil without any notable difference in the smell or color of coconut oil. The sensing mechanism is based on the sensitive dependence of the resonance peaks of a long period grating on the changes of the refractive index of the environmental medium surrounding the cladding surface of the grating. The modality of measuring is based on the shift of the attenuation bands. The wavelength shift of the attenuation bands of the long period grating was measured with the sensor immersed in a mixture of paraffin oil and pure coconut oil in different proportions. Detection limit of adulteration was found to be 3% for coconut oil-paraffin oil binary mixture.

^{13}C NMR Spectroscopy and ^{31}P NMR Spectroscopy

Nuclear magnetic resonance (NMR) techniques were used to quantify and profile refined and virgin oils by analysis of monoacylglycerides (MGs), diacylglycerides (DGs), sterols, free fatty acids (FFAs) and other minor components (Vlahov et al., 2003). These compounds are helpful in adulteration studies.

In one study (Dayrit et al., 2008), phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR) was used to differentiate virgin coconut oil from refined, bleached, deodorized coconut oil. MGs, DGs, sterols and FFAs in virgin coconut oil and refined coconut oil were converted into dioxaphospholane derivatives and analyzed by ^{31}P NMR.

As results, it can be mentioned that virgin coconut oil had 40% higher of 1-MGs content than refined coconut oil. On the other hand, virgin coconut had lower DGs content (1.5%) than refined coconut oil (4.1%). Total sterol contents in virgin coconut samples was approximately 0.096%, slightly higher compared to refined coconut oil (0.032%). The FFA contents showed that virgin coconut oil had higher FFA contents (0.127%) than refined coconut oil (0.015%). Principal component analysis showed that the DGs and FFA were the most important parameters for distinguishing virgin coconut oil from refined coconut oil.

^{31}P NMR is a useful method for the analysis of constituents in coconut oil and gives a valuable way of distinguishing virgin coconut oil from refined, bleached, deodorized coconut oil.

Another method to detect adulteration is ^{13}C NMR spectroscopy Application of ^{13}C NMR spectroscopy for detection of castor oil in different edible oils, such as palm oil, coconut oil, groundnut oil and mustard oil, was described (Husain et al., 1993). Quantitative^{13}C NMR spectra of oils were recorded in $CDCl_3$. Characteristic signals observed at different δ values related to some carbons of ricinoleic acid in castor oil were selected for distinguishing it from edible oils. The minimum detection limits for qualitative and quantitative analyses were in the range 2.0-3.0%.

CONCLUSION AND PERSPECTIVES

Virgin coconut oil could be considered functional food oil derived from the kernel of *Cocos nucifera L*. Therefore, the determination of coconut oil authenticity and the detection of adulteration are of great importance for food producers and consumers. The international standards for coconut oil are included in the Codex Alimentarius, the Asian and Pacific Coconut organization and in the national standards of countries that produce coconut oil (India, Malaysia, Philippines, Sri Lanka, Brazil, etc.). The identity and authenticity of the virgin coconut oil can be determined using standard chemical methods of analysis or instrumental analysis. Therefore, these methods can be used for detection of adulteration in virgin coconut oil. Several procedures for authentication of virgin coconut oil were developed based on refractometry, FTIR spectroscopy, differential scanning calorimetry, electronic nose, fiber optical sensor and NMR spectroscopy. For the analysis of data chemometrics techniques were employed. The best methods are able to detect adulterants in virgin coconut oil at levels below 1%. However, the improvement of current methods and the development of novel ones are expected in the future.

The future challenge could consist of developing, validating and harmonizing analytical methods and quality parameters related to authenticity issues. The possible applications are related to the blend of virgin coconut oil with soft treated coconut oil (i.e., soft deodorized) and the blend of virgin coconut oil with other vegetable oil or refined coconut oil.

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Chapter 8

THE EFFECTS OF FATTY ACID DERIVATES FROM CORN AND COCONUT OILS ON MICROBIAL PHYSIOLOGY

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ABSTRACT

Nowdays, green consumption" tendency promotes natural compounds, such as lipids and their derivatives, like fatty acids and formulated acylglycerols, as ingredients for many food and feed commercial products, nutraceuticals, pharmaceuticals or cosmetics products. Many studies demonstrated that the fats and their bioderivatives are important sources of biological active compounds that have influence on the cells metabolism and their physiology. The role of some fatty acids was intensely studied especially for their antimicrobial activity, in regards to natural microbiota and starter microorganisms development. The positive effects in cell's metabolism stimulation were less investigated but the preliminary results indicated good perspectives.

Keywords: coconut oil, corn oil, antimicrobial activity, microbial metabolism stimulation

INTRODUCTION

Lipids with biological activities have been extracted from various plant and animal species. It has been demonstrated that many lipids from different sources and also their derivatives (glycerides and fatty acids) exerted antimicrobial activity against a large spectrum of Gram-positive and Gram-negative bacteria, yeasts, fungi, viruses and parasites, that cause spoilage or various infections in both animals and humans (Glatz et al., 2015). Furthermore, fatty acids and derivated glycerides are important in the manufacture of emulsions (Thormar

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et al., 2006) and drugs (Thormar et al., 2006; Preston et al., 1996; Bergsson et al., 1999; Desbois and Smith, 2010; Kristmundsdottir et al., 1999) as alternative or adjuvant to antibiotic treatment used against resistant bacteria (Nakatsuji et al., 2009; Kitahara et al., 2004; Nair et al., 2005a,b). Nowadays, some fatty acids and their derivatives played an important role in food preservation and food safety assurance by producing natural food preservatives from vegetal and animal lipids (oils and fats) (Botterweck et al., 2000; Skandamis et al., 2001; Thormar, 2011). Beside the important role that fats and oils played in the diet, they are the main source of energy, have antioxidant properties and are also considered as raw materials for obtaining a wide range of products in the food, pharmaceutical, cosmetic and chemical industries (Kabara et al., 1991; Mandal et al., 2009).

Some tropical vegetable oils (coconut and palm oils) contain saturated fatty acids, while monounsaturated fatty acids are found prevalent in olive and corn oils whereas polyunsaturated fatty acids are found in corn, soybean and sunflower oils.

Coconut oil composition consists of 90% saturated fatty acids, 7.5% monounsaturated fatty acids and 1.8% polyunsaturated fatty acids (Zambiazi, et al., 2007) and it is as it follows: 1% caproic acid (C6:0), 2% caprylic acid (C8:0), 4% capric acid (C10:0), 50% lauric acid (C12:0), 18% myristic acid (C14:0), 9% palmitic acid (C16:0), 8% oleic acid (C18:1), 5% stearic acid (C18:0) and 2% linoleic acid (C18:2) (Paiwan et al., 2013). The structured lipids (24%), which usually are difficult to find in nature are obtained by crude oil biotransformation but another possibility to obtain them is by bioconversions in controlled conditions, in order to develop specific target compounds with biological activities that have different effects on microbial cells (Nugrahini et al., 2015).

On the other hand, corn oil composition consists of 14% saturated fatty acids, 24.76% monounsaturated fatty acids and 61.37% polyunsaturated fatty acids. The main profile of corn oil fatty acids is it follows: 10.47% palmitic acid (C16:0), 2% stearic acid (C18: 0), 24% oleic acid (C18: 1), 60% linoleic acid (C18: 2) and 1% linolenic acid (C18: 3) (Zambiazi, et al., 2007).

According to the literature, coconut and corn oils are recognized as the major sources of fatty acids, with active biological properties, that exert influences on microorganisms physiological activities, have microbiostatic and microbicide effects and also stimulate the metabolites production of the cells. (Desbois et al., 2010; DebMandal et al., 2011).

The antimicrobial activity of some fatty acids has been studied over several years through various research strategies in order to produce food additives that can inhibit or kill the microorganisms that have incidence in food spoilage and food safety assurance. The saturated fatty acids, (capric, caprylic, lauric, myristic acids) and the unsaturated fatty acids, (palmitoleic, linoleic and linolenic acids) that are found in the composition of coconut and corn oils have an important antimicrobial activity against a large number of pathogens (Agoramoorthy et al., 2007; Parfene et al., 2013).

The antimicrobial effect on bacteria and viruses of coconut oil is given by its high content of lauric acid (more than 50%), which *in vivo* is converted to monolaurin (Oyi et al., 2010; Parfene et al., 2013). The long chain fatty acids represented by oleic, linoleic and linolenic acids are more active against microorganisms compared to long-chain saturated fatty acids such as palmitic and stearic acid. Also, the medium-chain free fatty acids and their derivatives from coconut oil showed significant antimicrobial activity against Gram-positive and less significant to Gram-negative bacteria (Wille et al., 2003; Georgel et al., 2005; Skrivanova et al., 2006; Drake et al., 2008).

The positive effect of oils and their derivatives on microbial physiology was less studied, but the results obtained so far encouraged the research extention regarding the metabolism regulation, in order to improve the functionality and the biosynthesis potential of the cells (Azimatun Nur, 2015).

The main topic of this chapter is to review the *in vitro* and *in vivo* effects of coconut and corn oils and their derivatives (glycerides and fatty acids) on microorganisms' functionality in order to extend their benefits for increasing the quality of life.

ANTIMICROBIAL POTENTIAL OF VEGETABLE LIPIDS

Mechanism of Action

According to the literature, it was found a correlation between lipid's structure and antimicrobial activity of vegetable oils. Kabara and his collaborators (1978) concluded that, lauric acid, palmitoleic acid and linoleic acids have the most important antimicrobial activity from all the saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids, respectively. Their effect may be microbiostatic or microbicidal (Desbois & Smith, 2010).

It was also demonstrated that medium chain fatty acids and their monoglycerides are the most active in terms of antimicrobial effect than free fatty acids, particularly monolaurin acid. A few Gram-positive bacteria are more susceptible to the bactericidal effect of fatty acids compared with Gram-negative bacteria. On the other hand, the literature data showed that medium chain fatty acids (C10-C12) have a high antimicrobial potential against fungi (Kabara, 1978; Kabara, 1980) and monoesters of fatty acids are more active than diglycerides and triglycerides. It was found that, the antibacterial effect of short chain fatty acids (C6-C10) depends on the degree of their transformation and pH value. It seems like they are more active at pH 6.5 than pH 7.5 (Glatz et al., 2015).

Further, many studies have demonstrated that the antimicrobial potential of long-chain fatty acids increased significantly when the structure is changing. For example, one double bond in oleic acid (C18:1) increase the antimicrobial activity, comparing to the presence of three double bonds, which significantly decrease the antimicrobial potential (Kabara et al., 1972; Conley & Kabara, 1973).

Other studies showed that the fatty acids are able to break the barrier of the outer membrane of the cell wall allowing its access into the inner membrane (Thormar et al., 2006).

There are some differences in the degree of susceptibility of Gram-negative bacteria to fatty acids, especially *Helicobacter pylori* and enterobacteria (Bergsson et al., 2002). This may be due to differences in the bacteria outer membrane composition. For instance, the outer membrane of *Escherichia coli* and *Salmonella* spp. is composed only of lipopolysaccharide and proteins. The cell membrane surface contains hydrophobic chains represented by lipopolysaccharides which are making it difficult to be penetrated. Moreover, lipid molecules may have some difficulties to penetrate the bacteria membrane because of the low fluidity of lipopolysaccharide hydrocarbon chains and due to the strong interaction between lipopolysaccharide molecules. In contrast, some Gram-negative bacteria, particularly *Neisseria gonorrhoeae* (Preston et al., 1996) are easily damaged by medium chain fatty acids

and derivated monoglycerides. This is because the bacterial membrane is composed of a high amount of lipooligosaharides than lipopolysaccharides (Bergsson et al., 1999).

Another study conducted by Desbois and Smith (2010) showed the antimicrobial effect of some lipids against microorganisms. A series of inactivations or cells damage effects are caused by partial solubilization of cell membrane through action of fatty acids which determine the membrane proteins releasing. The antibacterial effect of fatty acids is carried out by penetration of the cell wall and the outer membrane of Gram-negative bacteria. As mentioned previously, the antimicrobial effect of lipids on Gram-negative bacteria takes place due to different outer membrane structure, which can act as a barrier and can provide protection to inner membrane of the bacterial cell.

In the inner membrane of bacteria there is located the electron transport chain with a number of carriers that pass electrons through each other in order to form water at the end of the process. This process generates a proton gradient and an increase in ATP synthesis by the enzyme, ATP synthase, through conversion of ADP. Medium and long-chain saturated and unsaturated free fatty acids after insertion in the cell wall or in the outer membrane of the Gram-negative bacteria are able to bind to the carrier of this chain of electron transport and to alter its function (Peters & Chin, 2003). There were proposed different mechanisms by which free fatty acids can alter the oxidative phosphorylation process as follows: by directly coupling to ATP synthase itself, by increasing the permeability of the membrane to protons or by decreasing the proton gradient and membrane potential (Boyaval et al. 1995; Wojtczak and Więckowski, 1998, 1999; Beck et al., 2007). The ATP production decrease because, the energy created through the electron transport chain is transformed into heat and not used for its production. In consequence, the cells are in the impossibility to achieve their vital functions and cells lysis is occurring (Chamberlain et al., 1991; Stulnig et al., 2001, Beck et al., 2007).

Antimicrobial potential of lipids and their compounds derivated after their hydrolysis depends on the microorganisms sensitivity. Some microorganisms are lyzed in a shorter period of time, at low concentrations of lipids, while others are destroyed after a longer period of time or at higher concentrations. The antimicrobial activity against bacteria, fungi or viruses depends on the lipids nature. In general, polyunsaturated fatty acids and saturated fatty acids with medium chain length are more active against pathogens (bacteria and viruses), while saturated fatty acid monoglycerides with medium chain are more active against fungi.

Antibacterial Activity

Medium chain fatty acids and their derivatives from coconut oil can destroy a wide range of bacteria after the degradation of bacterial cell lipidic bilayer. It was demonstrated the bactericidal effect against bacteria that cause various diseases, such as stomach ulcers, dental caries, food poisoning or urinary tract infections (Thormar, 2011).

The integration of unsaturated fatty acids into the bacterial membrane also causes an increase of its fluidity and permeability which leads to growth inhibition or bacterial cell death (Boyaval et al., 1995; Shin et al., 2007).

The antibacterial effect was demonstrated against both Gram-positive and Gram-negative bacteria. Moreover, the antibacterial effect of monocaprin and monolaurin against Gram-

negative bacteria (*Escherichia coli*) decreased at 30°C (Shibasaki & Kato, 1978) and at pH 7.0 (Bergsson et al., 2002). When *Escherichia coli* was grown in the presence of a mixture of lauric acid, monocaprin and monolaurin, at 50°C for 5 minutes, a strong inactivation of the bacteria function was observed. These results suggested that the temperature affected the external membrane permeability allowing the penetration of lipids in the cell wall of bacteria. When *Escherichia coli* cells were incubated in the presence of a mixture of citric acid and monolaurin, a large amount of monolaurin was found into bacterial cells (Thormar et al., 2006).

In a recent scientific report published by Oyi et al. (2010) it was shown that the coconut oil, in concentrations from 5% to 40%, has a bactericidal activity against *Pseudomnonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Bacillus subtilis* strains. Bactericidal potential against these bacteria was attributed to the complex of lauric acid and monolaurin (DebMandal & Mandal, 2011).

Literature data showed that the emulsions obtained with 1.25 mM monocaprin acid in citrate-lactate buffer at low pH values (4.0-5.0) caused a greater reduction in the number of viable cells (> 6 to 7·$\log_{10}$ CFU) of *Salmonella* spp., *Escherichia coli* and *Clostridium jejuni* (Thormar et al., 2006).

Lauric acid, which is the most abundant fatty acid in coconut oil is also present in breast milk and protect the newborns from the harmful action of pathogens. The coconut oil is a good source of fatty acids with bactericidal activity against pathogenic bacteria that cause infection and can be life threatening (Taheri et al., 2010).

Kabara and collaborators (1972) determined the minimum inhibitory concentrations (MIC) of the medium chain fatty acids against Gram-positive and Gram-negative bacteria, after 18 hours of incubation at 35°C. From all the saturated fatty acids (C6 to C18) that were tested, lauric acid had the greatest antimicrobial effect against Gram-positive bacteria. The most sensitive were the group A streptococci, while *Staphylococcus aureus*, *Staphylococcus epidermidis* and the streptococci from group D were the least sensitive (Conley & Kabara, 1973).

Some results presented the MIC of capric acid (C10:0), lauric acid (C12:0), linoleic (C18:2) and linolenic acid (C18:3) on 242 strains of *Staphylococcus aureus* and 117 strains of groups A, B and C of *Streptococcus* spp. (pathogenic strains). It was demonstrated that *Staphylococcus aureus* strains are less sensitive to fatty acids than other pathogenic species found in corn oil. Further, the lauric acid found in coconut oil showed the highest inhibitory action on the tested strains (Heckzko et al., 1979). Caprylic acid (C8:0), myristic acid (C14:0), lauric acid (C12:0), oleic acid (C18:1), monocaprylin (C8:0), monocaprin (C10: 0), monolaurin (C12:0), monomyristin (C14:0), monopalmitolein (C16:1) and monoolein (C18:1) exhibited a high antimicrobial potential against pathogenic strains from group A and group B streptococci and *Staphylococcus aureus* strains. Capric acid (C10:0) from coconut oil has antimicrobial effect against *Streptococcus* spp. (group A and group B), while the lauric acid (C12:0) from coconut oil and palmitoleic acid (C16:1) from corn oil has antimicrobial effect against *Staphylococcus aureus* strains (Bergsson et al., 2001). To determine which fat has the fastest bactericidal effect on Gram-positive bacteria, Bergsson et al. (2001) also tested the influence of time of incubation on antibacterial effect. It was demonstrated that after 10 minutes of incubation, in the presence of different fatty acids, the number of viable bacteria decreased up to 99%. Some bacteria that belong to *Streptococcus* genus, from the group A (GAS) and group B (GBS) have been cultivated for 10 minutes, in the presence of lauric acid

and palmitoleic acid, at 37°C. It was found that 5 mM lauric (C12:0) and palmitoleic acid (C16:1) are able to kill all the cells from tested cultures.

Capric acid showed a low antibacterial effect against *Staphylococcus aureus* strains at a 10 mM concentration. A similar effect has been demonstrated against *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* strains, which were incubated, for 1 minute, in the milk supplemented with 100 mM caprylic acid (C8:0) (Nair et al., 2005b).

It has been determined that the antibacterial activity of long-chain fatty acids is increasing when their structure changes by double bonds addition (Kabara et al., 1972; Conley and Kabara, 1973). In consequence, linoleic acid showed a strong antibacterial potential against Gram-positive bacteria, such as *Bacillus cereus* and *Staphylococcus aureus* strains. However, it is already known that the antimicrobial activity of linoleic acid can be also improved by the addition of emulsifying agents such as, monolaurin and monomyristin (Lee et al., 2002).

Other scientific reports proved the antibacterial activity of polyunsaturated fatty acids (PUFA) against some Gram-positive bacteria. *Lactobacillus acidophilus* and *Staphylococcus epidermidis* strains were inactivated after one hour of incubation at 37°C with 0.01 mM arachidonic acid (C20:4) (Knapp & Melly, 1986).

The antimicrobial action spectrum of monoglycerides was found to be more limited compared to those of free fatty acids. The glyceraldehyde minimum inhibitory concentration against a large range of Gram-positive bacteria as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* strains was also determined. From all of the monoglycerides tested, monolaurin was the most active. The antibacterial potential of monolaurin was somehow more emphasized than that of monocaprin. The studied *Staphylococcus pyogenes* strain was the most sensitive to the action of monocaprin, monolaurin and monomyristin. Monolaurin and monocaprin were the only monoglycerides that inhibited a strain of *Staphylococcus aureus*. Antibacterial potential of monoglycerides proved to be superior to that of free fatty acids (Guha et al., 2012).

Furthermore, monocaprin had the best bactericidal effect against *Staphylococcus aureus* from all the monoglycerides that were tested. It was also shown that the 1.25 mM monocaprin exhibited similar antibacterial potential against *Staphylococcus aureus* as monoglycerides that were introduced into the milk or milk formula (Guha et al., 2012).

Recent studies have been focusing on the antimicrobial effect of fatty acids with chain length from C6 to C18 (Guha et al., 2012). Thus, it was observed that, at low pH, the caprylic and capric acid were capable to inhibit and kill a considerable number of *Escherichia coli* viable cells. In contrast, the chain length of fatty acids from C2 to C6 and from C13 to C18 showed a negligible inhibitory effect against Gram–negative bacteria. This result was confirmed by studies conducted by Nair et al. (2005b) who showed that, the caprylic acid (C8:0) in concentrations from 25 mM to 100 mM when it was added into milk was able to reduce *Escherichia coli* cells.

In low concentration conditions, short incubation time (up to 30 minutes) and different physiological pH, fatty acid showed no bactericidal effect against *Escherichia coli* (Berbsson et al., 2002). On the other hand, 10 mM caprylic acid exhibited a significant reduction of *Campylobacter jejuni* cells, a pathogen responsible for food poisoning (Thormar et al., 2006). By measuring the minimum inhibitory concentrations (MIC), Skrivanova and collaborators (2006) demonstrated the inhibitory effect of 21 mM caprylic acid against *Escherichia coli* and

Salmonella spp. and of the 29 mM capric acid against *Escherichia coli*. Some reports have shown that lauric acid was the only fatty acid with antimicrobial activity against *Helicobacter pylori* (Petschow et al., 1996). The susceptibility of *Helicobacter pylori* to the action of saturated and unsaturated fatty acids was studied by Bergsson et al. (2001). They showed that 5 mM of lauric, capric and palmitoleic acids decreased the number of viable bacterial cells, after 10 min of incubation at 37°C. Also, they prove the antimicrobial effect of 1.25 mM lauric acid and 0.625 mM of palmitoleic acid against *Helicobacter pylori*, after 10 minutes of incubation (Bergsson et al., 2002).

Another Gram-negative bacterium, *Neisseria gonorrhoeae* was killed by a mixture of capric acid, lauric acid and palmitoleic acid. In addition, *Neisseria gonorrhoeae* was also inhibited by lauric and palmitoleic acids, after 1 minute of incubation (Bergsson et al., 1999).

Polyunsaturated fatty acids from corn oil, especially arachidonic acid (C20:4) has been proven to be effective in killing Gram-negative bacteria, *Neisseria* spp. and *Haemophilus* spp. Contrary, *Escherichia coli* strains showed no sensitivity during 6 hours of incubation with arachidonic acid (C20:4) (Knapp and Melly, 1986).

In agreement with the research conducted by Conley and Kabara (1973), di- and triglycerides of fatty acids showed no antimicrobial activity on Gram-negative bacteria (Kabara et al., 1972; Conley & Kabara, 1973). Similar studies carried out by Isaac et al. (1995) showed that the medium chain fatty acid monoglycerides were added to milk composition or breast milk formula in order to inhibit *Haemophilus influenzae*.

This effect was not available in the case of monoglycerides who had a less effective antibacterial action (Isaacs et al., 1995).

Some Gram-negative bacteria, such as *Escherichia coli* and *Salmonella* spp. strains, showed stability, at neutral pH, to all monoglycerides tested (Bergsson et al., 2002). Nevertheless, by using an acidic buffer (pH 5.0), monocaprin was highly effective on killing both types of bacteria (Thormar et al., 2006). Moreover, Nair et al. (2005a) have shown that, when 50 mM monocaprin is added in milk or 5 mM monocaprin, in apple juice, *Escherichia coli* viable cells were completely destroyed (Nair et al., 2005a,b). In addition, Preuss et al. (2005) demonstrated the bactericidal effect of essential oils, while monocaprin was totally ineffective to kill *Escherichia coli* and *Klebsiella pneumoniae* cells.

A study focused on hydrolyzed coconut fat microemulsions shown that the antimicrobial activity of microemulsion was higher compare to that of crude hydrolyzed extract obtained after enzyme hydrolysis (Parfene et al., 2010).

In conclusion, bactericidal and bacteriostatic effects differ because of the experimental conditions, regards to the microbial growth factors, the growth phases, the lipids type and their derivatives compounds concentration and also, the incubation time.

Antifungal Activity

Scientific reports have identified antifungal fatty acids that can combat fungal infections that affect plants, humans and food sources (Altieri et al., 2009; Thormar, 2011).

Fungal membrane plays an important role for maintaining cell integrity. Generally, fungal cell membrane represents the target for all the antifungal treatments (Avis, 2007). Avis and Belanger (2001) determined the general mechanism of how antifungal fatty acids interact with fungal cell membrane. The antifungal fatty acids which are naturally introduced in the

lipid bilayer physically disrupt the membrane increasing its fluidity. In consequence, the general disruption of the cell membrane leading to conformational changes in the membrane proteins, intracellular components releasing and cell lyses.

There are some factors, like chemical structure of fatty acids and medium pH, that plays an important role in the antifungal effect inhibition (Leyva et al., 2008).

Liu et al. (2008) showed that the fatty acids may replace some antifungal chemicals that are worldwide used in plant diseases combating. The effect of fungal cells life shortening was also studied and confirmed by Avis (2007).

Saturated and unsaturated free fatty acids may have antifungal potential, which effectiveness increases with their chain length (Kamem et al., 2009). An important role on microbial cells activity is played by hydrophobic groups of saturated fatty acids. The hydrophobicity increases with increasing the chain length, thus the solubility of fatty acids in aqueous environments decreases which prevents some interactions between these hydrophobic groups and the acyl chains of the membranes phospholipids.

Table 1. Antifungal activity of fatty acids from coconut oil

Fatty acid	Species	References
Butanoic (butyric) acid (C4:0)	*Alternaria solani*	Liu et al., 2008
	Cucumerinum lagenarium	Liu et al., 2008
	Fusarium oxysporum	Liu et al., 2008
	Myrothecium verrucaria	McDonough et al., 2002
	Trichoderma viride	McDonough et al., 2002
Hexanoic (caproic) acid (C6:0)	*Botrytis cinerea*	Leyva et al., 2008
	Cucumerinum lagenarium	Liu et al., 2008
	Fusarium oxysporum	Liu et al., 2008
Octanoic (caprylic) acid (C8:0)	*Alernaria solani*	Liu et al., 2008
	Cucumerinum lagenarium	Liu et al., 2008
	Fusarium oxysporum	Liu et al., 2008
	Kluyveromyces marxianus	Viegas et al. 1989
	Saccharomyces cerevisiae	Viegas et al. 1989
Decanoic (capric) acid (C10:0)	*Aspergillus nidulans*	Sjögren et al., 2003
	Aspergillus fumigatus	Sjögren et al., 2003
	Candida albicans	Bergsson et al., 2001
	Cucumerinum lagenarium	Liu et al., 2008
	Fusarium oxysporum	Liu et al., 2008
	Kluyveromyces marxianus	Sjögren et al., 2003
	Microsporum gypseum	Chadeganipour et al., 2001
	Myrothecium verrucaria	Gershon et al., 1978
	Penicillium commune	Sjögren et al., 2003
	Penicillium roquefortii	Sjögren et al., 2003
	Pichia anomala	Sjögren et al., 2003
	Rhodotorula mucilaginosa	Sjögren et al., 2003
	Saccharomyces cerevisiae	Stratford et al., 1996

Fatty acid	Species	References
Undecanoic acid (C11:0)	*Myrothecium verrucaria*	Gershon et al., 1978
	Saccharomyces cerevisiae	McDonough et al., 2002
	Trichoderma viride	Gershon et al., 1978
	Trichophyton rubrum	Peres et al., 2010
Dodecanoic (lauric) acid (C12:0)	*Aspergillus niger*	Altieri et al., 2007
	Blumeria graminis	Walters et al., 2003
	Candida albicans	Murzyn et al., 2010
	Colletotrichum gloeosporioides	Yenjit et al., 2010
	Cucumerinum lagenarium	Liu et al., 2008
	Fusarium avenaceum	Altieri et al., 2009
	Fusarium oxysporum	Liu et al., 2008
	Myrothecium verrucaria	Gershon et al., 1978
	Pythium ultimum	Walters et al., 2003
	Rhizoctonia solani	Walters et al., 2003
	Saccharomyces cerevisiae	McDonough et al., 2002
Tetradecanoic (myristic) acid (C14:0)	*Alternaria solani*	Liu et al., 2008
	Aspergillus niger	Altieri et al. 2007
	Candida albicans	Kabara et al., 1972
	Emericella nidulans	Altieri et al., 2007
	Fusarium oxysporum	Liu et al., 2008
	Penicillium glabrum	Altieri et al., 2007
	Penicillium italicum	Altieri et al., 2007
Palmitic acid (C16:0)	*Alernaria solani*	Liu et al., 2008
	Aspergillus niger	Altieri et al., 2007
	Aspergillus terreus	Altieri et al., 2007
	Cucumerinum lagenarium	Liu et al., 2008
	Emericella nidulans	Altieri et al., 2007
	Fusarium oxysporum	Liu et al., 2008
Oleic acid (C18:1)	*Crinipellis pernicosa*	Walters et al., 2004
	Pythium ultimum	Walters et al., 2004
Linoleic acid (C18:2)	*Alternaria solani*	Liu et al., 2008
	Candida albicans	Kabara et al., 1972
	Crinipellis pernicosa	Walters et al., 2004
	Fusarium oxysporum	Liu et al., 2008
	Pyrenophora avanae	Walters et al., 2004
	Pythium ultimum	Walters et al., 2004
	Rhizoctonia solani	Walters et al., 2004

Antifungal properties of fatty acids and their derivatives were studied a long time ago. In Table 1 and Table 2 there are presented the fatty acids from coconut and corn oils that shown antifungal activity against some pathogens.

Some studies showed antifungal potential of saturated fatty acids against *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp. The cultivation of fungi was performed on solid medium at 37.5°C, for 48 hours. All medium chain fatty acids (up to C14) and the unsaturated fatty acids were tested in terms of antifungal activity, in a pH range between 2.0 and 8.0. It was observed that, depending on the pH and chain length, all the tested fatty acids inhibited the molds growth. It was also proved that, the 6-carbon fatty acids were the most active at a pH value lower than 5.0, while the fatty acids with chain length between C7 and C12 were active at neutral pH. Isomeric forms of the unsaturated fatty acids have been shown to be less active than the corresponding fatty acids, but the degree of unsaturation increased the antifungal potential (Thormar, 2011).

Fungistatic effect of fatty acids was tested against *Trichophyton* spp. strains. The results showed that the antifungal activity of fatty acids increased with fatty acids chain length to the point when the solubility became the limiting key factor. Unsaturated fatty acids were more active than the saturated ones. It was noted that, for the optimal inhibitory activity against *Aspergillus* spp. the length of the chain should have 11 carbons, while for the *Trichophyton* spp., it should have 13 to 14 carbons. It was also observed that, particularly for short chain fatty acids, the antifungal activity increased by decreasing the pH till 4.5 (Peres et al., 2010).

Fungicidal and fungistatic differences were presented in various scientific reports but it seems like, these terms were used superficial in the past. According to this approach, fungistatic action is the one that inhibits or prevents fungal growth, while fungicidal activity destroys them (Murzyn et al., 2010). Medium chain fatty acids, by their mechanism of action developed an inhibitory effect on *Microsporum* spp. metabolic pathway, which was more effective with increasing chain length and decreasing the pH (Murzyn et al., 2010).

Capric acid (C10:0) and lauric acid (C12: 0) have antimicrobial activity against *Candida albicans* strains (Bergsson et al., 2001).

Table 2. Antifungal activity of fatty acids from corn oil

Fatty acid	Species	References
Palmitic acid (C16 :0)	*Alernaria solani*	Liu et al., 2008
	Aspergillus niger	Altieri et al., 2007
	Aspergillus terreus	Altieri et al., 2007
	Fusarium oxysporum	Liu et al., 2008
Oleic acid (C18:1)	*Crinipellis pernicosa*	Walters et al., 2004
	Pythium ultimum	Walters et al., 2004
Linoleic acid (C18:2)	*Alternaria solani*	Liu et al., 2008
	Candida albicans	Kabara et al., 1972
	Crinipellis pernicosa	Walters et al., 2004
	Fusarium oxysporum	Liu et al., 2008
Linolenic acid (C18:3)	*Crinipellis pernicosa*	Walters et al., 2004
	Pyrenophora avanae	Walters et al., 2004
	Pythium ultimum	Walters et al., 2004
	Rhizoctonia solani	Walters et al., 2004

Antiviral Activity

Coconut oil is very effective against a wide range of viruses, such as: *Epstein Barr* virus, influenza virus, leukemia virus and hepatitis C virus. It was demonstrated that the medium chain fatty acids from coconut oil kill these viruses by destroying their membranes. From all of the saturated fatty acids, lauric acid presents the greatest antiviral activity prior to capric acid (C10:0), caprilic acid (C8:0) and myristic acid (C14:0). It seems like, the monoglycerides from the composition of coconut and corn oils are more active against viruses compared to diglycerides and triglycerides. It was demonstrated that the monolaurin solubilizes the lipids and phospholipids from the membrane of the virus causing the disintegration of the viral body membranes (Arora et al., 2011).

Anti-Protozoal Activity

There are few reports which demonstrated the anti-protozoal activity of vegetable oils of *Hypericum* species (Moon, 2010; Zofou et al., 2011) based on the lipophilic components of the oil and its extract. A few studies have shown that long-chain fatty acids and β-sitosterol contributed to inhibit the protozoan organisms (Nweze et al., 2011).

The most important tropical parasitic disease is malaria. World Health Organization (WHO) estimates that there are around 300-500 millions clinical cases of malaria each year, and approximately 1 million deaths occur every year (Thayer, 2005). Malaria is caused primarily by four *Plasmodium* species, but *Plasmodium falciparum* is responsible for the most severe form of the disease, causing 90% of deaths in Africa (Thayer, 2005).

Antimalarial treatment is an area with a continue rising due to the limited number of drugs towards the parasites that are not resistant (Thayer, 2005). The fact that antimalarial fatty acids can inhibit fatty acid synthesis of *Plasmodium falciparum* was considered a pest control strategy.

Scientific results on antimalarial properties showed that the most effective activity in killing the parasite, *Plasmodium falciparum,* are polyunsaturated fatty acids (Kumaratilake et al., 1992). Therefore, with the increasing of unsaturated grade, it's also increasing their toxicity against *Plasmodium falciparum*. The order in which the fatty acids toxicity decrease is: docosahexaenoic acid (C22:6); eicosapentaenoic acid (C20:5); arachidonic acid (C20:4); oleic acid (C18:1); docosahexaenoic acid (C22:0). On the other hand, the concentrations of 20-40 mg/mL of docosahexaenoic acid (C22:6) were the most efficient in the breaking of the malaria parasite. The fatty acids methyl esters were reported as the effective free fatty acids in killing the parasite. These investigations led to the hypothesis that the lipid peroxidation is the responsible mechanism for the antiplasmodial activity of unsaturated fatty acids (Kumaratilake et al., 1992).

Other studies have also shown that the concentrations of 6.2 mg/mL of C18 fatty acids had an inhibitory effect against *Plasmodium falciparum*. The highest inhibitory effect against the parasites was depending on the degree of unsaturation and was given by the 96 µg/mL linolenic acid (C18:3), followed by 76 µg/mL linoleic acid (C18:2) and 23 µg/mL oleic acid (C18:1) (Kumaratilake et al., 1992). Corn oil is considered the most important source of polyunsaturated fatty acids, compounds that have inhibitory effect against *Plasmodium falciparum*.

These are important results because they highlight once again the highest potential of fatty acids with 18 carbons length on antimalarial activity. In consequence, further exploitation of antiplasmodial function compounds, from various vegetable oils and fats, is still needed to be made.

Practical Implications

Antimicrobial activity and low toxicity composition of fatty acids and their derivated compounds obtained after lipids hydrolysis suggest that they may have a wide range of applications, as ingredients in formulation of a large category of products, as food preservatives, in cosmetics and pharmaceutical industries and also as pests control products.

Antimicrobial compounds from vegetable oils have a great potential in obtaining personal hygiene products, like deodorants. For instance, the commensal microorganisms break down axial secretions in secondary compounds responsible for odors (Labows et al., 1999). Therefore, the use of antimicrobial lipids in antiperspirants composition may create an inhibitory effect on the action mechanism of these microorganisms, thus the amount of odor is significantly reduced.

Fatty acids and their monoglycerides were both used as food preservative and ingredients in cosmetic and pharmaceutical industries (Shibasaki et al., 1978; Bergsson et al., 2001; Drake et al., 2008). The advantage of using lipids and derivates with microbicide action in cosmetic products formula is that they cannot cause skin irritations. The main reason for their use as a preservative in cosmetics is the low toxicity and higher antimicrobial activity. There are many examples of using lipids as preservative system in cosmetics, such as shampoos, conditioners and moisturizers (Puri et al., 2009). Lipids were conventionally used as solvents in many pharmaceutical products composition, as oil phase in creams, as emulsifiers, antioxidants and stabilizers. Usually, these are used to facilitate penetration of the micro- and nano- drugs particles into the skin and as an secondary effect they can also exert antimicrobial activity and preservation strategies (Schafer-Korting et al., 2007).

The development and use of biodegradable packaging materials obtained from natural polymers are important growing tendencies, especially in the food industry. The concept of intelligent packaging with antimicrobial and antioxidative films based on natural polymers is an innovative one (Yam et al., 2005).

A combination of virgin coconut oil (VCO) and chitosan was used in the preparation of an edible composite film, with role in food industry, for biochemical and microbiological stability control, thus being used for packaging dry as well as moist foods (Binsi et al., 2013). The researchers evaluated the effect of VCO incorporation in different concentrations on chitosan emulsion system. Chitosan have different degrees of deacetylation thus, this biopolymer produces water-oil-water emulsions. The results of the study provided that, the addition of VCO in the system increased the film thickness and marginal reduction in film transparency (Binsi et al., 2013). On the other hand, between chitosan and the compounds from VCO different interactions were established, such as hydrogen/covalent bonding (between hydroxyl group of the aromatic ring of polyphenolic compounds and the reactive groups of chitosan) and electrostatically interaction (between short/medium chain fatty acids of VCO oil and cationic molecules of chitosan) (Rohn et al., 2002; Jumaa et al., 2002). The implications of VCO-polymer interactions are that the VCO may improve the barrier

properties of composite films, decrease the oxygen, carbon dioxide and water vapor permeability, reduce the moisture sorption value of chitosan film, improve the elongation at break values and film flexibility (Binsi et al., 2013). So, when there were combined the effects of medium chain fatty acids from coconut oil with those of chitosan it became possible to obtain an edible film used for packaging dry and moist foods, for spoilage control and food safety assurance.

A new field, in which antimicrobials compounds derivated from vegetal lipids could be used in infant formula (Issac et al., 1995). Presentlly the infant formula contains triglycerides, which are hydrolyzed in the gastrointestinal tract of children in order to produce antimicrobial fatty acids and their monoglycerides, in a manner similar to milk triglycerides (Canas-Rodriguez et al., 1966; Smith et al., 1966).

The antimicrobial activity of medium chain fatty acids (MCFAs) or their derivatives has been known for a long time (Kabara et al., 1972) but no experiments have been concentrated on their use as a potential source of *in situ* system antimicrobial compounds. Decuypere and Dierick (2003) proposed an interesting *in vivo* bioconversion process in order to obtain a progressive enzymatic release of MCFA in stomach and foregut. Following this approach it is easy to avoid taste aversion of non-esterified MCFA, given in high doses over a short period of time and the acid-base balance disturbance, in the animal. Further, a combination of triacylglycerols with an adequate lipase, used in a specific preparation, offers a gradual release, in the foregut, of MCFA, eventually together with their monoacylglycerols. This approach it seems to be preferable for a valuable alternative to in-feed antibiotics (Decuypere & Dierick, 2003). Also, the mixture can offer other advantages such as growth promotion and gastrointestinal disease treatment.

Parfene et al. (2013) also demonstrated the possibility to obtain food biopreservation as an effect of saturated fatty acids released from vegetal fats (coconut, palm and shea), by *in situ* solid state cultivation of *Yarrowia lypolitica* selected strains. There was studied the fatty acids production in restrictive environmental conditions, similarly with those applied for food preservation, i.e., at low temperature (4°C) and reduced level of water activity (a_w 0.93). The gradual release of fatty acids from lipids was demonstrated in very simple experimental conditions. These results developed new concepts in food preservation as a consequence of using natural preservatives, without influencing the sensorial and nutritive characteristics thus having a great impact on spoilage control and food safety assurance.

STIMULATIVE EFFECTS OF FATTY ACIDS ON MICROBIOTA

Gene Expression Regulation in Escherichia Coli by Fatty Acids

Studies about the regulation of genes from lipid metabolism by fatty acids were made in both unicellular and multicellular organisms. In *Escherichia coli*, fatty acids metabolism is tightly regulated in correlation with the environment changes. The model microorganism, *Escherichia coli* used long-chain fatty acids (LCFA) that are transported into the cell by the outer membrane protein FadL (Pech-Canul et al., 2011).

Medium (C7 to C11) or short (C4 to C6) chain lengths fatty acids cannot induce synthesis of the enzymes encoded by *fad* in *E. coli.* As a consequence, LCFAs represents the sole

carbon and energy sources and is coupled to the citric acid cycle via β-oxidation. Inside the cells, acyl-CoA synthetase enzyme, which is encoded by *fad*D gene, catalyze the formation of fatty acyl-CoA thioesters that are substrates for β-oxidation, which are further cleavaged into acetyl-CoAs (Campbell et al., 2003). Also, the fatty acyl-CoA thioesters are used in phospholipid biosynthesis and cellular signaling. In the first step of β-oxidation process, the acyl-CoA is converted to enoyl-CoA by FadE. The next steps in the fatty acids degradation are made by a tetrameric protein complex encoded by *fad*A and *fad*B genes, each having a double copy (Henry and Cronan, 1992; Campbell and Cronan, 2001). So, for the growth of *E. coli* cells in medium with LCFAs it is necessary that the transcription of genes from the *fad* regulon to be repressed. This function is made by the transcription factor FadR that is de-repressed (Gui et al., 1996). In consequences, FadR regulates both the conversion of fatty acids to acetyl-CoA and the utilization of this final product by the citric acid cycle.

Stimulative Effect of Polyunsaturated Fatty Acids on Lactic Acid Bacteria

The gastrointestinal tract (GI) has the most diverse microbiota that represents the largest source of non-self antigens in the body. The gut microbiota content can vary in the gut regions and can influence susceptibility to chronic disease of the intestinal tract. Probiotic microorganisms help in the maintenance of the digestive tract ecosystem and prevent the colonization with pathogenic bacteria at this level (Semih, 2014). Dysbiosis appears when an imbalance or an alteration of the intestinal gut microbiota function and composition are produced. Some factors such as age, diet, life style and disease are causing intestinal dysbiosis (Agans et al., 2011; Turnbaugh et al., 2009; Seksik et al., 2003).

Probiotics are „live microorganisms which, when administered in adequate amounts confer a health benefit on the host" (Schlundt, 2012). Data existing in the literature offers many information regarding the probiotic influences on population of beneficial microorganisms in terms of growth and stimulatory effect (Vandenbergh, 1993, Dima et al., 2014).

The lipids from diet specifically influence the population of lactic acid bacteria (LAB). *In vivo* studies on gnotobiotic piglets showed that, the oral administration of oils containing polyunsaturated fatty acids (PUFAs) enhances the number of *Lactobacillus paracasei* cells in the small intestine and inhibits digestive tract pathogens (Bomba et al., 2002).

Tween 80 is usually added to the growth media of lactobacilli in order to improve aerobic growth rates, glucosyltransferase secretion (Jacques et al., 1985) and glycine-betaine accumulation in LAB (Guillot et al., 2000). Tween 80 contains up to 90% oleic acid that is incorporated into LAB membrane, as it was experimentally proved (Johnsson et al., 1995; Partanen et al., 2001).

Corcoran and collaborators (2007) studied the effects of various C18 fatty acids with 0-2 bonds in the two configurations, cis and trans, on rate survival of a *Lactobacillus rhamnosus* GG strain, in the simulated acid conditions of gastric juice. The protective effects to the acidic environment was offered only by oleic and vaccenic acids [C18: 1 (11t)] among elaidic, linoleic and stearic acids and two conjugated linoleic acid (CLA) isomers (Corcoran et al., 2007; Kankaanpää et al., 2014). On the other hand, oleic acid increased the survival rate of a *Lactobacillus rhamnosus* strain, in 90 minutes after exposure to simulated gastric juice (by 1500-fold) compare to the control. Moreover, when the culture media was supplemented with

Tween-80, the content of cyclopropane fatty acids (CFAs) increased as a protective function to acidic environmental conditions (Budin-Verneuil et al., 2005; Klaenhammer et al., 2005). It seems like, an increased level of CFA in the membrane composition of lactobacilli is found in other stressful conditions for the cells, such as, higher concentration of NaCl (1M, for *Lactobacillus casei*) or the growth condition below 20°C or above 35°C (for *Lactobacillus fermentum*) (Suutari & Laakso, 1992; Machado et al., 2004). Among the utilization of exogenous oleic acid source by probiotic lactobacilli, in order to increase their acid survival, it was suggested that, the higher content of oleic acid found in the membrane is reduced to stearic acid (Corcoran et al., 2007).

Literature data specified that an important factor in the probiotic strains` growth is PUFA`s concentration levels. *In vitro* studies prove that a low concentration of gamma-linolenic acid and arachidonic acid (5 µg/mL) are essential for growth promoting and mucus adhesion of *L. casei* strains (Kankaanpää et al., 2001; Laparra & Sanz, 2010). The adhesion of probiotics depends on the hydrophobicity of the bacterial cell surface, being favorized the cells with a hydrophobic surface than with a hydrophilic one (Wadstrom, 1990; Kankaanpää et al. 2004). These are valuable information that can be used in order to increase the adhesion ability of bacterial cells if it is taken in account that the environmental growth conditions can affect bacterial membrane fluidity. So, by adhering to epithelial cells, the probiotics block the binding sites and protect the host from pathogens to initiate invasion.

In vitro studies on HT-29 epithelial cells were made in order to test the capacity of *L. johnsonii* strains to decrease *S. enterica* serovar *typhimurium* UK1-lux invasion (Muller et al., 2011). It was shown that the *L. johnsonii* proved its exclusion properties just when the probiotic bacteria are growing in minimal medium supplemented with fatty acids. In these conditions, *L. johnsonii* reduced significantly *Salmonella* spp. adhesion and invasion. If the *L. johnsonii* was grown in a medium supplemented with linoleic and linolenic acids, its adhesion was also possible even when the HT-29 cells were infected with *Salmonella* spp. (Muller et al., 2011). The mechanism proposed in order to explain how probiotics prevent pathogens from infecting cells is that they sterically hinder the adherence of pathogens (Fourniat et al., 1992).

The changes induced in the bacterial cells composition by PUFAs after their addition in the growth medium, can be correlated with the regulation of the fatty acids degree unsaturation, cyclization, proportions of CLA and PUFAs with C20 to C22 length (Kankaanpää et al., 2004).

In order to enhance the efficacy of probiotics, there were made combination between probiotics and components of natural origin, such as oligosaccharides, phyto-components, nutrients and growth factors, proteins, PUFAs, organic acids and bacterial metabolites (Pollmann et al. 1980; Galfi and Bokori, 1990; Gibson and Roberfroid, 1995; Yadava et al. 1995). It seems like the synergic effects of maltodextrin KMS X-70 and PUFAs (oil blend containing 0.1 g total n-6 PUFAs, 1.0 g total n-3 PUFAs, 2.6 g total monounsaturated fatty acids and 0.9 g total saturated fatty acids) is useful for potentiating the probiotic effect in the small intestine of gnotobiotic piglets (Bomba et al., 2002).

Implications of Fatty Acids in Rumen Methanogenesis

In vitro study on rumen fermentation microbiota and methanogenesis emphasized the synergistically influence of medium chain fatty acids, like capric acid (C10:0), lauric acid (C12:0) and myristic acid (C14:0). Lauric (C12:0) and myristic (C14:0) acids showed their potential to decrease rumen methanogenesis and the number of methanogens. When the two fatty acids were not combined the effects exhibited some limitations. *In vivo* incorporation of coconut oil in sheep diet caused a reduction from 26% up to 73% in the methane (CH4) emission and up to 39% after using a oil, in each day, in feed composition for beef heifers (Panyakaew et al., 2013).

Kongmun et al. (2011) investigated the effect of coconut oil and garlic powder supplementation on digestibility of nutrients, rumen fermentation, ecology and microbiota and also methanogens diversity. It was also observed that methane production was dramatically reduced while the number of amylolytic and proteolytic bacteria increased and the protozoal population decreased by 68–75%. The study demonstrated that the coconut oil could be efficiently used in the animal rumen after diet supplementation. Furthermore, coconut oil digestion could provide good fermentation end products and could also decrease the methane gas production without changing nutrient digestibilities.

Stimulation Effect of Corn Oil on Microorganisms' Stabilization

Corn oil was used in the preparation process of water-in-oil emulsions (W/O) with the aim of long-term stabilization of microorganisms by cells encapsulation. In these types of emulsions, there were included the larvacide *Lagenidium giganteum* and the green alga *Chlorella vulgaris* and it was observed that cells had the possibility to remain hydrated during storage and delivery, without being contaminated. The researchers proved that, during room temperature storage, the water-in-oil emulsion prolonged the viability of the microalgae and larvacide, *Lagenidium giganteum* (VanderGheynst, 2013). Water-in-oil (W/O) emulsions formula overcame the problems of industrial microbial agents regarding the contamination and also about the decreasment of cells viability during distribution, storage and delivery.

Effect of Polyunsaturated Fatty Acids and Enthomopatogenic Fungi

Linoleic and oleic acids, the two main components of corn oil can be used to enhance the thermotolerance of enthomopatogenic fungus (Kim et al., 2011) An entomopathogenic fungus is a fungus that kills the parasite of insect by its fixation on the external body surface of insects. The most important enthomopathogens that have been commercially produced were *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*. In the mode of action, these entomopathogens act on the cuticle of insects, penetrates it in order to proliferate, to produce the toxins and to kill the insect. It seems like the storage and applications of the fungi as biopesticides have a disadvantage related to exposure to high temperature conditions or to high humidity, which can drastically decrease the shelf life of those fungi. There were made attempts to increase their thermotolerance with unsaturated fatty acids (Kim et al., 2010). The results showed that, the treatments with unsaturated fatty acid from corn oil enhanced conidial

thermotolerance in a dosage-dependent manner. It means that, the more linoleic or oleic acids was added to ground corn, the better thermotolerance of conidia was obtained (Kim et al., 2010). So, from an industrial point of view, the development of a ground corn oil mixture, as a medium in order to obtain more thermotolerant conidia is an inexpensive approach that can allow commercial productions of fungal biopesticides to be more competitive than others.

Effects of Fatty Acids on Exo-Biopolymer Production

Exo-biopolymers produced by some fungi have been recognized as high value biomacromolecules from the last twenty years. The composition and the amount of exo-polysaccharides (EPSs) are genetically determined and are dependent to the culture medium composition and to the cultivation conditions during fermentation such as pH, temperature, oxygen concentration and rate of agitation.

The effects of some fatty acids, such as oleic, palmitic and stearic acids on exo-biopolymer production and mycelial growth of *Cordyceps militaris* were investigated (Park et al., 2002). *C. militaris* is the oldest source of some useful chemical constituents but with active principles used for the new herbal biotechnology. These compounds act as anti-inflammatory, anti-oxidant/anti-aging, anti-tumor/anti-cancer/anti-leukemic, anti-proliferative, anti-metastatic, immunomodulatory, anti-microbial, anti-bacterial, anti-viral, anti-fungal, anti-protozoal, hypolipidaemic, anti-angiogenetic, anti-diabetic, anti-HIV, neuroprotective, liver-protective, reno-protective as well as pneumo-protective (Das et al., 2010). A few long chain fatty acids (oleic, palmitic and stearic acids) with stimulatory effects on the mycelial growth were added in the medium in order to optimize the cost of the process. From the three fatty acids tested, the oleic acid was found to have the best stimulatory effect when added in the concentration of 0.2% (v/v). Palmitic and stearic acids also stimulated the growth mycelia compare to linoleic acid that drastically suppressed it (Park et al., 2002). Moreover, oleic and palmitic acids increased the exo-biopolymer concentration contrary to the effect of linoleic and stearic acids (Yang et al., 2000; Kendrick and Ratledge, 1996).

Enzymes Biosynthesis in Medium with Coconut Oil by Solid State Fermentation

Enzymes are indispensable components of all types of cells that have been extensively used in many industrial sectors and for environmental protection. Enzyme synthesis process was improved over the years in order to reach the best relation cost - product.

Coconut oil cake is a waste by-product obtained after the oil extraction from dried coconut. The major compounds from the coconut oil cake are short chain saturated fatty acids, which were proposed to be used as a carbon substrate source in solid-state fermentation (SSF) for enzymes biosynthesis. This approach in which the coconut oil cake represents a cheap way for the bioproducts synthesis is a new one and was adopted in the last years. The first report on enzymes synthesis using coconut oil cake was published by Ramachandran and coworkers, in 2004. The researchers optimized the production of α-amylase using a fungal culture of *Aspergillus oryzae* strain in SSF cultivation system on a medium based on raw coconut oil cake and it was obtained an α-amylase activity of 1372 U/g dw, in 24 h. Eight

years later, the enzyme, α-amylase was also produced in a SSF process based on the same substrate but using a *Streptomyces* spp. strain (Maheswari and Soundariya, 2012). Another enzyme, L-asparaginase, was obtained under SSF using *Serratia marcescens* (Ghosh et al., 2013). L-asparaginase is an important enzyme that catalyzes the hydrolysis of L-asparagine to aspartic acid and ammonia which was found to be an anti-cancer chemotherapy drug, thus being involved in the treatment of acute lymphocytic leukemia. The researchers used the response surface methodology in order to optimize the L-asparaginase production took into account four variables: substrate amount, moisture of substrate, temperature and pH. They concluded that, the coconut oil is an efficient substrate that can reduce the cost of L-asparaginase production, under optimal conditions (Ghosh et al., 2013).

Oxygenated Fatty Acids Synthesis by Microorganisms

In the industrial materials, the hydroxy, keto or epoxy fatty acids are used in many different types of products including resins, waxes, nylons, plastics, corrosion inhibitors, cosmetics, or coatings (Hou et al., 1998). Moreover, the hydroxy-fatty and keto-fatty acids are important as plasticizer, surfactant and lubricant. The oxygenated fatty acids can be obtained through the oxygen-dependent hydroxylations of some fatty acids, from vegetable oils, added in the growth media of specific microorganisms. A strain of *Torulopsis* spp. was able to hydroxylate palmitic, stearic and oleic acid to 15- or 17-hydroxy-fatty acids (Shanklin and Cahoon, 1998). In the hydroxylation process of palmitic acid by *Bacillus megaterium* ALA2 is involved Cytocrome P 450-dependent monooxygenase complex, in order to obtain three hydroxy palmitic acids, ω -1, ω-2 and ω-3 (Hou, 2005). Through β-oxidation, the yeast *Dipodascopsis uninucleata* converts linoleic acids (9Z, 12Z-octadecadienoic acid) to 3(R)-hydroxylated metabolites of shorter chain length (Venter et al., 1997). Further, through ω-oxidation, the enzyme system of *Pseudomonas oleovorans* strain is able to catalyze biotransformation of medium chain fatty acids, such as octanoate, decanoate, laurate and myristate or long-chain fatty acids, palmitic and stearic acids (Kusunose et al., 1964).

On the other hand, the enzymatic system of *B. megaterium* ALA2 is able to produce compounds with anticancer activity (two tetrahydrofuranyl fatty acids -THFAs), starting from linoleic acid (Hou et al., 1998; Gardner et al., 2000). So, the microbial conversion of medium and long chain fatty acids from vegetable oils sources, into sophorolipids gives new application possibilities in various sectors due to either their emulsifying, antimicrobial activity or other beneficial properties.

CONCLUSION AND PERSPECTIVES

Fatty acids and related metabolites derivated from coconut and corn oils are known to have an excellent biological activity with impact on microorganism metabolism, on cells nutrition and energy uptake. They are also recognized to have important antimicrobial activity. Their accessibility or their limitations, in time and space, in terms of cells contact have an important impact on biological processes regulation. The type and also the

concentration of fatty acids are important factors that influences various biological processes that take place into natural microbiota or in the starter cultures.

The antimicrobial potential of vegetable oils and their derivatives (acylglycerols and fatty acids) has practical importance in obtaining biopreservatives, as natural ingredients for commercial products, thus obtaining biochemical and microbiological stability and safety assurance. For practical applications is important to obtain active compounds using cheaper processing conditions like *in situ* bioconversions of lipids with enzymes or with whole cells. The positive effects of coconut and corn oils on microorganism's metabolism is prooved by *in vivo* applications, by stimulating the activity of different microbiota (gut microbiota, epithelial microbiota, rumen microbiota etc.) and also by *in vitro* approaches, in order to induce the biosynthesis of microbial metabolites, to produce active biofilms or to have surfactant role. The practical and scientific perspectives to use oils and bioderivates in microbiology or in applied biotechnology can also emphasize other new and modern practical applications.

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Chapter 9

NUTRITIONAL AND HEALTH BENEFITS OF COCONUT OIL

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ABSTRACT

Coconut oil is one of the most widely used natural oils which assure good health to mankind. It is rich in saturated fats, especially medium chain triglycerides that enhance digestion and other metabolic functions of the body. Applications of coconut oil extend from the kitchen to the dressing room; it is stable edible oil with unique medicinal properties. It gets easily absorbed in the body and is a nature mimic of the human breast milk fat and hence **it is** used in infant formulae. Coconut oil is extensively used to cure certain dietary disorders, dermatitis, psoriasis etc. The non-edible applications of coconut oil are in the soap industries, pharmaceuticals, cosmetics, rubber substitutes, synthetic resins etc. After a period of controversy, researchers concluded that pure coconut oil promises a healthy heart to the consumers by increasing the total HDL (High-density lipoprotein, 'good cholesterol') content of the body. Thus, coconut oil is a pure food that can play an important role in a well-balanced diet.

Keywords: coconut oil, saturated fats, nutritional benefits, cholesterol

INTRODUCTION

The coconut (*Cocosnucifera*) is the most extensively grown palm with great importance. Coconut tree meets almost all basic needs of mankind including food, shelter, clothing,

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health, wealth, etc. and for this reason, they are known as ‘Kalpavirksha’ or ‘Tree of Life’ (Lombard, 2001). Edible product of dried fruit (copra) of coconut is coconut oil which is equally popular just as other coconut products such as coconut water and meat; and indeed, it is the chief source of cooking oil consumed in many South-east and East Asian regions. Additionally, it has also found wide applications in traditional medicines and as carrier oil in pharmaceutical industries besides being widely used in pharmaceuticals, cosmetics andbaking industries. Moreover, it is one of the main ingredients in soap making and infant formulae.

Nutritious products, other than coconut oil, obtained from coconut tree include virgin coconut oil and Neera. Virgin coconut oil (VCO) differs from commercial coconut oil in the way it is processed. The latter is produced from copra or dried coconut meat and undergoes refining processes to make the oil edible. The refined oil produced is called RBD (Refined, Bleached and Deodorized) coconut oil, which is largely used as cooking oil. VCO production does not subject the oil to refining processes since the oil produced is already edible. In effect, the term Virgin refers only to the process and not the chemical properties which are essentially the same in both RBD oil and VCO. Their effect on health would likewise be the same, given the same medium chain fatty acid (MCFA) compositions. Neera is the sweet, oyster white colored sap, tapped from the immature inflorescence of coconut. It is a delicious health drink and a rich source of natural sugars, minerals and vitamins.

Dietary fats and oils are the most concentrated form of energy as they yield 9 calories/g, whereas, carbohydrates and proteins yield only 4 calories/g. The significance of dietary oils is that they provide essential fatty acids (EFA), as their name defines, and are absolute essential nutrients required by the body (Baba et al., 1982). Coconut oil is a dense source of energy; 100g oil provides about 862 kilocalories of energy. Its unique combination of fatty acids can have profound positive effects on health. Coconut oil has low polyunsaturated fatty acid; it is very stable and resistant to oxidation. This makes it excellent cooking oil, thereby protecting our cells against damage. Today, coconut oils available in the market vary dramatically in terms of quality. Low-quality coconut oils, which should be avoided, are processed by chemical extraction, using solvent extracts and chemical residues. Many of them are hydrogenated, bleached and deodorized which produce higher yields and are quicker and less expensive. High-quality coconut oil is a completely different product and is truly the healthiest oil we can consume (Carandang, 2008). It is a much safer alternative to other popular oils such as canola oil, where most of its omega-3s are transformed into trans-fats during the deodorization process, which increases the dangers of chronic diseases.

PHYSICAL CHARACTERISTICS

Coconut oil is a colorless to pale brownish yellow oil and is white in its solid form. Unrefined coconut oil melts at 24-25°C (76°F) and smokes at 177°C (350°F), while refined coconut oil has a higher smoke point of 232°C (450°F). Though coconut oil is known as triglyceride or lipid, it also contains minor proportions of mono- and di-glycerides and has the highest content of glycerol (13.5% to 15.0%). Coconut oil is a highly saturated oil (about 90%), and 60% of its total fatty acid composition are medium-chain fatty acids (MCFA) with a chain length of 6 to 12 carbon atoms (Bach &Babayan, 1982; Bhatnagar et al., 2009; Gopala Krishna et al., 2010; Rosell et al., 1985). The majority of fats in the human diet are

composed almost entirely of long chain fatty acids (LCFA), making coconut oil unique among other dietary fats. Among the most stable of all oils, coconut oil is slow to oxidize and thus resistant to rancidity, lasting up to two years due to its high saturated fat content (Lauretes et al., 2002).

HEALTH AND NUTRITIONAL ASPECTS

Coconut oil is one of the few foods that can be classified as a "super food or functional food." The health and nutritional benefits derived from coconut oil are unique and compelling (Enig, 1996; Dayrit et. al, 2008), th**ey** had stated that medium chain triglyceride, a fraction of coconut oil, has been identified as an important, medically efficacious food.

Increases Digestion and Nutrient Absorption

Most of the fatty acids in the diet are long-chain fatty acids (LCFA) whereas coconut oil is predominantly rich in medium-chain fatty acids (MCFA). They are low molecular weight compounds and are highly soluble in biological fluids. These properties make them unique.Medium chain fatty acids are metabolized differently compared to LCFA (Fushiki, et al., 1995). They are absorbed directly into the portal circulation without re-esterification in intestinal cells (Ferreira et al., 2012). The MCFA are partly independent of the carnitine transport mechanism into the mitochondria of the liver and are rapidly oxidized for the production of energy (Rubin et al., 2000) In contrast, the long-chain fatty acid (LCFA) commonly found in most diets are incorporated into chylomicrons after being absorbed in the intestine where they are subjected to re-esterification and then reach the bloodstream via the lymphatic system (Ferreira et al., 2012). Most LCFA are stored in the adipose tissue (Rego Costa et al., 2012). As a result, coconut oil is used in special food preparations for those who suffer digestive disorders and have trouble in digesting fats (Hoahland& Snider, 1943).

Table 1. Fatty Acid Composition of Coconut Oil

Name of fatty acid	Percentage	Type of fat
Lauric acid	45% to 52%	Saturated fat
Myristic acid	16% to 21%	Saturated fat
Caprylic acid	5% to 10%	Saturated fat
Capric acid	4% to 8%	Saturated fat
Caproic acid	0.5% to 1%	Saturated fat
Palmitic acid	7% to 10%	Saturated fat
Oleic acid	5% to 8%	Monounsaturated fat
Palmitoleic acid	in traces	Saturated fat
Linoleic acid	1% to 3%	Polyunsaturated fat
Linolenic acid	up to 0.2%	Unsaturated fat
Stearic acid	2% to 4%	Saturated fat

Medium-chain fatty acids also improve the absorption of many other nutrients. The absorption of minerals (particularly calcium and magnesium), B vitamins, fat soluble vitamins (A, D, E, K and beta-carotene) and also amino acids **has** been found to increase when infants are fed a diet containing MCFA. In addition, coconut oil can be digested by the salivary lipase, getting absorbed very fast to give energy like carbohydrates. All other fats need the pancreatic lipase for digestion that the infants do not have (Murthi et al., 1987).

Reduces Cardiovascular Diseases

Cardiovascular diseases (CVD) are one of the most common diseases in different parts of the world especially in developing countries. Earlier it was believed that as coconut oil contains a high amount of saturated fats, it initiates a rise in blood cholesterol levels and promotes heart disease. Later,Kurup and Rajmohan(1995) conducted a study on 64 volunteers and found no statistically significant alteration in the serum total cholesterol or LDL cholesterol from baseline values. Kaunitz and Dayrit (1992) reviewed epidemiological and experimental data regarding coconut-eating people and noted that the population studies show that dietary coconut oil does not lead to high serum cholesterol or to high coronary heart disease mortality or morbidity.The research over four decades concerning the benefits of coconut oil in controlling heart disease is quite clear; coconut oil has been shown to be beneficial. The saturated fats in most hydrogenated oils are of a far worse kind. These are high chain triglycerides that the body cannot break down as efficiently as medium chains fatty acids. This means that they build up as fatty deposits around heart and arteries increasing the risk of coronary heart disease. Replacing these oils with coconut oil therefore actually decreases the risk of heart disease (Bellenand et al., 1980; Halden&Lieb, 1961; Nagaraju&Lokesh, 2008; Tsuji et al., 2001). Finally researches concluded that natural coconut oil significantly increases HDL(good cholesterol) which promotes healthy heart but the use of hydrogenated coconut oil may increase LDL(low density lipids, "bad cholesterol") (Harris et al., 1993; Dayrit, 2003).

Neurological Effects

The brain is the functionally and metabolically active organ of the body (Fernstrom, 2000). When the blood glucose levels fall, the brain requires an alternative source of fuel instead of glucose for its function. Ketones are high-energy fuel produced in the liver, specifically to nourish the brain. A common feature found in Alzheimer's disease and many other neurological disorders is chronic inflammation which interferes with normal glucose metabolism. This defect in energy conversion starves the brain cells causing them to degenerate and die. Therefore, if enough ketones are available on a continual basis, they could satisfy the brain's energy needs. However, ketones are only produced when food, particularly carbohydrate, consumption is very low. When coconut oil is consumed, a portion of the MCFA is automatically converted into ketones, which in turn, enhance the functioning of the brain. Case histories of Alzheimer's patients receiving coconut oil have demonstrated that it is possible not only to stop the progression of the disease, but also to bring about significant improvement.

Improves Immune Support

Maintaining a healthy diet, results in a well-balanced immune system. Including unsaturated oils in the diet disturbs the smooth functioning of the immune system. Here, antioxidants are frequently used up to remove free radicals produced by these oils which ultimately slowdown the immune system. In the case of natural coconut oil, saturated fatty acids are stable and do not oxidize easily, which in turn, reduce the use of antioxidants. Caprylic and Capric fatty acids in coconut oil, when broken down, are converted into specific antibodies that enhance the body's defenses against a range of diseases, both bacterial and viral in nature. Having the right antibodies to fight specific bacteria is central to body's well-being. Hence, including coconut oil in the diet is an easy way to ensure good health. Consumption of coconut oil enhances the metabolic rate, which in turn, accelerates healing processes, cell regeneration and smooth functioning of the immune system. When applied to infected areas, coconut oil forms a chemical layer that protects the infected body part from external dust, air, fungi, bacteria and viruses. Coconut oil is highly effective on bruises because it speeds up the healing process of damaged tissues.

Coconut oil has been demonstrated to have anti-inflammatory, analgesic and fever-reducing properties. This plays a good role in removal of toxins from cells. It is thought to curb inflammation of cells by improving cellular function. The cells become more efficient in removing toxins. Bruce Fife (Fife, 2013), in"Coconut Cures" lists fifteen toxins neutralized in part or whole by coconut oil. These include aflatoxin, *E. coli*endotoxin, and MSG. Thus in different aspects coconut oil supports the immune system and is an ideal food for immune suppressed individuals.

Enhances Antimicrobial Activity

Major saturated fatty acids in coconut oil include 48% lauric acid (an 12 chain saturated fat), 7% capric acid (an 10 chain saturated fat), 8% caprylic acid (an 8 chain saturated fat) and 5% caproic acid (an 6 chain saturated fat) which enhances the antimicrobial properties of coconut oil. Several reports state that these saturated fatty acids and their monoglycerides kill or inactivate microorganisms by lysing the phospholipid bilayer of plasma membrane, interfering in signal transductions, virus reproduction etc. (Kabara, 1978; Hierholzer&Kabara, 1982). Monolaurin, the monoglyceride of lauric acid,possesses antiviral and antibacterial properties. They kill the enveloped bacteria and viruses by solubilizing their phospholipid bilayers. Evidences suggest that MCFA are effective in destroying bacteria like *Staphylococcus*, *Helicobacter Pylori*, *Chalamydia trachomatis*, *Streptococcus*, *Neisseria* etc., fungi like Candida and yeast, protozoans like giardia, viruses including herpes virus, influenza, Epstein-Barr virus, hepatitis C virus, human immunodeficiency virus(HIV), and others (Isaacs et al., 1992).

Anti-Cancer Effects

Coconut oil resists cancer and tumors to a greater extent compared to other unsaturated oils. The protective effect of coconut oil against chemically induced colon and breast cancer

is more profound than that of other oils. They inhibit the carcinogenic agents of colon and mammary glands. In 1987 Lim-Sylianco (Lim-Sylianco, 1987) published a 50-year literature review showing the anti-cancer effects of coconut oil. Growing evidences show that cancer is a metabolic disease characterized by cellular mitochondrial respiratory insufficiency. Cancer cells can only survive and thrive off of glucose and amino acid fermentation. A ketogenic cleanse has been proposed as a means of starving off cancer cell development (Seyfried, 2012).The ideal fuel source for the ketogenic cleanse is coconut oil and virgin coconut oil with its powerful immune boosting properties. The metabolism of fatty acids in coconut oil results in the liberation of ketones that normal cells can utilize but cancer cells cannot. These ketone bodies provide a great anti-inflammatory fuel source for the body that also starves the cancer cells from their nutrient demands.

Good for Diabetics

Recent studies show that coconut oil provides a good protection against insulin resistance, a major cause of diabetics. Medium chain fatty acids in coconut oil put a lowerdemand on the enzyme production of the pancreas. This lessens the stress on the pancreas during meal time when insulin is produced most heavily, thus allowing the organ to function efficiently. MCFAs in coconut oil have a greater ability compared to other oils in developing binding affinity between insulin and cells (Yost et al., 1998) which improves the secretion of insulin, which in turn, controls blood sugar. It also helps in effective utilization of blood glucose.

Combat Tooth Decay

Dental caries is a commonly overlooked health problem, affecting 60 to 90 per cent of children and the majority of adults in industrializedcountries. Present studies by researchers of Athlone Institute of Technology in Ireland shows that coconut oil can act as a natural antibiotic and helps to fight against the sugar loving bacterium that causes tooth decay. Coconut oil which had been treated with enzymes stopped the growth of Streptococcus bacteria, a major sugar loving bacterium that causes tooth decay. With this, coconut oil finds its way into toothpaste and mouthwash as an active ingredient. Since coconut oil facilitates absorption of calcium by the body, it also helps in getting strong teeth (Southward, 2011).

Reduces Body Weight

Coconut oil is a low calorie fat and helps control body weight. In addition, coconut oil stimulates metabolism to get itself metabolized fast to supply quick energy unlike other fats. This also helps control body weight. Most of the coconut oil is medium chain fat, it gets absorbed and metabolized so fast that it rarely gets transported to fat depots like other fats, altering the lipoprotein fractions of blood– another great boon. Changing the food fat to coconut oil could help reduce weight in obese individuals. Additionally, research has demonstrated that due to its metabolic effect, coconut oil increases the activity of thyroid

which is one of the reasons why some people are unable to lose weight (Geliebter et al., 1983; Kaunits, 1970).

Hair Care

Coconut oil is rich in nutrients that are required for hair growth.Regular massage of the head with coconut oil ensures growth of healthy and shiny hair. Coconut oil is a good conditioner and enhances re-growth of damaged hair. It is therefore used for the manufacture of herbal oils, baby shampoos, conditioners and various anti-dandruff products (Rele&Mohite, 2003).

Strengthens Bones

Coconut oil improves the ability of the body to absorb important minerals. This includes calcium and magnesium which are necessary for the development of bones and also reduces the oxidative stress within the bone, which may prevent structural damage in osteoporotic bone. Thus, coconut oil is very useful to women who are prone to osteoporosis (a disease of bone that can cause fractures) after middle age (Hegde, 2006; Hegde, 2009).

SKIN CARE

Coconut oil is anexcellent moisturizer for the skin. It prevents the formation of destructive free radicals and can prevent skin from developing blemishes caused by aging and overexposure to sunlight. When oil is applied over theskin, it aids in exfoliating the dead skin cells which make skin smoother and also reduces wrinkle formation. Coconut oil forms a protective coating over the skin which guards against bacteria. Different formulations of coconut oils protect skin from various skin problems like psoriasis, dermatitis, eczema and other skin infections. Using its antimicrobial property, it also protects the skin from microbial infections. Therefore, coconut oil is one of the basic ingredients in many of the body care products like soap, body lotions, baby oils, sun screens etc. (Gopala Krishna et al., 2010)

Prevents Acne or Pimples

Acne is common during the stage of adolescence due to some hormonal changes experienced in puberty. One of these hormonal changes is the abnormally excessive secretion of oil (sebum) by the sebaceous glands. When bacteria mix up with sebum, there is acne eruption. Coconut oil contains medium chain fatty acids that boost the metabolic processes in the body. Ingesting coconut oil can improve metabolism that enhances cell and hormonal functions. As a result of better digestion, there is regulated excretion of oil by the sebaceous glands (Vala&Kapadiya, 2014).

Eczema

Coconut oil cure can also be true to eczema. Eczema, an inflammation of the epidermis, is caused by hereditary factors, allergens like dust, pets, or plant pollens and irritants like smoke or solvents. As a result, a person experiences itching, flaking, dryness, or even bleeding of the skin. Moisturizers are very important treatment for eczema and coconut oil is best for lasting moisture for the skin (Rethinam, 2013).

CONCLUSION AND PERSPECTIVES

Coconut oil contributes to good health and nutrient benefits to mankind. It is one of the healthiest dietary oils on earth. As a functional food, coconut oil has a long shelf life and is used for the manufacturing of various dairy and other food products. It is one of nature's richest sources of medium chain triglycerides with antiviral, anti-bacterial and anti-fungal properties. Recently, the western world has discovered coconut oil for its ability in promoting weight loss and preventing premature aging. Coconut oil contains short and medium-chain fatty acids which enhance digestion and support healthy functioning of the thyroidgland and enzymesystems. Its antioxidant properties have been shown to slow the aging process. It is safe for cooking at high temperatures.

The regular use of coconut oil in the diet would regularize blood fats and is known to increase the HDL cholesterol fraction while decreasing LDL and triglycerides.Coconut oil contains so many anti-oxidants that it resists oxidation even if it is preserved for as long as a year whereas all other fats would have been already oxidized and have become trans-fatty acids by the time they come on the food store shelves! Coconut oil resists oxidation even on boiling at 76 degrees centigrade. So, there are no trans-fats in coconut oil. While fried foods are not good for health, if fried in coconut oil, fried foods are not that bad, after all. With all these health, nutritional and body care benefits that onegets from coconut oil, there is no doubt as to why this has been hailed as the "miracle oil."

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Chapter 10

BENEFICIAL EFFECTS OF FATTY ACIDS FROM COCONUT OIL ON HUMAN METABOLISM AND HEALTH

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ABSTRACT

The aim of this chapter was to underline the most important beneficial aspects of the fatty acids found in the coconut oil on the human metabolism and, as a consequence, on health. Medium-chain triglycerides (MCT) are the most representative fatty acids from coconut oil that are digested in the gastrointestinal tract, and then subjected to the absorption process, easier than long chain fatty acids. Medium-chain fatty acids are faster oxidized in the liver, being an important source of energy. They increase the energy expenditure and reduce the fat mass. Thus, they have been proposed for usage in the obesity prevention and treatment, and also in the prevention of insulin resistance development through the Peroxisome Proliferator-Activated Receptors (PPARs) activation. Medium-chain fatty acids stimulate the gut hormone, grelin,that is acylated by ghrelin-o-acyltransferase, which acts as a stimulus that sends the information regarding the presence of a calorie-rich environment to the central nervous system, in order to optimize the lipid storage. *In vivo* and *in vitro* experiments have shown that fatty acids from coconut oils decrease the inflammatory response in patients with inflammatory bowel disease and that they may reduce the side effects related symptoms of chemotherapy on women diagnosed with breast cancer. Nonetheless, the MCTs are considerated to be therapeutic agents used not only in the inhibition of tumoral cells development. Moreover, they have also been proposed to usage in the diet formula of patients with type 1 diabetes and Crohn's disease. Furthermore, they are able to improve the cognitive function and to support the synaptic transmission in case of acute hypoglycemia, without increasing the blood glucose levels.

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Keywords: medium-chain fatty acids, coconut oil, metabolic syndromes, therapeutic agents

INTRODUCTION

Coconut oil has been used for many years in different countries, for edible and non-edible interests. The beneficial aspects of coconut oil, which distinguish it from other oils, derive from its chemical composition that consists of a high content of short (SCFAs) and medium-chain fatty acids (MCFAs). Most of the fatty acids of coconut oil are saturated (90%) and are represented by 47% of lauric acid (C12:0), 18.1% of myristic acid (C14:0), 8.8% of palmitic acid (C16:0), 7.8% of caprylic acid (C8:0) and 6.7% of capric acid (C10:0) (Rossell, 1985). The medium-chain fatty acids (C8-C10) are metabolized differently from the long-chain fatty acids (C14 or longer) and this process leads to significant pharmacological effects.

Usually, the intestinal digestion of long-chain fatty acids begins with the emulsification process through the bile salt action, which allows lipases to hydrolase lipids into free fatty acids and monoglycerides. Medium chain triglycerides (MCTs) are rapidly subjected to hydrolysis without the necessity of bile emulsion, but through the action of the intestinal and pancreatic lipases which generate the MCFAs and the specific monoglyceride (Ramirez et al., 2001).

The absorption process is carried out through passive diffusion because the MCFAs have low affinity for fatty acid binding proteins (Bach & Babayan, 1982). The diffusion of MCFAs into portal blood is associated with the albumin that is used for the transport directly to the liver, without the reesterified process (Bloch, 1974; Guillot et al., 1993; Guillot et al., 1994). For these reasons, medium-chain triglyceride has been used as energy source in syndromes having pancreatic-enzyme deficiency, such as cystic fibrosis (Jensen et al., 1995).

In contrast, LCFAa are incorporated into chylomicrons, which are transported through the lymphatic system before hepatic oxidation and then assembled into more complex triglycerides.

The proved potential of MCFAs is that of reducing body fat by reducing lipoprotein secretion and of protecting the intestinal mucosa from injury. On the other hand, it has been demonstrated that the substitution of LCT with MCT acts on energy metabolism and promotes weight reduction. Further, the MCTs enriched diet is a good strategy for patients with inflammatory bowel disease because the medium chain fatty acids decreases the proinflammatory activity and the symptoms. The clinical symptoms of those patients are the results of an excessive expression of the pro-inflammatory cytokines IL-1, IL-6, and IL-8.

This chapter focuses on the changes induced by the MCFAs in the mitochondrial metabolism and on the oxidative stress, changes that confer beneficial metabolic reactions when MCFAs are used in diets conferring the hypocholesterolemic effect. It also presents the remarks made by the researchers in the field regarding the functions of saturated fatty acids in relation to anti-diabetic properties, intestinal inflammation, thrombogenic and atherogenic risk factor improvement, immune system activation, the inhibition of tumor proliferation and the functions of the nervous system.

EFFECTS OF MEDIUM CHAIN FATTY ACIDS ON HUMAN METABOLISM AND OBESITY

Fat is a valuable macronutrient in human health. The types of fatty acids are important because each of them biologically affects the body in different ways. Vast and long-term researches have been carried out on the impact of specific fatty acid groups on public health (American Dietetic Association, 2007).

Metabolic syndromes (Grundy et al., 2004) include a group of metabolic disorders that contribute to increased cardiovascular morbidity and mortality. The plethora of disorders is represented by abdominal obesity, atherogenic dyslipidemia, hypertension, insulin resistance, and/or glucose intolerance, proinflammatory and prothrombotic states (Grundy et al., 2004; Nagao & Yanagita, 2010). The International Diabetes Federation defines a person as having metabolic syndrome if that person presents abdominal obesity waist circumference, WC ≥88cm), plus two of the following values: higher triacylglycerol level (≥150 mg/dL), reduced HDL-cholesterol (80 mg/dL in males and <50 mg/dL in females), higher blood pressure (systolic ≥ 130mmHg or diastolic ≥ 85mmHg), and higher plasma glucose (≥100 mg/dL).

Assunção et al., (2009) studied the effects of dietary supplementation with coconut oil (for a period of 12 weeks) on women with abdominal obesity. The selected women were aged between 20 and 40 years old (pregnant woman excluded), had a low income family (USD$1/day) and suffered from abdominal obesity. The diet consisted of the ingestion of 30 mL of coconut oil /day, under specific nutritional recommendations and under a fitness trainer's supervision. The authors concluded that the ingestion of coconut oil offers the reduction of body mass index, hip circumference, total abdominal fat, visceral fat, and waist circumference and that it induces an increase in peripheral insulin resistance, when compared with the control group (Assunção et al., 2009; Crozier et al., 1987; Geliebter et al., 1983; St-Onge et al., 2003; Aluko, 2012).

Other experimental studies have demonstrated that dietary MCFAs/MCTs inhibit fat deposition through enhanced thermogenesis and fat oxidation in animal and human subjects. An open-label pilot study has been carried out by Liau et al. (2011), in order to assess the efficacy of virgin coconut oil (VCO) on lipid profile of some obese volunteers, for a four weeks period. The researchers concluded that VCO is helpful for WC reduction, being proposed for safe use in humans.

On the other hand, studies on human subjects have shown that, by replacing dietary LCT with MCT, the daily energy expenditure increases from 100 kJ (Flat et al., 1985) to 669 kJ (Hill et al., 1989) in men and to 138 kJ/day (White et al., 1999) in women. The MCTs were proposed to be used in the treatment or prevention of human obesity because their energy expenditure released after ingestion was higher, in comparison with LCT (Scalfi et al., 1991; Seaton et al., 1986; Dulloo et al., 1996).

For a 4 weeks period, Onge and Jones (2003) examined the relationship between the composition of the body and the thermogenic responsiveness to MCTs, represented by 55% octanoic and 44% decanoic acids, extracted from coconut oil. The body weights have been measured using a standard scale and the body composition has been assessed using magnetic resonance imaging (MRI). Their research showed that, when dietary LCT is replaced with MCT, the energy expenditure and fat oxidation increases and the body composition changes

in a beneficial way. Therefore, MCTs improve the clinical nutrition of patients suffering from malabsorption because they supply both energy and essential fatty acids.

The question "How does MCTs act on these two factors in order to obtain good results for health?" arises. It seems that the fat mass is being reduced through the down-regulation of adipogenic genes as well as through the t factor PPARγ (Peroxisome Proliferator-Activated Receptor γ, as known as NR1C3) transcription (Han et al., 2003). PPARγ, a member of the nuclear hormone receptor superfamily is highly expressed in adipose tissues (white and brown), especially during adipogenesis and is activated by NEFA (Non-esterified fatty acids).

A more recent study supports the idea according to which dietary with SCFAs induces PPARγ to switch from lipid synthesis to hydrolysis. By decreasing the adipose and the hepatic PPARγ expression and activity, it increases the expression of uncoupling protein 2 (UCP2) and stimulates the oxidative metabolism in these tissues, via AMP-activated protein kinase (den Besten et al., 2015).

An important role in the MCFAs metabolism was directed to cytochrome P450 omega hydroxylase (CYP4), which catalyzes the ω-hydroxylation of saturated, branched chain or unsaturated fatty acids that are further metabolized to dicarboxylic acids (DCA), which are metabolized by the peroxisome β-oxidation system (Figure 1).

Under normal conditions, (5–10%) fatty acids are converted to dicarboxylic acids. The short chain fatty acids are transported to the mitochondria for complete oxidation after the peroxisome β-oxidation (L-PBE) process that prevents DCAs accumulation and, consequently, liver failure through the inflammation and fibrosis induction (Hardwick, 2008).

Therefore, the MCFAs increase the energy expenditure, reduce the fat mass and prevent the development of insulin resistance through the PPARs activation (Figure 1).

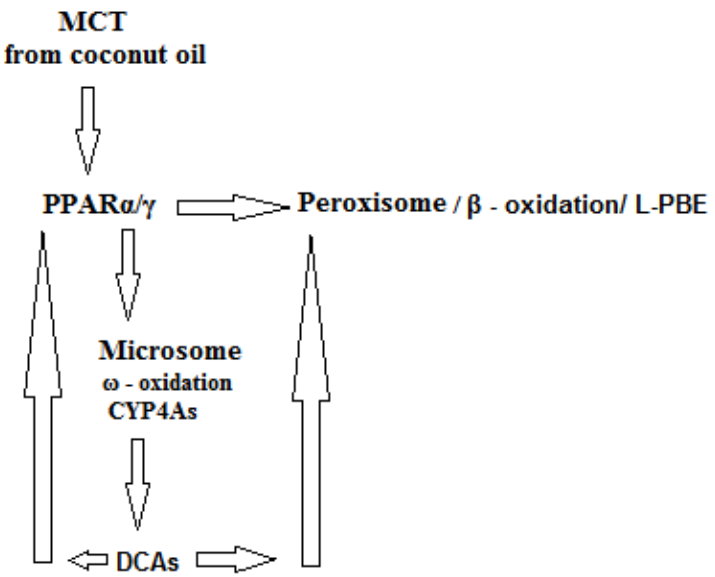

Figure 1. Schematic representation of dicarboxylic fatty acids (DCAs) synthesis and their degradation through L-PBE peroxisomal system, after medium chain triglycerides (MCT) liver absorption (adapted from Hardwick, 2008; Ding et al., 2013).

The uncoupling proteins (UCPs) are located in the inner mitochondrial membrane and are involved in different processes, such as energy metabolism regulation, body weight control, and glucose level control (Echtay, 2007).

They have been recently found to produce a biological impact on the areas of degenerative, neurological, circulatory, and immunological diseases and aging. The sequence analysis indicated that UCPs are mitochondrial anion carriers. The uncoupling protein-1 (UCP1) is a brown adipocyte-specific protein not coupled to ATP synthesis, but involved in obesity and in energy expenditure modulation.

The UPC2 and UPC3 are expressed in several cell types participating in fatty acid metabolism: adipose cells, skeletal muscle, and macrophages. More exactly, the UCP2 is found in all mammalian tissues, except parenchymal hepatocytes (Fleury et al., 1997) and UCP3 is found in skeletal muscles and brown fat (Boss et al., 1997).

Because the Ucp2 and Ucp3 genes are placed in a region with genes that encodes proteins involved in the hyperinsulinemia and obesity control (Fleury et al., 1997; Pecqueur et al., 1999) it seems that the fatty acids increase the expression of Ucp2 and Ucp3 (Brand and Esteves, 2005). In a few studies, Robbins and Zhao have suggested that the malfunction of the UCP isfollowed by neurodegenerative disease, atherosclerosis, and cancer. Thus, targeting the UCP contributes as a promising experiment for cancer prevention (Robbins and Zhao, 2011).

In order to reach the mitochondrial matrix, MCFAs do not need carnitine palmitoyl transferase (CPT); however, they do require medium-chain acyl CoA synthases in order to be rapidly oxidized. It has been observed that, after high MCT diets consumption, it increases the hepatic lipogenesis, it down regulates the activity of CPT1 and the MCFAs bypass the intramitochondrial transport process. In a human volunteers study, Labros et al. have shown that, under the condition of hyperinsulinemia-hyperglycemia, the oxidation of fatty acids depends on the rate of fatty acid entrance into the mitochondria (Labros et al., 1996). The difference in the oxidation pattern of the LCFAs and MCFAs is just the requirement of the former for CPT I, in order to reach access into the mitochondria, observation that has been proved after the CPT1 inhibition. Their results suggest that glucose plus insulin directly regulate the fatty acid oxidation by controlling the rate of the LCFA entrance into the mitochondria, the MCFAs entrance not being affected (Labros et al., 1996). Nagasaki et al. (2012) also identify the physiological roles of GPR84 in adipose tissue along with its specific ligands, the medium chain fatty acids. GPR84 is a member of the G protein – coupled receptors (GPCRs) that are remarkable molecules, activated by a spectrum of ligands. GPRs represent the largest family of membrane proteins in the human genome.

In 2006, a group of researchers showed that the orphan G protein-coupled receptor, GPR84, functions as a specific receptor for MCFAs with carbon chain lengths of C9–C14 (Wang et al., 2006), the most potent agonists of GPR84 being C10:0, C11:0, and C12:0. The researchers found that the GPR84 is involved in the inflammatory changes that occur in the adipocytes of the person that suffers from insulin-resistance syndrome (Nagasaki et al., 2012).

In the adipose tissue, the adiponectin and the TNFα are important control factors through the MCFA GPR84 systemthat is involved in the use of excess calories and in the control of the insulinresistance syndrome. In the inflammation process of the adipose tissue, which also increases the GPR84 mRNA expression, some specific products such as tumor necrosis factor (TNF)-α, interleukin-6 (IL-6) and adiponectin are secreted.

On the other hand, MCFAs (such as C10) suppress the expression of adiponectin mRNA in the TNFα-primed adipocytes (Nagasaki et al., 2012). Adiponectin is a 29-kDa protein, expressed exclusively in adipocytes that have an antagonist function with the TNFα.

INSULINOTROPIC EFFECT OF MEDIUM CHAIN FATTY ACIDS

Medium chain fatty acids can also act as ligands on two other G protein-coupled receptors: GPR120/FFAR4 and GPR40/FFAR1. These fatty acids receptors are involved in the mechanism that links obesity and type 2 diabetes(T2D). Because the GPR40 is abundantly expressed in the pancreatic β-cells, after its activation, the signal is transduced and the secretion of insulin is stimulated. Insulin acts in many stimulatory and inhibitory ways in different tissues mediated by a complex intracellular signaling pathway. Insulin maintains the glucose homeostasis by decreasing the blood glucose levels and by increasing its uptake into various organs. The mechanism by which the MCFAs enhance the glucose-stimulated insulin secretion takes into account the coupling of GPR40 - Gq/11, resulting in the stimulation of protein kinase C activity and in the enhancement of intracellular calcium (Covington et al., 2006).

In addition, signaling through phospholipase C (PLC) and diacylglycerol (DAG) may contribute to exocytosis of insulin in the pancreatic β cells and its release. Some of the relative insulinotropic effects of the fatty acids may also be exerted via an extracellular mechanism. Thus, when the glucose concentration in the blood increases, the transcription of the GPR40 gene is also intensified which transforms these receptors into a new target for the type II diabetes treatment (Kebede et al., 2012). Fatty acids, including the medium chain fatty acids, may act as extracellular signaling molecules at G protein – coupled receptors, to regulate the β - pancreatic cells, in addition to their effects mediated through their intracellular metabolism.

In 2012, Pu and Liu published a very interesting study about the mechanism through which palmitic acid stimulates the glucose uptake and the GLUT 4 translocation from the citosol to the plasma membrane, in rat skeletal muscle cell line L6. The stimulatory effects of palmitic acids were comparable to those of the insulin. The researchers proved that, in the glucose uptake process, the palmitic acid stimulated Akt (protein kinase B) phosphorylation in a manner depending on the palmitic acid concentration. They also tested the activity of some signaling molecules, such as AMP-activated protein kinase (AMPK) and extracellular signal related kinase (ERK1/2), that were activated through the palmitic acid treatment. It seems that after the increase of the palmitic acid level,the glucose uptake occurs via activation of the AMPK and ERK1/2, which leads to GLT4 translocation (Pu & Liu, 2012). The results obtained by the two researchers are physiologically significant in the obesity treatment because they explain how the fatty acids level induces an earlier Akt phosphorylation, leading to abnormal glucose uptakes, in the case of the obese patients (Pu & Liu, 2012).

The anti-diabetic properties of MCTs have been reported both in humans and animals studies. Yost and Eckel have analyzed the effects of a hypocaloric diet containing MCTs and LCTs on obese women. The rate of weight lost, the amount of ketones from the serum, and the nitrogen balance did not show differences between the groups. Anyway, the patients whoconsumed MCTs needed a glucose increase in order to maintain euglycernia during the

exogenous insulin administration after weight loss (Yost & Eckel, 1989). In general, it appears that the MCTs improve the body tolerance to carbohydrate. In a similar study, Eckel et al. have reported the possibility of improving the insulin-mediated glucose metabolism in patients with diabetes mellitus, but non-insulin-dependents, using a 40% fat diet containing 77.5% medium-chain triglycerides (Eckel et al., 1992). The conclusion was that MCT could increase the insulin-mediated glucose metabolism in diabetic patients, effect that appeared to be mediated by the increases in the insulin-mediated glucose disposal (Eckel et al., 1992).

The insulinotropic property was also attributed to lauric acid from coconut oil *in vitro* experimental conditions, with isolated islets of mice. The synergic effect of the lauric acid combined with the polyphenolic content of the coconut oil offer a reduction in blood sugar. When the lauric acid is in the same percent, the hot extracted virgin coconut oil appears to have a better hypoglycemic effect, compared to the cold extracted virgin coconut oil. The result offers a good perspective on the protective effect of the polyphenolic compounds found in the two oils on pancreatic beta cells from apoptosis (Siddalingaswamy et al., 2011).

In a recent study, Iranloye and colleagues have also proved the hypoglycemic effect of the virgin coconut oil in an induced type I diabetes male rats. The diabetes was induced by alloxan, which generates reactive oxygen species, causing pancreatic cells death. The treatment with a dose of 10 mL/kg body of VCO for a four weeks period has a beneficial effect on hyperglycemia, improving both glucose tolerance and insulin secretion (Iranloye et al., 2013). On a long term, the rats' diet with MCT also improved both insulin sensitivity and glucose tolerance, in comparison with the LCTs diet (Han et al., 2003).

MEDIUM CHAIN FATTY ACIDS AND THE SATIETY EFFECT

Hunger and satiety are mechanisms that govern the energy intake. Satiety is a stage of satiation where hunger decreases because of food ingestion and is influenced by the distribution of the dietary macronutrients. Satiety is influenced by the fatty acid chain length and by the intake mode (oral vs. gastro-intestinal infusions). The published results have shown the fact that the triacylglycerols of the medium chain are more satiating than the triacylglycerols of the long-chain (Onge et al., 2003, Samra, 2010). Moreover, when the MCT is taken orally in the case of humans, the satiating effect is more marked than long chain triglycerides (Onge et al., 2003; Samra, 2010). An interesting study tested the effect of a meal rich in medium-chain SFA (from coconut oil: 0.5% caproic acid, 8% caprylic acid, 6% capric acid , 47.5% lauric acid, 18% myristic acid, 9% palmitic acid , 3% stearic acid, 6% oleic acid cis-9-18:1, 2% linoleic acid cis-9, cis-12-18:2.) on postprandial triglyceridemia (PPL) in seventeen relatives (REL) of patients with type 2 diabetes, compared with 17 controls (CON) (Pietraszek et al., 2012). It also evaluated the effects of these fatty acids on incretin, on ghrelin responses, and on the expression of some genes involved in lipid and glucose metabolism in muscle and adipose tissues.

Glucagon-like peptide-1 (GLP-1) is a hormone secreted by the intestinal L cells. It is involved in glucose homeostasis, in improving insulin secretion and the pancreatic beta-cell function. On the other hand, the ghrelin is a hormone that is synthesized in the human stomach, being involved in meal initiation. The biological functions of ghrelin are: to

stimulate the appetite and the food intake, to balance the gastric acid secretion and motility, and to modulate the endocrine and exocrine pancreatic secretions (Delporte, 2013).

The results obtained by Pietraszek and co-workers have shown that, during the first 2 hours after the meal, the TG level did not increase and the insulin, the postprandial incretin and ghrelin responses did not differ significantly between REL and CON (Pietraszek et al., 2012).

Ghrelin was discovered by Nishi et al. (2005) as a gut hormone stimulated by the ingestion of medium-chain fatty acids or by medium-chain triglycerides. It seems that these lipids are the direct source of the fatty acids that induce the acyl modification of the ghrelin. The acyl modification mechanism of the ghrelin after ingesting MCT appears to be slightly different than the one used after administrating high-fat diet (Nishi et al., 2005) and is catalyzed by the Ghrelin-O-acyltransferase (GOAT). The GOAT acylates ghrelin with fatty acids ranges from C7 to C12 and it is believed that the process occurs in the endoplasmic reticulum. The GOAT acylates ghrelin reacts more efficiently with octanoyl-CoA than the decanoyl-CoA at Ser3 residue (Akamizu et al., 2012). The ghrelin acylation, via GOAT, is involved in eating behavior. When the food intake is conducted by necessity, due to energy deficiency, the GOAT mice models can show similar or opposing phenotypes, depending on the type of diet. The GOAT knockout mice fed with medium-chain triglycerides rich diet displayed lower body weight and fat mass, despite the increased food intake (Kirchner et al., 2009).

Thus, the main role of the ghrelin–GOAT system is to send information about lipids to the central nervous system and to act as stimulus, in order to alert the same system about the presence of a calorie-rich environment, and to optimize the lipid storage (Janssen et al., 2012).

On the other hand, the medium-chain acyl molecules modify the stomach ghrelin peptides and are easily displaced by ingested MCFAs or MCTs. Therefore, it might be possible to treat appetite and control obesity by simply ingesting MCFAs or MCTs (Nishi et al., 2012). The adipose tissue gene expression can also be modulated by the type of dietary fat. Some differences in gene expression between patients with type 2 diabetes and their relatives were also found (Pietraszek et al., 2012). In the muscle tissues of the patients with type 2 diabetes, the following genes were upregulated: ACSL1 (coding of the long-chain acyl-CoA synthetase), ACCB (coding of the acetyl-CoA carboxylase beta), LIPE (coding the Hormone-sensitive lipase), and UCP3, as a response to the meal reach in medium chain fatty acids.

The protein encoded by the ACSL1 gene is an isozyme of the long-chain fatty-acid-coenzyme A ligase family, being involved in the conversion of the free long-chain fatty acids into fatty acyl-CoA esters. It also has a decisive function in lipid biosynthesis and in fatty acids degradation. Acetyl-CoA carboxylase beta is supposed to be involved in the regulation of fatty acid oxidation, rather than fatty acid biosynthesis. Hormone-sensitive lipase, in the short form, has a key function in the mobilization of the stored fat inhibited by insulin. Altogether, the data suggest that the MCFAs might represent a potential therapeutic instrument for the clinical manipulation of energy metabolism, through the modulation of ghrelin activity.

The effects of MCFAs on some genes expression were also investigated during rat pregnancy, in order to test the susceptibility of offspring to later-life obesity (Dong et al., 2011). The results obtained showed a down–regulated ACC1 (acetyl-CoA carboxylase) and

fatty acid synthase (FAS) genes expression in the liver of the offspring rat from the MCFA group, accompanied by a decrease in body weight and body fat content. Equally, the mRNA and the protein expression of the adenosine monophosphate (AMP)-activated the protein kinase (AMPK), and thecarnitine palmitoyltransferase 1 and the UCP3 were increased (Dong et al., 2011). The researchers' conclusion was that, during pregnancy, the diet rich in MCFAs might prevent later-life obesity in the offspring because of the programming fatty acid metabolism gene expression (Dong et al., 2011). To sum up, the dietary supplemented with MCFAs is able to decrease the basal insulin level, to equilibrate the blood glucose level, and to attenuate the symptoms of the metabolic syndrome (Hajri et al., 2001) by gene expression regulation.

THE EFFECTS OF THE MEDIUM CHAIN FATTY ACIDS ON INTESTINAL INFLAMMATION

Inflammatory bowel disease (IBD), alongside Crohn's disease (CD) and ulcerative colitis are characterized by the inflammation of the gastrointestinal tract. The etiology of IBD is induced by a combination of factors that contributes to this pathogenesis.

Even if the etiology of IBD is not yet known, the existing experimental data indicate that dietary fat may play important roles in the pathogenesis and clinical display of the IBD.

The role of MCT in this disorder was proved in various experiments carried out *in vivo* and *in vitro*. It was suggested that the MCTs decrease the inflammatory response and they were proposed for usage in patients with IBD. The clinical symptoms derived from the excessive expression of pro-inflammatory cytokines such as IL-1, IL-6, and IL-8.

In order to assess the mucosal inflammatory responses of the fat, some researchers have comparatively evaluated the effects of the MCFAs and LCFAs on the interleukin (IL-8) secretion in the intestine-407 cells (Andoh et al., 2000). IL-8 is a human signaling molecule that initiates the inflammatory responses when its expression increases by 10 to 100 fold, in response to some proinflammatory factors such as tumor necrosis factor (TNF)-a / IL-1, bacterial or viral products, and cellular stress. Moreover, in the intestinal lesions of the IBD, the neutrophils level is increased because of the IL-8 action.

It was proved that the absorption process of the MCFAs did not affect the basal secretion of the IL-8 in the intestine cells in comparison with LCFAs, results of clinical importance in the maintenance of the intestinal inflammation (Andoh et al., 2000). The replacement of dietary LCT with MCT is a good strategy for CD patients because MCTs decrease the proinflammatory activity and because they reduce "both steatorrhea and diarrhea as well as fecal electrolyte excretion in patients with a reduced small intestinal mucosal area" (Andoh et al., 2000; Jeppensen and Mortensen 1998).

Another chemical property of the MCT, water solubility, makes the MCT colon absorption possible, a very important process for the CD patients. Medium chain fatty acids are also energy sources for the patients which suffer from ulceration and inflammation in the intestine (Andoh et al., 2000). The nutrition plays an important role in the management of patients with IBD. It seems that a dose of MCT oil up to 50 g/day can be a good dose for patients with ileitis or an extensive resection of the small intestine, as osmotic diarrhea (Eiden, 2003).

In another study, it has also been proved that the MCT rich diet induced a relative decrease in the IL-6 levels, factor that is significantly increased in the serum of the CD patients (Papada et al., 2013).

EFFECTS OF MEDIUM CHAIN FATTY ACIDS ON THE IMMUNE SYSTEM

Recent works have demonstrated that fatty acids play a key role in immune responses. This effect can be obtained in different ways, for instance, by modulation of the intestinal permeability or by influence on intestinal epithelial cytokine expression (Yoshida et al., 2001; Hoshimoto et al., 2002). As presented before, the mRNA of GPR84 is mainly found in brain, heart, muscle, colon, thymus, spleen, kidney, liver, intestine, bone marrow and leukocytes (Wittenberger et al., 2001; Venkataraman and Kuo, 2005). So far, it was reported that the MCFAs activated the GPR84 and stimulated the IL-12p40 production in monocytes/macrophages and neutrophils (Wang et al., 2006), its function being distinguished in the immune system, more exactly in the modulating leukocyte functions and host defense.

Il-12p40 is included in the Interleukin-12 (IL-12) family of cytokines, which also includes important mediators of inflammatory disease such as: IL-12, IL-23, IL-27 and IL-35. The IL-12 (also known as IL-12p70) is composed of two subunits: p35 and p40. The p40 (IL12-p40) subunit can exist and act as an antagonist monomer of the IL-12 function. IL-12p40 operates as a chemoattractant for macrophages, being associated with several pathogenic inflammatory responses. The proinflammatory cytokine IL-12 induces and maintains T helper1 (Th1) responses and inhibits T helper 2 (Th2) responses. Because the MCFAs stimulate the GPR84, it is possible to affect the Th1/Th2 equilibrium and could give important information about the correlation between the metabolic and autoimmune diseases (Venkataraman and Kuo, 2005).

EFFECTS OF THE MEDIUM CHAIN FATTY ACIDS ON CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is a substantial issue in most of the developing regions of the world. Cardiovascular disease is a general term that includes ischemic myocardium, myocardial infarction, cerebrovascular disease, hypertension, peripheral arterial disease, arrhythmias, congenital heart disease or rheumatic heart disease (Mendis et al., 2011). Traditionally, it has been estimated that low density lipoprotein cholesterol (LDL-C) is an important marker in assessing the risk of evolving CVD. As it was presented, medium chain fatty acids results after coconut oil digestion. In order to produce energy, they are directly transferred through the portal vein into the liver, where they are oxidized. Because the MCFAs are not incorporated into chylomicrons, they do not contribute to hyperlipidemia. Furthermore, the consumption of coconut oil has not been associated with heart attacks or other forms of cardiovascular disease.

Among all saturated fatty acids, the stearic acid (C18:0) has a unique characteristic from a nutritional point of view, even though it is classified as a saturated fatty acid. In 2001, Kelly and coworkers showed that there is no correlation between the rich stearic acid diet (19 g/day)

and the risk of CVD or for those associated with thrombosis. Two years later, Mensink et al. evaluated the effects of different fatty acids on the ratio of total to high density lipoprotein cholesterol (HDL-C) and on serum lipoproteins. Their results suggested that the stearic acid lowered the cholesterol level, and that the lauric acid, the major compound of the coconut oil, increased the cholesterol level, especially the HDL cholesterol. *In vitro* and *in vivo* studies have consistently shown that the HDL molecules have multiple functions which include: capacities to protect vascular endothelium through effluxing the cellular cholesterol, to decrease vascular constriction, to reduce the inflammatory response, to protect against the pathological oxidation, and to improve the glucose metabolism (Chapman, 2005; Francis, 2010; Navab et al., 2009; Khera et al., 2011; Navab et al., 2011).

Other studies have demonstrated that the stearic acid had a neutral effect on blood total and low density lipoprotein (LDL) cholesterol levels. Yu et al. (1995) developed predictive formulas to evaluate the effect of the stearic acid on blood lipid levels. The analyses revealed that the stearic acid had no effect on blood total, LDL-C and HDL-C levels in adults.

The mechanisms through which stearic acid lowers the plasma cholesterol levels are still debatable. One of these is that the absorption of stearic acid is lower than that of other saturated fatty acids, being a very poor substrate for TG and cholesteryl ester formation (Bonanome et al., 1992; Woollett et al., 1992).

Another hypothesis supports the idea that stearic acid is converted by the Stearoyl-CoA-Desaturase (SCD) enzyme into oleic acid, which does not affect blood cholesterol levels (Pai et al., 1997). Moreover, it seems that stearic acid does not suppress LDL receptors activity and, consequently, the plasma cholesterol level decrease. *In vitro* experiments have shown that stearic acid reduces cholesterol absorption by altering the synthesis of bile acids and cholesterol solubility.

Kelli et al. (2001) compared the thrombosis potential of diets rich in stearic acid with palmitic acid rich diets by measuring the platelet aggregation, the platelet volume and other key components of the haemostatic pathways. The researchers found out that the mean platelet volume, the coagulation factor FVII activity and the plasma lipid concentrations were significantly decreased on diet with stearic acid. The FVII factor is one of the proteins that have been proven to be associated with blood clot in conjunction with the tissue factor that is located outside the blood vessels. Following the same idea, Cocchi and coworkers analyzed the biochemical and functional aspects of the stearic acid on platelets in different population groups: supposedly healthy, depressive, young adult, young children, ischemic and pigs (Cocchi et al., 2009). It seems that the stearic acid protects the platelets aggregation, having a protective role in controlling the ischemic stroke, and being a marker for the ICD (Kelli et al., 2001; Cocchi et al., 2009).

Other several studies have consistently shown that the virgin coconut oil (VCO) decreases the lipid levels and the thrombotic risk factors in rats fed with VCO, compared to copra oil and sunflower oil (Nevin & Rajamohan, 2008; Marina et al., 2009). Furthermore, the high density lipoprotein cholesterol in animals fed with VCO was increased, while low density lipoprotein cholesterol levels were significantly decreased, when compared to copra oil (Nevin & Rajamohan, 2004).

In their next study, Nevin and Rajamohan (2006) offered a new perspective on the beneficial aspects of the VCO. They proved that the VCO has an antioxidant property, distinguished by an increased catalase and superoxide dismutase activities and an increased total glutathionine content, the last one being a sensitive indicator of the antioxidant status.

The phenolic content of the VCO can provide disease prevention because it inhibits the formation of free radicals. It is already known that free radicals are responsible for causing a great number of diseases, including cardiovascular disease and atherosclerosis.

THE EFFECTS OF MEDIUM CHAIN TRIGLYCERIDE ON TUMOR CELLS

Nowadays, cancer research is one of the topics that have priority in the world of science. Many experimental protocols have been designed in order to find the key that turns a normal cell into a tumor and to discover the way in which the body can be precisely determined to cure it. Some investigators have shown that there is a bridge between the ketogenic diet and some antioncogenes, although the correlation between the energy cell metabolism and the genes responsible for the tumor development is not completely understood (Otto et al., 2008; Ramanathan et al., 2005). Cancer is caused by multiple factors which affect the normal cell processes such as cellular proliferation, differentiation and death. Otto and colleagues, for example, have compared the effects of a nutritionally balanced carbohydrate - restricted diet, supplemented with oil extracts displaying different levels of polyunsaturated omega-3 fatty acid and MCT with those of a standard diet, on the growth inhibiting tumors of the human gastric adenocarcinoma cell line 23132/87, in a xenograft model (Otto et al., 2008). Their data suggested an antitumor effect for both omega-3 fatty acids and MCT in patients and experimental models, results that were comparable with those obtained by other researchers (Wolters et al., 1994; Wolters et al., 1994; Nebeling & Lerner, 1995).

Other several studies have shown that coconut oil has an inhibitory effect on the carcinogenic agents used to induce tumors in the colon and mammary in the case of test animals (Reddy & Maeura, 1984; Cohen et al., 1986). In their study, Reddy and Maeura (1984) compared the effect of dietary with corn oil, sunflower oil, olive oil, coconut oil and medium-chain triglycerides on 20 mg azoxymethane/kg body weight, factor that induced colon tumors in F344 female rats. The animals were fed 5% of oils 2 weeks before, during, and 1 week after the azoxymethane injection and after that; for some groups, the dose was increased with 23.52% for oils and 17.64% for MCT. It seems that diets containing high levels of olive oil, coconut oilor MCT had no promoting effect on the colon tumor incidence, compared to the other tested oils, the fatty acid composition / the type of fat being an important factor in the determination of the promoting effect in colon carcinogenesis (Reddy & Maeura, 1984). *In vivo* studies on the N-methylnitrosourea-induced rat mammary tumor model have proved that diets containing MCT: com oil (3:1) can decline the mammary tumor-promoting effect (Cohen et al., 1984). The study shows that the diet containing high levels of medium chain fatty acids does not induce the development of mammary tumors, in comparison with the diet containing long chain fatty acids from corn oil (Cohen et al., 1984).

Other *in vivo* studies have been made on Yoshida-sarcoma-bearing rat model, in order to prove that the fatty acids composition is essential for the inhibition of the tumor growth (Ling et al., 1991). The researchers fed the animals the same fat carbohydrate ratio, with the exception of dietary fat type LCT or 60% MCT /40% fish oil, and analyzed the changes in the tumor volume, body weight, urinary nitrogen, whole-body and tissue protein kinetics (Ling et al., 1991). The results have demonstrated that the feeding rich in MCT/fish oil correlated with

the TNF treatment leads to a tumor growth inhibition, less total urinary nitrogen excretion and a decreasing tumor protein synthesis in Yoshida-sarcoma-bearing rats (Ling et al., 1991).

Medium-chain fatty acids are adjunctive therapeutic agents for the treatment of colorectal cancer. Fauser et al. (2013) treated the Caco-2 and IEC-6 intestinal cell lines with lauric acid and butyrate, in order to compare their potential for apoptosis induction, glutathione levels modification, and changes of the cell cycle phase.. The results presented were promising in terms of antineoplastic properties of the lauric acid. It was shown that this medium chain fatty acid is capable to induce the death of the cells, to decrease glutathione concentration, to generate reactive oxygen and to block the tumoral cells in the S and G2/M phases or to reduce them in G0/G1. Compared with the butyrate, the lauric acid displayed anti-tumoral properties (Fauser et al., 2013).

For a normal cell, G0 is the phase of the cell cycle where the cell stopped dividing. G1 (Gap1) belongs to the Interphase and is a checkpoint control mechanism that guarantees that the DNA synthesis is ready to start. In the Synthesis (S) phase the DNA replication occurs, followed by G2 (Gap 2), another checkpoint that ensures that the cell is ready to enter the Mitosis (M), thus being ready to divide into two daughter cells (figure 2).

The inhibitory effect on the growth of colorectal cancer cells could be explained by the potential of the lauric acid to down-regulate the genes that are involved in the control of cell cycle and to up-regulate the genes that are correlated with the apoptosis process. Apoptosis is an essential physiological process for the homeostasis maintenance in multicellular organisms through cell death programming. Moreover, apoptosis is a coordinated process in which a group of cysteine proteases (caspase) is activated in a cascade of events that have, as final goal, the demise of the cell. Caspase-8 is an initiator that activates caspase-3, which is considered to be the most important element of the caspases (figure 3). Its role is that of activating the endonuclease CAD (Caspase-activated DNase) and of degrading the chromosomal DNA and the chromatin condensation (Martinnvalet et al., 2005) (figure 3).

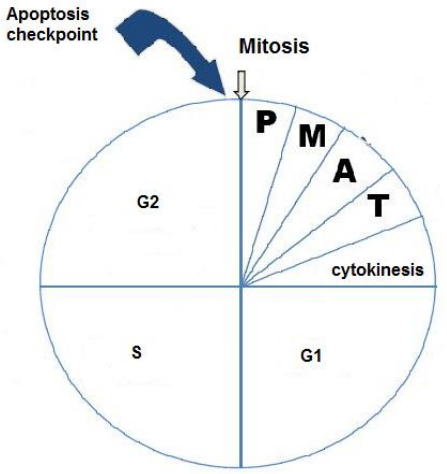

Figure 2. The cell cycle. P – Prophase, M-Mitosis, A-Anaphase, T-Telophase; G1- Gap1; S- DNA Synthesis; G2-Gap.

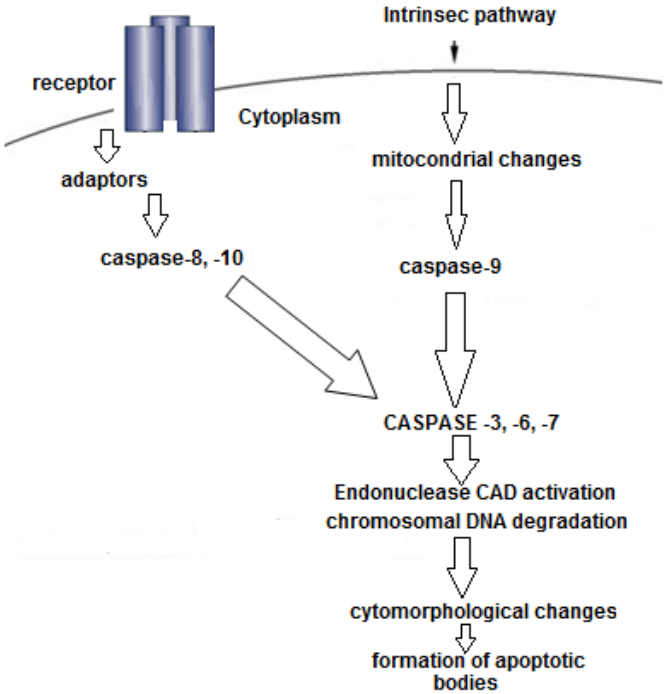

Figure 3. Schematic representation of caspase pathways activation in mammalian cells.

Furthermore, the ketogenic diet has been proven to reduce the tumor growth by lowering the levels of circulating glucose and by reducing the blood insulin levels. A ketogenic diet composed of 60% MCT, 20% proteins, 10% carbohydrates and 10% other dietary fats was divided into five or more small feedings and was given to a selected group of children with cancer, for a period of 7 days (Nebeling and Lerner, 1995). The researchers checked the influence of a ketogenic diet on the tumor glucose metabolism. After this period, the blood glucose level decreased to low-normal levels and the blood ketones were elevated 20 to 30 fold (Nebeling & Lerner, 1995). The obtained results promote MCT on a diet formula for pediatric patients with cancer, who are not under chemotherapy treatment or having other compromising nutritional status (Nebeling & Lerner, 1995).

This could be an interesting approach for cancer therapy, if it is considered that the Warburg effect, a phenomenon with elevated aerobic glycolysis, is based on the metabolic alteration in cancer cells.

Another proven benefit of the VCO was the reducing of symptoms related to the side effects of chemotherapy on persons diagnosed with breast cancer influencing the global quality of life (QOL) (Law et al., 2014; Richardson et al., 2007; Lemieux et al., 2008). Chemotherapy is used in order to inhibit metastasis and to prolong the survival rates but it is linked with many uncomfortable effects such as vomiting, hair loss/ alopecia, fatigue, anxiety, and depression. The research conducted in 2014 by Law et. al was based on some questionnaires, all documented, in order to measure QOL in cancer patients regarding the global health status, the functional status, and the cancer-related symptoms (Law et al., 2014). Thus, after the consumption of VCO (10 mL twice/day) throughout six cycles of

chemotherapy, the women diagnosed with breast cancer had an improved functional status and global QOL, with decreasing systemic therapy side effects.(Law et al., 2014).

EFFECTS OF MEDIUM CHAIN TRIGLYCERIDE ON THE NERVOUS SYSTEM AND ITS DISORDERS

The nervous system is a complex system that, structurally, has two components: the central nervous system and the peripheral nervous system. Between these two there is a complicated network of signals, that are responsible for all the activities, senses, speech, memories and emotions that a human can have. The human brain, being the most metabolically active organ in the body, requires large amounts of energy in order to develop its functions.

Because in normal conditions the most important source of energy for the brain is glucose, in some unfavorable cases, such as a defective capacity of metabolizing glucose or if the glucose supply is broken, the brain is put at risk. Severe hypoglycemia is a cause for cognitive dysfunctions, including disturbances in the cerebral metabolic rate of glucose (CMRglu) which is implicitly a cause for the deterioration of the cognitive function, without warning symptoms. In some diseases, in the type 1 diabetes, the brain can use alternative fuels, such as monocarboxylic acids, lactate, and ketones.

Medium-chain fatty acids can generate ketones after the oxidizing process that takes place in the brain and can offer type 1 diabetic patients a prophylactic treatment strategy and a normal function of the brain during hypoglycaemic episodes, without increasing the blood glucose levels.

A group of researchers (Page et al., 2009) has shown that MCFAs improve the cognitive function in patients treated for type 1 diabetes and also support the synaptic transmission in the case of acute hypoglycemia. They have observed that the most beneficial effects of the MCFAs ingestion were on verbal memory, which is under hippocampus control (Page et al., 2009).

Moreover, the researchers have checked *in vitro* the hypoglycaemic conditions of the hippocampal synaptic transmission of β-hydroxybutyrate and octanoate. It seems that, in these conditions (*in vitro* hippocampal slice preparations and low-glucose conditions), the β-hydroxybutyrate is involved in the synaptic transmission. Instead, octanoate is involved in the synaptic function recovery after the normalization of the glucose concentration (Page et al., 2009). The study brings valuable results to the diabetes management field, offering patients a normal functioning of the brain during the hypoglycaemic episodes (Page et al., 2009).

The favorable effects of the MCFAs on brain homeostasis in patients with mild cognitive impairment (MCI) were also demonstrated by Rebello and collaborators (2015), on six patients enrolled in a randomized placebo-controlled experiment. MCI is one of the earliest stages of Alzheimer's disease (AD) that was characterized, in incipient stages, by learning and memory dysfunctions.

In AD there are a number of associated abnormalities on the cellular and subcellular level that include dystrophic neurites, the activation of genes from the signaling pathways or the deficiency of the mitochondrial function. Diet supplementation with MCT oil favors the increase of the circulating ketone bodies, which are a good secondary energy sources for the

brain when glucose concentration is decreased, situation that has been observed in persons with AD.

In the appearance and development mechanism of the Alzheimer's disease, the presence of apolipoprotein Eε4 allele (ApoE4) seems to play a major role. The evaluation test was designed for a period of 24 weeks on serum β-hydroxybutyrate (BHB) concentration, apolipoprotein-E4 status and cognitive performance in MCI subjects (Rebello et al., 2015). The consumption of 56 g/day of MCT oil seems to have a positive effect on the memory of people with negative or positive ApoE4 status, based on the increased concentration of BHB proving that ketones are able to offset the effects of the cerebral energy metabolism.

The effect of the capric acid (C8:0) was checked on 152 subjects diagnosed with mild to moderate AD in a 90 days study (Henderson et al., 2009). The MCT and the capric acid were introduced in a normal diet as it follows: 10 g/day in the first 7 days and 20 g/day, for the next days. The results presented proved the increase of the serum ketone body, in the next two hours after the administration. More exactly, the mean of the BHB concentration increased from the 0.09 mmol/L in the first day to 0.039 mmol/L, in the last day of the study. This effect was more obvious in ApoE4 negative subjects (Henderson et al., 2009).

The efficacy of the extra virgin coconut oil, whose predominant component is MCT has also been checked in subjects with moderate to severe AD (Gandotra et al., 2014). The test has been carried out on 31 patients who were daily given 20 g extra virgin coconut oil, on a 6 weeks trial. The researchers have evaluated the primary cognitive end points using the Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog), coupled with the Clinicians' Interview based on the Impression of Change Plus Caregivers input (CIBIC-Plus) (Gandotra et al., 2014). Gandotra et al. (2014) have obtained the same favorable effect as other researchers who have tested the MCT oils on cognitive performance in subjects with AD, by using the equivalent amount of virgin coconut oil.

In vivo experiments have demonstrated that ketones offer neuroprotection in the case of cell culture models of the two most common degenerative neurological diseases, Alzheimer's and Parkinson's and they have also proved that the ketones may offer neuroprotection in the treatment or prevention of both diseases.

Genetic and environmental factors (toxins or infections) may cause the Parkinson's disease, which is characterized by muscle rigidity, bradykinesia, eosinophilic Lewy-body inclusions and the death of the dopaminergic neurons in the pars compact of the substantia nigra (Jankovic, 2008).

In the investigation of Parkinson's disease it has been found out that the complex I of the electron transport chain from the mitochondria is defective. The role of the NADH formed in the citric acid cycle is to send electrons to complex I and further on, the electrons are sent to complex IV undergoing complex III. The gradient proton is produced and induces the formation of ATP, which is used by the cells in every physiological step that requires energy. Thus, the inadequate function of complex I reduces the ATP formation and causes the oxidative stress (Maalouf et al., 2009). Furthermore, by using a ketogenic diet it is possible to fuel the electron transport chain with electrons to the complex II from the FADH2, which results from the fatty acids oxidation.

Consequently, an enriched MCT diet may slow down the progression of the Parkinson` disease, allowing those patients to have normal lives. Another proper aspect of the ketogenic diet is its usage in therapies for dementia, in dietary management of multiple sclerosis and epilepsy (Maalouf et al, 2009).

CONCLUSION AND PERSPECTIVES

The basis of this chapter was the plenitude of information that exists in literature regarding the beneficial properties of the fatty acids from coconut oil on human health. *In vivo* and *in vitro* studies on both human and animal metabolism offered valuable experimental data on the positive effects of the enriched MCT diets, more precisely on the fact that MCFAs inhibit fat deposition, enhance thermogenesis, fat oxidation, and daily energy expediture, respectively. The chapter also emphasizes the fact that the unique physicochemical characteristics of these fatty acids offer facilities in their absorption mode, transport, oxidation degree and utilization. These data provide new perspectives on the mechanisms that contribute to the supportive effects of the MCFAs on energy homeostasis and insulin action. There are highly available energy substrates in different diseases such as type 2 diabetes, Alzheimer's and Parkinson's disorders, in which the hypoglycaemic phases affect the brain function. The anticancer activity of some MCFAs was also proven on cultured human colorectal, skin and breast cancer cells. Many research studies mention the necessity of more genomic experiments, that would offer comprehensive information about the effects of these fatty acids in relation to the anticancer mechanism.

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