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# Phytochemicals Components as Bioactive Foods

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## 1. Introduction

Phytochemicals in plant material have raised interest among scientists, food manufacturing and pharmaceutical industry, as well as consumers for their roles in the maintenance of human health. Phytochemicals are the bioactive, non-nutrient, and naturally occurring plant compounds found in fruits, vegetables, and whole grains. They can be categorized into various groups, i.e., polyphenols, organosulfur compounds, carotenoids, alkaloids, and nitrogen-containing compounds (Lampe & Chang, 2007).

Many phytochemicals are potent effectors of biologic processes and have the capacity to influence disease risk via several complementary and overlapping mechanisms (Lampe 2007). Further some phytochemicals undergo bacterial modification to produce metabolites that are more biologically active than the parent compounds (Lampe & Chang, 2007).

At present, one of the main objectives of the industry is to identify plant matrices rich in these compounds.

The goal of this chapter is devoted to the description of four classes of phytochemical compounds founded in oil-seeds such as: Sterols, Aliphatic alcohols (e.g. Policosanol), Squalene, Phenolic acids (e.g. resveratrol) and their content in oil-seeds. We will also evoke the physiological impact of these nutraceuticals on human health.

## 2. The main phytochemical compounds: structures and sources

The chemical compounds of oilseeds can be divided into two groups: the saponifiable fraction, which accounts for almost the entire weight of the oil (98–99% of total weight) and the nonsaponifiable fraction, which represents 0.5–2.0% of the total weight and is constituted of diverse components that are of great importance in terms of its biological value (Samaniego-Sanchez, 2010).

This fraction is used when we wish to study the characteristics and authenticity of oil or fat, due to its varied composition, including phytosterols, policosanols, hydrocarbons, polyphenols, tocopherols, triterpenic alcohols and volatile products.

## 2.1 Phytosterols

### Structure

Phytosterols (plants sterols) are a group of naturally substances occurring in plants as bioactive minor constituents (up to 5%). They are derived from 3-hydroxylated polycyclic isopentanooids which have a 1,2-cyclopentanophenanthrene structure with a 5,6- double bond (Fig. 1) (Abidi, 2004, 2001). These compounds contain a total of 27-30 carbon atoms (the number of carbon atoms in the biosynthetic precursor squalene oxide) and that of the side chain attached at the carbon-17 position can be equal to or greater than seven (Abidi, 2004). In addition to the free form (FS), phytosterols occur as four types of “conjugates” in which the 3 $\beta$ -OH group is esterified to a fatty acid (SE) or a hydroxycinnamic acid (HSE), or glycosylated with a hexose (SG) or a 6-fatty-acyl hexose (ASG) (Moreau et al., 2002). In free phytosterols (FS), the 3 $\beta$ -OH group on the A-ring is underivatized, whereas in the four conjugates the OH is covalently bound with another constituent. In the case of SE, the OH group is ester-linked with a fatty acid and linked with a 1-O -  $\beta$ -glycosidic bond with a hexose (most commonly glucose). In ASG, there is an addition of a fatty acid esterified to the 6-OH of the hexose moiety. In the fourth group the sterol 3 $\beta$ -OH group is esterified to ferulic or *p*-coumaric acid especially in rice and corn seeds (Moreau et al., 2002).

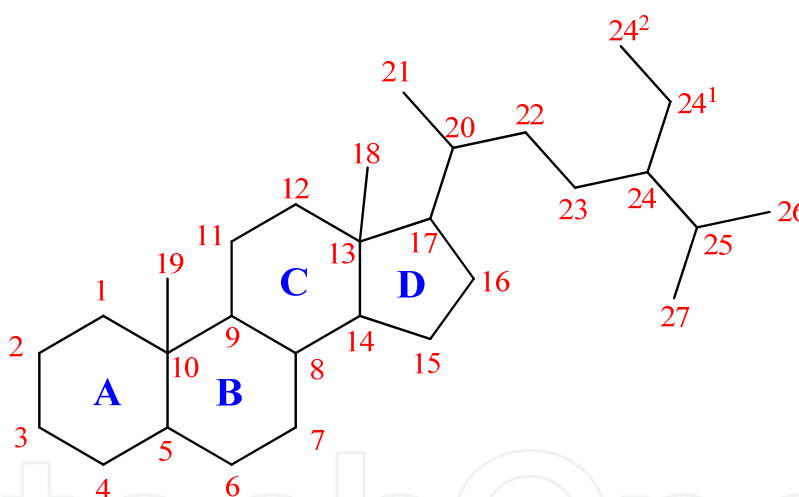


Fig. 1. Structure of a steroid numbered.

Phytosterols have been recognized into three subclasses (Fig 2); on the basis of the number of methyl groups at the C<sub>4</sub> position: none (4-desmethylsterols or sterols), one (4 $\alpha$ -methylsterols) and two (4,4'-dimethylsterols or triterpenes alcohols (Cherif, 2011a; Moreau, 2002; Benveniste, 2002) (Fig. 2). These later two groups are metabolic intermediates in the biosynthetic pathway leading to the end-product, 4-desmethylsterols, but they are usually present at lower levels than 4-desmethylsterols in most plant tissues (Cherif, 2011a; Moreau, 2002; Grunnwald, 1975). According to the position of the double bond at C<sub>5</sub> or C<sub>7</sub> in A ring (Fig. 1); 4-desmethylsterols are present in two groups which are  $\Delta$ <sub>5</sub>-desmethylsterols and  $\Delta$ <sub>7</sub>-desmethylsterols. The most proportion of 4-desmethylsterol is  $\Delta$ <sub>5</sub>-desmethylsterol which contained mainly  $\beta$ -sitosterol,  $\Delta$ <sub>5</sub>-avenasterol, campesterol and stigmasterol (Pardo, 2007).

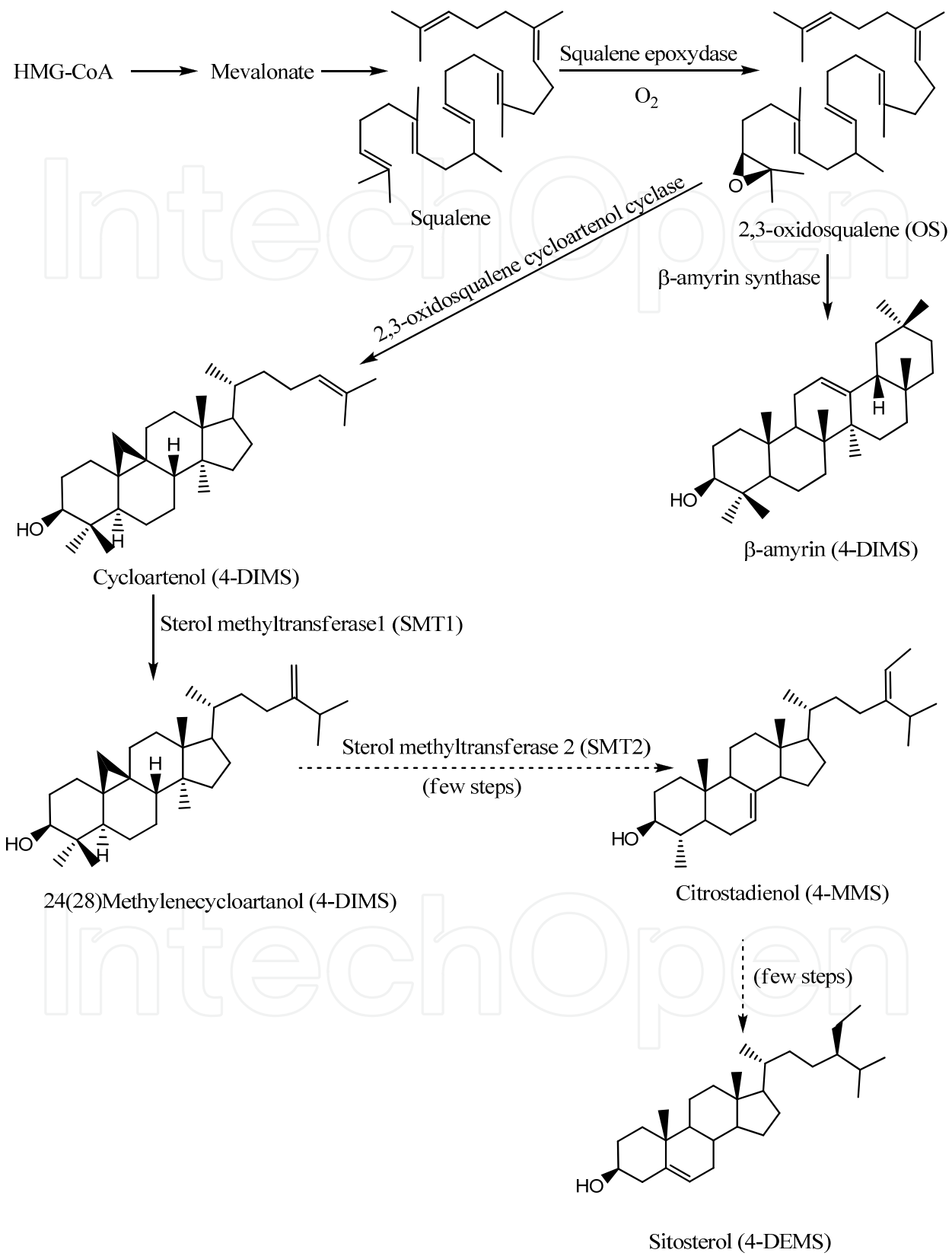


Fig. 2. The sterol pathway for higher plants.

Indeed, 250 types of phytosterols are actually reported in the literature, nutrition research has focused mostly upon the unsaturated  $\beta$ -sitosterol (24 $\alpha$ -ethylcholest-5en-3 $\beta$ -ol), campesterol (24 $\alpha$ -methyl-5-cholesten-3 $\beta$ -ol), stigmasterol (5,22-cholestadien-24 $\alpha$ -ethyl-3 $\beta$ -ol) and  $\Delta^5$ -avenasterol (24-ethyl-cholesta-5,24 (28)Z-dien-3 $\beta$ -ol) (Kochar, 1983).

Compounds 4-DEMS	MW	Mains fragmentation ions (M/Z) and others
Campesterol	472	382, 343, 255, 129
Stigmasterol	484	394, 255, 175, 129, 83
$\beta$ -sitosterol	486	396, 381, 357, 255, 129, 73
$\Delta^5$ -avenasterol	484	469, 386, 379, 355,296, 129, 55
Fucosterol	484	469, 386, 296, 129, 55
$\Delta^7$ -sitosterol	486	303, 255, 229, 213
$\Delta^7$ -avenasterol	486	386, 343, 255, 213

MW: Molecular Weight (g/mol)

Table 1. Retention time and mass spectrometric data for trimethylsilyl derivatives of 4-desmethylsterols identified by GC-MS (Cherif, 2011a)

Compounds 4-DIMS and 4-MMS	MW	Mains fragmentation ions (M/Z) and others
Obtusifoliol	498	.498 ; 483 ; 393 ; 109
$\beta$ -amyrin	498	483 ; 408 ; 393 ; 218 ; 203.
$\alpha$ -amyrin	498	483 ; 408 ; 393 ; 218 ; 203.
Gramisterol	484	469 ; 393 ; 357 ; 254 ; 206 ; 83 ; 41.
Cycloartenol	498	483 ; 408 ; 393 ; 365 ; 271 ; 189 ; 69
28-methyleneobtusifoliol	512	497 ; 407 ; 309 ; 295 ; 281 ; 255 ; 55
24-methylenecycloartanol	512	497 ; 422 ; 407 ; 379 ; 353 ; 255 ;73.
Citrostadienol	498	400 ; 357 ; 264 ; 250 ; 83 ; 47.

MW: Molecular Weight (g/mol)

Table 2. Retention time and mass spectrometric data for trimethylsilyl derivatives of 4,4'-dimethyl and 4 $\alpha$ -monomethylsterols identified by GC-MS (Cherif, 2011a)

Effects

Phytosterols exist in all foods of plant origin and are known to have several bioactive properties with possible benefits for human health (Cherif et al.,2010a).They contribute to lowering serum cholesterol levels (Cherif, 2010a), and are also considered to have anti-inflammatory, anti-bacterial, anti-artherosclerotic, anti-oxidative, anti-ulcerative, anti-tumor properties in humans (Lagarda, 2006; Moreau, 2002; Beveridgs, 2002), as well as contributing to the oxidative and thermal stability and shelf-life of vegetable oils (Przybylski, 2006). Additional, they are useful emulsifiers for cosmetic manufacturers and supply the majority of steroidal intermediates and precursors for the production of hormone pharmaceuticals (Grunnwald, 1975). Phytosterols might also prevent the development of colon cancer and benign prostatic hyperplasia (Awad, 2000). Among different plant sterols,  $\beta$ -sitosterol has been most intensively investigated and has been shown to exhibit anti-inflammatory; antineoplastic, antipyretic, and immunomodulating activities (Careri, 2006). Also,  $\beta$ -sitosterol, campesterol and stigmasterol exert antioxidant effects. Indeed,  $\Delta^5$ -avenasterol has an essential

antipolymerization effect, which could protect oils from oxidation during heating, exposure to ionizing radiation, light, chemical catalysts, or enzymatic processes (Nasar, 2007).

Titerpenes compounds and 4 $\alpha$ -monomethylsterols are also important bioactive secondary metabolites, due to the wide range of their biological activities. They show mainly antimicrobial, cytotoxic, antitumoral, antiviral, anti-inflammatory, hepatoprotective, antifeedant and insecticidal activities ( Alvarenga, 2005). These methylsterols have been used as markers to characterize and to detect admixture of vegetable oils (Cert, 1997; Jimenez, 1996).

It is noteworthy that the compositional distributions of phytosterols in certain vegetable oils have been used for their identification (Abidi, 2001). Hence, phytosterols and other non-saponifiable compounds in oils are often used as markers for the assessment of adulterated oils (Abidi, 2001). Recently, plant sterols have been added to margarine and vegetable oils as examples of successful functional foods and used as food ingredients in modern formulations (Fernandes, 2007). Reported phytosterols data for some oilseeds have shown that (Lin, 2004)

Vegetable oils	PS totaux	$\beta$ -sitosterol	Campesterol	Stigmasterol	$\Delta$ 5avénasterol
peanut	315	203	52	28	22,8
Linseed	471	226	120	38	70,7
Olive	177	141	9	4	22,9
corn	843	519	184	56	41,4
grapes	215	169	24	24	nd

nd : not detected

Table 3. The major phytosterols components content in selected vegetable oils (mg/100g of oil) (Lin, 2004)

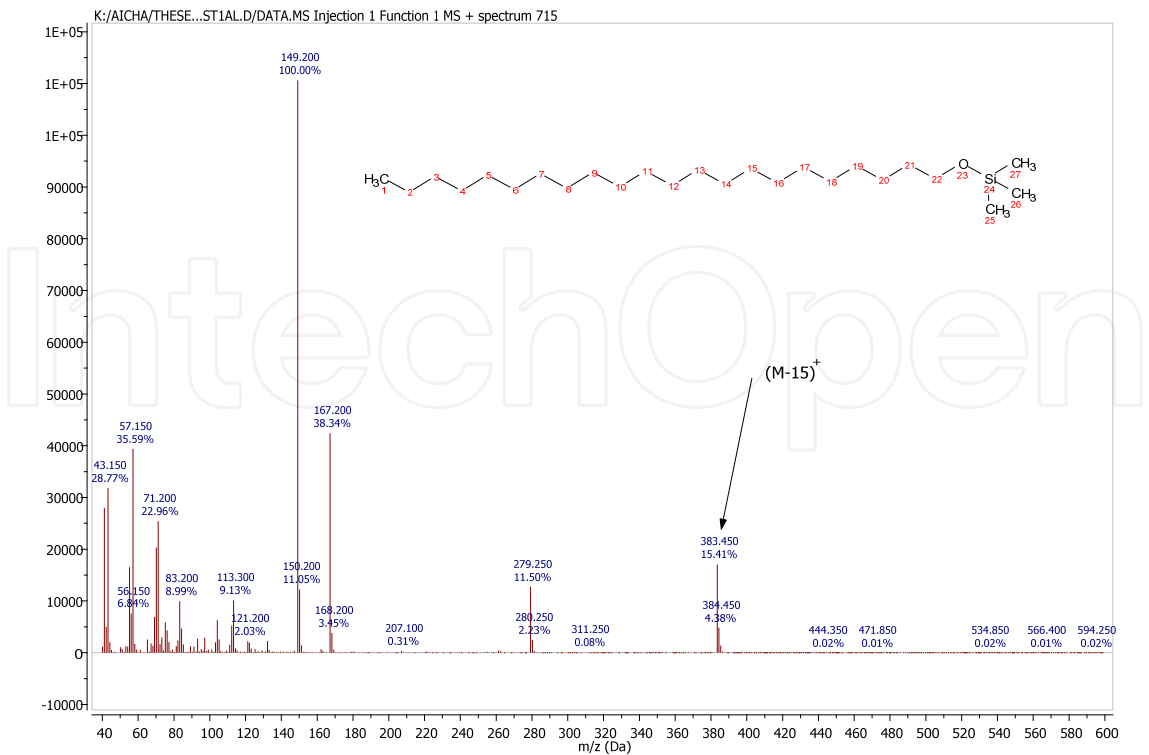


Fig. 3. Mass spectrum of Docosanol (Cherif, 2011b)

2.2 Aliphatic alcohols (e.g. Policosanol)

Structure

POLICOSANOL (PC) is a trivial name of a mixture of high molecular weight (HMW) aliphatic primary alcohols (C<sub>20</sub> to C<sub>36</sub>), originally isolated from sugar cane (*Saccharum officinarum* L.) (Cherif, 2010b).

It is also found from a diversity of other natural substances, such as bee wax, rice bran and wheat germ (Lin, 2004). These plants represent good sources of PC with its health-enhancing components. PC is present also in the fruits, leaves, and surfaces of plants and whole seeds (peanut seeds) (Cherif, 2010b). It is containing mainly docosanol (C<sub>22</sub>), tetracosanol (C<sub>24</sub>), hexacosanol (C<sub>26</sub>), octacosanol (C<sub>28</sub>) and triacontanol (C<sub>30</sub>) (Irmak, 2006).

The analysis of individual policosanol components required mass spectrometric for tentative identification of the chromatographic peaks (Cherif, 2010b). Several components have been identified in the literature. The majorities of them are saturated n-alcohols, but we also note the presence of some unsaturated alcohols as in the case of peanut (Cherif, 2010b).

M <sup>b</sup>	[M-15] <sup>+</sup> (m/z)	Alcohol <sup>1</sup>
286	271	Tetradecan-1-ol
300	285	Pentadecan-1-ol
314	299	Hexadecan-1-ol
328	313	Heptadecan-1-ol
340	325	(Z) Octadec-9-en-1-ol
342	327	Octadecan-1-ol
356	341	Nonadacan-1-ol
368	353	Eicosen-1-ol
370	355	Eicosan-1-ol
382	367	Heneicosen-1-ol
384	369	Heneicosan-1-ol
396	381	Docosen-1-ol
398	383	Docosan-1-ol
412	397	Tricosan-1-ol
424	409	Tetracosen-1-ol
426	411	Tetracosan-1-ol
440	425	Pentacosan-1-ol
454	439	Hexacosan-1-ol
468	453	Heptacosan-1-ol
482	467	Octacosan-1-ol
510	495	Triacontan-1-ol

<sup>b</sup>Mass of the trimethylsilylated alcohol. In all cases the mass spectrum exhibited a major peak due to CH<sub>3</sub> loss, [M-15], and a peak characteristic of the Trimethylsilyl group on a terminal ether site, m/z =103 [(CH<sub>3</sub>)<sub>3</sub>SiOCH<sub>2</sub>]<sup>+</sup>

<sup>1</sup>Compounds tentatively identified.

Table 4. Retention indices and mass spectrometric data for trimethylsilyl derivatives of aliphatic alcohols identified by GC-MS (Cherif, 2010b).



Beneficial effects of policosanol

Several researchers have reported the biological effects of active compounds extracted from sugar cane crude wax (CW) such as long chain *n*-alcohols, fatty acids or ethanolic extracts, which have application to artherosclerotic vascular, coronary heart diseases, and dermatologic diseases (Lucas, 2007).

In fact, policosanol has been shown to decrease platelet aggregation, endothelial damage, and foam cell formation. The effectiveness of policosanol as a lipid-lowering agent, in several different populations has been extensively (Irmak, 2006). PC is supposed to downregulate the cellular expression of hydroxymethylglutamyl coenzyme Z (HMG-CoA) reductase; the rate-limiting enzyme in the cholesterol synthesis, rather inhibit the enzyme activity (Lin, 2004). So inhibiting cholesterol biosynthesis and enhancing low-density lipoprotein (LDL) decatabolism (Menendez, 1994). Similarly, PD improves protection of lipoproteins against lipid peroxidation, both in the lipid and the protein moieties (Menendez, 1999).However, Franci-Pesenti et al (Francini-Pesenti, 2008) have reported that the mechanisms by which PC improves plasma lipid profile are unclear, and there is a continuing debate about the exact effect of PC. Nevertheless, PC was successful worldwide, and it is sold as a lipid-lowering supplement in more than 40 countries (Cherif, 2010b). The majority of these supplements are prepared from bees wax or sugar cane extracts.

Marcelleti et al. reported that docosanol inhibits replication of herpes viruses in vitro and in vivo. While, octacosanol might be oxidized and degraded to fatty acids via  $\beta$ -oxidation in mammals (Menendez, 2005).

Samples	C <sub>22</sub>	C <sub>24</sub>	C <sub>26</sub>	C <sub>28</sub>	C <sub>30</sub>	PC
Wheat germ (mg/kg)	2,8	1,4	nd	2,9	2,5	10,0
Beeswax (g/kg)	0,06	2,6	1,7	2,0	5,7	12,0
Sugar cane (mg/kg)	0,92	1,68	0,9	10,0	1,0	17,4
Policosanol <sup>TM</sup> (mg/comprimé)	7,1	14,8	8,5	8,8	8,3	53,6

Policosanol <sup>TM</sup>: commercial dietary supplement; nd : non détecté.

Table 5. Policosanol content of some natural sources rich in PC in compared with that PC supplement (Cherif, 2011b)

2.3 Squalene

Structure

In virgin oil, the major hydrocarbon is the squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,20-tetracosahexaene) (C<sub>30</sub>H<sub>50</sub>), a terpenoid hydrocarbon occurring in high concentrations (800-12000 mg/kg which can constitute up to 90% of the hydrocarbon fraction of olive oil and pumpkin oils (Fig .4) (Tuberoso, 2007). It is also present in all plant oils and fats except flaxseed, grapeseed, and soybean oils (Tuberoso, 2007). It is accompanied by *n*-alkanes in the range of C<sub>8</sub>-C<sub>35</sub>, being the more abundant the



comprehended between  $C_{21}$  and  $C_{35}$ , in which alkanes with an odd number of carbon atoms predominated over those of even numbers (Cert, 2000).

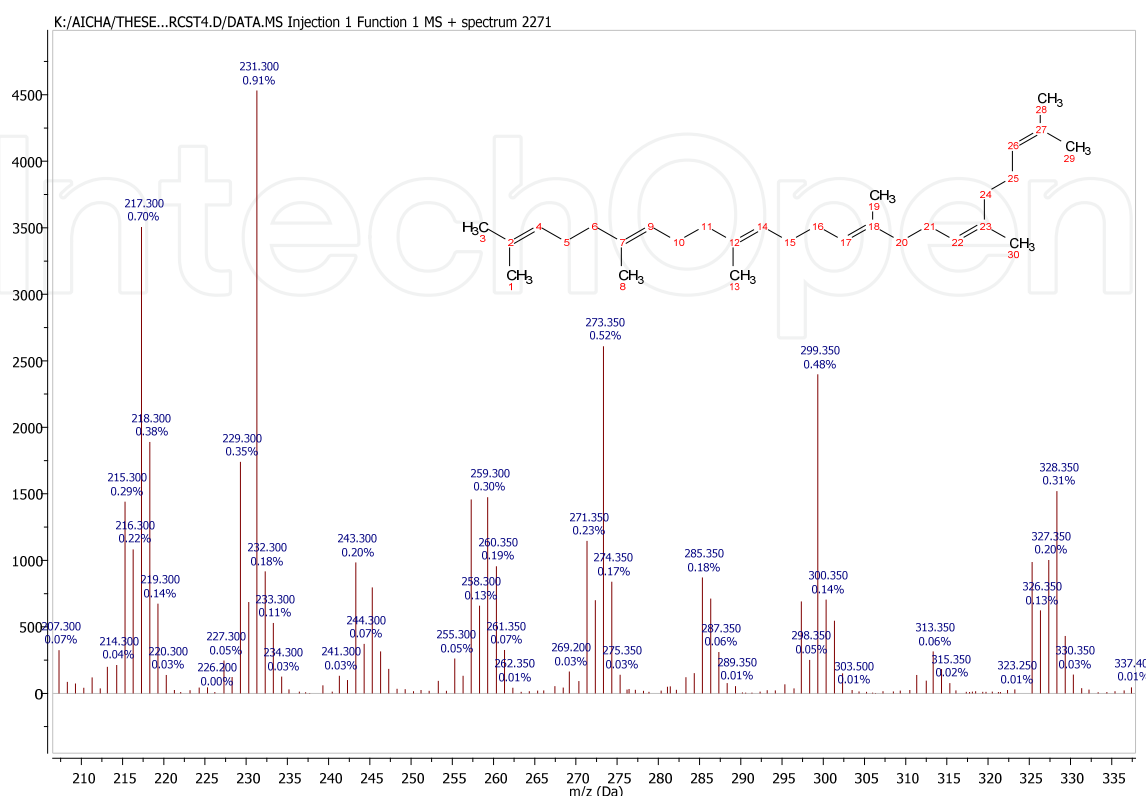


Fig. 4. Mass spectrum of squalene

Squalene is built up from a universal C5 building block isopentenyl diphosphate (IDP or IPP). The IPP in the classical Bloch-Lynen pathway; is formed from three molecules of acetyl-CoA via the mevalonate (MVA pathway). Then the squalene epoxidase catalyzes the epoxidation of squalene to 2,3-oxidosqualene, which is the substrate for formation of sterols and a wide range of triterpenoids (Volkman, 2005).

In humans, squalene is synthesized in the liver and the skin, transported in the blood by very low density lipoproteins (VLDL) and low density lipoproteins (LDL), and secreted in large quantities by the sebaceous glands (Reddy, 2009).

#### Beneficial effects of squalene

Squalene is also regarded as one of the compounds responsible for the beneficial effects against certain type of cancers (Tuberoso, 2007). Antioxidant activity of squalene is demonstrated against polyunsaturated fatty acid (PUFA), and it is secondary to that of phenols and tocopherols (Tuberoso, 2007). Its antioxidant activity can reinforce the endogenous antioxidant system against oxidative damage (Tuberoso, 2007). Together with other components of the non-saponifiable fraction, it prevents also the autooxidation process and thus contributes to the stability of the oil and its aroma and flavor characteristics (Tuberoso, 2007). It is normally used in its natural or hydrogenated form (squalene) as a moisturizing or emollient agent in cosmetic preparations (Moreda, 2001). Squalene is widespread in nature, especially among olives, liver oil, wheat germ and rice bran.

Oilseeds	Squalene
peanut	1276.0±27.8
olive	5990.0±95.1
maize	338.7±11.5
sunflower	170.5±6.4
pumpkin	3529.9±61.4
rapeseed	437.4±18.3

Table 6. Squalene content of selected vegetable oils (mg/kg, mean±SD) (Tuberoso, 2007)

2.4 Polyphenols (e.g. resveratrol)

The polyphenols are some of the most studied compounds and can be further divided into flavonoids (including flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavones), phenolic acids, stilbenes, coumarins, and tannins (Lampe, 2007).

Structure

*Trans*-Resveratrol or Res (trans-3,5,4'-trihydroxystilbene) (Fig.5) (Lin 2007) and its many derivatives (pterostilbene, piceatannol) are naturally occurring phytoalexins; a class of antibiotic compounds, produced in a select number of plant species as part of the plant's defense system (Bolivar, 2007).The terminal enzyme in the production of resveratrol is resveratrol synthase, which condenses *p*-coumaroyl-coenzyme A and three malonyl-coenzyme A molecules to form resveratrol (Bolivar, 2007).This enzyme is highly regulated by elicitors and general plant defense compounds in an effort to protect the plant. Resveratrol exist as both the *trans*- and *cis*-isomers but the most active for is the *trans* one.

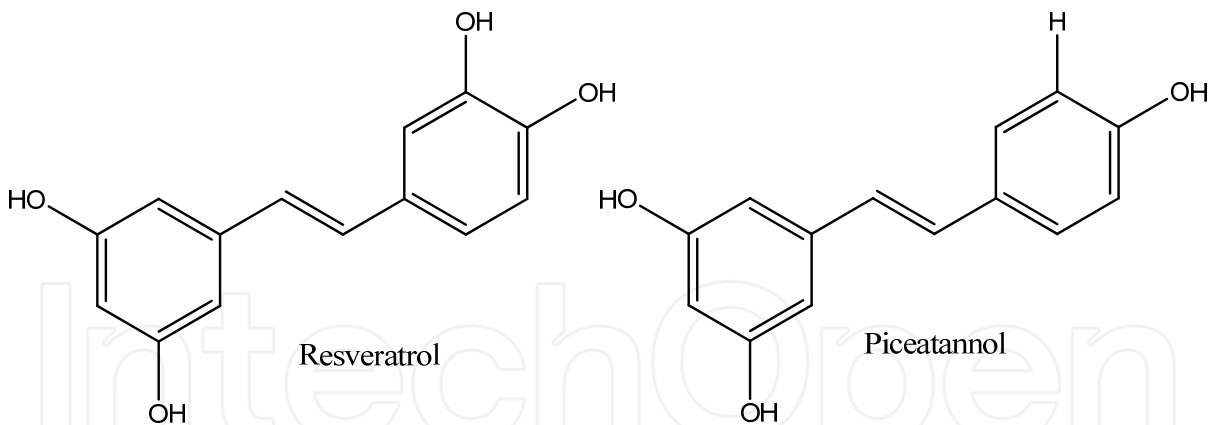


Fig. 5. The chemical structure of *trans*-resveratrol and *trans*-piceatannol (Lin, 2007)

Resveratrol and effects

Res and its derivatives like piceatannol, has similar structure (Fig Lin, 2007), but piceatannol has much potent biological activities than resveratrol. Piceatannol is more efficient inducer of apoptosis (Lin, 2007). Other reports, suggested that both piceatannol and resveratrol are able to induce apoptosis in many cancer cell lines, but to different extent (Lin 2007).Lin et al. reported that piceatannol is more effective in cancer prevention and could be an anticancer compound while resveratrol is a chemoprotective one. In fact, resveratrol and piceatannol become promising natural compounds in cancer treatments (Lin, 2007), they blocks the carcinogenesis stages of initiation, promotion and progression (Lu, 2009).

In plants, the amount of piceatannol is much lower than that of Res. Lu et al reported that roots can produce higher levels of phytoalexins in response to environmental stimulations like injury or fungal attack (Lu, 2009).

Besides, its strong anticarcinogenic effect, Res decreased coronary heart diseases mortality (Lu, 2009), modulates lipid metabolism, protects low-density lipoproteins against oxidative and free radical damage (Lu, 2009), and inhibits platelets activation and aggregation (Lu, 2009), and anti-aging benefits (Bolivar, 2007)

A number of taxonomically unrelated plant families (72 plant species) have been reported to produce marked levels of resveratrol including: peanuts, several types of berries, some pine trees and most recently tomato fruit skin (Ragab, 2006).

The highest level of piceatannol and resveratrol was detected in roots due to their consistent exposure to the microorganisms in soil.

### 3. Conclusion

Phytochemicals in plant material have raised interest among scientist, producers, and consumers for their roles in the maintenance of human health and in assessing the protective status of people from chronic degenerative disorders. Given the importance of dietary habit and food components to health, the provision of phytochemical information of a range of foods is vital to support the future work in assessing the protective status of people from chronic degenerative disorders. Food-based approaches would be essential for sustainable solutions to combat the alarming prevalence of chronic cancer, coronary heart diseases and diabetes. The health benefits of these foods may prompt research onto the assessment and determination of potential rich sources of phytochemicals compounds in agricultural produce.

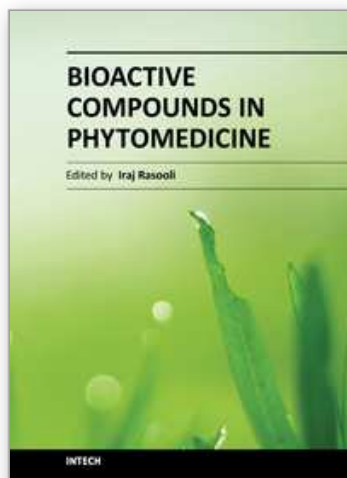
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## **Bioactive Compounds in Phytomedicine**

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There are significant concerns regarding the potential side effects from the chronic use of conventional drugs such as corticosteroids, especially in children. Herbal therapy is less expensive, more readily available, and increasingly becoming common practice all over the world. Such practices have both their benefits and risks. However, herbal self-therapy might have serious health consequences due to incorrect self-diagnosis, inappropriate choice of herbal remedy or adulterated herbal product. In addition, absence of clinical trials and other traditional safety mechanisms before medicines are introduced to the wider market results in questionable safe dosage ranges which may produce adverse and unexpected outcomes. Therefore, the use of herbal remedies requires sufficient knowledge about the efficacy, safety and proper use of such products. Hence, it is necessary to have baseline data regarding the use of herbal remedies and to educate future health professionals about various aspects of herbal remedies.

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