UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

# ADVANTAGES OF EXTRA VIRGIN OLIVE OZONIDE OIL COMPARED TO OTHER LIPID MATRICES

PhD in clinical pharmacology and medical therapy
Biomedical Sciences and Human Oncology Department

Dott. Angelo TARULLO

Oxygen: the element most present and active in the mechanisms that govern life

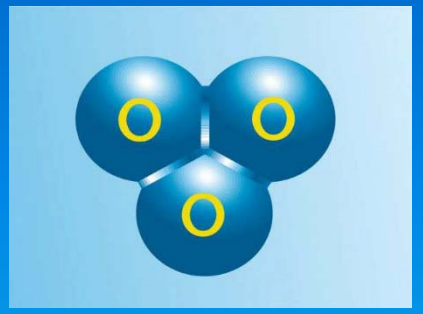
-It is one of the fundamental constituents of living matter;

-It is the parent of all chemical reactions of higher organisms;

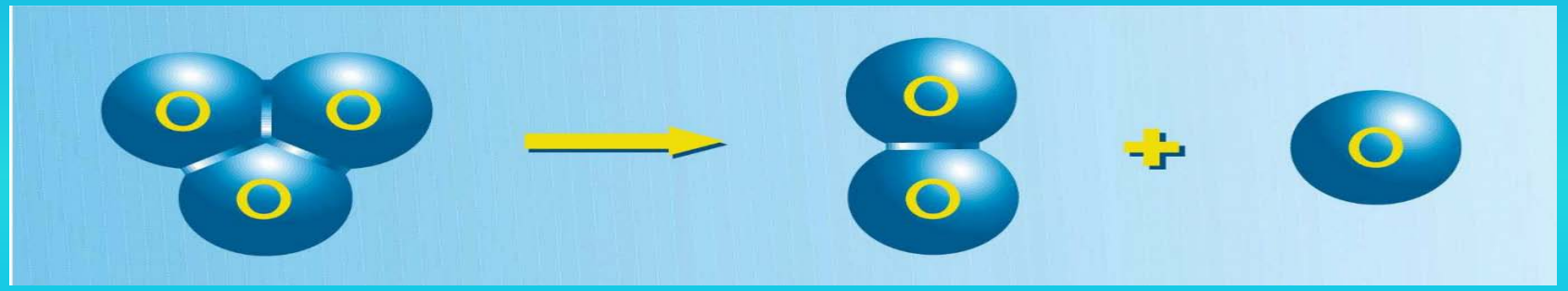
-It is the power source that enables the continuous renewal of our cells;

The Ozone

Ozone is a gas formed by three atoms of oxygen



It is very unstable and tends to decompose in an infinitesimal fraction of a second, releasing more stable molecular oxygen and highly reactive atomic oxygen

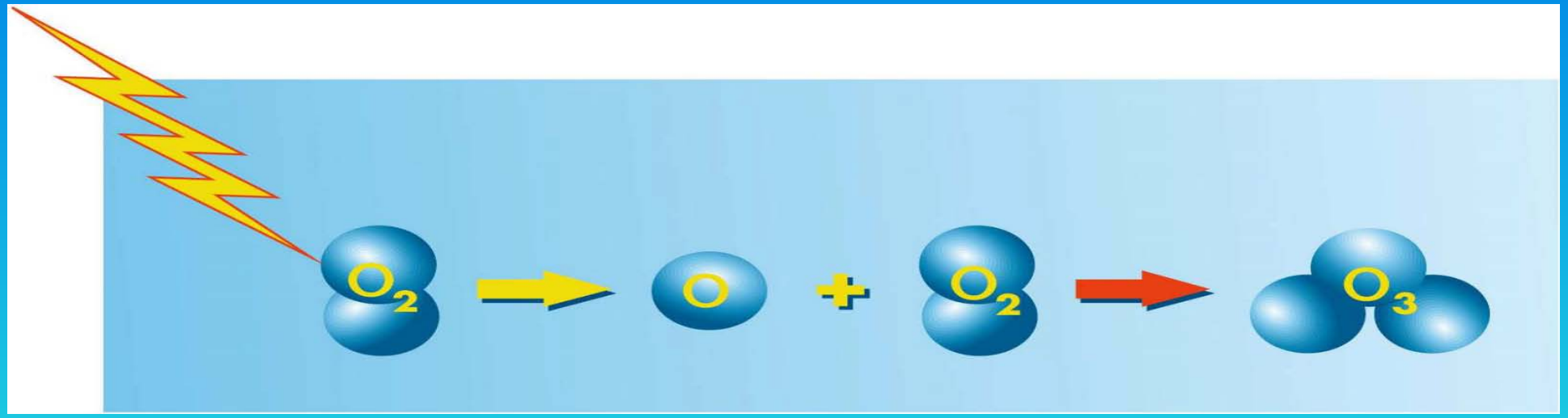


Rappresentazione grafica REAZIONE DECOMPOSIZIONE OZONO

# The Ozone

Ozone is formed during thunderstorms due to electric shock

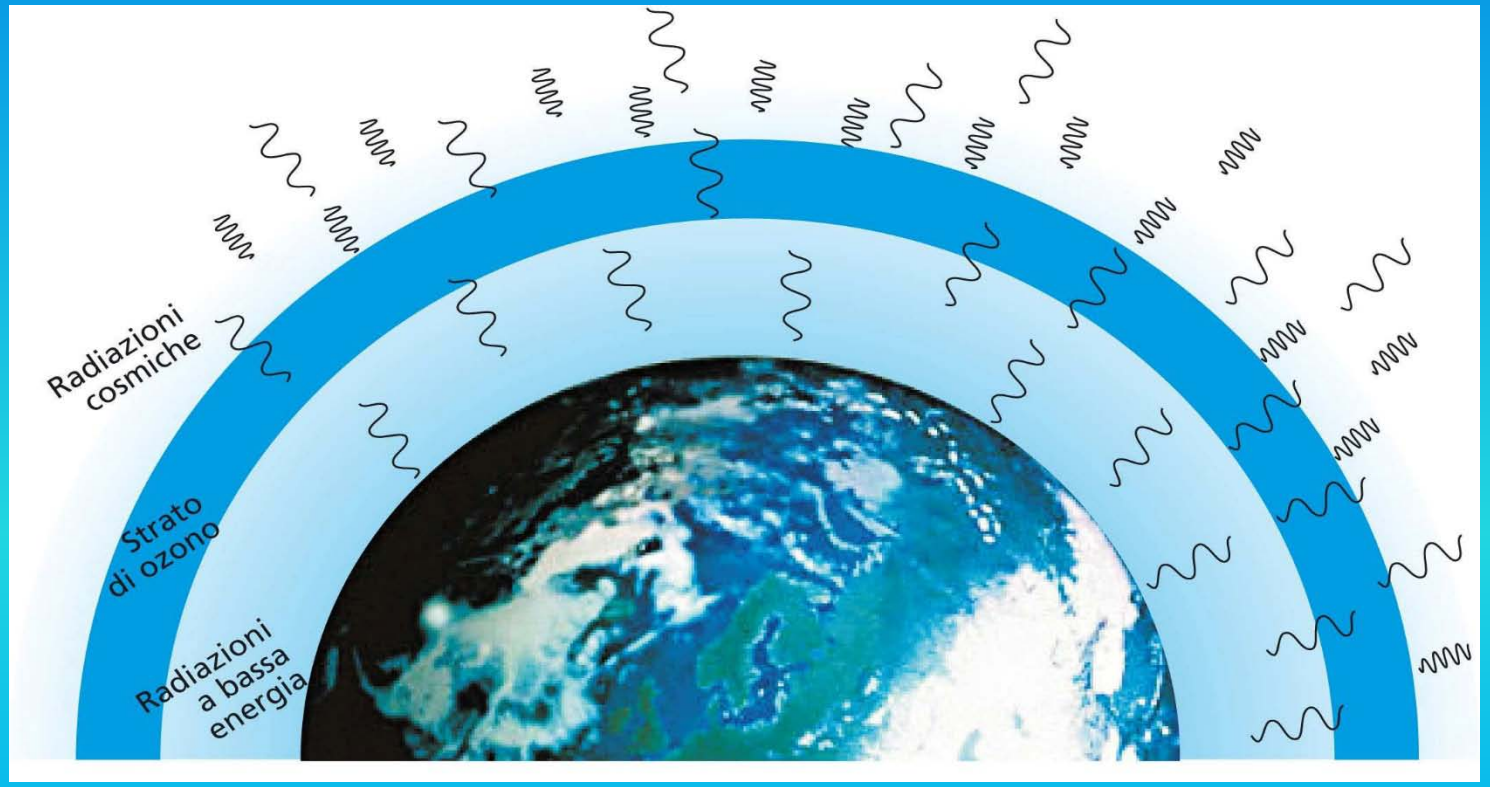
and its typical smell is felt easily




Ozone gas is perfectly tolerated by the skin on which it pours its beneficial, energizing and sanitizing properties

Ozone: the "gas of life

A layer of ozone gas in the upper atmosphere allows the existence of living organisms because of its ability to absorb the most energetic and dangerous radiation from space (cosmic radiation, gamma radiation and UVB-UVC)



# The first medical applications

- Oxidant and disinfectant properties are at the basis of research into therapeutic applications;

- In the twentieth century, particularly during World War I, ozone was used for disinfecting wounds and for water purification.

# Ozonetherapy in the XX century

The first applications of Ozone, produced by using electric arcs, were limited to skin contact through special waterproof casings that surrounded and closed areas to be treated;

Later on, the mixture of oxygen enriched with Ozone was directly injected into the blood

# The Ozonetherapy gas phase: its limits

Topical applications of ozone or plasma treatment with ozone require:

- Equipment capable of generating ozone gas from photovoltaic shots;
- Specialized personnel and appropriate tools;
- The need for the patient to travel to the clinic where the treatment is made.

In practice, no home care is possible and the costs are very high.

# The big challenge: to trap the Ozone

Great instability:

-Difficulty in practicing the Ozonetherapy;

-need to "store" this molecule.

Now

Trapping Ozone: the chalenge has been won

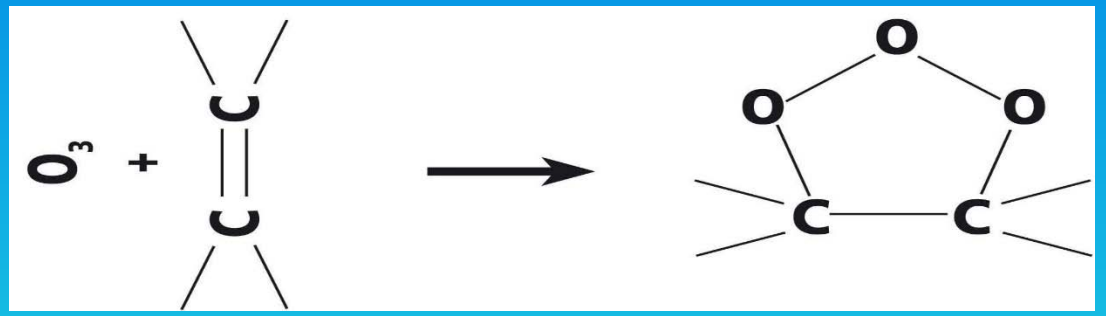
Ozone prefers the double bonds of the unsaturated lipid chains

SO

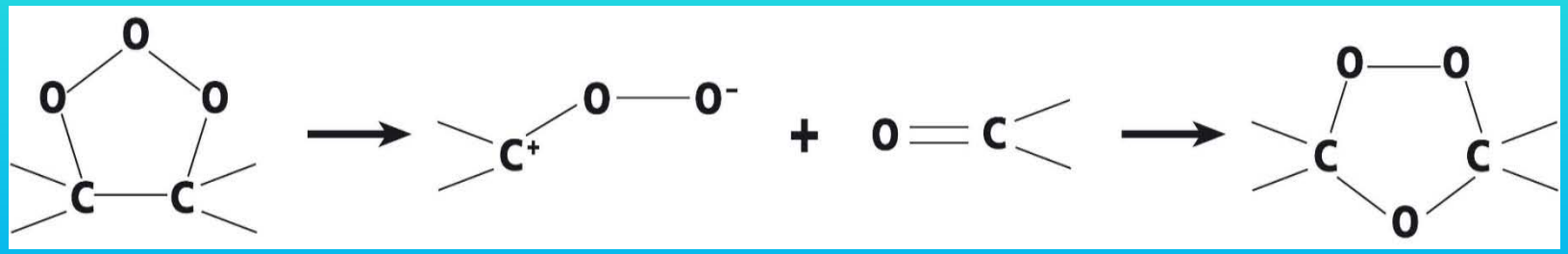
Extra virgin olive oil "reservoir" for the three oxygen atoms of ozone

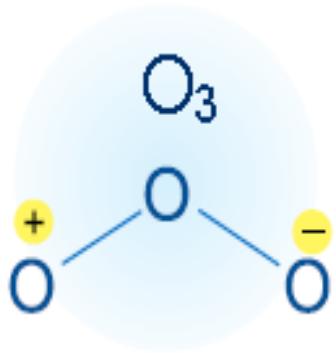
# The structure of Ozonides

The reaction that takes place between the gaseous ozone and oils with high level of unsaturation is an addition of the lipid chains to the double bond carbon-carbon

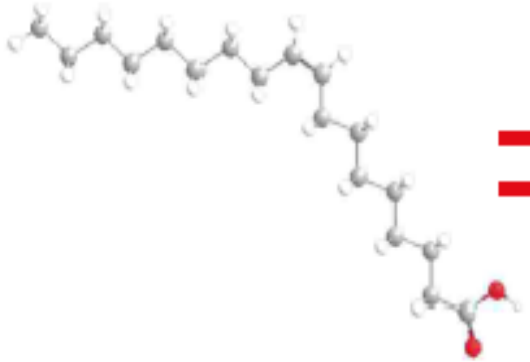



The formation of primary unstable Ozone is an intermediate step of very short duration. Through a rearrangement a much more stable Ozonuro results

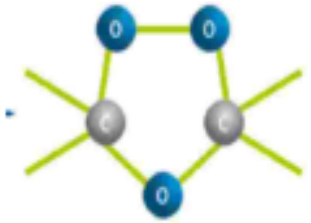





OZONE

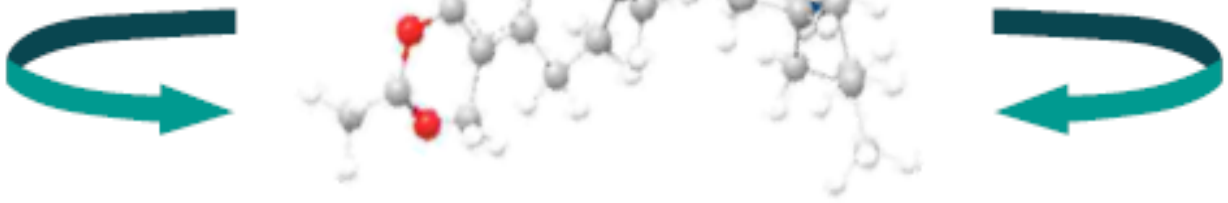


OLEIC ACID




OZONIDE

BIOPEROXIDES

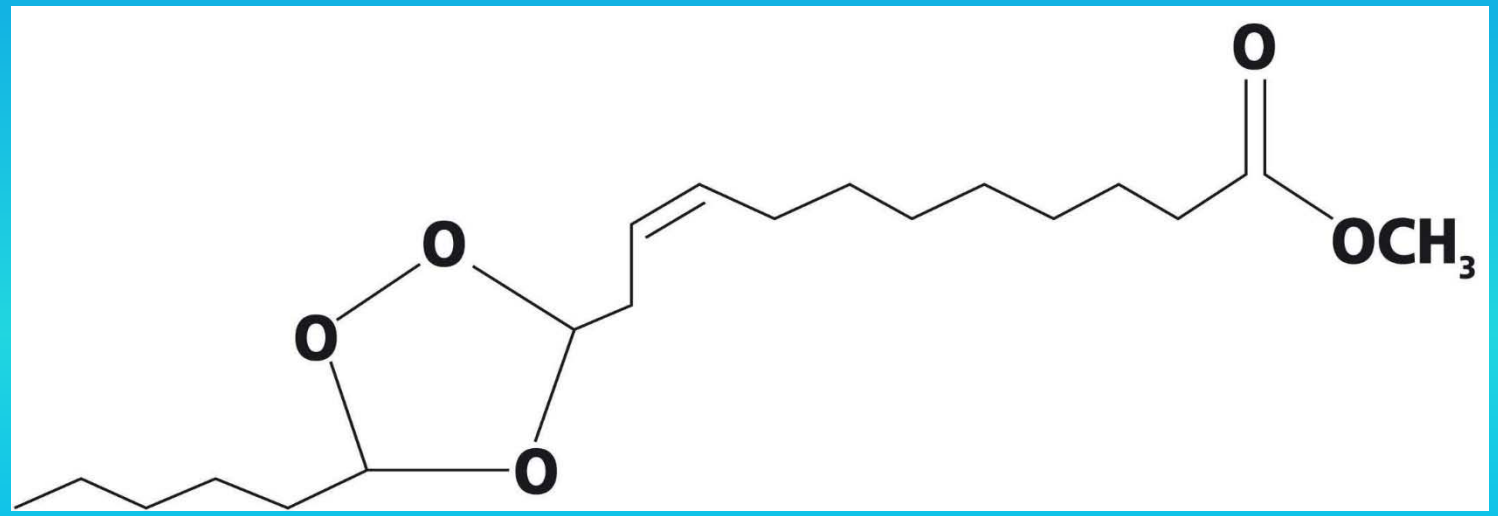


ACETATE E VITAMINE

# The extra virgin olive oil: chemical characterization

The structural identification of molecules of Ozonides by means of GC / MS Gas chromatography-mass spectrometry and NMR was conducted by the Institute of Organic Chemistry of the University of Bari and Lecce.

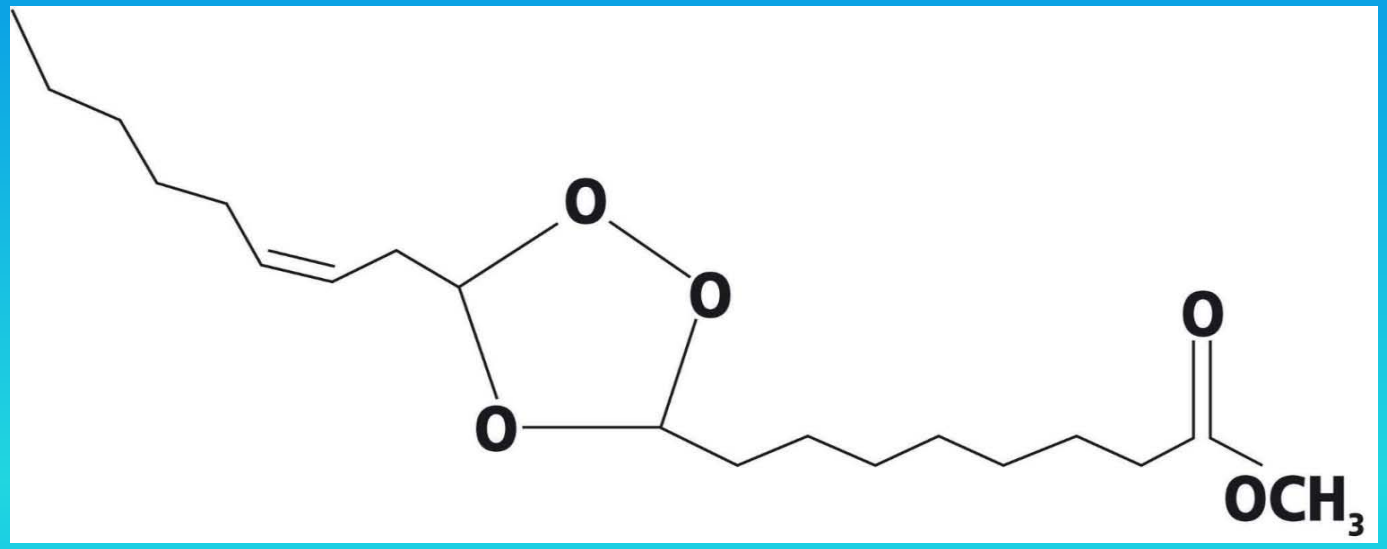
The linolenic and linoleic acids molecules, with the double bonds saturated with 3 oxygen atoms, are to be highlighted in particular




Stereochemical structure of linolenic acid ω3 ozonated Monozonuro in A12

# The extra virgin olive oil: chemical characterization

Its chemical characterization highlights the ozone loading in the double bond of linoleic acid




Stereochemical structure of linolenic acid ω6 ozonized Monozonuro in A9

# The Ozonides:

Molecules of fatty acids in which the double bond is saturated by three oxygen atoms of ozone.

- "Transporters" of ozone: they imprison it, keep it stable and make it available and ready for use EVEN FOR HOME CARE;

- They are incorporated into a composition with Vitamin E and alpha-lipoic acid. This way functional products for topical applications are obtained.

# Toxicological and dermatological evaluations

The Ozonides obtained by selective catalysis, as well as being studied in their chemical structure, have been widely tested in their pharmacology and toxicology and in their dermatological compatibility.

**Toxicological and dermatological information:**

| | |
|---|---|
| **Toxicity:** | |
| Acute oral/Swiss mice (Parma Univ. Italy) | LD50 > 16 000 mg/kg (non toxic) |
| Sub acute oral/Swiss mice (5 weeks)(Parma Univ. Italy) | LD50 > 3 000 mg/kg (non toxic) |
| Intraperitoneal/Swiss mice (Parma Univ. Italy) | LD50 > 1 600 mg/kg (non toxic) |
| Mutagenicity/Wistar rats, intragastric | not mutagenic, nor citotoxic |
| Teratogenicity/Wistar rats, intragastric | not teratogenic |
| Muscle histology/Wistar rats | no effect |
| **Animal dermatological tests:** | |
| Dermic sensibilization (ISO-10993) (Guinea pigs) | none |
| Dermic fotosensibilization (Nagamuna, M. Shiseido Labs. Jap.) (Male New Zealand White rabbits) | none |
| Ophthalmic irritation (Draize) (Male New Zealand White rabbits) | <20 (pass test) |
| Vaginal irritation (ISO-10993) (Female virgin New Zealand White rabbits) | Med. (pass test) |
| **Human dermatological tests:** | |
| Open epicutaneous irritation test (Ferrara Univ., Italy) | Non irritant |
| Dermal patch irritation test (Ferrara Univ., Italy) | Non irritant |

# Chemical composition of fats and oils of both plants and animals

| Oils and Fat | Saturated | Monounsaturated | Polyunsaturated | Smoke point | |
|---|---|---|---|---|---|
| | | | | A[65] | B[66] |
| Peanuts Oil | 14.5-26.9 | 37.1-69.4 | 14.0-43.1 | 232°C | 198°C |
| Coconut Oil | 82.0-100 | 5.4-8.3 | 1.0-2.3 | 180°C | 194°C |
| Sunflower Oil | 9.2-16.4 | 14.0-39.8 | 48.3-74.2 | 232°C | 209°C |
| Corn oil | 9.9-21.9 | 20.2-42.7 | 39.9-64.0 | 232°C | |
| Olive Oil | 8.0-26.1 | 55.0-86.9 | 3.5-21.0 | 210°C | |
| Palm Oil | 45.0-57.0 | 36.0-44.0 | 6.5-12.5 | 180°C | 223°C |
| Palmist Oil | 77.0-87.0 | 12.0-19.2 | 1.0-3.7 | | |
| Soy Oil | 11.1-20.3 | 17.7-26.7 | 55.3-66.6 | 232°C | 213°C |
| | | | | | |
| Butter | 53.2-67.5 | 20-27 | 3.4-5.5 | 149°C | |
| Margarine | 33.8-71.5 | 36.78 | 17.64 | 170°C | |
| Lard | 43 | 43 | 12 | 191°c | |

# Differences in fatty acid composition between the various vegetable oils

**Extra virgin olive oil**

- Oleic acid: **73.6** %
- Linoleic acid (omega 6): 7.85 %
- Linolenic acid (omega 3): 0.99%

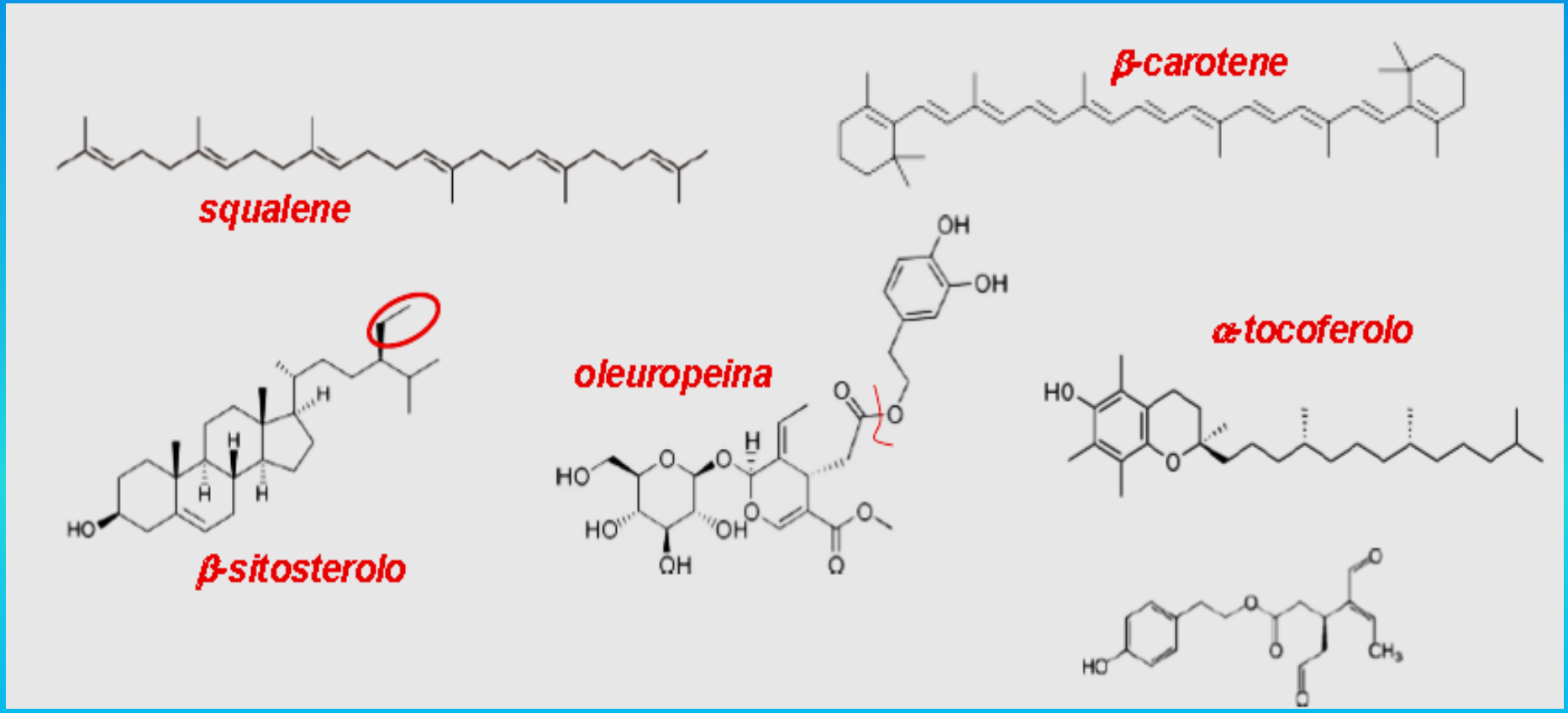
**Corn oil**

- Oleic acid: 29.9 %
- Linoleic acid (omega 6): **49.8** %
- Linolenic acid (omega 3): 0.6 %

**Sunflower oil**

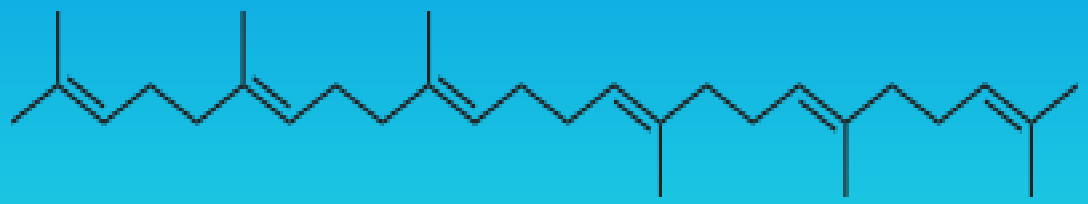
- Oleic acid: 32.9 %
- Linoleic acid (omega 6): **49.9** %
- Linolenic acid (omega 3): 0.33 %

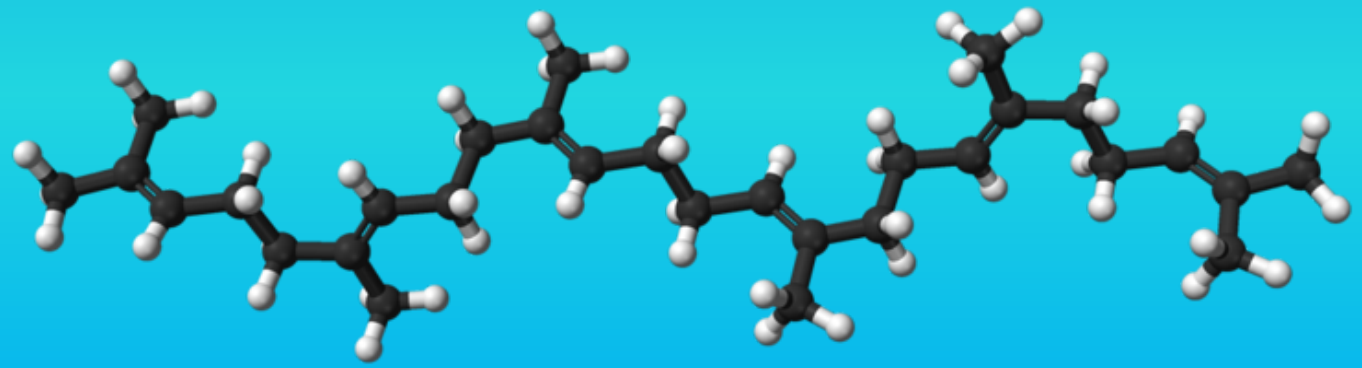
# Substances present in 2-3% unsaponifiable fraction



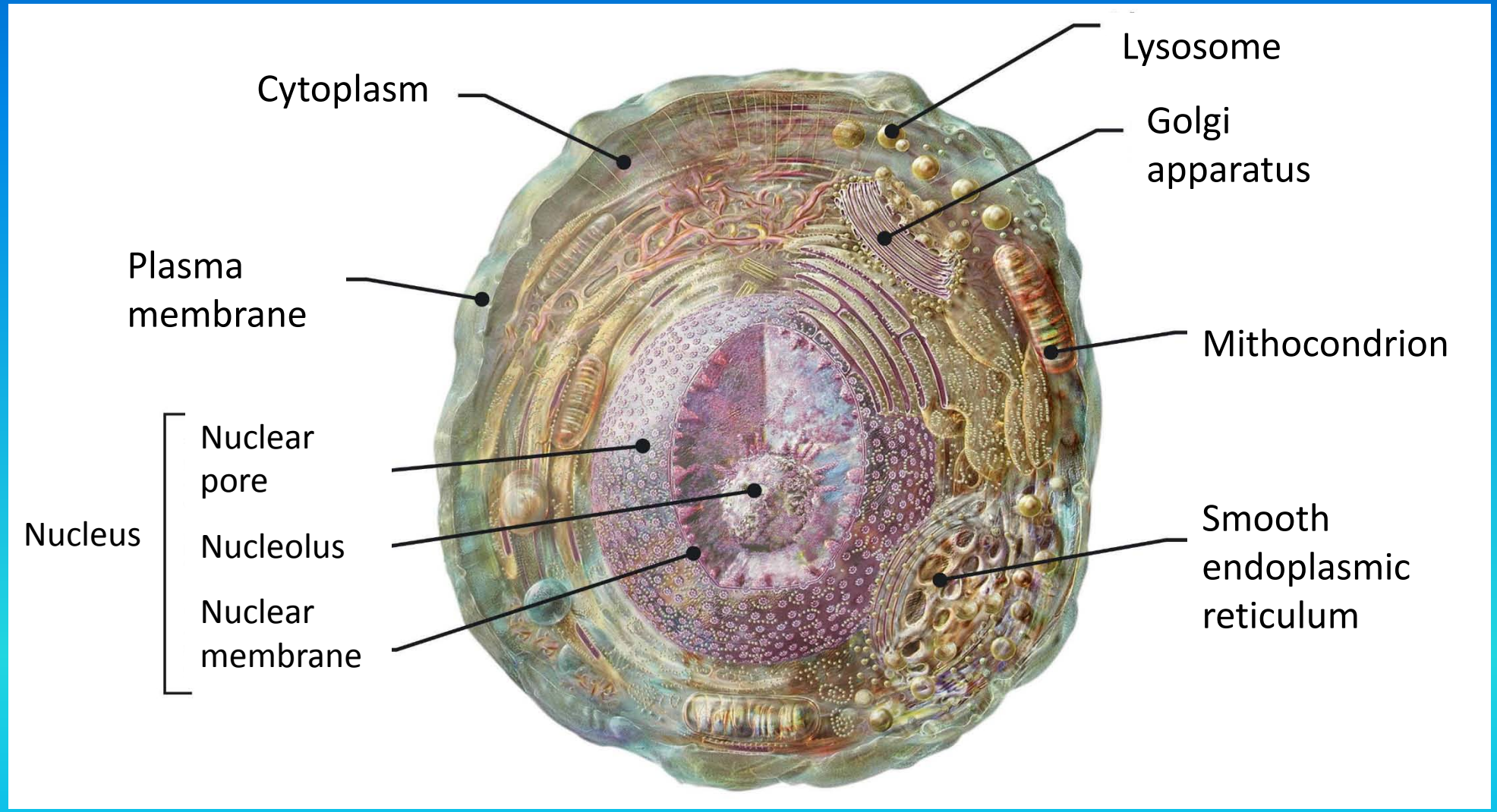
# Squalene

It 'a triterpene with formula C30H48. Vegetable Squalene in EVO is found in percentage of 400mg / 100g of oil. Some studies attribute to the Squalene antioxidant properties associated with a protective effect against cancer of the skin, thanks to its ability to scavenge free radicals and absorb harmful solar radiation. Starting from Squalene β - sitosterol is formed in not less than 93% of the total.

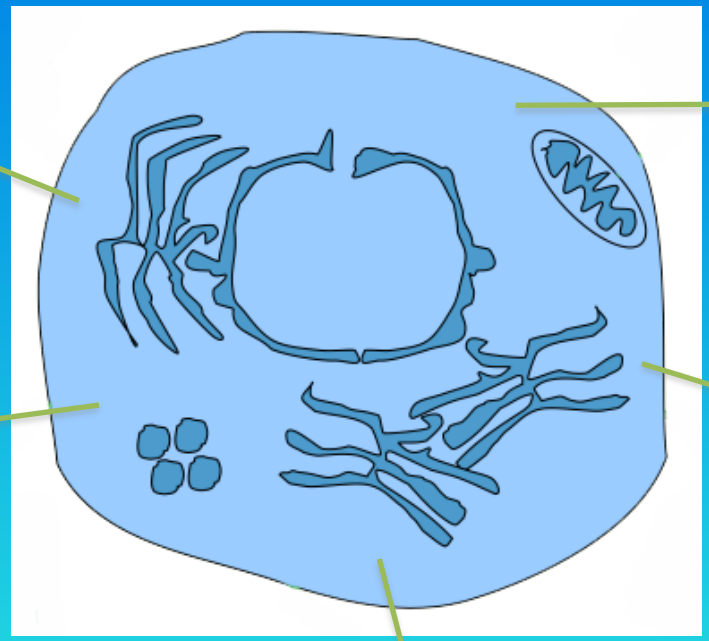




# The human cell



# The antioxidant system is regularly distributed in the cell



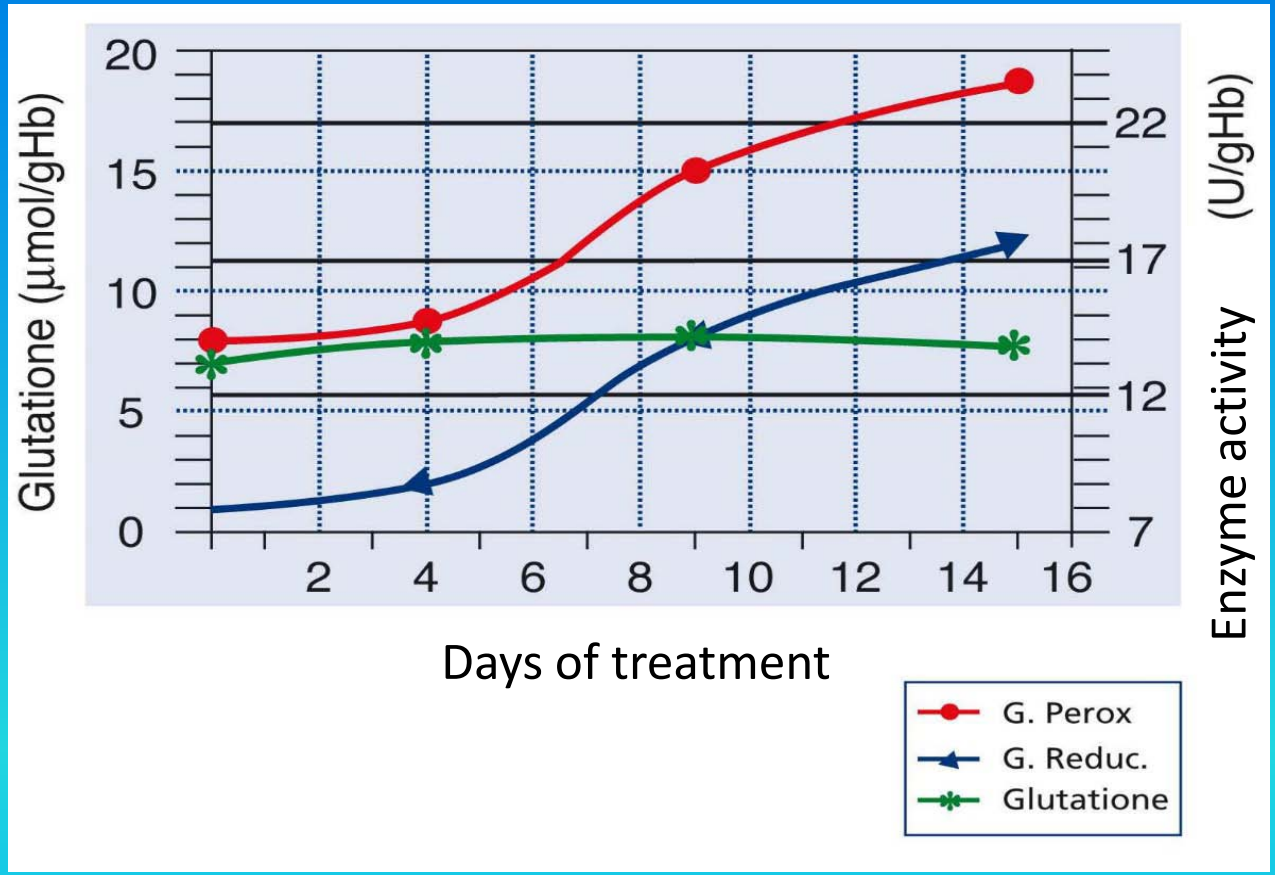

The lipophilic antioxidants present in the membranes are the first line of defense against ROS

# The enzyme levels of the blood and the antiradical effect :

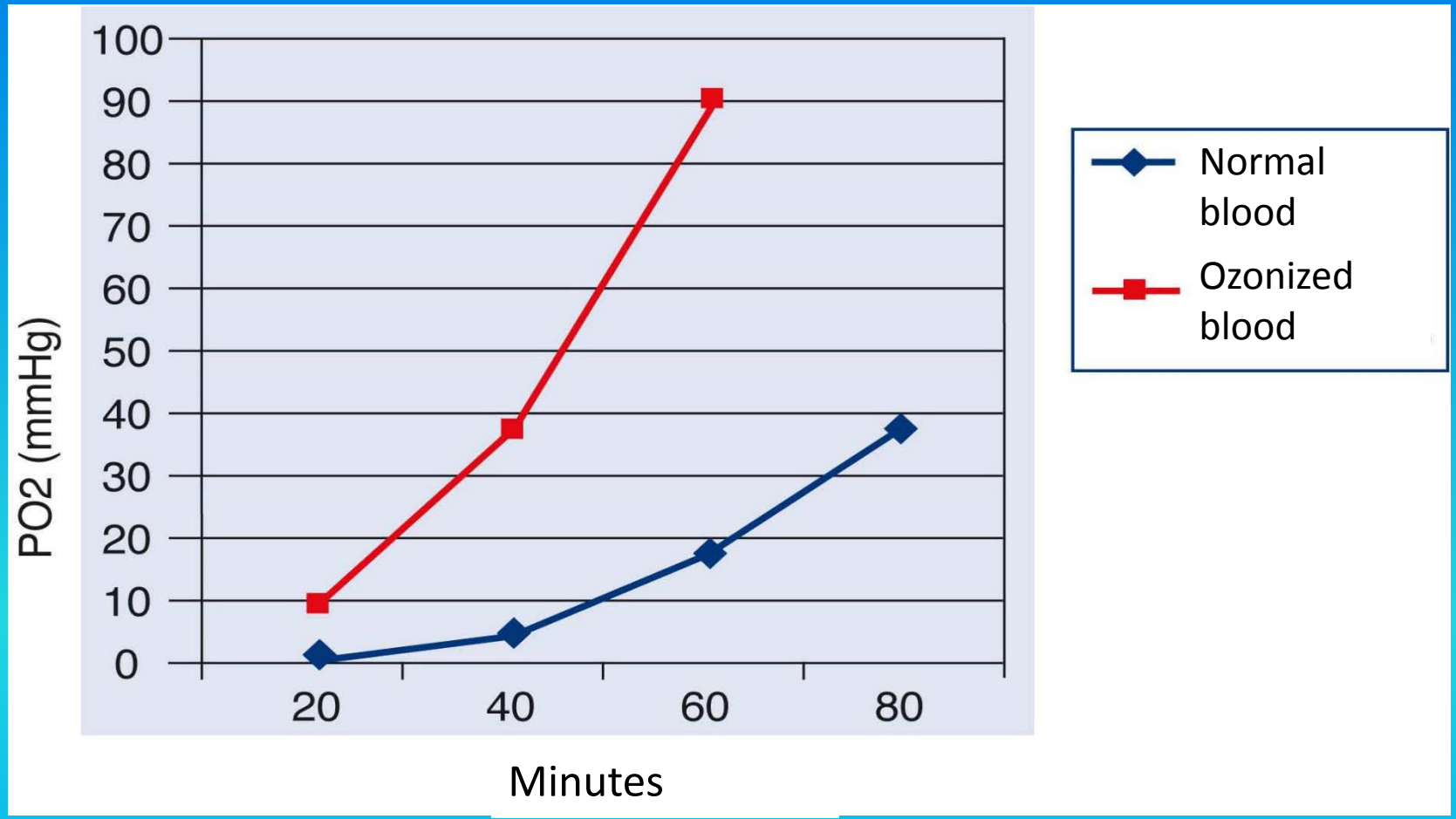
## scientific tests on human plasma

The increase in the capacity of defense by rise of enzyme values of the cell has been experimentally demonstrated in human plasma after treatment with Ozone.

The content of the enzymes involved in the destruction of free radicals increases.



# Ozone and oxygen in the blood - scientific tests



The bioavailable Ozone :

the answer to tired cell (anti-radical action)

The Ozonides easily penetrates the cell membrane

RESULT:

- Acceleration of glycolysis;
- Increased availability of energy for the cells;
- Increased values of enzymes responsible for the neutralization of toxic substances.

A mechanism of penetration of Ozonides through the cell membrane has been hypothesized, that has three basic conditions:

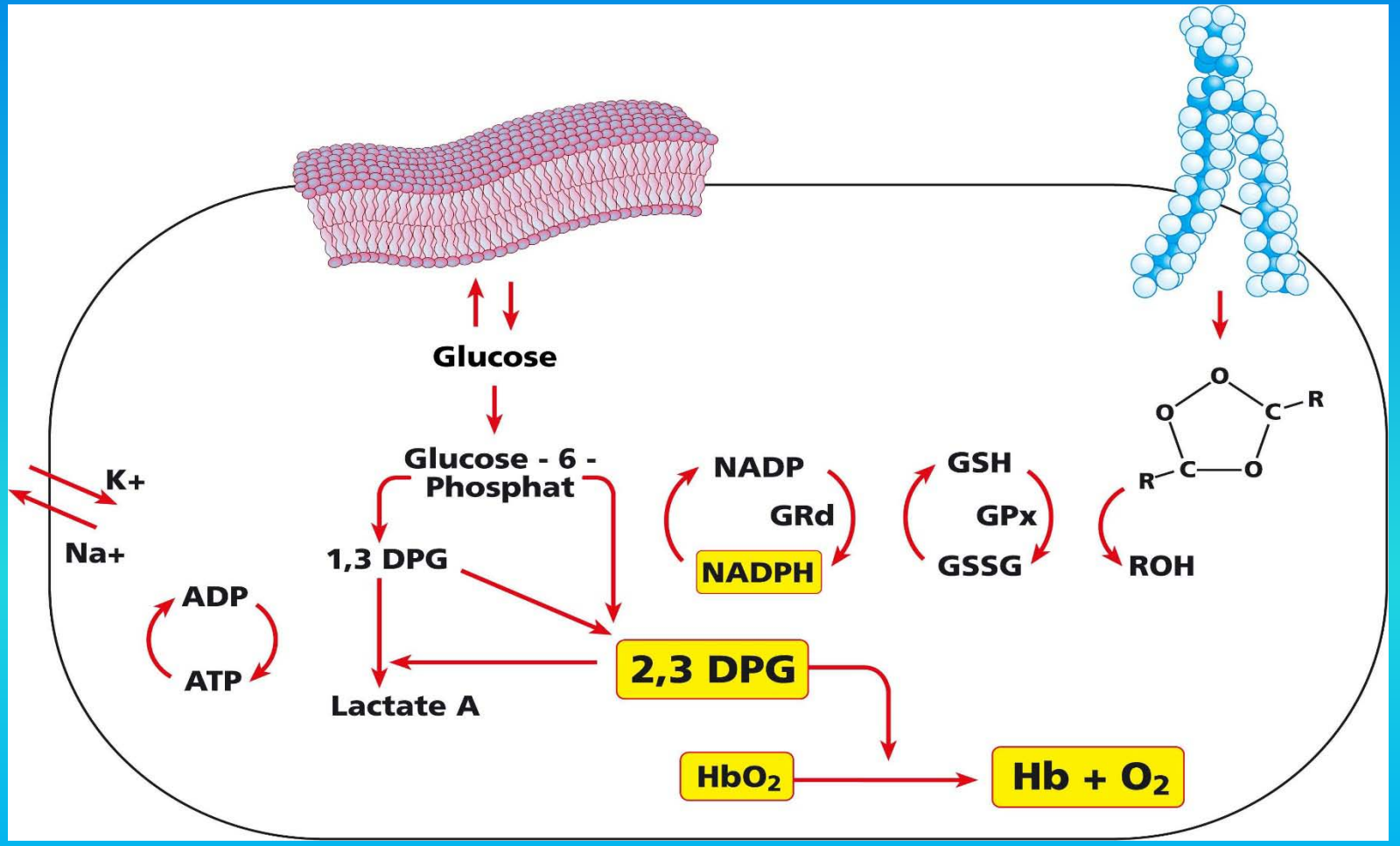
- low molecular weight (less viscosity);
- short chain length of unsaturated fatty acids linked to ozone (easy penetration);
- hydrophilic molecule merging with the cell wall can flow into the cell cytosol.

**The action of Ozonides is expressed both on Coenzymes, both at the level of all the three fundamental Metabolic lines :**

- **Carbohydrate metabolism:** The transformation of peroxides in alcohols and the split of GSH (glutathione) in GSSG (glutationedisulfile) has action on the shunt of the pentoses with an increase of glycolysis. By speeding glycolysis an increase of the energy released from the aerobic glucose demolition is obtained, used for the synthesis of ATP which in fact increases.
- **Lipid Metabolism:** by reacting with ozone in a waterside setting, the lipids form peroxides which lead to hydrolytic cleavage of the same, losing their hydrophobic character they are transformed into hydrophilic components. Direct effect on lipolysis and on the breakdown of fats which release energy.
- **Protein metabolism:** Action on amino acids (methionine and tryptophan) or sulfur-containing (cysteine) whose degradation is protected by glutathione and by coenzyme NADH and NADPH. Interaction with the phospholipids of the cell erythrocyte membrane - Increased deformability (electrostatic effect) leading to the increase in glycolysis (greater presence of the intermediate metabolite of erythrocyte glycolysis 2,3 diphosphoglycerate) **Facilitating the release of O2 by hemoglobin through the following scheme:HbO2 + 2.3 * Hb.2,3 DPG DPG + O2 (Bohr effect)**

# The mechanusm of action in the cell

The Ozonides stimulate the cell's natural defense from harmful substances and increase their energy as ATP



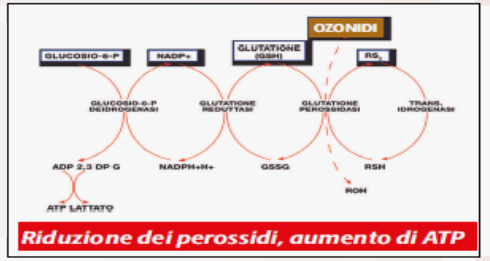
# Ozonides:

## CARRIER ACTION

*Gli Ozonidi veicolano ossigeno legato con biodisponibilità immediata attraverso la membrana plasmatica, facilitano il passaggio dei principi attivi*

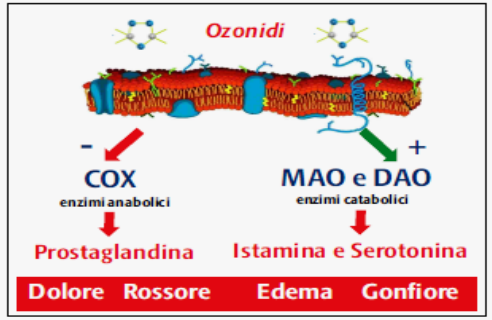

## FREE RADICAL SCAVENGER ACTION

It triggers a series of reactions related to the processing of peroxides in alcohols which combined with the disruption of the GSH-GSSG accelerate glycolysis



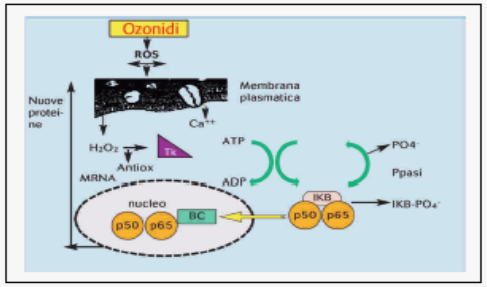

## ANTI-INFLAMMATORY ACTION

The concentrations of inflammatory mediators decrease, so decreasing symptoms and signals induced by inflammatory state




## IMMUNO-MODULATING ACTION

The Ozonides exert a stimulating effect on the production of cytokines and interleukins involved in the mechanisms of cellular communication at the level of the immune system



# To summarize:

**The application of Ozonized Oil on the wounds:**

- **It speeds up the repair process;**
- **increases the average number of fibroblasts;**
- **increases the average number of blood vessels making the skin tissue more organized and sprayed;**
- **red cells are arranged neatly along the axis of the vessel to form a stack (effect Fahraeus - Lindquist)**

# Resultsi:

**+ Natural defenses**

**+ Energy to fibroblasts**

**+ Collagen production**

**+ Speed in the processes of repair**

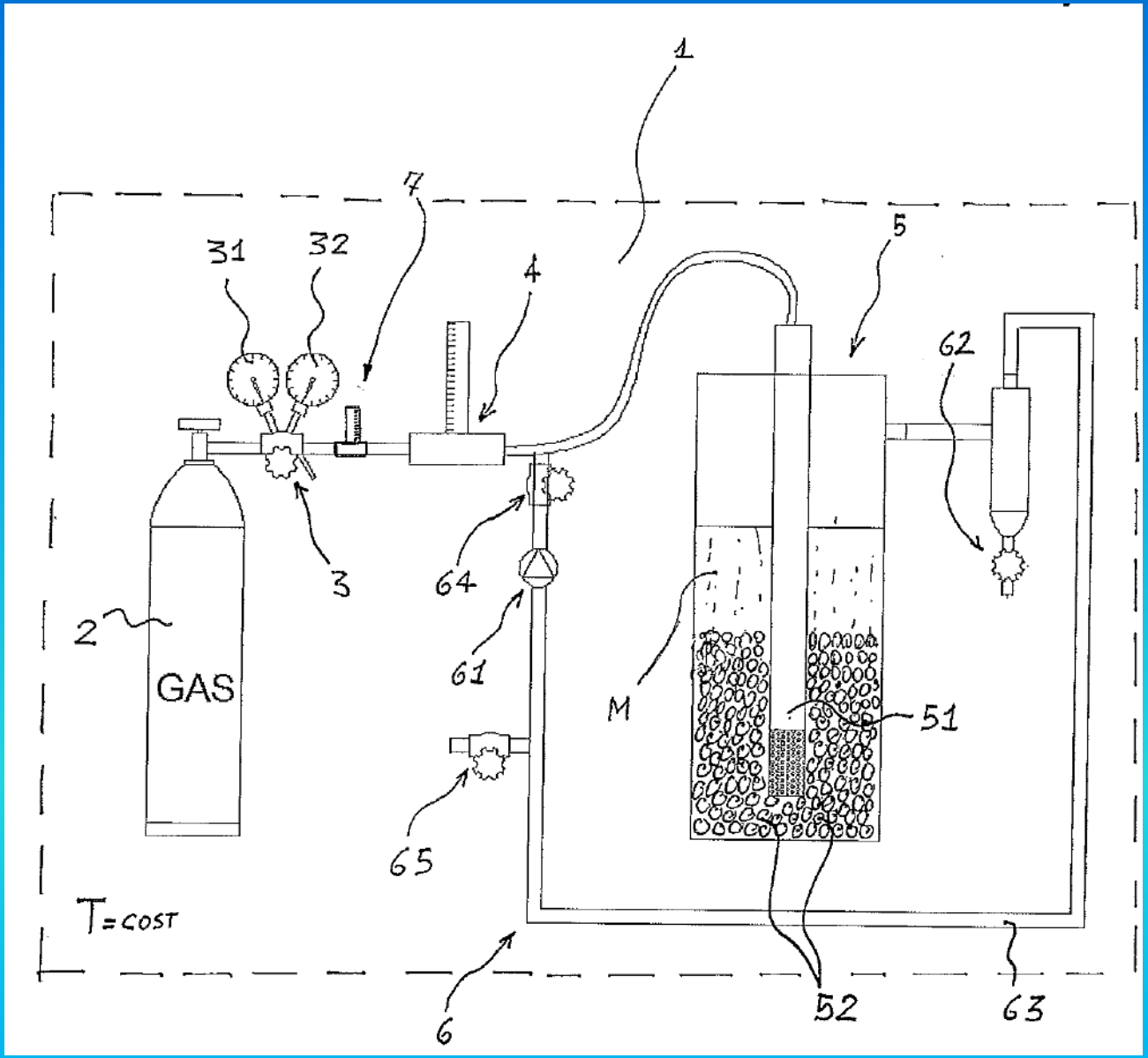
**+ Health for the skin**

# Useful substances to improved olfactory

| Volatile substance | Scent |
|---|---|
| **2-methyl-propanole** | Like ethyl acetate |
| **1-penten-3-ole** | Wet ground |
| **1-penten-3-ole** | Sweet, strawberry, tomato, green |
| **3-pentanone** | Sweet |
| **2-methyl-1-butanole** | Fish oil |
| **(Z)-2-penten-1-olo** | Banana |
| **esanale** | Green, apple, cut grass, sweet |
| **(E)-2-esanale** | Almond, green, fruity |
| **(Z)-3-esanale** | Banana, freshly cut grass, green |
| **(E)-2-esenole** | Green sliced fresh, sweet, floral, fruity, tomato |
| **1-esanolo** | Fruity, aromatic, soft |

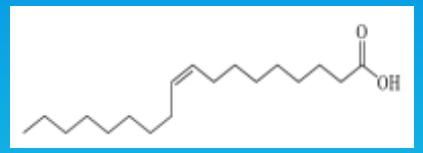
**Morales et al. 1995. J. Agric. Food Chem, 43:2925-2931**

# NOTES ON PATENT



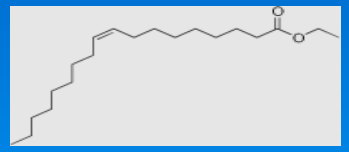
In the nineties, several studies have been conducted by an Italian industrial chemist **Prof. Walter Valeri** on chemical and pharmacological comparative aspects of ozone reacted with some lipid biological matrices.

These investigations were concluded with a list of three substances that react more effectively obtaining the ozonides with improved pharmacological characteristics:

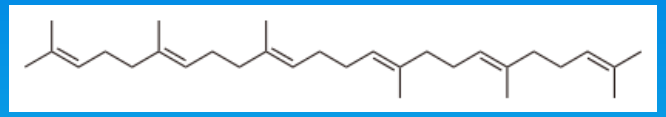
**First** 


**Oleic Acid** that for purely steric reasons (length of the molecular chain - low molecular weight) favors the reaction with ozone compared with a triglyceride unsaturated, obtaining preparations much more stable. Another important characteristic considered was the much lower viscosity than other fats and oils. In fact, to proceed to an pushed ozonation as in the method of our patent, other oils (castor oil or sunflower) significantly increase their viscosity forming polymers which, in association to the length of the molecular chain and the increase of molecular weight, play an unfavorable role on the pharmacological and absorption.

**Second**



**Ethyl oleate** which is obtained by the condensation of Oleic acid with Ethyl Alcohol (ethanol). It is formed naturally in our body after strong ingestion of ethanol. Its effectiveness depends on the fact that the carboxylic group of the oleic acid is involved in the ester bond with the alcohol group, resulting in a lower surface tension, greater reactivity with ozone, lower intermolecular forces, facilitating the absorption, compounds devoid of odorosity .

**Third** 

**Terpenes and their derivatives** without doubt the best ever, given their great affinity to water, low molecular weight, greater ratio active oxygen / moles of substance, great ease of spreading in tissues, increased reactivity with biological matrices.

# CONCLUSIONS

Let's recap the main points of this work:

- The high content of oleic acid in olive oil, the lower steric impact, the low molecular weight, 282.45 Daltons, and the lower intermolecular forces enhance and facilitate the absorption of ozonides;
- Best olfactory performance for the presence of hexenal;
- Massive presence of Squalene triterpene (400mg each 100 g of EVO);
- The new method (patented) makes ozone saturation of active lipid matrices (greater ratio O2 / moles of substance, a low molecular weight) faster and quantitatively more saturated. Furthermore the incorporation of Vitamin E and lipoic acid produces more stable secondary Ozonides, that is essentially pharmacologically more active.

- The high content of polyphenols with antioxidant, anti-inflammatory and stabilizing activity contributes, together with the Ozonides, to the formation of cytokines and interleukins having immunostimulating action, which, decreasing the superoxide and increasing the ATP, will trigger the process that limits and blocks Lipo- and Cyclooxygenase.

This, in turn, acting on the formation of leukotrienes and prostaglandins, will lead to a reduction in pain, swelling and inflammation.

These actions, when added to increased oxygenation and antibacterial, virustatic, antifungal action, will produce an early and safe healing, free of side effects.

In the light of these comparative studies, we can state that:

among the several lipid matrices up today ozonated with classical methods, those obtained by the new methodology from cold pressed virgin olive oil, ensure a higher content of Ozonides stabilized and pharmacologically active

# OLIVE WOOD ART

# Thanks for your attention

+