Ozonoids in Medical Practice

Authors; Dr Julian Holmes and Gijs Kort, South Africa, 2006.

Title;Ozonoids and Omega 3, 6 and 9 Fatty Acids in Skin Care; an Innovative Perspective.

Abstract; There is renewed interest in plant and vegetable extracts for use in the medical, pharmaceutical and cosmetic industries. These extracts offer opportunities to chemists and researchers to find novel products that mimic synthetic additive without unwanted side effects. These extracts meet the current trends demanded by consumers towards a more natural and holistic approach to health care. Consumers are keen to avoid the need for animal testing. This has to be balanced with research to show potential toxicity and microbiological resistance. Ozonated plant and vegetable extracts have been researched in various countries. They offer good anti-microbial activity, show no tendency to produce micro-biological resistance, and show no harm to the patient or operator. This paper seeks to explain why the current oils used in the ozonation process may not be the best choice, and research should be directed towards alternative oils. The concept of the 'Potential Ozonoid Index' (POI) is introduced and discussed. The POI can be used to select vegetable and plant extracts for ozonation.

Introduction;

There is a great deal of interest in plant and vegetable extracts for use in the medical, pharmaceutical and cosmetic industries. These extracts could potentially provide a number of novel natural products that mimic synthetic additives and antibiotics, without unwanted side effects. The various industries that research and manufacture products for the public need to be aware that there is a current trend by the consumer towards a more natural and holistic health-care product range. These consumers are also seeking to avoid the need for animal testing. Of prime importance in the pharmaceutical industry is the potential for microbiological resistance and the absence of harm to the end user.

Ozonated plant and vegetable extracts have been researched in various countries, notably Cuba where original research has been published for the last 40 years on the effects of ozonated sunflower oil. These ozonated oils offer good anti-microbial activity, show no tendency to produce micro-biological resistance, and show no harm to the patient or operator (Sechi LA, et al 2001).

The word for ozone comes from the Greek word "ozein" which means "to smell". Ozone was first noticed because of its characteristic pungent odor. The odor is detectable in air at levels of about 0.1 parts per million, and exposure to ozone becomes fatal to humans at around levels of 100 ppm for 10,000 minutes or 10,000 ppm for 30 seconds. Ozone, O3, is a blue-coloured gas at ambient temperatures, but this colour is not noticed at the low concentrations at which it is usually generated. In the liquid and solid states, ozone is dark blue. Liquid ozone boils at -111.3 ˚C and solid ozone melts at -192.5 ˚C.

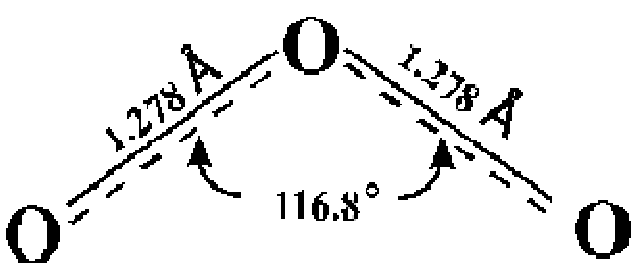

Fig 1. Atomic structure of Ozone (O_3)

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Ozone has the unique feature of decomposing to harmless, non-toxic and environmentally safe oxygen (O2). Ozone is one of a number of oxygen species generated by exposure of a stream of oxygen to a high voltage creating a corona discharge, the most common way of creating ozone for use. Ozone is the tri-atomic state of oxygen, symbol O3, and is one of many oxygen species formed in a corona discharge. Described in 1785 by Van Marum and named in 1840 by Shonbein (although some historical authors have suggested another German scientist, Christian Fernandez should be credited with the discovery of ozone)

‘ozone’, from the Greek word “ozein”- to smell, it was not until a reliable and cheap generator was invented by Werner Von Siemens in 1857 did the medical significance of this gas come to the fore. The chemical nature of this new gas was studied in Oxford in 1872. At that time these scientists were working on the chemical properties of oxygen calling oxygen the “Hero of Chemistry”. When they began to study ozone they were not willing to give it a separate identity, so they called it ozonated oxygen.

It has had a chequered history in medical and dental usage. In the early 20th century, the USA and Germany led the research into the pharmacological effects of ozone with numerous studies and books being published. However, despite a plethora of text books and clinics throughout Europe and the United States from the 1930’s through to the 1950’s, the use of ozone in medicine and dental care has now virtually disappeared. The cheap production of chlorine (developed as part of a chemical warfare research project in the First World War) was found to be more economical for water sterilisation, and in medicine, the development of antimicrobials and antivirals pushed the research into ozone to the doldrums.

In contrast, in the water industries around the world, ozone sterilisation has become the treatment of choice for potable water supplies, and has been used in waste water management since the late 19th Century (Dickermann et al 1954). Chlorine has been found to produce a number of carcinogens, and is now added in far smaller quantities than before.

The current resurgence in interest in ozone in medicine has been pioneered mainly in Cuba (Castañeira ET et al 1995), Russia and Europe, with additional research in South Africa. Professor Bocci from Milan, Italy has published data that shows that ozone should form part of modern medicine and clinical management (Bocci V 1992, 1994,1996,1996,1999, 2004) Professor Bocci has also shown that ozonated extracts have a part to play in the modern medical management of a number of medical conditions (Bocci et al, 2005).

In Europe, research in dentistry has shown that ozone can eliminate biofilms in water pipes (Abu-Naba'A L et al, 2002) in dental and medical equipment. Ozone treatment allows a holistic approach to the management of dental decay (Baysan et al 2000, Holmes 2003) without the need to amputate large volumes of tooth tissue. Professor Bocci has also argued that the toxic effects of ozone discussed by Menzel (Menzel, 1970) should not prevent it being used in a safe way as an adjunct to modern pharmacology (Bocci, 2004).

The process of bubbling ozone through plant and vegetable oils was first practiced as part of the protocol for the inhalation of ozone. Ozone was bubbled through olive oil, and the fumes that formed inhaled for lung infections and disorders in the 19th Century. It was observed that over a period of time the ozone reacted with olive oil, leading to the formation of a thick ‘cream’, or petroleum jelly like product. These were called ‘ozonated oils’. With the development of modern chemistry the structure of these products was determined. They were named ‘ozonoids’. The chemistry of ozone, and how it reacts with plant and vegetable oils is complex.

As a result of its dipolar structure, the ozone molecule may lead to 1-3 dipolar cyclo addition on unsaturated bonds, with the formation of primary ozonide (I) corresponding to the reaction shown in Fig 2. This is called Cyclo addition, also known as the Criegee mechanism, and results in a primary ozonoid.

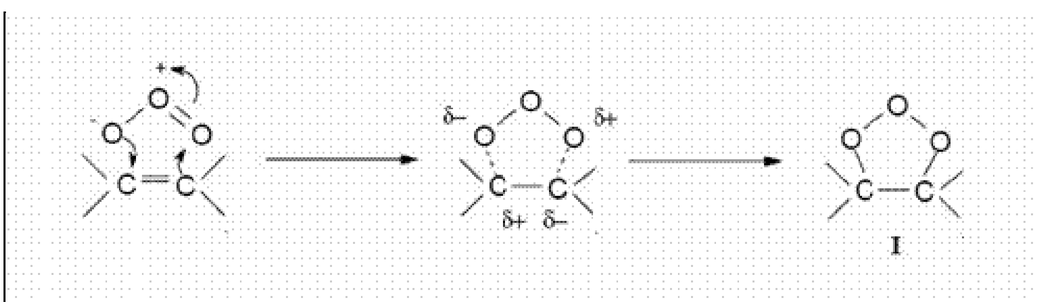

Fig 2.
Primary ozonide formation

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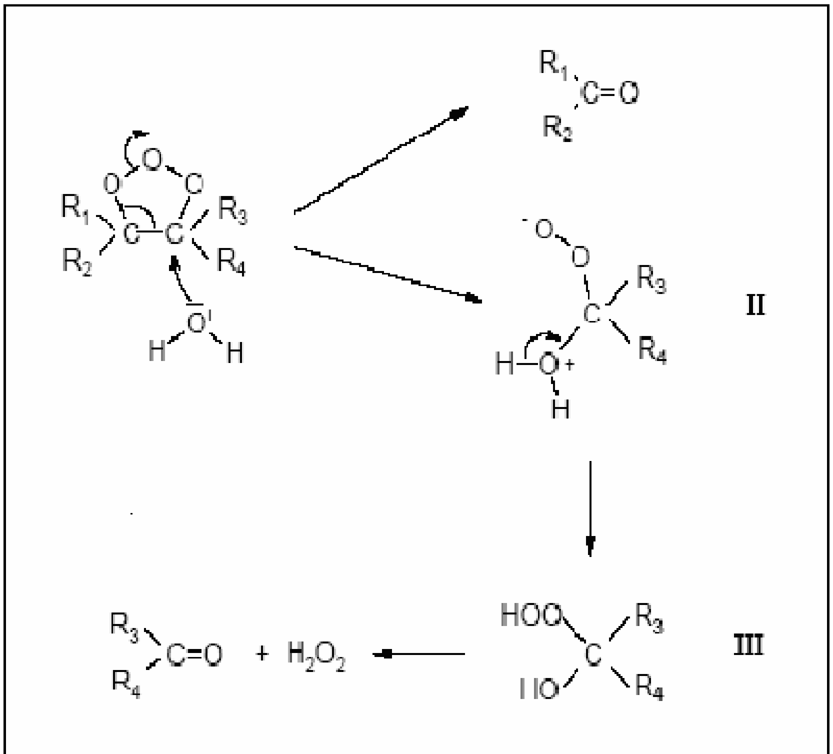

Fig 3.
Primary ozonide decomposition into a carbonyl compound (aldehyde or ketone) and a zwitterion (II) leading to a hydroxy-hydroperoxide (III) stage that decomposes into a carbonyl group

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The Use of Ozonoids in the Cosmetic Industry;

Díaz M (Díaz M 2004) discussed the ozonation of plant and vegetable oils in a 2004 paper. When vegetable oils, which consist fundamentally of triglycerides, are ozonised, ozonides, aldehydes, and peroxides are formed. Diaz reviewed past research from Cuba and Russia that showed these products are related with the observed biological effect of these oils.

In the cosmetic industry, ozonated olive, sunflower, jojoba and caster oils are recognised in the International Cosmetic Ingredient Dictionary and Handbook (Issue 1191). These oils are classified chemically as ‘esters’ and their function as ‘Skin Conditioning Agents and Miscellaneous’. Esters area chemical group of substances that are distinguished by a carbonyl group, where R 1-4 are carbon groups at either end of this chain. The primary ozonide shown in Fig 2 decomposes into a carbonyl compound (aldehyde or ketone) and a zwitterion (II) that quickly leads to a hydroxy-hydroperoxide (III) stage that, in turn, decomposes into a carbonyl compound and hydrogen peroxide, as shown in Fig 3.

Díaz M et al (Díaz M et al 2004) reported that cosmetic creams with active oxygen as part of their chemical ingredients, have a moisturiser and conditioner effect. Therefore, skin oxygenation and microcirculation are increased preventing cutaneous aging. These ozonated oils super oxygenate the skin providing enhanced energy leading to increased metabolic rate. This promotes rapid cell renewal and rapid healing. Additional metabolic pathways that protect, repairs and rejuvenate skin tissue are positively influenced. The ozonation of these extracts takes place at the double bonds in unsaturated oils. Oils containing omega 3, 6 and 9 fatty acids are better, in that they are the essential fatty acids which have a positive beneficial action on the epidermis in terms of their anti-inflammatory action and that they are superior in slowing down trans-epidermal water loss.

Plant and vegetable extracts are rich in Omega 3, 6 and 9 oils. These oils have in their chemical structure double bonds that ozone will react with, and hence are suitable for ozonation. Omega 3 is Linolenic acid with 3 double bonds in its carbon chain. Omega 6 is Linoleic acid with 2 double bonds in its carbon chain.

Omega 9 is Oleic acid with 1 double bond in its carbon chain

Ozonoids have a ring-type structure, and result from the breakdown of the double carbon bonds found in linolenic acid, linoleic acid and oleic acid, natural components of plant and vegetable extracts (Roehm et al 1971, Lynch E et al 2003). Ozone attacks these double carbon bonds, and inserts an ozonoid structure. As olive oil was used in the first protocols, it continued to be used as the oil of choice in the manufacture of ozonoids. It produces a stable product, with a longevity exceeding 15 years.

However, olive oil is one of many commercially available plant and vegetable extracts that contain omega oils. If the concentrations of linolenic, linoleic and oleic acids are known for each plant and vegetable extract, it is possible to calculate a 'Potential Ozonoid Index' (POI) for each extract, and then select the most appropriate oil for ozonation.

The molecular mass of omega 3, 6 and 9 oils are within 4g of each other, and hence are not significantly different in weight. Therefore a fixed weight of any ratio of these fatty acids would remain constant. To calculate a factor proportional to the number of double bonds could therefore be computed by multiplying the relative omega fatty acid content by its relative number of double bonds. i.e. % omega 3 X 3, % omega 6 X 2, % omega 9 X 1

The POI is calculated by adding the sum of the 3 as the combined factor for that particular oil. The higher the combined factor or POI, the higher the number of double bonds (ie reactive sites) as targets for ozonation.

Table 1 shows the POI for a number of commercially available plant and vegetable extracts. Average omega 3, 6 and 9 values are shown to account for seasonal variations in their concentrations. The omega oil contents are referenced from the World Wide Web internet site, www.queenhill.demon.co.uk/seedoils/oilcomp.htm. The data in Table 1 is ranked according to the POI in the extracts selected, and a range from 221 to 92 is shown. Olive oil has a POI of just 92, the lowest in this table. Sunflower oil has a POI of 153, showing the availability of more sites for the formation of ozonoids, and hence a potential higher ozonoid content. This would account for the positive results the Cuban researchers have obtained in their published studies.

PLANT OIL	OMEGA 3%	OMEGA 6%	OMEGA 9%	COMBINED FACTOR
Flax (linseed)	58	14	19	**221**
Hemp (c. sativa)	20	60	12	**192**
Evening Primrose	0	81	11	**173**
Safflower	3	75	13	**172**
Chia	30	40	0	**170**
Kukui (Candle Nut)	29	40	0	**167**
Perilla	55	0	0	**165**
Grape Seed	0	71	17	**159**
Pumpkin Seed	8	50	34	**158**
Sunflower	0	65	23	**153**
Walnut	6	51	28	**148**
Soybean	7	50	26	**147**
Corn	0	59	24	**142**
Wheat Germ	5	50	25	**140**
Rape (Canola)	7	30	54	**135**
Sesame	0	45	42	**132**
Cotton Seed	0	50	21	**121**
Rice Bran	1	35	48	**121**
Beechnut	0	32	54	**118**
Sweet Almond	0	17	78	**112**
Olive	0	8	76	**92**

<center**Table 1: Omega 3, 6 and 9 oil content of plant & vegetable extracts</center**

However, it can be seen that a number of alternative extracts have higher POI's. If nut extracts are excluded on the grounds of potential allergy considerations, flax, hemp (canabis sativa), evening

primrose, safflower, and chia have POI's at 170 or above, perilla has a POI of 165, and grape seed, pumpkin seed and sunflower POI's of 159 to 153.

The most obvious choice of extracts to ozonate are those extracts with the higher POI's – flax (POI=221), hemp (canabis sativa) (POI=192), evening primrose (POI=173), safflower (POI=172) and chia (POI=170). This has to be balanced with the cost of these extracts, and both flax and hemp (canabis sativa) are relatively inexpensive, as well as being readily available.

Flax oil – (POI 221). Flax oil derived from the flax plant Linum usitatissimum and is known as linseed oil. Flax oil is actually one of the oldest and one of the original "health foods," treasured because of its healing properties throughout the Roman Empire. It was one of the original "medicines" used by Hippocrates.

Besides being the best source of omega 3's, flax oil is a good source of omega 6, or linoleic acid (LA). Sunflower, safflower, and sesame oil are greater sources of omega 6 fatty acids but they don't contain any omega-3 fatty acids. Flax oil is 45 to 60 percent the omega-3 fatty acid alphalinolenic acid (ALA). Flax oil can be used for patients who have dry skin or eczema, or whose skin is particularly sun-sensitive. A theoretical problem is the potential for the oil to set solid as ozone is bubbled through it and reacts with the oil. A possibility to be explored is the blending of Flax with Hemp oils to counter this.

Hemp (Cannabis sativa) oil – (POI 192). Hemp oil is derived from the Cannabis sativa plant. Hemp seeds contain 40% fat in the form of an oil. It has a remarkable fatty acid profile, being high in the desirable omega-3s and also some GLA (gamma-linolenic acid). Hemp oil contains 57% linoleic (LA) and 19% linolenic (LNA) acids.

Hemp oil simulates growth of hair and nails, improves the health of the skin, and can reduce inflammation. The intoxicating properties of Cannabis sativa reside in a sticky resin produced most abundantly in the flowering tops of female plants before the seeds mature. The main psychoactive compound in this resin is tetrahydrocannabinol (THC). Strains of hemp grown for oil production have a low resin content to begin with, and by the time the seeds are ready for harvest, resin production has dropped even further. Finally, the seeds must be cleaned and washed before they are pressed. As a result, no THC is found in the final product.

Discussion

A number of plant and vegetable extracts have been identified as having a higher POI than the traditional olive and sunflower oils used in the manufacturing of ozonated products. These ozonated oils have a role in the cosmetic industry as moisturisers, conditioners, and regeneration products, as well as microbial effects that have been published in the literature. Díaz M (Díaz M 2004) discussed the use of ozonated sunflower, coconut and theobroma oils, and their use in Dermatology, Parasitology and Cosmetology. Lynch E et al (Lynch E et al 2003) showed that the treatment of vegetable oils with ozone gave rise to the consumption of polyunsaturated fatty acids present. Signals present in the 5.10-5.25 ppm regions of ozonated grape and sunflower seed oils were assignable to the ring protons of ozonides. Further ozone-induced modifications to the oils included the production of aldehydes, i.e. -CH2CHO aldehydic group, terminal products arising from the decomposition of ozonides.

Published research has demonstrated that the application of ozone gas to skin tissue has the potential to result in tissue damage (Thiele JJ et al 1997, Valacchi G et al 2005). Ozone exposure causes damage to cutaneous lipids, an effect which can be attenuated by vitamin E application (Thiele JJ et al 1997). The use of ozone gas has been shown to produce a progressive depletion of antioxidant content in the stratum corneum and this can then lead to a cascade of effects resulting in an active cellular response in the deeper layers of the skin. Using an in vivo model Valacchi G et al (Valacchi G et al 2005) have shown an increase of proliferative, adaptive and proinflammatory cutaneous tissue responses.

Díaz M et al (Díaz M et al 2004) reported that cosmetic creams with active oxygen as part of their chemical ingredients have a moisturiser and conditioner effect. Therefore, skin oxygenation and microcirculation are increased preventing cutaneous aging. The recently published paper by Valacchi G et al (Valacchi G et al 2005) showed that ozonated extracts have been shown to be safe for use on skin tissue. The use of these ozonated extracts and blends are wide; from medical treatment of infections (bacterial, viral and fungal infections eg; gingivitis, athletes foot and herpes), skin conditions (eczema) and wounds (insect bites & stings, cuts, burns, leg ulcers, bed sores).

Ozonated extracts could be combined with other products to form a new range of products in the cosmetic industry for skin tissue protection, repair, maintenance and rejuvenation.

Ozonated plant and vegetable extracts should be farmed from organically farmed and sustainable crops to avoid contamination by pesticides, fertilizers, and cold pressed to avoid contamination by solvent chemicals used to scavenge the extracts from the plant or vegetable pulp during processing. Once ozonated, these extracts can be blended with other products that have important vitamins and trace minerals, as well as their own innate pharmacological properties.

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Authors;

Dr Julian Holmes, BA SocSci, BDS; Ozone Researcher, South Africa.
Gijs Kort, Senior Development Chemist, Incorporated Laboratories Pty Ltd, South Africa