the generation of ozone due to lightning strikes or formation of ozone in the upper atmosphere through reaction of high energy solar plasma with oxygen in the troposphere. However, ozone can also be generated from more manmade sources including, in particular, urban smog, and many cities actually monitor urban ozone levels as an indicator of smog.\(^2\) Because ozone is a key component of human-produced smog, it has been studied extensively for its respiratory health effects, particularly for people prone to asthma and people living in urban populations. More recently, it has now been recognized that ozone has detrimental effects on skin.\(^3\) In particular, it has been established that ozone, while it can only penetrate a few microns into the stratum corneum, will almost instantly react with vitamin C and vitamin E reservoirs in the skin, rapidly depleting these important antioxidants. In addition, it has been shown that ozone will also rapidly convert skin and cellular lipids into lipid peroxides that lead ultimately to membrane and skin degradation. Studies have also demonstrated that ozone can oxidize important proteins and nucleic acids.\(^4\)

**INCI Name:** Saccharomyces Ferment Lysate Filtrate  
**SAP Code#:** 137180  
**CLS Code#:** 520402-42

**Key Product Attributes:**  
- Environmental Pollution Protection  
- Lipid Barrier Protection  
- Lipid Barrier Repair  
- DNA Protection  
- Skin Firming and Smoothing  
- Anti-Wrinkle  
- Ozone Protection Factor  
- Prevention of AGE Formation

**Product Information**

Ozone \((O_3)\) is a highly reactive form of molecular oxygen in which normal molecular oxygen combines with highly reactive oxygen radicals to form transitory, but somewhat stable molecules containing three oxygen atoms.\(^1\) Sources for ozone can be completely natural such as...
On the other hand, ozone is also known as a safer alternative to chemicals in regards to disinfectant properties, sanitizing or purifying everything from medical equipment to food, water and even air! Topical ozone skin treatments have reached popularity, touting to promote exfoliation and skin whitening.

For all of these reasons, ozone became a target for our Biodynes™ Yeast Platform.\(^2\) The theory being that if ozone was applied to the growing microorganisms, a 'stress' would be created on the yeast such that the yeast would need to respond with protective agents to help it survive against the stress.\(^{21,24}\) It was based on this theory, that Biodynes™ \(O_3\) was developed, using the influence of ozone to create an anti-aging active for both hair and skin.

**Manufacturing Process**

**Yeast Lysate Created by Ozone**

Biodynes™ \(O_3\) is manufactured using fermentation biotechnology in which yeast are fed a nutrient media essential for development of the yeast. During the cellular growth phase of the yeast, a sublethal dose of ozone is applied to the yeast generated via a commercial ozonator. The ozone is applied for a specific duration of time while maintaining a viable cell count in the fermentation process. Upon completion of the ozone application, the yeast cells are then lysed to break open the cellular membrane and the key constituents of the interior of the cell, including material from the cytoplasm and the nuclear materials, are isolated.

**2-D Electrophoresis of the Lysate**

After isolation of the lysate, the changes that occurred in the protein composition of the yeast were examined using 2-Dimensional Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis (2D SDS-PAGE).\(^{25}\) The images of an unstressed (left) and an ozone stressed yeast lysate (right) are shown below (Figure 1). Careful examination of the two gels side by side shows that the application of the ozone does indeed cause up-regulation and down-regulation of certain proteins.

In Figure 1, visually obvious changes in protein composition as a result of ozone stress are noted in the green and red circles (the yellow circle represents an internal standard that is used in SDS PAGE to indicate reproducibility of the electrophoresis). The gel on the left was not stressed, the one on the right was stressed for fifteen minutes with ozone. The green circles show examples of proteins that were present in the unstressed yeast (left) that visually faded as a result of ozone stress, (right, a down regulation of these proteins). The red circles in the ozone-stressed yeast lysate indicate proteins that are upregulated as a result of the ozone stress compared to the unstressed gel on the left. Many other less obvious examples exist in these two gels, the examples above are only intended to demonstrate that indeed ozone stress can influence the production of proteins within a living system. More in-depth computer aided analysis of these gels can help to identify the bulk of the changes that have occurred.

**In Situ and In Vitro Testing Summary**

Figure 2 outlines several studies which were conducted to evaluate the benefits of Biodynes™ \(O_3\) both in situ and in vitro. A fundamental in situ study confirmed that Biodynes™ \(O_3\) provides an oxidative protective mechanism against ozone preventing the degradation of a brilliant blue, ozone sensitive dye, carmine. A 2% level of Biodynes™ \(O_3\) successfully protected the dye from fading to a light blue color, during an ozone purge of the dye solution.

Testing on a full thickness tissue model showed that Biodynes™ \(O_3\) very effectively protects the lipid bilayer from cholesterol degradation following ozone exposure. The results from this study were so compelling that Biodynes™ \(O_3\) appeared to actually promote cholesterol synthesis. A follow-up cell culture study confirmed that a 1% Biodynes™ \(O_3\) treatment did promote cholesterol synthesis, actively repairing or rebuilding the lipid bilayer.

Additional testing on a full thickness tissue model generated data substantiating that Biodynes™ \(O_3\) offers DNA protection by reducing both 8-oxoguanine and TT Dimer formation following exposure of the tissue model to ozone. At a 5% test level of Biodynes™ \(O_3\), complete DNA protection was achieved in the 8-oxoguanine study.

<table>
<thead>
<tr>
<th>Study</th>
<th>Level of Biodynes™ (O_3) tested</th>
<th>Biodynes™ (O_3) Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carmine Dye Test</td>
<td>2%</td>
<td>Protected dye from degradation</td>
</tr>
<tr>
<td>Cholesterol Protection (^{16,19,26})</td>
<td>5%</td>
<td>Offered complete protection and appeared to promote cholesterol synthesis</td>
</tr>
<tr>
<td>Cholesterol Synthesis (^{27})</td>
<td>1%</td>
<td>Promoted Cholesterol synthesis</td>
</tr>
<tr>
<td>DNA Protection 8-oxoguanine as marker (^{24})</td>
<td>1% and 5%</td>
<td>1% reduced DNA damage, 5% offered complete protection</td>
</tr>
<tr>
<td>Prevention of TT Dimer formation and 8-oxoguanine (^{25})</td>
<td>1%</td>
<td>Reduced levels of both, more effective at reducing 8 oxoguanine</td>
</tr>
</tbody>
</table>

In Figure 2, In Situ and In Vitro Testing Summary of Biodynes™ \(O_3\)
Environmental Ozone Protection Factor (OPF)
The data generated from the DNA Protection Study provides us with an opportunity to develop a new definition of skin protection called the "Ozone Protection Factor" or OPF. The results of this study can be directly correlated to the ability of Biodynes™ O₃ to protect skin from environmental ozone exposure. The control cells that were not treated with Biodynes™ O₃ represent an OPF of 0%; as a significant increase in 8-oxoguanine results from direct ozone exposure. Incorporation of 1% Biodynes™ O₃ offers the cells a moderate OPF in the range of 50%; as the presence of Biodynes™ O₃ effectively reduces the level of 8-oxoguanine compared to the control. Likewise, 5% treatment of Biodynes™ O₃ offers a high OPF in the range of 100%. These values are plotted below as the anticipated performance of various concentrations of Biodynes™ O₃ to protect the skin from a day of ozone exposure, Graph 1.

An in vitro study was done on full thickness tissue (Epiderm EFT 200) to evaluate the effects of ozone on influencing AGE production. As demonstrated in Figure 3, compared to non-ozonated tissue (UT-Ozone), tissue treated with a short exposure to ozone shows a very significant increase in AGE production (UT + Ozone). In this study, some well known antioxidants, vitamin C (sodium ascorbate, NaAsc) and 70% EGCG Green Tea Polyphenols (EGCG), were compared against Biodynes™ O₃ to determine the ability of the products to prevent the formation of AGEs. As shown in Graph 2, all the antioxidants provide some protection against AGE formation, but Biodynes™ O₃ was superior when compared to equal quantities of 70% EGCG and about equal to vitamin C as a protectant.

In Vitro AGE Prevention – Full Thickness Tissue Model
Advanced Glycation Endproducts, or AGEs, form when oxidatively damaged proteins such as collagen, elastin, keratin, fibronectin, etc. react with sugars present in the skin to initially form some intermediate products called Schiff Bases and Amadori products, but ultimately form irreversible products called Advanced Glycation Endproducts. It is generally felt that accumulation of AGEs in the skin is a measure of protein breakdown due to oxidative stress and levels of AGEs have been shown to be high in people with photoaged skin versus people with less photoaged skin. Also, it has been demonstrated that aged cells such as fibroblasts will have higher levels of AGEs than young cells. Therefore, the formation of AGEs is an indication of skin aging and extracellular matrix breakdown, which can ultimately lead to sagging and wrinkles.

Change in Autofluorescence with Ozone Exposure (AGE Measurement)

A 60-person, double-blind, placebo controlled study was conducted for a 90-day evaluation. The study was monitored by a skilled dermatologist who examined the participants throughout the course of the evaluation.

The participants were broken down into three groups of twenty. One group was the placebo control group who used an SPF 8 lotion that contained none of the test material. The second group used the same lotion with 3% Biodynes™ O₃ added and the third group applied a 5% Biodynes™ O₃ lotion. Each participant had a three day wash out period prior to starting the study in which they were asked to stop using their standard moisturizers and were asked to wash their faces and arms with a non-conditioning bar soap. The participants were not allowed to use any skin-effecting pharmaceuticals during the course of the study.
On Day 90, at completion of the test period, the participants were tested in the following ways: Facial moisturization using corneometer, forearm barrier integrity using TEWL, wrinkles using SilFlo® replicants and digital analysis and dermatologist assessment of skin firmness, overall facial elasticity and skin appearance.

Ozone Levels Throughout the 90 Day Study
Ozone levels in São Paulo, Brazil from July to November 2005 were monitored weekly. The data is shown in Graph 3.

Graph 3
Weekly ozone levels in São Paulo, Brazil from July through November, 2005, as monitored by CETESB (Companhia de Tecnologia de Saneamento Ambiental).

Results of In Vivo Testing
Facial Moisturization
Changes in skin moisturization were determined using corneometric analysis. Results are shown in Graph 4.

% Increase in Facial Moisturization Compared to Baseline

From the data it can be seen that after the 90-day treatment regimen, the Biodynes™ O₃ at 3% provided an 11% increase in moisturization compared to an 8% improvement noted for the placebo-treated group.

Forearm Barrier Integrity
Forearm barrier integrity was measured using transepidermal water loss. Graph 5 shows the data for this study after the 90-day treatment.

Average TEWL Readings

From the data, it can be seen that after 90 days, the placebo-treated control group showed a 92% decrease in barrier integrity compared to 20% improvement in barrier integrity for the participants using the lotion containing 5% Biodynes™ O₃.

Wrinkle Study
At the end of the 90-day treatment period, participants were analyzed for wrinkle effects using Silflo replicants and digital photography to assess overall wrinkle appearance. The data indicates that 34% of the participants using 5% Biodynes™ O₃ showed a statistically significant improvement in overall wrinkles compared to the placebo-treated controls. A 5% treatment level showed the best performance in cutaneous relief, improvement in skin roughness, and improvement in deep wrinkles.

Dermatological Assessment Facial Skin Firmness
After the 90 day treatment period, the participants were examined by a dermatologist who assessed their skin for facial firmness. The data from this examination is provided in Graph 6.

Dermatological assessment indicated a statistically significant improvement in skin firmness after 90 days, with a 72% improvement in the group using 5% Biodynes™ O₃ versus a 38% improvement for the placebo-treated control group.
AGE Study
Each participant in the study was tape stripped at baseline and at the end of the 90 day treatment period, and tape strips were analyzed for the presences of AGEs (Advanced Glycation Endproducts). The data in Graph 7 indicates a dose response for reduction in AGEs going from the placebo formulation [No Active] to 3% and then to a 5% Biodynes™ O3 treatment. The linear reduction in AGE formation in the skin over the 90-day test period confirms what was found in vitro on the full thickness tissue model.

Dermatological Assessment of Skin Elasticity
Following the 90-day treatment, the participants were assessed by a dermatologist for changes in their facial skin elasticity. The results of this assessment are shown in Graph 8.

Dermatological Assessment of Overall Skin Appearance
After 90 days, the participants were assessed by a dermatologist for their overall facial skin appearance. Results are shown in Graph 9.
Dermatological assessment after 90 days indicated that at a level of 3% Biodynes™ O3 there was a statistically significant 76% increase in overall skin firmness versus 30% for the placebo controls.

**Conclusion**

Biodynes™ O3 is a yeast extract that offers one of the first, scientifically-designed, topically applied defenses against ozone, which is rapidly becoming a well recognized threat against skin health, aging and well being. In vitro studies confirm that Biodynes™ O3 is effective at protecting fragile cellular nucleic acids, including nuclear and mitochondrial DNA and RNA, and skin lipids, critical for proper functioning of the skin’s lipid structure, from the detrimental effects of ozone. *In vitro* studies also confirm Biodynes™ O3 prevents the formation of Advanced Glycation Endproducts as effectively as green tea and vitamin C.

*In vivo* clinical studies substantiate the use of Biodynes™ O3 as an active affording anti-wrinkle, skin firming claims as well as reducing trans-epidermal water loss and enhancing skin moisturization, and reducing AGE formation.

Biodynes™ O3 allows a formulator to literally “dial in” a factor of ozone protection (OPF) into their product depending on anticipated exposure times that the consumer may need the protection. Biodynes™ O3 can help assure the consumer that they are receiving steps towards proper care and safeguards for the skin from harmful effects of the environment; it truly is the pollution solution!

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**Typical Properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, yellowish liquid</td>
</tr>
<tr>
<td>pH (Direct @25°C)</td>
<td>4.0 – 7.0</td>
</tr>
<tr>
<td>Non-Volatile Matter (1g · 1hr · 105°C)</td>
<td>1.0 – 4.0%</td>
</tr>
<tr>
<td>Residue on Ignition</td>
<td>1.5% Maximum</td>
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<tr>
<td>Nitrogen (Kjeldahl)</td>
<td>0.1 – 1.0%</td>
</tr>
<tr>
<td>Microbial Content</td>
<td>100 opg Maximum; No pathogens</td>
</tr>
<tr>
<td>Preservative System</td>
<td>0.9 – 1.1% Phenoxethanol</td>
</tr>
<tr>
<td>Recommended Use Level</td>
<td>0.5 - 3.0%</td>
</tr>
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</table>

**Product Safety Review**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi-Ocular – MTT Viability Assay</td>
<td>Minimal/to mild</td>
</tr>
<tr>
<td>[Product tested at 100%]</td>
<td></td>
</tr>
<tr>
<td>Epi-Derm – MTT Viability Assay</td>
<td>Minimal/to mild</td>
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<tr>
<td>[Product tested at 100%]</td>
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</tr>
<tr>
<td>RIPT</td>
<td>Non-sensitizing</td>
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</tbody>
</table>
Ozone Exposure Induces Antioxidant/Stress Related Atherosclerotic Arteries.

Lerner RA. Evidence for Ozone Formation in Human Substrates. as an Oxidizing Agent to Evaluate Antioxidant Activities of Natural Substances.


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