



ZipZyme™

OMEGA-3 SUPERFOOD

by PHYT●SMART

Preamble:

ZipZyme™ is an innovative product created to enhance the supplemental marine omega-3 realm. We craft our ZipZyme™ products with a commitment to sustainability, employing methods that avoid harmful oil extraction. Instead, ZipZyme™ offers unadulterated whole-cell algae-derived omega-3 nutrition devoid of additives or synthetic components. Moreover, our production process retains the protective enzymes naturally present in algae.

While our current products cater to the pet industry, we have a pipeline of additional products for agriculture, aquaculture, aquariums, and humans. This paper delves into the advantages of omega-3's for pet health and addresses the limitations of current market solutions for delivering these essential fatty acids.

ZipZyme™: An Innovation in Omega-3 Nutrition

ZipZyme™ is an innovative product created to enhance the supplemental marine omega-3 fatty acid realm. We craft our ZipZyme™ products with a commitment to sustainability, employing methods that avoid harmful oil extraction. Instead, ZipZyme™ offers unadulterated whole-cell algae-derived omega-3 nutrition devoid of additives or synthetic components. Moreover, our production process retains the protective enzymes naturally present in algae.

The Crucial Roles and Oxidative Vulnerability of Omega-3 Fatty Acids

Omega-3 fatty acids are long-chain polyunsaturated fatty acids that play pivotal roles in human health, participating in myriad biochemical processes and modulating gene expression across the lifespan. Robust scientific evidence suggests that these essential fats can prevent or alleviate symptoms associated with many chronic diseases and may offer protection against age-related concerns.

These essential fatty acids contain multiple double bonds, rendering them vulnerable to oxidation, which can occur following exposure to oxygen, heat, light, and other reactive substances. Oxidation involves the loss of electrons from a molecule, promoting the formation of free radicals, a class of highly reactive molecules that can damage cells and other molecules, including proteins, lipids, and DNA. Omega-3 fatty acid oxidation alters their chemical structure, driving the formation of various toxic oxidation end products, including:

- Hydroperoxides, primary oxidation products that can further decompose into secondary products.
- Aldehydes and ketones, highly reactive hydroperoxides that contribute to off-flavors and odors in foods.
- Malondialdehyde, a marker for lipid oxidation that serves as an indicator of oxidative damage to omega-3 fatty acids.

Additionally, omega-3 fatty acids are precursors to anti-inflammatory molecules called eicosanoids, including prostaglandins and leukotrienes, but oxidation can alter these compounds, diminishing their beneficial effects.

It is noteworthy that the oxidation process can trigger a chain reaction where one oxidized omega-3

molecule can, in turn, oxidize others, leading to rapid, diffuse oxidation, particularly in concentrated omega-3 oils. Therefore, preventing or mitigating oxidation is crucial when working with products containing these essential fatty acids.

Health effects of ingesting oxidized omega-3s

Ensuring the quality of omega-3 supplementation for pets is crucial for their overall health and well-being. The consumption of oxidized omega-3 fatty acids can lead to adverse health effects and severe consequences for pets, including:¹

- **Nutrient deficiency:** Oxidized omega-3 fatty acids have lower nutritional value than their fresh counterparts. The oxidation process degrades the fatty acids' quality, diminishing their effectiveness in delivering intended health benefits. Consequently, pets may not receive the full advantages associated with omega-3 supplementation.^{1,2}
- **Increased inflammation:** Oxidized omega-3 fatty acids may promote inflammation, counter to omega-3's anti-inflammatory purpose. This increase in inflammation could be counterproductive, particularly for pets with inflammatory conditions or diseases.³
- **Digestive concerns:** Ingesting oxidized omega-3s can promote digestive disturbances in pets, manifesting as diarrhea, vomiting, abdominal discomfort, or loss of appetite. These disturbances disrupt normal digestion and nutrient absorption, potentially promoting malnutrition or weight loss over time.¹
- **Oxidative stress:** Oxidized omega-3s can induce oxidative stress, characterized by an imbalance between the production of free radicals and the body's capacity to neutralize them. This imbalance can lead to cellular damage, inflammation, and an increased susceptibility to chronic diseases.¹
- **Impaired organ function:** Prolonged consumption of oxidized omega-3s may adversely affect pet organ function. For example, oxidized fats can impair liver and kidney function, hindering detoxification and waste product elimination, compromising well-being.¹

Mitigating Omega-3 Oxidation with ZipZyme™ Products

To combat the oxidation of omega-3 oils, supplement manufacturers commonly employ synthetic tocopherols, molecules designed to oxidize more readily than omega-3 molecules. While oxidized tocopherols are considered relatively stable radical molecules compared to oxidized omega-3 molecules, they still have the capacity to induce oxidative damage in the body.

PhytoSmart, the creators of ZipZyme™, considers this approach less than ideal because it not only encourages the intake of radicalized tocopherols but also because many omega-3 oils exhibit elevated levels of toxic oxidation even before consumers open the packaging.^{4,5} Consequently, PhytoSmart has chosen a safer strategy for addressing oxidation with ZipZyme™ products.

ZipZyme™ offers comprehensive microalgal omega-3 nutrition derived from whole algae cells. These microalgae naturally contain protective anti-oxidative enzymes that are critical in mitigating oxidation by catalyzing specific chemical reactions that either directly neutralize reactive oxygen species or indirectly hinder the formation and progression of oxidative chain reactions, providing a superior, safer product for pets.

The Superiority of Phospholipid DHA

Docosahexaenoic acid (DHA) is an omega-3 fatty acid produced by certain species of marine microalgae and

found in the fish and crustaceans that consume them. It is also widely available as a dietary supplement. DHA can be found in a variety of forms, including free fatty acid, ethyl ester, triglyceride, and phospholipid, each affecting physiology differently.

Phospholipid DHA stands out among these forms due to its unique structure and distinctive interactions with cell membranes. It exhibits superior bioavailability in both humans and pets, ensuring that a greater percentage of the consumed phospholipid DHA is effectively utilized by cells. As a result, DHA is swiftly integrated into cell membranes, particularly within neural and retinal tissues. This integration supports cell membranes' structural integrity and fluidity, promoting optimal cell function and potentially enhancing cognitive function, memory, and vision support. ZipZyme™ contains a notably high proportion of phospholipid DHA.

Immune Function: A Specialized Role for DHA

DHA plays a vital role in immune function, encompassing a range of mechanisms, from reducing inflammation to regulating immune cell expression and function. Understanding the significance of DHA in immune response is essential for appreciating its broader health implications. DHA influences immunity in many ways, including:

- **Anti-inflammatory effects:** Chronic inflammation can gradually weaken the immune system, rendering the body more susceptible to infections. DHA demonstrates robust anti-inflammatory properties, bolstering immune system responses and effectiveness.⁶
- **Cell membrane fluidity:** Cell membrane fluidity and flexibility influence how immune cells move, interact with pathogens, and effectively execute their functions. DHA preserves the fluidity and flexibility of immune cell membranes.^{6,7}
- **Phagocytosis enhancement:** Phagocytosis involves the immune cells' capacity to engulf and digest pathogens, a process undertaken by cells like macrophages and neutrophils. DHA may augment phagocytic activity, enabling immune cells to eliminate invading microbes effectively.⁶
- **Immune cell regulation:** DHA influences the production and activity of various immune cells, including B cells, T cells, and natural killer cells, ultimately regulating immune responses, ensuring they are appropriate and not overly aggressive.⁶
- **Inflammation resolution:** Following an infection or injury, the immune system must "switch off" the inflammatory response once the threat is vanquished. DHA, alongside other omega-3s, can facilitate this resolution phase, averting chronic inflammation.
- **Gut health:** A balanced gut microbiome is indispensable for immune system function because a substantial portion of the immune system resides in the gut. DHA contributes to a healthy gut microbiome by fostering the growth of beneficial bacteria while curtailing the proliferation of harmful ones.
- **Antioxidant properties:** DHA boasts antioxidant characteristics, protecting immune cells from oxidative stress and the detrimental effects of free radicals. This safeguarding enables immune cells to operate at their prime.

DHA Synthase Enzymes

ZipZyme™ is produced in a manner that retains the integrity of microalgae enzymes. Many species of marine microalgae convert the precursor molecule alpha-linoleic acid (ALA) into DHA. This process occurs in many organisms (including humans and pets); however, it is largely an ineffective mechanism to provide adequate DHA. The microalgae used in ZipZyme use DHA synthase enzymes to convert small-chain carbon

molecules more directly to DHA and do not require ALA as a precursor molecule. As such, these microalgae produce extraordinarily high concentrations of DHA.

By preserving enzyme functionality, PhytoSmart, the creator of ZipZyme™, hypothesized that DHA synthase enzymes might continue producing additional DHA after ingestion. To explore this hypothesis, PhytoSmart conducted two initial studies – one involving salmon and the other layer hens. The outcomes were remarkable, indicating DHA accumulations in salmon and egg production that exceeded the premanufactured levels present in ZipZyme™ products. This preliminary research drew the attention of the National Oceanographic and Atmospheric Administration (NOAA), leading to the award of a Phase I Small Business Innovation Research grant in 2022. Building on this success, NOAA granted Phase II funding in 2023, with a focus on the commercialization of DHA synthase products like ZipZyme™.

ZipZyme™: A Comprehensive Health Companion for Pets

ZipZyme's™ DHA plays a multifaceted role in promoting the well-being of pets. It serves as a cornerstone for various aspects of their health, from cognitive function and vision to joint mobility, skin health, heart function, and immune support. Understanding the diverse benefits of DHA underscores its significance in ensuring the overall vitality of companion animals.^{8,9}

- **Brain:** DHA is critical in developing and maintaining a healthy pet brain. It supports optimal cognitive function, enhances learning abilities, and aids memory retention. Ensuring adequate DHA levels during early development can improve trainability and overall mental acuity in pets.
- **Eyes:** DHA is a vital component of the retina in the eyes. Incorporating DHA into pets' diets promotes healthy vision and visual development, which can be particularly important for breeds prone to eye-related issues.
- **Joints:** Pets, especially aging ones or those with joint conditions like arthritis, can benefit from the anti-inflammatory properties of DHA. DHA helps reduce joint inflammation, alleviate discomfort, and enhance mobility.
- **Skin and coat:** DHA is pivotal in maintaining healthy skin and promoting a glossy coat in pets. It alleviates itching, dryness, and flakiness of the skin. Additionally, DHA contributes to a soft, shiny coat and aids in managing conditions such as dermatitis and allergies.
- **Heart:** DHA and other omega-3 fatty acids support cardiovascular health in pets by promoting proper heart function, reducing blood pressure, and moderating inflammation. This fosters a healthier heart and circulatory system, contributing to overall well-being.
- **Immune system:** DHA exhibits immunomodulatory properties in pets, helping regulate immune system responses. This, in turn, bolsters the immune system's strength and enhances resistance against certain diseases and infections.

Sustainability Concerns in Fish and Krill Oil Production

Fish and krill oils are typically sourced from wild ocean fisheries, but the prevalent issue of overfishing has become a significant concern. Many of the world's wild fish stocks are presently exploited at unsustainable rates despite attempts to compensate for declining traditional species with new ones. This has resulted in an annual global wild fish harvest of approximately 80 million tonnes, remaining relatively constant for nearly four decades and unable to meet the growing consumer demand for fish.

Traditionally, fish oil has been obtained from various wild fisheries, but the depletion of these sources has led to a shift towards the Patagonian anchovy fishery as the primary fish oil supplier. Krill oil, on the other

hand, is derived from small shrimp-like crustaceans primarily found in the Antarctic. However, fish and krill oil harvesting and production face significant sustainability challenges.

Notably, the fish oil supply from the anchovy harvest has considerably diminished due to the increasing inclusion of younger anchovies with lower oil content.¹⁰ This pattern indicates overfishing and mirrors the collapse in fisheries like the Atlantic cod. The decline in the anchovy fishery places additional pressure on other wild omega-3 sources, especially krill, which occupies a vital position in the marine food chain, serving as a primary food source for an entire ecosystem. Overfishing of krill threatens the fragile oceanic balance, potentially driving cascading downstream effects for numerous marine species, including whales, seals, penguins, and fish.^{11,12}

Furthermore, krill fishing often unintentionally captures non-target species, called bycatch. Selectively harvesting krill without affecting other marine organisms proves difficult, resulting in the incidental capture of various fish species, squid, and marine invertebrates.¹³ Some non-target species hold ecological significance and may experience harm or mortality.

In addition, the regulation of krill fishing lags behind that of other fisheries, needing robust management measures and quotas, exacerbating sustainability concerns. A pressing need for improved monitoring and regulation of krill fishing practices is apparent to address these issues and reduce harm to the marine environment.

In contrast to traditional fish and krill oil sources, ZipZyme™ offers a genuinely sustainable solution. Our whole-cell microalgae product is cultivated entirely separate from the marine ecosystem in a land-based facility in Maine, USA, utilizing proprietary technology. We source nearly all of our microalgae's diet from industrial waste streams and employ water reclamation technology to recycle water within our production facility. These practices make ZipZyme™ a truly sustainable source of omega-3s, ensuring minimal environmental impact.

About Phytosmart and the Beginnings of ZIPZYME™

In late 2016, PhytoSmart collaborated with Bigelow Laboratories in Boothbay, Maine, to conduct DHA lipid concentration profile studies on a number of marine microalgae species. Following this preliminary assessment, we identified the microalgae with the highest net omega-3 content to concentrate our research efforts on.

Over the next five years, PhytoSmart developed its patented sterile bioreactor growing technology, SPAGS (Sterile Plastic Algal Growth System), to address the heterotrophic nutrition needs of these species and associated contamination concerns. In 2017, PhytoSmart secured funding from the Maine Technology Institute to maximize yield parameters for this species.

Initially, PhytoSmart intended to extract DHA oil with cleaner, low-temperature methods using supercritical CO₂ extraction technology to maintain the integrity of DHA synthase enzymes in the spent biomass. The business's initial direction was B2B, focusing on developing large-scale production capacity using SPAGS.

However, a twist of fate led PhytoSmart to pivot its approach. Recognizing the imperfections of all extracted omega-3 products due to their susceptibility to oxidative damage, the company shifted its focus to producing ZipZyme™ Omega as a whole-cell fresh food source of omega-3 DHA. This innovation not only made the product impervious to oxidative damage, a common issue with extracted oil omega-3 products, but it also preserved the activity of the native synthase enzymes.

PhytoSmart's inaugural product, ZipZyme™ Omega, boasts a high concentration of phospholipid DHA and

preserves the algal protective anti-oxidative and metabolic DHA synthase enzymes. This shift from extracted oil to whole cell biomass transformed PhytoSmart from a pet food supplement company into an innovative food technology firm, with plans for future applications across all sectors within the omega-3 nutrition space.

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