



# TetraSOD<sup>®</sup>, plant-based ingredient

to combat oxidative stress, the pathologies derived from it and as a modulating element of the immune system

TetraSOD<sup>®</sup> is a plant based ingredient obtained from the sustainable and controlled cultivation of the marine microalgae *Tetraselmis Chuii*. With an antioxidant and anti-inflammatory function, it prevents oxidative damage, but also stimulates numerous mechanisms that strengthen health and the immune system. It induces the endogenous production of the main antioxidant enzymes at the cellular level, increasing the defense power of them and of the tissues and organs that compose it. After demonstrating its efficacy in a wide variety of pathologies and endorsed by clinical studies and scientific publications, it has become an ingredient present in formulations of nutritional supplements aimed, among others, at joint, auditory, cardiovascular, cognitive, skin, and fertility health, among others. also to improve athletic performance and recovery.

## THE CONCEPT OF OXIDATIVE STRESS

The cells produce reactive oxygen species (ROS) as a result of the metabolic processes themselves, with the superoxide anion being the precursor to all other ROS [14]. In vivo, the three main sources of superoxide anion are mitochondrial respiratory chain complexes, nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase and xanthine oxidase. ROS can cause damage to proteins, lipids and DNA when antioxidant capabilities are overcome by the burden of ROS, a state known as "oxidative stress" (Figure 1) [18].

Under healthy physiological conditions, ROS production is balanced with enzymatic defenses and different types of antioxidant agents. On the contrary, oxidative stress is a condition of imbalance between ROS production and the set of all antioxidant defenses,

To neutralize ROS, cells have developed an elaborate antioxidant defense system in which superoxide dismutase (SOD) represents the first line of enzymes involved in the elimination of ROS. This enzyme reduces superoxide anion to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and subsequently the enzymes glutathione peroxidase (GPx) and catalase (CAT) convert H<sub>2</sub>O<sub>2</sub> into water (H<sub>2</sub>O) and oxygen (O<sub>2</sub>). (Figure 2).

In recent years, many scientific studies have shown that daily exposure to the characteristic factors of modern life is related to an overproduction of ROS and a physiological alteration of the mechanisms of protection against endogenous antioxidants. ROS can stimulate signal transduction cell pathways, resulting in changes in gene expression that can mediate a number of responses with a profound impact on cellular function and survival. The onset of oxidative stress as a result of both endogenous metabolism and exogenous factors (diet, contamination, UV radiation, smoking, mental stress, etc.) can cause oxidative damage at the molecular level (DNA, proteins, lipids) if cell repair processes are insufficient. All of these ROS-induced changes can have an impact over time on aging processes as well as age-related diseases such as cancer, atherosclerosis, diabetes and chronic inflammation, and neurodegenerative and cognitive disorders may also occur [4]. It is important to consider ROS production to be a powerful antimicrobial weapon and an important component of

FIGURE 1. ROS generation and degradation that contribute to oxidative balance

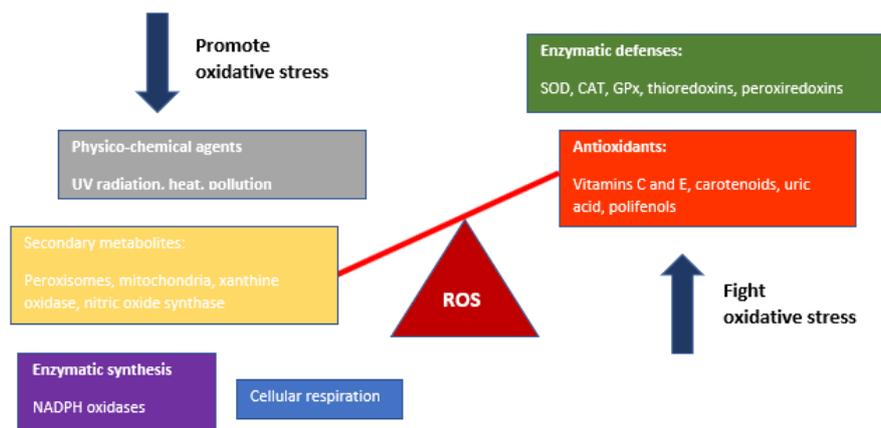
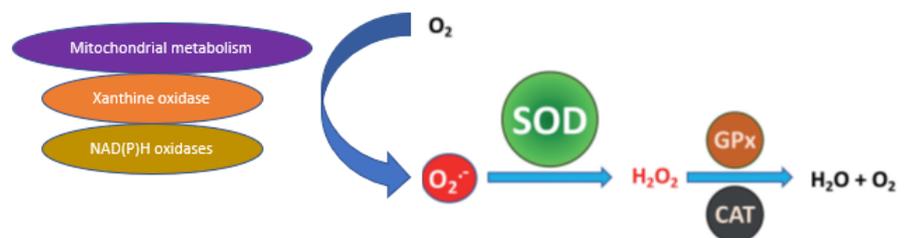


FIGURE 2. Action of SOD to control superoxide anion



innate immune defense against bacterial and fungal infections. Defects in ROS production allow bacteria to repeatedly survive and colonize tissues, as well as cause sepsis [9]. In this context, ROS can be generated by different types of immune cells such as innate phagocytes, specifically polymorphonuclear leukocytes (PMNs). At the site of infection, PMNs express a large number of cell surface receptors that recognize the presence of pathogens or other markers of the inflammatory environment. The activation of these receptors in PMNs triggers a variety of intracellular signaling pathways that support an efficient antimicrobial response, including ROS production, and promote an inflammatory environment.

### BREATHING BURST

The human immune system is composed of innate and acquired defense mechanisms.

Phagocytosis is an innate process, which interconnects these two systems, since the processing of pathogens by those known as "professional phagocytes" (a term that includes not only PMNs, but also monocytes/macrophages and dendritic cells) is a fundamental stage for the production of antibodies. During phagocytosis the production of ROS is induced, which lead to the formation of powerful bactericidal compounds to combat microorganisms [1].

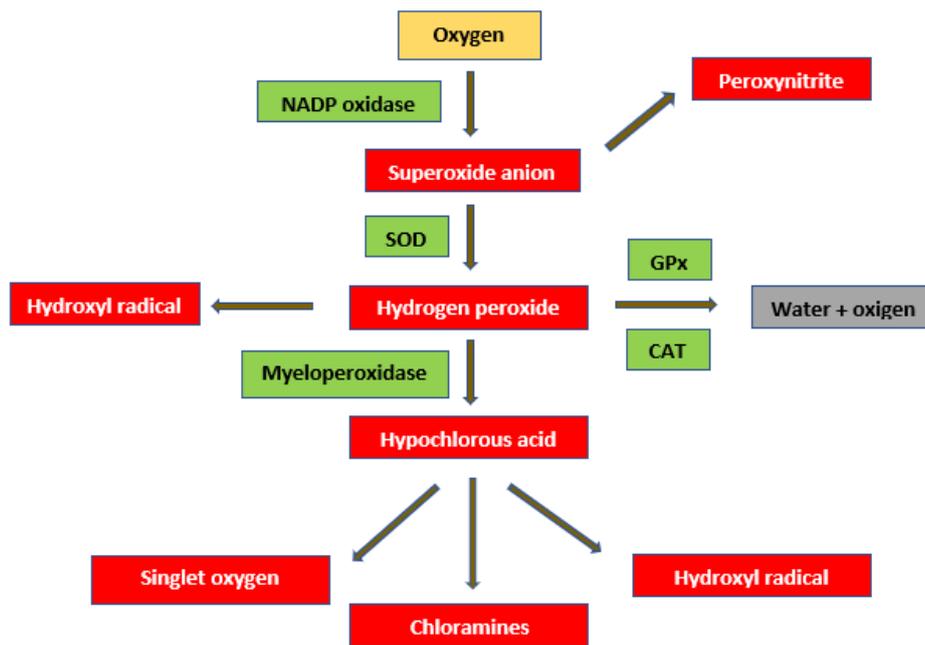
The importance of ROS in innate immunity was first recognized in professional phagocytes experiencing a "respiratory burst" when activated. The excessive oxygen consumption that occurs during this respiratory burst is related to a superoxide anion-generating enzyme, NADPH phagocytic oxidase. After proper stimulation of macrophages,

phagocytosis is associated with ROS production. More specifically, oxygen reduction, catalyzed by NADPH oxidase, results in superoxide anion. The antioxidant enzyme SOD converts the superoxide anion into hydrogen peroxide, from which they are metabolites in phagocytes such as hydroxyl radical, hypochlorous acid and singlet oxygen (Figure 3).

Different ROS, generated in huge amounts by the action of NADPH oxidase and other enzymes such as SOD or myeloperoxidase, can kill pathogens directly-mind by causing oxidative damage to biocomposites, or also indirectly by stimulating the elimination of pathogens through various non-oxidative mechanisms, including cytokine production, pattern recognition receptor signaling (PRR), autophagy and T-cell-mediated responses. From he-cho, ROS derived from the action of NADPH oxidase are necessary for the processing of antigens by dendritic cells and can interfere with adaptive immunity, increasing the immunogenicity of proteins, inducing physiological changes in dendritic cells or influencing the polarization of lymphocytes during specific antigen responses [11].

The production of ROS within the phagomes of immune system cells is critical for antimicrobial activity and for the correct processing of antigens, and influences signaling pathways that direct the host's responses to infection and inflammation. Because excess oxidizers can cause tissue damage and oxidative stress, phagocytes must accurately

FIGURE 3. Oxygen metabolites of phagocytes



monitor both the location and timing of NADPH oxidase activation [10]. Although ROS, which act as microbicide agents, are generated in phagocytic vacuoles, a significant amount crosses into the extra-cellular and extracellular environment. To prevent this damage, cells and organisms need to improve their defenses against ROS to prevent their own cellular structures from collapsing as a result of oxidative stress [2]. However, excessive activation of NADPH oxidase in phagocytic cells results in excessive production of ROS, which are released into the external environment, which contributes to tissue damage. In fact, excessive production of ROS by neutrophils is thought to be involved in various inflammatory diseases such as acute respiratory distress syndrome, rheumatoid arthritis, arterosclerosis, ischemia-induced tissue injury, hypertension, or diabetes [3]. It is important to note that during aging there is an increase in oxidative bursting,

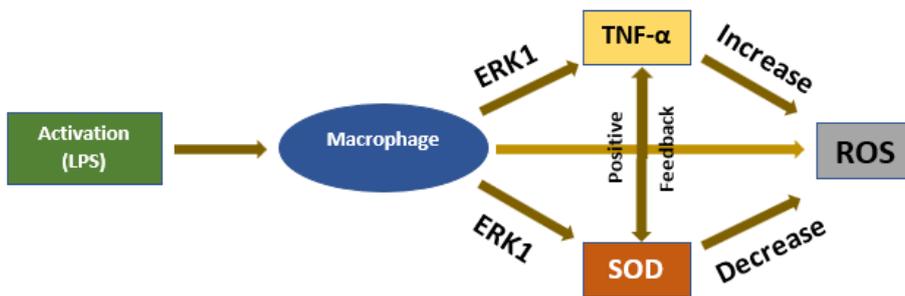
resulting in a higher incidence of pathologies that occur with chronic inflammation. Aging cells decrease the production of antioxidant enzymes such as SOD, CAT and GPx, thus increasing the accumulation of ROS.

In short, it is clear that, during the respiratory burst, the generated ROS triggers a process of elimination of pathogens to control infection. But, on the other hand, excess ROS (produced when antioxidant defenses decrease with age, for example) has been shown to induce chronic inflammatory processes that cause different age-related health problems.

## SOD AND THE IMMUNE SYSTEM

The gene expression of SOD, a key enzyme in the neutralization of the superoxide anion, is regulated by various factors, including microbial surface antigens such as LPS [20], and also by immune system regula-

FIGURE 4. Positive feedback control of TNF- $\alpha$  and SOD level in activated macrophages



Such changes in SOD expression levels indicate that this gene may play an important role in immune responses to infection. For example, SOD induction has been shown to empower macrophages to cope with increased production of superoxide anion, while allowing for increased macrophage activation and increased secretion of TNF- $\alpha$  pro-inflammatory cytokine (Figure 4) [8].

Hydrogen peroxide and ROS are agents that are commonly produced during inflammatory processes. ROS, and specifically hydrogen peroxide at relatively low concentrations, serve as messengers that directly or indirectly mediate the activation of transcription factors and, as a result, induction of several pro-inflammatory genes as well as SOD, which in turn allows the cell to manage toxic concentrations of ROS.

However, at higher concentrations, ROS are toxic and should be eliminated by the cell's antioxidant defense mechanisms. The SOD pathway eliminates such high concentrations of toxic ROS while allowing for greater metabolism and cytokine synthesis [8].

On the other hand, the powerful antioxidant function and antibacterial activity of SOD has been analyzed comparing the gene expression of healthy tissues and stimulate-two tissues with various molecular patterns associated with pathogens using an animal model (fish). In this sense, it has been observed that the expression of SOD significantly delays the growth of both large-positive and large-negative bacteria. Therefore, SOD acts as an antioxidant enzyme and is in turn involved in the immune response [16]. In addition, SOD has been observed to be involved in the

## SOD IS DIRECTLY AN IMMUNITY RELATED ACTIVATOR THAT PROTECTS ORGANISMS AGAINST PATHOGENS

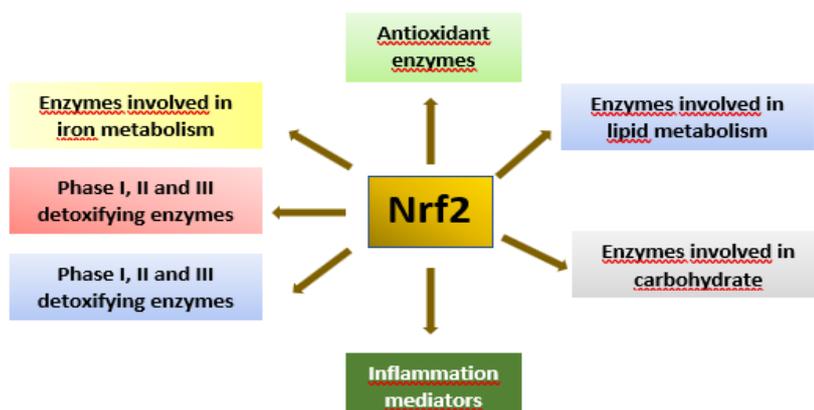
immune response induced by different viruses in aquatic organisms. For example, SOD is activated in hemocytes and the hepatopáncreas when cells become infected with white spot syndrome virus [24], and also after infection by the pathogenic bacterium *Vibrio alginolyticus* [23].

In short, SOD acts not only as an antioxidant enzyme, protecting the body from ROS produced during immune system activation, but is also directly an immunity-related activator that protects organisms from pathogens (both bacteria and viruses), acting as a regulator of cytokine production.

### TetraSOD® AS IMMUNE SYSTEM MODULATOR

Oxidative stress is not only related to tissue damage, but also to cellular signaling pathways that strictly control cell division, migration and production of mediators that ultimately regulate various cellular functions. In fact, ROS maintains their own production and induces the release of cytokines, adhesion molecules and lipid mediators, and also induces the activation of the "nuclear factor kappa B" (NF- $\kappa$ B). NF- $\kappa$ B is a transcription factor that is also involved in biochemical pathways in the context of inflammation [22].

FIGURE 5. Nrf2 plays a central role in the regulation of a multitude of physiological processes



When an inflammatory process is deregulated it can cause persistent tissue damage, various pathological disorders, or even death. Activation of "factor 2 related to nuclear factor erythroid 2" (Nrf2) is essential for the breakdown of this circle, regulating redox balance and cellular response to stress. In fact, the Nrf2 cell signaling pathway is key to the antioxidant response to oxidative stress, regulating more than 600 different genes, of which at least 200 encode proteins with a cytoprotective role (Figure 5). In vivo studies have shown that Nrf2 signaling plays an essential role in limiting neuropathies, arthritis, colitis, pneumonia, pulmonary fibrosis, skin diseases, liver and kidney damage, as well as affecting tumor development. Nrf2 regulates the expression of a plethra of genes involved in the response to oxidative stress caused by inflammation, aging and tissue damage, among other pathological conditions. Deregulation of this cytoprotective system can also interfere with innate and adaptive immune responses [21].

When immune system cells such as monocytes/macrophages detect the presence of a pathogen thanks to molecular receptors present on its surface, the inducible expression

of the enzyme nitric oxide nitase and transcription factor NF- $\kappa$ B is triggered. The NF- $\kappa$ B signal transduction pathway also triggers the expression of FAgocytic NADPH oxidase, which finally promotes the expression of Nrf2. In addition, NF- $\kappa$ B is also able to directly induce the expression of Nrf2 [17]. In this sense, it is well known that Nrf2 modulates resistance to infections caused by viruses (such as Influenza or hepatitis C), bacteria (such as Salmonella), and protozoa (such as Plasmodium or Leishmania).

In addition, Nrf2 is also a key regulator of inflammatory response [19] and has recently identified its role in the suppression of inflammatory genes in macrophages. In fact, two important effects have been described: first, Nrf2 inhibits the expression of pro-inflammatory cytokine genes, including IL-6 and IL-1, in a process independent of ROS, and second, Nrf2 is the key regulator of intracellular ROS by activating the expression of detoxifying antioxidant genes such as HO-1 or NQO-1 [7]. Other inflammatory mediators and enzymes that are inhibited by Nrf2 include: (i) cell adhesion molecules (E-selectin, VCAM-1, ICAM-1), which regulate the migration and infiltration of inflammatory cells into inflamed tissue, (ii) matrix metalloproteinase (MMP-7, MMP-9), proteins present in the extracellular matrix that are involved in physiological and pathological processes such as proliferation, cell migration and differentiation, tissue repair, angiogenesis, programmed cell death or apoptosis, and tumor metastasis, and (iii) cyclooxygenase 2 (COX2) and the inducible form of nitric oxide synthase (iNOS), proteins that mediate pro-inflammatory processes in a multitude of tissues.

Finally, it is important to mention that Nrf2 plays a leading role in differentiating T cells into a state (Th2) in which low levels of gamma interferon cytokine (IFN) occur, which plays an essential role in antiviral defense [13]. Interestingly, this interferon pathway is presented as a promising strategy for preventing SARS-CoV-2 infection, the etiological agent responsible for COVID-19 coronavirus disease. The spike (S) protein in the coronavirus facilitates viral entry into target cells, using angiotensin-converting enzyme 2 (ACE2) as a receptor. SarS-CoV-2 ACE2 receptor is an interferon-stimulated gene in human upper respiratory epithelial cells. That is, SARS-CoV-2 uses a host receptor that increases with the body's own response to a viral infection [25]. Since the activation of Nrf2 in T cells inhibits the production of IFN, this key transcription factor is presented as a molecular target that could undoubtedly have a positive effect on the prevention of COVID-19. TetraSOD<sup>®</sup> is a unique natural ingredient of marine origin that is grown using a technology designed by the company Fitoplancton Marino, S.L. (El Puerto de Santa María, Cádiz). Using this technology, the company is able to produce biomass from the Tetraselmis chuii microalgae with the highest activity content of the antioxidant enzyme SOD that can be found in any product present on the market (>30,000 U/g). In vivo, dietary administration of TetraSOD<sup>®</sup> induces antioxidant capacity by activating SOD, CAT and GPx enzyme activities in an animal model [15]. Similar effects have also been observed in vitro in human cells, occurring in parallel with increases in enzymatic activities positive regulation in the expression of genes encoding these

## **Nrf2 MODULATES RESISTANCE TO INFECTIONS CAUSED BY VIRUSES, BACTERIA AND PROTOZOA**

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enzymes [12]. More importantly, TetraSOD® exercises its antioxidant capacity through the activation of the gene expression of factor Nrf2 (Figure 6) [12].

This is a crucial finding because it implies that TetraSOD® not only exerts its antioxidant activity by direct inactivation of ROS (direct antioxidant), but also activates the body's internal antioxidant system (indirect antioxidant) via route Nrf2. Thanks to this regulatory role of Nrf2, TetraSOD® is therefore presented as a dietary supplement with a potential role modulating immune re-setting at various levels :

-Acting as a regulator of intracellular ROS during phagocytosis and infection processes.

-Modulating the inflammatory response.

-Limiting the proliferation of infections induced by different pathogens (viruses, bacteria, protozoa, etc.).

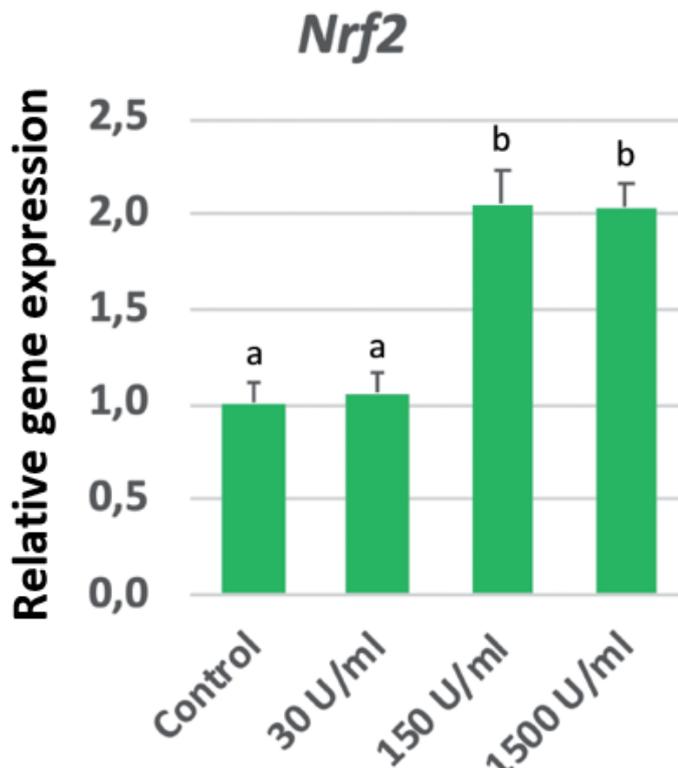
-Activating immune cells like Th cells.

-Controlling one of the viral enhancer mechanisms of CO-VID-19 by reducing the stimulation of the viral input receptor ACE2, by decreasing the production of IFN.

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FIGURE 6. TetraSOD® is capable of modulating the expression of Nrf2



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The advertisement features a woman in a white dress and straw hat standing on a beach with her arms raised, looking out at the ocean. The background is a soft-focus view of the sea and sky. The product logo, 'TetraSOD', is prominently displayed in the center, with 'Tetra' and 'SOD' in green and a registered trademark symbol. Below the logo, the text 'Tetraselmis chuii HIGH SOD' is written in a smaller green font. At the bottom of the image, the phrase 'FOR GOOD HEALTH' is written in a bold, grey, sans-serif font.