Tasco®'s Antioxidant Action

By F.D. Evans, PhD

A by-product of normal biochemical reactions that occur in all living animals is the production of damaging free radicals. Examples of these free radicals or "reactive oxygen species" (ROS) are superoxide anion (O^{2-}), hydroxyl radical (OH^-), singlet oxygen ($^1O^2$) and hydrogen peroxide (H_2O_2). Energy is normally produced in living cells in the mitochondria when energy rich electrons are passed down the electron transport chain. When under high metabolic activity, such as during high productivity, growth or exercise the high levels of ROS occur when electrons escape from the electron transport chain during energy production in mitochondria (Muller, 2000). Another source of damaging ROS occurs when an animal is stressed or diseased and the immune system releases superoxide as a tool to kill invading microorganisms and clear damaged tissue. In fact, ROS increases during any stress, disease state, during high rates of production and as a result of the natural aging process of living cells (Li et al., 2013). These ROS interact with tissues by oxidizing biochemical pathways, damaging enzyme systems and the biochemical machinery necessary for normal metabolic function within the cells. These damaged biochemical components must then be cleared by the immune system and replaced, using valuable stores of energy and protein that have to be diverted from normal growth processes. Anything that we or the animal can do to mitigate the production of ROS damage prevents premature aging, an overloaded immune system and compromised growth processes.

Tasco®, a new generation functional feed ingredient, is a proprietary, patent protected, branded product, produced by Acadian Seaplants Ltd., manufactured from a pure source of a single species of macroalga from managed, natural beds called *Ascophyllum nodosum*. It is dried by a unique, low temperature process (sundried) to retain all of the bioactivity and nutrition. Tasco® has been found to be a potent source of antioxidant protective compounds. These protective antioxidants, some uniquely found in Tasco®, include compounds such as the polyphenolics, flavonoids and fucoxanthin.

Antioxidant type actions are associated with many constituents and chemical entities that are found in food and feed. For example, L-ascorbic acid, commonly called vitamin C is well known for its antioxidant properties. When measured by an *in-vitro* chemical assay, L-ascorbic acid is considered to have a highly effective ROS scavenging ability even at low concentrations (Figure 1). Tasco®, either as a simple water extract or methanol extract also exhibits highly effective scavenging ability of ROS at only slightly higher concentrations than the positive reference compound, L-ascorbic acid, as detected by changes in flores-

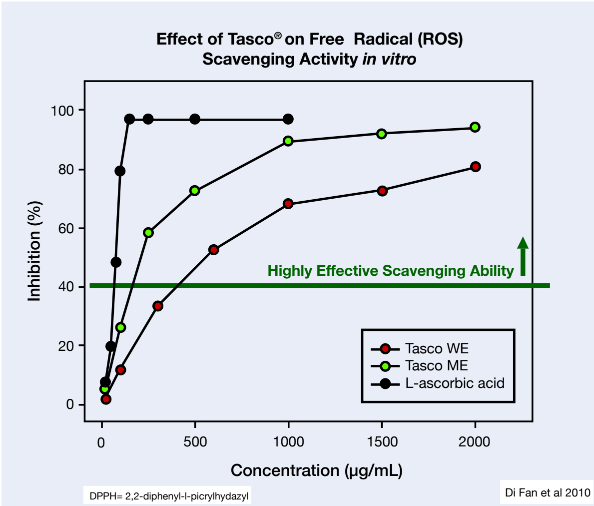

Figure 1 – Effect of standard reference compound L-ascorbic acid and Tasco® as a water extract (Tasco-WE) or methanol extract (Tasco-ME) on effectiveness on free radical scavenging activity (ROS) in a simple in-vitro assay system.

cence of the metabolic dye, 2,2-diphenyl-1-picrylhydazyl (DPPH). Although chemical assay systems such as these show powerful effectiveness of antioxidant constituents, some argue that these systems are not particularly relevant to biological processes since similar reactions do not occur in isolation like in the laboratory (Patras et al. 2013).

To address the concern of assay systems useful to measure biologically relevant antioxidant action, Oxygen Radical Absorbance Capacity (ORAC) type assays have been studied. In these assay systems the compound in question is evaluated for its effects on preventing oxidation of a fluorescence compound and quantified as equivalence compared to the antioxidant Trolox, a standard vitamin E analogue (Ou et al. 2001). This assay system is an improvement over the one measuring gross antioxidant action by DPPH in that it accounts for samples with and without lag phases of their antioxidant capacities as contained in complex food-type ingredients with both slow and fast acting antioxidants. In this system, Tasco® exhibits a ROS scavenging ability similar in Trolox equivalence to a well-known human nutraceutical, *Ginko biloba* (Gb). Interestingly, in the same study, when compared to other macroalgae sources of species harvested for generic kelp meal purposes, Tasco® performs considerably higher and shows its unique antioxidant capability (Figure 2).

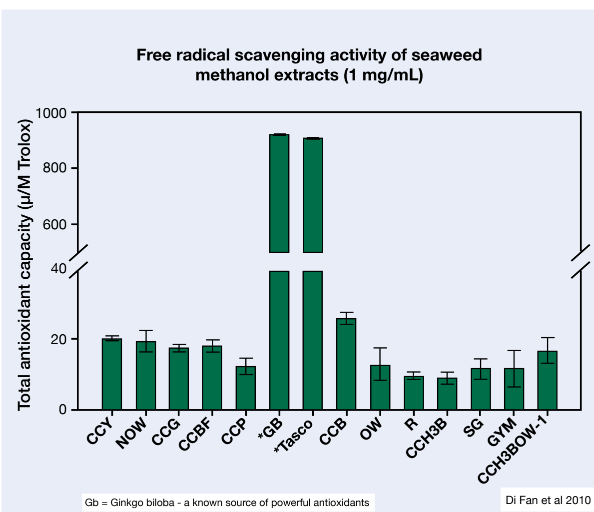

Figure 2. ORAC type assay of Tasco® versus the positive control, Ginko biloba, *known for its high ROS scavenging ability. Other common species of macroalgae harvested for kelp meal purposes do not show the same anmtioxidant capacity as Tasco® does.*

Despite the usefulness of the ORAC system of measuring the antioxidant capacity of complex food type ingredients and mixtures of food/feed stuff, recent criticism of the assay has resulted in new approaches to the measurement of antioxidant capabilities of food stuffs. This criticism stem from the idea that, while the antioxidant capacity can be measured *in-vitro* by this method, it bares no similarity to the kinds of actions required of compounds to the biological processes of intestinal uptake, transport to metabolically active tissue within the circulatory system and *in-situ* participation in tissues that are metabolically active. With this new understanding, use of ORAC values has been discouraged by both the Food and Drug Administration of the USA (FDA), as well as European Food Safety Authority (EFSA) unless additional physiological evidence exists to imply an antioxidant benefit (Patras et al. 2013).

In order to address the physiological relevance, a living, metabolically active whole animal model must be used to measure ROS scavenging ability. One of the most suitable animal models that can be used to study such responses is the *Caenorhabditis elegans* (*C.e.*) or nematode; a microscopic soil worm that can be easily grown in the laboratory in large numbers, with a total lifespan of about a week (Figure 3). This animal, not only has about 66 percent of the same genes as occurs in human beings or other higher animals, but it also has the distinction of being the very first animal on earth where each and every gene has been sequenced and thus we know what each gene does and what role it plays in the animal's metabolic pathways. The use of *C.e.* as a model animal for higher animals such as pets and agricultural species has been recently reviewed (Ewart 2015) and the conclusion of the reviewers is that the model is not only scientifically appropriate but economical and endorsed by the scientific community for use in metabolic and aging studies for application to higher animals.

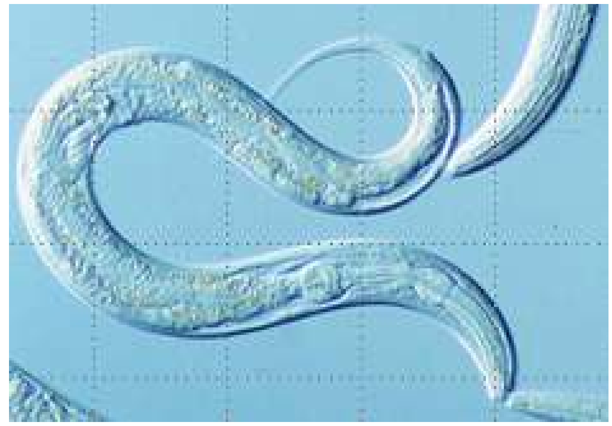

Figure 3. A picture of C Caenorhabditis elegans *as seen under a microscope. Notice that this animal is completely transparent which allows the researcher to see organelles function in* vivo *in real time as treatment is applied directly to the live animal (Di Fan et al., 2010).*

When *C.e.* is subjected to stressors in the environment, they react like all animals by metabolic responses to counter the effects of the stress on the normal functioning of the cellular processes. In a heat stress situation, the heat damage causes denaturation of proteins and enzymes vital to the normal function of cellular processes with the result that the animal's health is compromised and lifespan is reduced. Figure 4 shows the viability measurement of *C.e.* animals after Control or Tasco® treatment when subjected to acute heat stress. The Tasco® treated animals are better able to tolerate the heat stress since less metabolic damage is done due to the ROS scavenging capacity of the Tasco® (Figure 4.).

One of the issues in the scientific literature that has arisen when discussing the subject of the protective effects of antioxidant compounds from different sources is that although many food constituents show antioxidant capacity when measured *in vitro*, not all can be considered to act *in vivo* in a biologically relevant manner. Although antioxidant capacity can be demonstrated in the isolated raw food, these constituents must be digestible, taken up by the cells lining the digestive tract and then transported to the metabolically active tissues while still maintaining their reactive oxygen species (ROS) scavenging ability. To test this, the *C.e.* whole animal model can be used in combination with a metabolic dye, 2,7-dichlorofluorescein diacetate (DCF-DA). Since *C.e.* is transparent animal, and the molecular size of the dye is small, when added to the environment of the nematode, the dye penetrates all tissues of the animal and reacts to tissues concentrations of ROS by changing florescence. If the *C.e.* animal is subjected to acute heat stress as shown by the blue bars in Figure 5, ROS content of metabolically active neuronal tissue in the head of the animal shows a precipitous rise with time. Associated with the rise in ROS content will be protein denaturation and metabolic damage that eventually results in the high mortality for the Control treatment as seen in Figure 4.

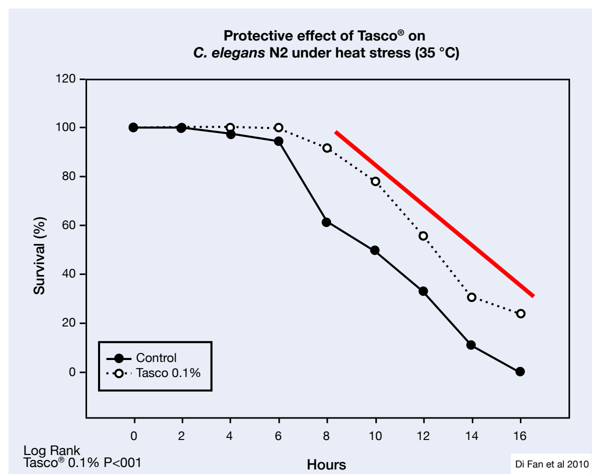

Figure 4. When Tasco® is administered at a low dose prior to the imposition of an acute heat stress, its antioxidant action appears to reduce the effects of that heat damage to cellular systems and so allows the animal to maintain health and viability longer than control treated animals. Treatment differences under the red line are significantly different than controls (P<0.001)

However, when Tasco® has been added to the feed of *C.e.* under the same acute heat treatment conditions, Figure 5, yellow bars, ROS content as measured *in situ* by the

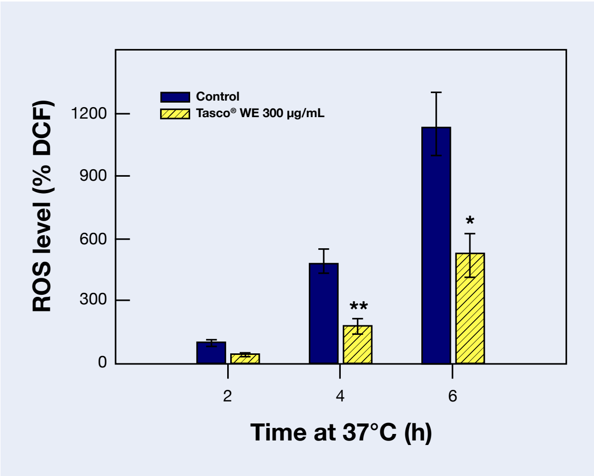

Figure 5. When C.e. under control conditions are exposed to heat stress as shown by the blue bars measuring ROS content by changes in DCF-DA florescence, ROS increases precipitously with time. When Tasco® is administered prior to the imposition of an acute heat stress, its antioxidant action appears to significantly reduce the increase in ROS content with time in vital neuronal tissue located in the head of the nematode as shown by the yellow bars.

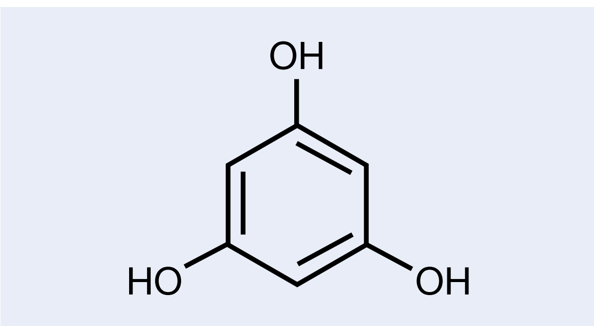

Figure 6. Phloroglucinol is a simple polyphenolic compound that forms the base consituent of other complex phenolic consituents found only in brown seaweeds of marine plant origin.

DCF-DA metabolic dye shows that the potential for ROS damage is greatly reduced (Kandasamy et al., 2011). This shows that the antioxidant constituents in Tasco® that act to scavenge the ROS not only can be digested by the nematode from its digestive tract but is readily taken up by the nematodes circulatory system and transported to metabolically active neuronal tissue in its head whereupon it protects the tissue from oxidative damage. Thus, Tasco® acts not only in a chemical capacity to scavenge ROS but in a biologically relevant manner to protect metabolic pathways and cellular components from oxidative damage.

Tasco® contains significant quantities of constituents that have been shown to have this potent antioxidant action. The most potent of these is probably the polyphenolic compounds. In fact, the type of polyphenolic compounds that occur in Tasco® are only found in brown seaweeds of marine plant origin such as *Ascophyllum nodosum* from which Tasco® is manufactured. The polyphenolic compounds in Tasco® are composed of a simple polyphenolic compound called phloroglucinol as shown in Figure 6. These phloroglucinol molecules are found in Tasco® as complex polymers called phlorotannins as shown in Figure 7. The dry weight of Tasco® contains about 5 to 8% of these phlorotannins and is a significant and potent source of ROS scavengers (Holdt et al. 2011).

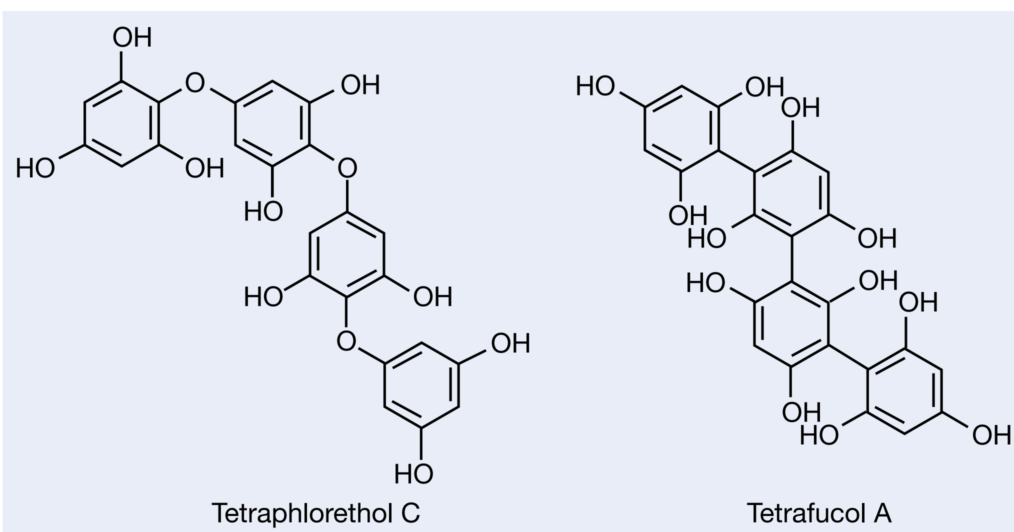

Figure 7. Tasco® contains 5% to 8% dry weight of complex polyphenolic compounds called phlorotannins made from various configuration of a simpler molecule called phloroglucinol. These compounds all have significant ROS scavenging capacity and are found only in brown seaweeds of marine origins such as Ascopyllum nodosum from which Tasco® is manufactured.

Other antioxidant compounds are also found in Tasco® (Holdt et al., 2011). Fucoxanthin is a xanthophyll pigment with the formula $C_{42}H_{58}O_6$. It functions within the seaweed as an accessory pigment and gives the seaweed its brown or olive-green color. It has been reported to have many bioactivities including strong antioxidative ability against ROS. Flavonoids are also reported to be present in seaweeds from which Tasco® is manufactured. Flavonoids are small 15-carbon molecules that are secondary metabolite pigments, generally yellow in color. Although flavonoids are reported to have a wide array of functions it is not clear from the scientific literature whether when eaten by animals if the antioxidant action acts directly from the compound or from metabolic derivatives (Lotito et al. 2006). More research is required to determine the exact role and importance of these compounds in the ROS scavenging ability of Tasco®.

In summary, Tasco® has been shown to have significant antioxidant capacity that has been demonstrated to play a significant role in protecting animals from ROS damage. This protection is most important when the animal is utilizing high amounts of energy, for example when under stress, during disease outbreak or under high growth or productive output. Tasco® can help maintain productivity of animals by protecting important metabolic pathways from ROS damage. This allows the animal to better tolerate the stress, better recover from the disease or difficult situation and helps maintain high levels of productivity. For this reason, Tasco® belongs in the feeding program of all animal species where it has the potential to maximize productivity and help maintain high levels of production despite insult from both biotic and abiotic stressors.

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