layn Natural Ingredients

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POLYPHENOL-RICH BOTANICAL EXTRACTS IN PET NUTRITION AND HEALTH

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Oxidation in pet food is an important topic as consumers are increasingly demanding healthier, higher quality food for pets. This scientifically supported white paper covers important findings for natural, botanical antioxidants to replace synthetic ingredients.

Learn about the mechanisms of technologic antioxidation, science behind polyphenol-rich botanical antioxidants, and findings on which natural ingredients are the best candidates to deliver natural antioxidation to meet today's growing consumer demands.

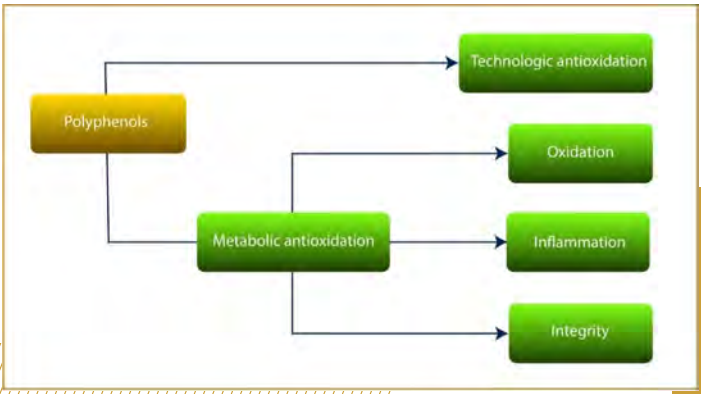
Polyphenols are secondary plant metabolites, and scientists know over 8,000 different substances with potential nutrition and health benefits.

Names like quercetin, rosmarinic or gallic acids, oleuropein, or resveratrol define chemicals that belong in this class of compounds.

We can classify the potential benefits of polyphenols into two main areas:

A. Technological oxidation or prevention of the degradative process of fat and oils added to pet diets.

B. Metabolic oxidation, an internal process that is intimately linked to, and is thought being the cause of oxidative processes, inflammation and decreased gut integrity.



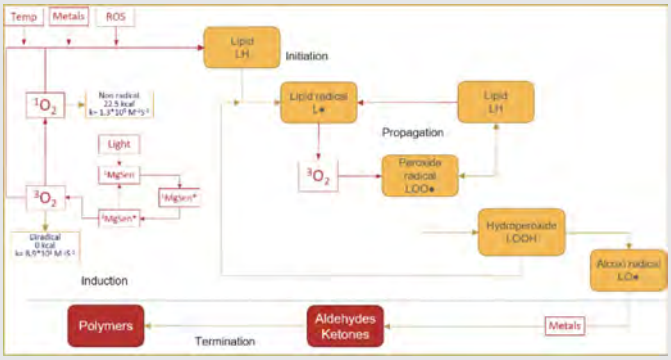
TECHNOLOGIC ANTIOXIDATION

The main objective of this application is avoiding the autoxidation of fats and oils, source of energy and essential nutrients in pet diets.

Autoxidation starts with ground state oxygen, or triplet oxygen, acting upon intact lipids with the help of metals, temperature or reactive oxygen species present in the lipid mass, starting the degradative process.

Another way of starting autoxidation is triplet oxygen changed into singlet oxygen due to the activity of light and some metal sensitizers, e.g. Mn from chlorophyll. Singlet oxygen does not need double bonds to initiate degradation, and it reacts with lipids 1,000 times faster than ground state oxygen.

Lipids degrade by hydrogen abstraction from a double bond, turning the molecule into a free lipid radical.



This radical combines with oxygen and form a peroxide radical. Peroxides abstract a hydrogen from intact lipid molecules, turning them into free radicals that close the cycle, and giving rise to hydroperoxides.

Hydroperoxides are unstable and decompose into alcoxi radicals. These, through metal interactions, originate termination phase chemicals, like aldehydes, ketones and polymers, which change the organoleptic nature of fats and oils.

Stable fats and oils are essential components in pet foods, contributing positive outcomes regarding freshness, flavor, nutrition and taste.

However, when these fats and oils are subject to oxidation, the result is rancidity, reduced nutrition and bioavailability, and off-putting odors consumers ultimately notice and interpret as spoiled or low quality food. The task of an antioxidant is avoiding the radicalary reactions.

Generally, oxidation starts by hydrogen abstraction from a bis-allyl bond in a fatty acid. Bis-allylic bonds contain two contiguous double bonds in the molecule, and their abstraction energy is the lowest among the chemical bonds.

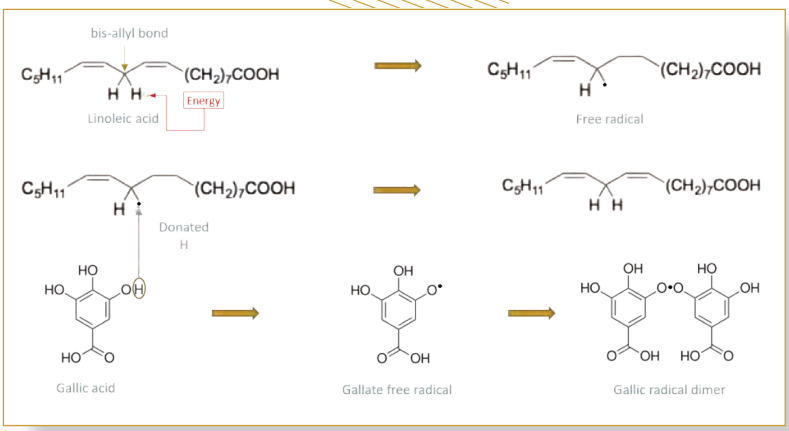
The fatty acid molecule loses a hydrogen, transforms into a free radical, and thus initiate the radicalary reaction.

Antioxidants lend a hydrogen to the fatty acid radical, stabilizing it and avoiding the reaction to progress. In the process, they also turn into a free radical. Free electron delocalization or dimerization are mechanisms antioxidant radicals undergo to avoid them behaving as pro-oxidants.

From the variety of natural antioxidants, we use two chemicals families from botanical extracts to formulate antioxidants: diterpenes from rosemary extract, and catechins and gallic acid derivatives. Antioxidant capacity is associated to substances having phenol rings with substitutes containing hydrogen atoms that can be lent. Many polyphenols contain one or more phenol rings, and those rings possess one or more hydrogen with potential use as antioxidants in fats and oils.

Rancimat is an automated OSI system that allows comparing antioxidant activity by accelerated lipid degradation. Our Research group compared the antioxidant capacity of

formulated antioxidants based on catechins and gallic acid - CGA, or rosemary diterpenes- RD, to the synthetic antioxidants BHA and BHT at the same dose.



CGA or RD antioxidants performed better than synthetic antioxidants irrespective of the instauration degree of the lipid used as experimental substrate. In some cases, the relative activity was over three times that of synthetics.

Comparing CGA or RD to tocopherol-based solid antioxidants, same dose achieves superior protection than tocopherols. CGA in liquid form performs much better than competing tocopherol-rosemary extract based antioxidants used in fat and oils for pet diets.

Regarding cost in use, polyphenol rich TruGro AOX by Layn botanical solutions are applied to the same dosage as the most common synthetic antioxidant on the market, which makes this range of botanicals very cost competitive. These botanical solutions can also be applied to higher inclusion rates than synthetic antioxidants, making these products much more competitive in terms of performance.

The pattern of antioxidant use in the industry would call for protecting fats and oils, or lipid-containing meals, at the renderer's, and using complementary dose thereafter in the final food manufacturer. This will allow achieving the required shelf life for these pet food matrices.

METABOLIC ANTIOXIDATION

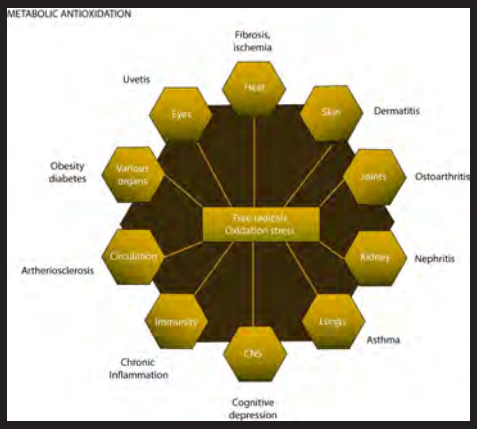
Recent studies suggest most chronic, and some acute inflammatory processes originate because of excess free radicals in the body.

For example, research now considers asthma and obesity, two previously unrelated conditions, as caused by surplus reactive oxygen species.

Many organic inflammatory conditions, ranging from dermatitis to chronic inflammation, associate with ROS, and control of oxidative status of the animal may be a method to avoid pathology typical of mature pets.

Energy metabolism is the main source of free radicals and the superoxide radical its primary by-product. The enzyme superoxide-dismutase (SOD) turns it into hydrogen peroxide. This chemical is also very reactive and the enzyme catalase (CAT) decomposes it into water and oxygen.

Glutathione also decomposes hydrogen peroxide into water, with the aid of a pair of enzymes glutathione peroxidase and



glutathione reductase (GPx-GR).

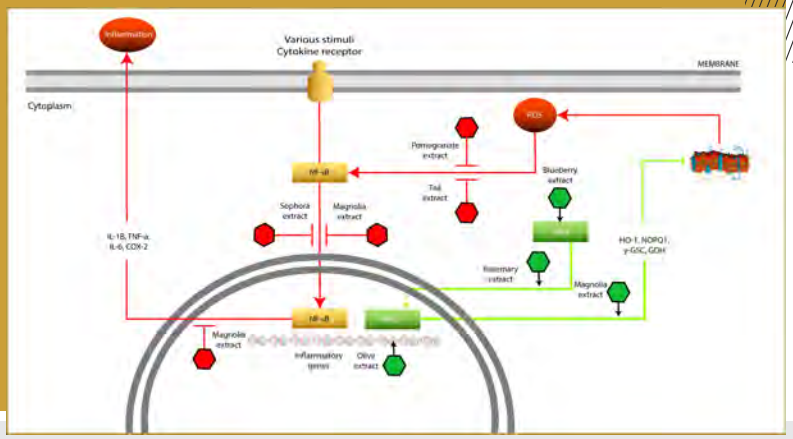
In presence of iron, however, hydrogen peroxide originates hydroxyl radicals that may interact with membrane lipids creating peroxide radicals and malonaldehyde (MDA). This latter compound can bind to DNA bases, altering protein transcription at nucleus level.

Polyphenols can avoid ROS buildup by scavenging free radicals, as described for tea, rosemary or grapeseed extracts. In addition, they increase the transcription of antioxidant enzymes, as reported for hibiscus extract. Finally, activation of the Nrf2 pathway would process heme through a joint mechanism between heme-oxidase and ferritin, avoiding iron impact on the progress of metabolic oxidation.

INFLAMMATION

Metabolic oxidative processes influence most inflammatory conditions in pets. From dermatitis to IBD, one can find ROS in the onset or progress of the condition.

Inflammation initiates and progresses following detailed metabolic pathways. Various stimuli acting upon cytokine



receptors activate the Nuclear Factor kappa B (NF-kB) pathway. Some elements in this pathway translocate to the cell nucleus and activate inflammation genes to produce interleukins 6 and 1β (IL), Tumor Necrosis Factor-α (TNF), and cyclooxygenase (COX-2), that cause inflammation. In addition, excess ROS in metabolism also activates the NF-kB pathway.

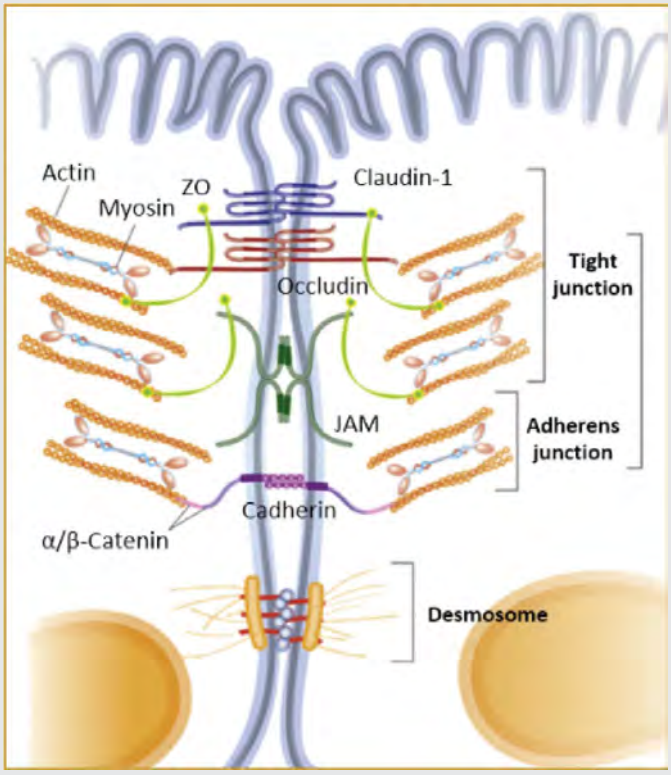
Polyphenols from tea or pomegranate are free radical scavengers that block ROS interaction with the NF-kB pathway. Polyphenols from Magnolia, or Sophora avoid NF-kB pathway derivatives translocation into the nucleus and curb the inflammatory genes activation.

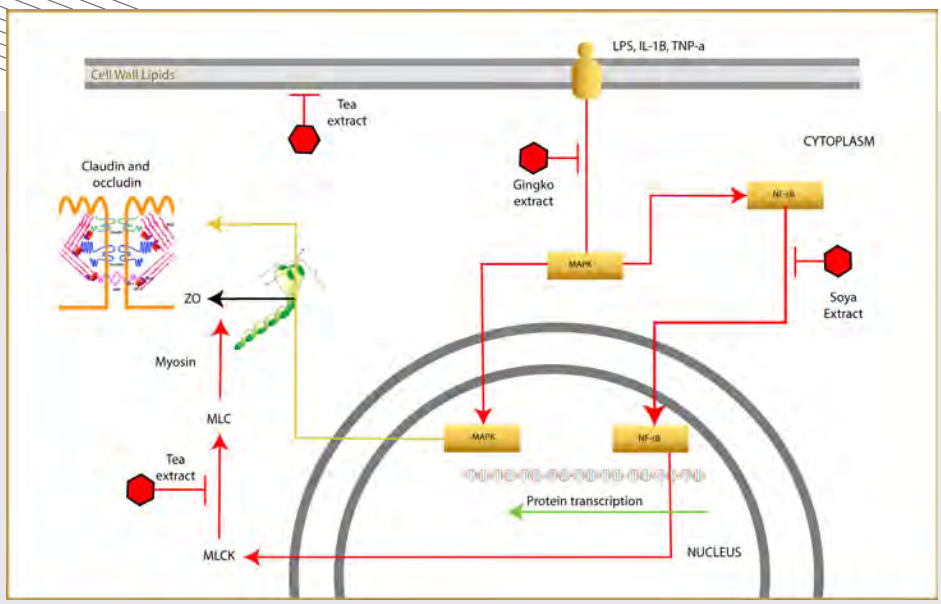
Blueberry or olive polyphenols activate the Nrf2 pathway. This results in increased transcription of antioxidant enzymes, such as heme- oxidase, reducing the amount of ROS build-up and their contribution to inflammatory substances transcription.

INTESTINAL INTEGRITY

The digestive function depends on the integrity of intestinal structures.

For perspective, the gut consumes 12% of the protein and 20% of the energy from the diet, just to maintain its metabolic function. In addition, the gut is the major immunological system in the body, being the first defense against infections.





Nutrient absorption and thus growth, depends on villi and crypt status, and on barriers being undamaged. Barrier alterations may happen in acute or chronic gut pathologies.

Barrier function in the intestine depends on a set of structures located between enterocytes. Three main proteins form the tight junction: claudin and occludin, which open or close the junction; and zonula occludens, which anchors the former to the acto-myosin cytoskeleton.

In nominal status, the barrier prevents passage of macromolecules or microorganisms to the lamina propria and inflammatory or immunitary processes do not exist. When barrier function is altered, noxious substances and even microorganisms may cross it, creating inflammation or immunitary reactions in the intestine or beyond it.

Barrier function depends on the quantity and quality of proteins that make it, and a transcriptional process from the cell nucleus regulates these proteins. Bacterial lipopolysaccharides may interact with a membrane receptor and stimulate the Mitogen-Activated Protein Kinase pathway (MAPK).

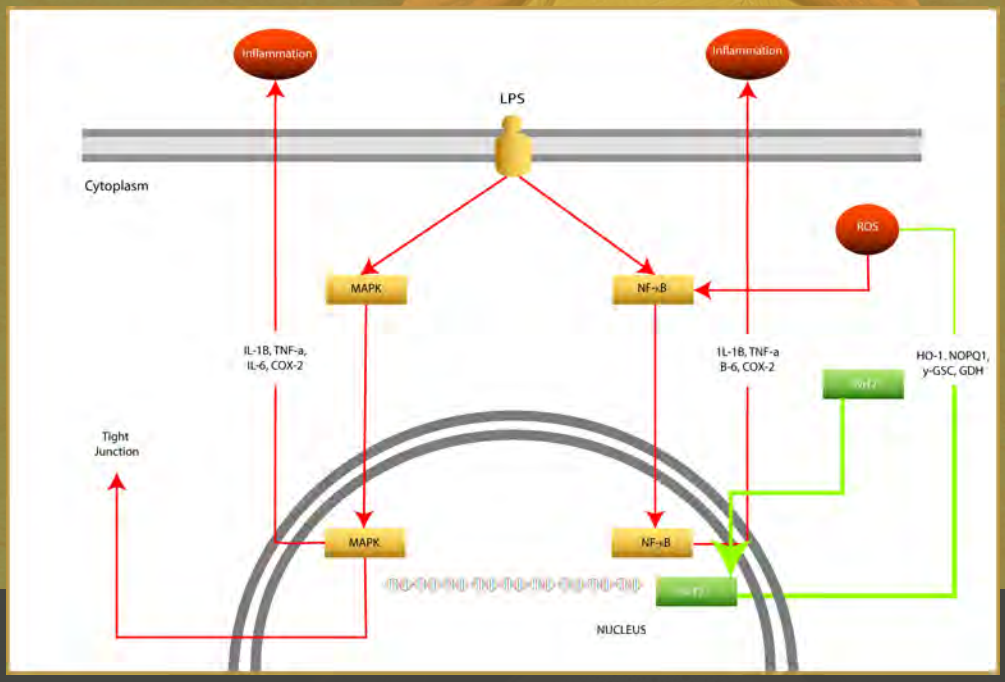
Elements following this pathway translocate to the nucleus, altering protein transcription and reducing tight junction protein synthesis.

Elements from MAPK pathway activate the NF-kB pathway, which translocate into the nucleus and stimulates transduction of Myosin Light Chain Kinase (MLCK), a protease that degrades myosin and weakens tight junction structure.

Tea polyphenols improve cell wall structure protecting lipids from oxidative degradation. Tea polyphenols Also, avoid MLCK activity on myosin, preserving its anchor function to the tight junction.

Polyphenols from Gingko and soya extracts block progression of MAPK and NF-kB, thus avoiding negative influence on tight junction structure and functionality.

Experiments performed by our Research group have demonstrated that providing specif c polyphenol-rich extracts to the diet of test animals increased the relative abundance of claudins 1 and 5, occludin, and zonula occludens above the control animals levels, thus demonstrating the ability of polyphenols to increase intestinal barrier integrity.



CONCLUSIONS

Metabolic oxidation is the root cause of many conditions, and it is linked to inflammatory processes of various nature. Oxidative-inflammatory conditions involve activating two metabolic pathways, MAPK and NF-kB through external conditions, or internal, ROS-mediated stimulation. Through nuclear translocation, this activation results in synthesis of inflammatory proteins, and reduced synthesis of tight junction proteins. The Nrf2 pathway acts as an internal buffer, increasing the synthesis of antioxidant proteins to block ROS action.

TAKE-HOME MESSAGES

For use as technological antioxidants, polyphenols are natural substances with high antioxidant capacity that protects pet food fats and oils to meet shelf life requirements.

For metabolic antioxidation, polyphenols scavenge free radicals from energy production, and avoid the influence of excess ROS on inflammation and related conditions.

Inflammation derives from the activation of specific pathways by external stimuli, or metabolic ROS build-up. Polyphenols either block pathway progression, or scavenge free radicals to reduce their influence on inflammatory protein synthesis.

Finally, research shows that polyphenol-rich botanical extracts supplied through the diet increased the relative abundance of tight junction proteins, thus improving gut integrity and barrier function.

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Layn Natural Ingredients is one of the world’s largest innovators of natural botanical extract ingredients and solutions serving the biggest brands in food, beverage, flavor, nutraceutical, personal care, animals and pets for over 25 years. TruGro™ by Layn is Layn’s speciality business unit providing botanical ingredients and solutions for pet and animal nutrition.

Truly vertically integrated, Layn offers nearly three decades of experience in providing a fully secure, manufacturer-direct, transparent and scalable supply chain. From seeds and agronomy, to extraction and formulation, Layn is committed to quality, innovation and sustainability.

Its world-class R&D operation processes more than 90 million kilos of biomass annually and includes global innovation centers throughout the world to conduct research, ensure quality, and provide formulation and application guidance.

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