Vibrant Carotenoids

By Steve Myers, Senior Editor

One of the greatest outdoor showings is the annual fall leaves spectacle, but carotenoids, which deliver a yellow, red and orange spectrum, have more to offer than mere aesthetics, as formulators continue to flock to these ingredients that offer deep antioxidant protection to areas like the heart and the eyes. Based on the variety of products that now incorporate carotenoids, from supplements to skin care, it might seem there growth has no limit; however, these compounds do present formulation challenges, the greatest of which may be stability concerns. Working together on ways to meet these challenges has positioned suppliers and formulators to both perpetuate and innovate this market segment.

Science-Driven

The use of carotenoids in formulations is propelled by the science behind their health benefits, which focuses on antioxidant protection in various body systems. These fat-soluble compounds are found in the leaves and tissues of plants, helping chloroplasts collect light during photosynthesis and protecting them against damage from excess ultraviolet (UV) sunlight; when chlorophyll breaks down in autumn leaves, the vibrant colors of carotnenoids take over. They are also found in algae and some marine life further down the food chain from algae.

Animals are unable to manufacture carotenoids, making the diet the primary source. Beta-carotene and alpha-carotene are found in orange plants including carrots, sweet potatoes and pumpkins; astaxanthin, which is commercially sourced from alga, and lycopene reside in red plants such as tomatoes and grapefruits, as well as in some fish (salmon) and crustaceans; and lutein and zeaxanthin can be sourced from dark, leafy greens (spinach and kale) as well as some flowers.. There are some “colorless” carotenoids (phytoene and phytofluene) that absorb only UV light and are found in algae, fungi and various plants.

Of the hundreds of known carotenoids in nature the most common fall into one of two major classifications. Beta-carotene, alpha-carotene and beta-cryptoxanthin are considered provitamin A carotenoids because they can be converted in the body to the vitamin A form retinol, although each carotenoid has its own conversion efficiency. In fact, the Institute of Medicine (IOM) has noted anywhere from 26 percent to 34 percent of vitamin A consumed in the United States is in the form of provitamin A carotenoids. These carotenes are also called non-oxygenated carotenoids. Oxygenated carotenoids are also known as the non-provitamin A group, which includes lutein, zeaxanthin, lycopene and astaxanthin.

The highest concentration of carotenoids in the body are in the liver, ovaries, testes and adrenal glands, although their presence and dominance in areas such as blood lipids and the retina have drawn much of the recent spotlight. Due to their composition, carotenoids protect lipids in the body from peroxidation, a leading factor in cardiovascular diseases (CVDs). Lipid peroxidation occurs when free radicals steal electrons from a lipid, most commonly polyunsaturated fatty acids (PUFAs) that feature highly reactive hydrogens. This process creates new radicals and goes unchecked until antioxidants slow or stop the proliferation. Rampant lipid peroxidation can cause damage to cell membranes and can cause health problems.

Lycopene protects against lipid peroxidation with a resulting anti-atherosclerotic benefit. A 2010 review article noted lycopene dosages of 25 mg/d or greater resulted in significant reductions (10 percent, comparable to statins) in total serum cholesterol and low-density lipoprotein (LDL) cholesterol, whereas lower dosages did not produce such significant results.[1] Further, lycopene significantly reduced systolic blood pressure.

Additionally, research has shown lycopene can preserve myocardial antioxidant status and significantly inhibit lipid peroxidation resulting from myocardial ischemia-reperfusion injury.[2] However, researchers have increasingly focused on lycopene in the form of tomatoes, finding tomato supplementation (60 days) can improve levels of serum antioxidant enzymes and decrease lipid peroxidation rate in coronary heart disease (CHD) patients.[3] Pretreatment with a tomato extract also helped protect against oxidative damage and myocardial necrosis in a heart attack study.[4] In fact, in one study comparing tomato juice to lycopene alone, both interventions reduced lipid peroxidation, but only the juice improved post-ischemic ventricular function and reduced heart attack damage.[5]

Astaxanthin has made a similar mark on lipid peroxidation in cardiovascular health. A 2009 review cited at least eight human clinical trials showing astaxanthin pretreatment (intravenously and orally) protects against myocardial ischemia and reperfusion injury, in addition to reducing markers of oxidative stress and inflammation, and improving blood flow.[6] Astaxanthin can also reduce plasma levels of nitric oxide (NO) end products and lipid peroxidation, resulting in improved arterial structural and function in hypertensive subjects.[7]

Other research discovered high levels of plasma beta-cryptoxanthin and lutein were associated with a decreased risk of acute myocardial infarction among hundreds of cases of acute myocardial infarction, after adjustment for multiple risk factors for CHD.[8]

Despite the apparent group effort against lipid peroxidation in cardiovascular (CV) health, recent reports suggest not all carotenoids act the same. Researchers at Elucidia Research in Beverly, MA, measured the effects of astaxanthin, zeaxanthin, lutein, beta-carotene and lycopene on lipid peroxidation using model membranes enriched with PUFAs.[9] They found non-polar carotenoids including lycopene and beta-carotene disordered the membrane and promoted hydrogen peroxidation, while astaxanthin (polar) preserved the membrane and showed significant antioxidant activity.

Many factors such as delivery form, dosage and combinations may have contributed to conflicting or inconclusive results on carotenoids and lipid peroxidation in CV disease, but the positive results show great potential as much as the less-positive results show the situation isn't as straight forward as perhaps first thought.

Lipid peroxidation is not limited to cardiovascular health. In the brain, levels of antioxidant carotenoids including lutein, zeaxanthin, beta-cryptoxanthin, lycopene and beta-/alpha-carotene are lower in patients with dementia or Alzheimer's disease (AD).[10] A 2007 trial compared plasma levels of several carotenoids (lutein, zeaxanthin, beta-cryptoxanthin, lycopene, alpha-carotene and beta-carotene) and performance on a range of cognitive tests in 1,300 healthy elderly subjects.[11] They found participants in the lowest quartile of cognitive function had low levels of lycopene and zeaxanthin.

The same group that found astaxanthin reduced NO and lipid peroxidation in hypertension also found astaxanthin supplementation reduced blood pressure and delayed incidence of stroke in stroke-prone

rats.[12] They suggested a NO-related mechanism behind this effect and further noted astaxanthin appeared to improve memory in vascular dementia.

Lutein has been shown protective against peroxidation of phospholipids, which are important components of the brain, eye and liver. A 2009 research report noted supplemented lutein incorporates into erythrocytes (human red blood cells) where it decreases phospholipid hydroperoxides (PLOOHs), which are increased in dementia patients.[13] Lutein appeared to act on PLOOHs in erythrocyte membranes, but not plasma.

However, lutein's biggest role may be in the eye, specifically the retina, where it pairs with zeaxanthin as the primary macular pigments. People with the highest dietary intakes of both carotenoids have a reduced risk of AMD compared with those with the lowest intakes.[14,15] From another perspective, people with high plasma zeaxanthin can have as much as a 93-percent reduced risk of age-related maculopathy (ARM), while high plasma levels of lutein can result in about a 69-percent reduction in risk of ARM.[16]

The Lutein Antioxidant Supplementation Trial (LAST), which involved 90 patients with atrophic age-related macular degeneration (AMD), found 10 mg/d of lutein resulted in a 36-percent increase in macular pigment optical density (MPOD).[17]

Formulating nutritional products for babies might not be the biggest market segment, but a 2010 study report detailing the eye health benefits of increased lutein in preterm babies shows the importance of this carotenoid in early ages.[18] Oral lutein was well-absorbed and increased plasma lutein concentration in preemies, which researchers said may impact macular development and visual function.

"Liver loves lutein" may roll off the tongue, but it is not mere word play. In one trial, lutein protected against tissue damage caused by hepatotoxins, a benefit credited to antioxidant mechanisms.[19] A 2010 publication detailed how both in vivo and in vitro evaluations revealed lutein increased the activities of antioxidant enzymes in both blood and liver, with resultant potential for affecting related degenerative diseases.[20] In this same study, lutein inhibited generation of superoxides in macrophage immune cells.

True to its letter group, the liver appears to also love lycopene. A Turkish trial found lycopene administration protected against methanol-induced hepatic injury by reducing the increase of lipid oxidation.[21] Another 2010 trial confirmed lycopene protects against oxidative damage in the liver, calling the carotenoid a potent free radical scavenger.[22] 2010 results out of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, showed lycopene and tomato extract administered to rats with nonalcoholic steatohepatitis (NASH), a liver disease that occurs in subjects without a history of alcohol abuse, reduced high-fat-diet-induced lipid peroxidation in the liver.[23] Ultimately, the researchers concluded the two lycopene-based interventions inhibited NASH-promoted hepatocarcinogenesis via reduced oxidative stress, which could be fulfilled through different mechanisms.

Carcinogenesis, or the development of cancer, is not a comfortable area for natural products formulators or manufacturers. Still, the results on various carotenoids and cancer highlight the powerful immune health benefits carotenoid ingredients can bring to a formula.

Mexican researchers reported supplementation with lutein decreased tumor expression of several genes during both prevention and treatment stages of colon cancer in an animal trial.[24] Astaxanthin also made inroads on colon cancer research, with one study showing the algae *Haematococcus pluvialis*, the primary commercial source of astaxanthin, protected against colon cancer in a human cell line,[25] while a 2011

Indian trial concluded astaxanthin exhibited anti-inflammatory and anti-cancer effects by inducing apoptosis in rat colon carcinogenesis.[26]

Lycopene has a long, winding journey with cancer research. In 2000, Israeli researchers reported the inhibitory effects of lycopene (as Lyc-o-Mato®, from LycoRed) on cancer cell growth was not due to the toxicity of the carotenoid, but was due to an interference in IGF-1 receptor signaling and cell cycle progression.[27] Then in 2004, research presented at the 95th Annual Meeting of the American Association for Cancer Research in Orlando revealed lycopene (as Lyc-o-Mato) may help prevent cancer by stimulating the body's antioxidant response element (ARE), which activates cancer-preventive enzymes called phase II detoxification enzymes.

For a while, lycopene was promoted as a men's health powerhouse due to its researched protective benefits in the prostate. Epidemiological evidence linked tomatoes and then lycopene to reduced risk of prostate cancer, so more detailed trials ensued. The original theory focused on lycopene's antioxidant properities, but a 2009 report concluded while a substantial portion of lycopene's anticancer effect can be linked to antioxidant action, other mechanisms are likely involved; they found effects on inflammatory pathways.[28]

A number of studies reported lycopene inhibited the proliferation of several prostate cancer cell lines,[29,30] A 2009 review acknowledged a chemopreventive benefit of lycopene supplementation and confirmed a number of trials showed reduced cancer symptoms, but they said there is insufficient evidence to support a firm conclusion on lycopene's benefit to prostate cancer patients.[31] And results from a 2011-reported nested case-control study showed serum lycopene levels were not associated with prostate cancer risk in participants of the Prostate Cancer Prevention Trial (PCPT).[32] They even reported a slight increase in cancer risk correlated to a slight increase in lycopene. Of course, the landscape is still unsettled, as another 2011 report concluded lycopene can affect gene expression relative to cancer development.[33] The researchers noted the benefit from lycopene was the same regardless if in a food matrix or a purified extract.

Where formulators are concerned, FDA squashed most of the health claims requested in a 2005 petition case, approving only a very limited (and wordy) set of qualified health claims for tomato and tomato sauce and reduction of prostate cancer. The agency generally cited a lack of intervention studies on lycopene and many other cancers, including lung, breast and colon, and said observational evidence did not support the proposed claims.

Another carotenoid batted back and forth over the years as a cancer fighter, beta-carotene has found some positive trends in skin health research. Ten weeks of beta-carotene supplementation may provide protection against sunburn, according to a 2008 meta-analysis of trials.[34] Animal research highlighted this carotenoid's ability to accumulate in the skin where it protects against UV-A-related oxidative damage.[35] While beta-carotene was found in one trial to interact with UVA in skin cells, protecting them from photo-aging-associated mitochondrial DNA mutation,[36] other research found the ingredient failed to modify the severity of photodamage in normal individuals to a clinically relevant degree.[37]

As was the case with beta-carotene and cancer, skin researchers debated whether the carotenoid has antioxidant or pro-oxidant actions in skin health. In 2010, researchers from Seoul National University Boramae Hospital, Korea, discovered beta-carotene's antioxidant benefit was tied to dosage, with 30 mg/d demonstrating the most efficacy in preventing and repairing photoaging.[38]

Researchers have also looked at mixtures of carotenoids on skin health, including a 2006 study involving two different antioxidant formulas, one including lycopene, lutein and beta-carotene, and the other including beta-carotene and lycopene, along with other non-carotenoid antioxidants.[39] Researchers found both formulas increased serum levels of the antioxidants and improved roughness and scaling.

Astaxanthin has also made good strides in skin health. Japan scientists reported in 2010 results of their trial on human dermal fibroblasts suggested astaxanthin would have a significant benefit on protecting against UVA-induced skin photo-aging such as sagging and wrinkles.[40] Similarly, an Italian study on cultured human dermal fibroblasts found uptake of astaxanthin was higher than uptake of two other carotenoids, beta-carotene and canthaxanthin, and showed a preventive effect towards photo-oxidative changes in the cell culture.[41]

Carotenoids in Demand

Global Industry Analysts Inc. released a new market report in late 2010, detailing the rising demand for carotenoid ingredients, which the analysts said is being driven by increased demand for natural colors and fortified foods. They reported beta-carotene is the most popular carotenoid, due to health benefits and its popularity as a colorant, but also called canthaxanthin, also extensively used as a food color, an up-and-comer in the global carotenoids market. Overall, they said the antioxidant properties of carotenoids are creating more demand for their use in food products. According to the report, the global carotenoid market—an estimated $1.07 billion currently and projected to reach $1.2 billion by 2015—is dominated by the United States and Europe, with DSM and BASF the top leaders and major players including Carotech , Chr. Hansen A/S, Cognis Group, Cyanotech Corporation, Kemin Industries, LycoRed and Valensa International.

In terms of growth, Gary Howe, vice president of operations, Food Business Unit, DSM, said beta-carotene used for coloring is leading the carotenoids pack. “There has been an uprising around artificial color that started in Europe and led to reformulation of many products,” he explained. “This trend is beginning to hit North America, with the Canadian government looking at labeling regulations for colors, and FDA looking into the issue as well. A lot of companies are reformulating.”

The number of different applications for carotenoid ingredients is continuing to rise. Bob Capelli, vice president of sales and marketing for Cyanotech, said the list of application and products continues to grow as more research on health benefits emerge. Where astaxanthin is concerned, “There are many different benefits derived from taking natural astaxanthin, some are well documented with extensive human clinical research and some have only one or two human studies, while some are still emerging,” he said. “The most researched applications correspond with the most widely used formulations: joint and tendon health, skin health including internal sunscreen (Cyanotech holds a patent for this use), eye health and sports nutrition.” He noted these health conditions currently have the most products, but CV health and immune system enhancement are catching up.

Capelli said the most interesting recent carotenoid-containing product is OmegaAstin, which combines 8 mg of pure Hawaiian astaxanthin per serving with all-vegetarian sources of Omega-3s, -6s, and -9s. “This is a great alternative to krill oil and fish oil products for vegetarians.”

Supplements still rule the carotenoid roost, but food and beverage products are increasing in both demand and production. Incorporating carotenoids into these new product types brings some challenges formulators should consider. As lipid-soluble nutrients, carotenoids fare better in oil-based mediums,

making their incorporation in liquids a difficult enterprise. Microencapsulation has helped overcome this challenge. Another challenge with liquid formulations is the natural color of carotenoids, which can overpower the colors of other ingredients.

Gus Castro, senior technical marketing manager, beverages, DSM, said for almost any application a customer suggests, DSM has a format that would work, from dry powders to emulsions and various encapsulations. He noted DSM has recently released a Beta-Carotene 15% LCS for formulators that seek the pro-vitamin A properties, but not the rich, typical reddish-orange color of the carotenoid.

Some manufacturers such as Fuji Health Science have come up with versions that don't affect a product's color. Fuji's AstaREAL Clear 75 is a water-soluble natural astaxanthin ingredient designed for ready-to-drink (RTD) functional beverages. According to the company, the ingredient mixes with any aqueous system and remains clear with no separation or residual film. Its AstaREAL P2AF is a cold water-dispersible powdered astaxanthin extract that is directly compressible and suitable for powdered drinks, two piece capsules and tablets.

LycoRed has invested in the recent years special developments forms of delivery systems for a variety of food and beverage applications to enable it to be stable in a wide range of pH, light and heat. The company said its Lyc-O-Mato natural tomato complex can be used in dairy application, breakfast cereals and even for functional water or juices. LycoRed also offers a cold water-dispersible powdered version of its Beta-Cote™ beta-carotene ingredient; it is intended for use as a colorant and for pro-vitamin A fortification in food systems. Addressing the most common carotenoid formulation challenge, stability in the face of oxygen, LycoRed offers a beadlet form for both its lycopene and beta-carotene ingredients. The beadlet protects the carotenoids from oxygen and from leaking after tableting.

As potent antioxidants, carotenoids are highly reactive with oxygen, making them sensitive cargo in any formulation. Valensa has addressed this situation with its O2B® Peroxidation Blocker technology, an application-specific formulation that combines highly effective botanical ingredients. Omniactive uses a patent-pending vegetarian beadlet technology to encapsulate its Lutemax® Free Lutein and Lutemax® Lutein Esters ingredient for improved stabilization, in addition to the beadlets and cold water-dispersible powders often found in the top carotenoid suppliers. Kemin addresses stabilization by using a patented microencapsulation for one of its FloraGlo Lutein ingredients intended for tablets, and is vegetarian-grade, kosher, and GMO-free. FloraGlo is commercialized globally by DSM Nutrition Products.

Udi Alroy, vice president of global marketing and sales for LycoRed, said his team sees supplementation via science-backed ingredients and formulations for skin, prostate, cardiovascular health will become key ingredients for growth in the marketplace. "The second largest is the personal care and beauty from within category, focusing on beautifying the skin and protecting it from environmental stress in both oral supplementation and topical applications," he said.

Alroy reported LycoRed have developed a new formulation for topical applications and have invested recently in clinical research for topical benefits of the natural tomato complex. Its Lyc-o-Mato lycopene complex was recently featured in a new anti-wrinkle line of cosmeceuticals from Dr.Fischer's "The topical formulation enhances the protection of the skin from environmental damages," he explained. "Combining the oral and topical products the skin will carry a stronger defense mechanism against those aspects."

Based on other introduction in the category, carotenoids have indeed become a sought-after ingredient in cosmeceuticals, either as beauty from within or in topical creams and lotions. For example, Chrysantis

offers its marigold-derived zeaxanthin-lutein ingredient Easy Eyes™ in oil suspension forms for use in cosmetics. And Capelli noted BioAstin astaxanthin has been used in both oral and topical sunscreens; in fact, the ingredient can be found in a line of anti-aging and moisturizing creams and lotions from Derma e®, as well as similar creams from Vera Botania and DermaSource. .

The quality carotenoid suppliers are offering formulators a variety of the most common and researched carotenoids, although many suppliers have focused on specific carotenoids backed by specific research, such as in the case of lutein/zeaxanthin and eye heath. Working with a quality carotenoid manufacturer that can educate on the ingredient technology available and help tie dosage and form to both research and finished product goal will be the ideal route for formulators looking to make an efficacious, targeted and high-quality carotenoid product, be it a supplement, functional food/beverage or a cosmeceutical.

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