Antibacterial effects of HIDROX™

Executive Summary

- The safe and effective elimination of food borne pathogens represents an important unmet need. Multiple lines of converging evidence point to the use of olive juice and olive oil polyphenols, particularly HIDROX™ and hydroxytyrosol, to protect foods against pathogens.
- HIDROX™ and hydroxytyrosol are natural products that exert significant antibacterial effects against food borne bacteria.
- Multiple studies have confirmed that HIDROX™, hydroxytyrosol, and olive juice and olive oil polyphenols have prominent effects against at least 14 different bacterial strains including *E. coli*, *Salmonella enterica*, and *Staphylococcus aureus*.
- HIDROX™’s safety has been formally acknowledged by the United States Food and Drug Administration through its GRAS certification. HIDROX™ has an extremely strong safety profile; HIDROX™ and hydroxytyrosol’s safety have been confirmed in a large number of experimental and human clinical trials.

HIDROX™

HIDROX™ is CreAgri’s trademarked name for hydroxytyrosol, which is produced using a unique, patent protected process. Hydroxytyrosol is a natural, potent, and protective polyphenol derived from olive juice and olive oil. However, olive juice has over 300 times the level of antioxidant polyphenols compared to olive oil.

Hydroxytyrosol is scientifically recognized for its powerful antibacterial, anti-inflammatory, antioxidant, and cardioprotective health benefits. Independent laboratory analyses have demonstrated that hydroxytyrosol is one of the most potent natural antioxidants yet discovered with the highest level of free radical protection activity ever reported for any natural antioxidant compound. Hydroxytyrosol’s therapeutics effects have been demonstrated in a large number of published scientific articles in internationally-recognized journals.

Dr Roberto Crea is the Chairman and Chief Executive Officer of CreAgri. Dr. Crea has over 30 years of experience in the biotechnology field as a scientist, investor, and entrepreneur. He is one of the scientific co-founders of Genentech, one of the leading global biotechnology companies. Dr Crea is also the founder of Creative Bio Molecules, Inc (1982), Creagen, Inc (1992), CreAgri (1998), Bioren (2002) and ProtElix (2005). Creagen, Inc was merged into Neurex in 1994, which was then sold to Elan Pharmaceuticals for over $700 million. Pfizer successfully acquired Bioren in 2005. Dr. Crea is the author of more than 25 international patents and has co-authored more than 50 scientific articles.

Broad Spectrum Antibacterial Effects of HIDROX™

Olive juice and olive oil polyphenols represent an effective and compelling means of preserving food products to prevent the growth of food borne pathogens or to delay the onset of food spoilage; HIDROX™
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and hydroxytyrosol are natural products that have been shown to inhibit or delay the growth of various bacterial and fungal strains.

The powerful antibacterial effects of olive juice polyphenols have been demonstrated in a series of experiments using HIDROX™. Researchers assessed HIDROX™'s antibacterial effects on a variety of foods including lettuce, beef, pork, chicken, and bologna (Ravishankar et al., 2010).

Lettuce was treated for 5 minutes with HIDROX™ and studied for bacterial growth in the following 24 hours; compared to control lettuce samples, HIDROX™ treated lettuce had significantly reduced levels of *E. coli*.

After HIDROX™ was applied to contaminated uncooked beef, pork, chicken, and bologna, meat samples treated with HIDROX™ were shown to have considerable reductions in *E. coli*, *Salmonella enterica*, and *Listeria monocytogenes* after 0 to 3 days. HIDROX™'s antibacterial effects were also assessed in cooked beef; infected beef samples were cooked following an application of HIDROX™. Compared to cooked beef samples in the absence of HIDROX™, those prepared with HIDROX™ had a 10 million fold reduction in the number of *E. coli* bacteria. A separate study of hydroxytyrosol corroborated these findings by demonstrating that the agent significantly inhibited the growth of *E. coli*, *E. faecalis*, and *E. aerogenes* (Medina et al., 2007).

An additional study by Capasso and colleagues (1995) has also successfully showed that hydroxytyrosol exerts notable antibacterial effects; hydroxytyrosol inhibited bacterial growth by 73%. Furthermore, the same study determined that hydroxytyrosol had an exceptionally strong safety profile by exerting 0% toxicity against human cells. Hydroxytyrosol’s lack of phytotoxicity has also been demonstrated, which further supports the agent’s strong value proposition as a key food safety and preservative agent (Bartolini et al., 1994). Collectively, these findings suggest that HIDROX™ derived from olive juice and hydroxytyrosol are well positioned for agricultural use as antibacterial agents given their effectiveness and safety.

Medina and colleagues (2006) investigated the antibacterial effects of a range of olive oils and isolated polyphenols. Virgin olive oils, which are known to be rich in polyphenols with high levels of hydroxytyrosol, were shown to have powerful broad spectrum antibacterial effects against 14 different strains of bacteria including *E. coli*, *Salmonella enterica*, and *Staphylococcus aureus*. These important effects were observed after only 60 minutes. Additionally, the antibacterial effects observed were directly correlated to polyphenol concentrations; virgin olive oils had the greatest effects while sunflower and corn oils, which have no or minimal polyphenols, exerted no antibacterial effects.

In a separate series of experiments the same investigators isolated specific polyphenol compounds from virgin olive oil to further identify the cause of these significant antibacterial effects of polyphenol-rich olive oil. Hydroxytyrosol was shown to be a powerful antibacterial agent by significantly reducing the survival of *E. coli* and *Salmonella enterica* bacterial cells.

Additional experiments have also isolated polyphenols from olive oil extracts to demonstrate their antibacterial effects. Hydroxytyrosol had antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*; high levels of hydroxytyrosol present in one particular olive oil extract were also attributed to its strongest antibacterial effects on *Pseudomonas aeruginosa* (Obied et al., 2007).
Evidence also suggests that hydroxytyrosol exerts important effects against human microbial infections. Furneri and colleagues (2004) demonstrated that an extremely low concentration hydroxytyrosol inhibited 25 strains of mycoplasma. On the basis of these findings, the authors of this study proposed that hydroxytyrosol showed strong potential as an antimicrobial agent for treating human infections and that daily consumption of the supplement could reduce the risk of important human infections.

In summary these findings demonstrate the significant antibacterial effects of HIDROX™ derived from olive juice and hydroxytyrosol. Olive juice contains over 300 times the level of polyphenols compared to olive oil. HIDROX™ is a natural product, and its rapid and powerful effects against multiple strains of bacteria in a broad range of foods reinforce the agent’s effectiveness as a key food safety product.

**Additional beneficial effects of HIDROX™**

The additional therapeutic effects of hydroxytyrosol and HIDROX™ are wide ranging. A large number of experimental studies of hydroxytyrosol have shown that the agent has multiple cardiovascular and anti-inflammatory benefits. Hydroxytyrosol reduces blood pressure, provides an important safeguard against potentially lethal blood clots, improves blood cholesterol and prevents the formation of plaques within arteries. Clinical trials of HIDROX™ have demonstrated that the agent reduces important biological markers of inflammation in patients suffering from rheumatoid arthritis, improves mobility and quality of life in patients suffering from osteoarthritis, and improves the cholesterol profile of patients suffering from hyperlipidemia. Hydroxytyrosol has also been shown to raise levels of vitamin C in the body.

**Strong Safety Profile of HIDROX™**

The safety of HIDROX™ has been formally acknowledged by the United States Food and Drug Administration (FDA); HIDROX™ has been granted GRAS certification. GRAS (Generally Recognized As Safe) is a designation granted by the FDA and states that a chemical or substance added to food is considered safe by experts. GRAS certifications are granted to substances that are generally recognized, among experts qualified to evaluate product safety, as having been adequately shown through scientific procedures to be safe under the conditions of their intended use.

A large number of studies also strongly suggest that daily consumption of polyphenol compounds, including hydroxytyrosol and HIDROX™, is free from major adverse events. Extensive toxicology studies of HIDROX™ have revealed an extremely strong safety profile; HIDROX™ administered in single or multiple dosages of up to 2000 mg/kg/day resulted in no adverse clinical, haematological, biochemical, or reproductive effects (Christian et al., 2004). Furthermore, HIDROX™ has been successfully used in a number of human studies, with no major adverse events reported.
Research on the Antibacterial Effects of HIDROX™, Hydroxytyrosol, and Polyphenols


Obied HK, et al. (2007). Bioscreening of Australian olive mill waste extracts: Biophenol content, antioxidant, antimicrobial and molluscidicidal activities. Food and Chemical Toxicology, 45, 1238-1248.


Ravishankar S, et al. (2010). Research on antimicrobial effects of HIDROX™ on contaminated raw and cooked meats and on lettuce. CreAgri-funded research and development in collaboration with the University of Arizona.


Research on the Additional Therapeutics Effects of HIDROX™ and Hydroxytyrosol


Bitler, CM et al. (20xx). Effects of hydrolyzed vegetation water on serum LDL levels and antioxidant capacity in male and female subjects.


González-Santiago M et al. (2006). One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. *Atherosclerosis*. 188(1), 35-42.


Biography

**Dr. Matthew Killeen** - CreAgri’s Scientific Affairs Advisor. Dr. Killeen received his undergraduate degree in pharmacology with highest honors and was awarded a Ph.D. in cardiovascular electrophysiology from the University of Cambridge. At Cambridge he studied the mechanisms underlying sudden cardiac death and identified a number of effective pharmacological treatment strategies. Following his Ph.D., Dr. Killeen was awarded research fellowships at Harvard Medical School and the Massachusetts General Hospital. Dr. Killeen has authored 19 peer-reviewed publications in leading international journals. He is a member of the Cardiac Safety Research Consortium, a collaborative initiative between the FDA and Duke University. Dr. Killeen developed the concept for and co-chaired an FDA Think Tank on pediatric drug safety. Dr. Killeen has also previously worked for Eli Lilly and he is the author of the forthcoming book, “Cardiac Drug Safety: A Bench to Bedside Approach,” which will be published in 2011.