

From waste to elixir

▶ **Biotechnology meets process innovation to convert toxic olive press byproducts into a therapeutic ingredient.**



▶ Roberto Crea, president and chief scientist, Creagri Inc., Hayward, CA

These are dynamic times in understanding how the human body stores and metabolizes food, though much remains to be learned about the critical molecules and enzymes in the food itself. Discoveries involving Omega-3 fatty acids, for example, or the recovery of valuable proteins in whey that used to be spread as fertilizer, have occurred. A molecule that has appeared on the radar in recent years is hydroxytyrosol, a polyphenol found in abundance in the waste stream of the olive oil milling process. Early clinical trials suggest hydroxytyrosol imparts numerous therapeutic effects, working as an anti-inflammatory, an antibacterial, a blood-cholesterol reducer and an anti-clotting agent. Even anti-aging benefits have been ascribed to hydroxytyrosol, though no human trials support these claims.

A powerful antioxidant with high free-radical scavenging properties, hydroxytyrosol is found only in trace amounts in olive oil; it is concentrated in the juice, which typically is discarded during the extraction process. To recover and stabilize the compound, a California manufacturer with its own organic olive grove developed a process that removes the pit, separates the oil from the juice, then acidifies the juice and incubates it, sometimes for months, to provide time for the oleuropein in the water to convert to hydroxytyrosol. A dried extract containing as much as 95 percent of the active ingredient is then produced.

The creator of hydroxytyrosol is Creagri Inc., founded in 1999 and one of several entrepreneurial ventures of Roberto Crea. The native of Italy received a doctorate in biological chemistry from the University of Pavia and was an associate professor of DNA chemical synthesis at Leiden University in the Netherlands before immigrating to California's Bay Area in 1977 to become one of the four original scientific directors at biotechnology pioneer Genentech Inc. After helping guide the development of Humulin, a synthetic human insulin, Crea struck out on his own, founding six biotech firms, successfully spinning off most and retaining two, including Creagri.

FE: When did you focus your research on the olive's components?

Crea: About 18 years ago, researchers started to identify polyphenols as the key to healthy outcomes associated with the Mediterranean Diet and the low frequency of cancer and longer life of those who followed it. Until then, oleic acid was thought to be the active element, but oleic acid is found in concentrations similar to olive oil in sunflower and canola oils. We began our research into polyphenols about 10 years ago, which was still ahead of the curve. We began to focus on hydroxytyrosol, a new and exciting molecule that is very small and simple in structure, similar to dopamine, which is a key to brain activity in mammals. A shortage of dopamine in the body is associated with Parkinson's disease and other dysfunctions.

Hydroxytyrosol is present in olive oil but in very small amounts, less than 2 percent. We were the first to propose extraction of this fraction from the juice, which contains 300-fold more hydroxytyrosol than you find in the oil. Others were looking at ways to isolate this molecule in the oil, but the processes were expensive and tedious, and the recovery rate was very poor. We realized that the majority of the polyphenols were being thrown away.

FE: Were processors deriving any by-products previously from the juice?

Crea: No. In fact, many small farmers in Italy have stopped growing olives because the juice, or vegetation water, quickly becomes toxic and poses waste disposal problems they can't cope with. We came up with



► **Research Associate Tatiana Odinena at work in Creagri's laboratory in Hayward, CA. Biotech and nutrition research is ongoing to support human health claims for hydroxytyrosol.** Source: Creagri Inc.

the regions of the brain, which may produce a positive impact on brain activity.

FE: What is your involvement with Colant One, the olive de-pitting machine developed in Italy?

Crea: Although I immigrated to the US in the 1970s, I often return to Italy to see my parents and family. About 10 years ago, I was looking for something to do while I was there, and, as a good Italian boy, I got involved with olive oil production. People always are looking for ways to produce better olive oil, and friends in Rome directed me to efforts to remove the pits before the olive is crushed.

I found a group with a prototype machine that could separate the stones from the meat and decided to finance the completion of their project. Besides equity in the company, I bought 80 percent of the machinery production. As a result, I received the first machine produced, and I put it into production in California. In Italy, there must be a hundred producers who now use this technology because of the higher quality oil it helps them produce.

FE: A collaboration between a biotech firm and a machine builder is unusual, isn't it?

Crea: It was serendipitous, but I've always been fascinated with the ability of technology to solve problems. And I've been an entrepreneur for 20-plus years, though the work at Creagri was a bit of a divergence from the other enterprises.

The process we developed was the first time the fruit had been separated into four fractions: the stone, the oil, the liquid fraction and the solid particles. This technology changes the whole paradigm of olive oil productions because we don't produce any waste. All the fractions are recovered and sold. The stones are sold as fuel for stoves. Solids are used as animal feed.

a way to avoid toxic reactions and quickly preserve the juice.

FE: How does your process differ from conventional olive oil production?

Crea: Typically, the olive, including the pit, is crushed to produce a thick paste, and the paste is squeezed mechanically to extract the oil. The pulp and the stone are rich in phenolic compounds, including oleuropein, but most of those compounds are washed out during malaxation, the continuous washing with water that the crushed olive undergoes.

Our process begins by removing the pit from the pulp and then separating the water from extra virgin olive oil. Less than 1 percent citric acid is added to the juice, lowering the pH and preventing oxidation, polymerization and bacterial growth. Oleuropein, the precursor to hydroxytyrosol, is present in high concentrations in the water. It is a big molecule that won't cross the gastrointestinal tract, so there's little bioavailability. Therefore we incubate the acidified vegetation water, allowing the breakdown into the much smaller hydroxytyrosol molecule. This molecule is small enough to cross through

FE: How is this ingredient delivered for human consumption?

Crea: We're looking for many different ways to deliver it. If you want the full beneficial effect, you should take it in a capsule. It's a soluble powder, as well, and can be delivered with a beverage. It's GRAS certified, and a company in Europe will be putting it into spring water.

Early in the development process, we tried to make a beverage of lemon juice with this, but it immediately turned brown because of bacteria and oxidation. That's why we looked at citric acid and added an incubation period to allow greater conversion to the small molecule.

FE: How much scientific evidence exists to support the claims for hydroxytyrosol's effectiveness?

Crea: Several human and animal trials of the safety and efficacy have been conducted. In a 105-subject clinical study conducted by researchers at Arizona State University, for example, people with osteoarthritis and rheumatoid arthritis showed significant decreases in serum homocysteine levels after eight weeks, compared to a control group. Rheumatoid arthritis subjects also had statistically significant decreases in serum homocysteine, which is associated with cardiovascular events. In another study, putting hydroxytyrosol into the diet of fruit flies extended their lives 40 percent. We hope to conduct a clinical study involving humans to determine the impact on aging and the immune system.

We're committed to a high degree of research integrity about the effectiveness of this molecule and backing health claims with supporting evidence. It's fascinating from a research standpoint.

FE: Having isolated hydroxytyrosol from a fraction of the olive, are you considering fractionating the olive pit to see if those elements have therapeutic benefits?

Crea: The stone is another world, particularly with all the proteins and enzymes present. Pharmaceutical manufacturers isolate some of those enzymes to help metabolize lipids. That's a whole different field of research. ❖