Nature and nanotechnology join forces

A defining characteristic of nature is its lack of uniformity and predictability. Nanotechnology offers a way to smooth these rough edges in order to harness the power of essential oils to control post-harvest diseases in citrus.

1 Prof. Sandra Combrinck, project leader.
2 Scanning electron microscope (SEM) images of lemongrass-encapsulated silver nanoparticles. The light areas on the left indicate the presence of the nanoparticles.

THE USE OF fungicides to combat pathogens has contributed greatly to sustaining food quality and food security. However, this contribution is threatened by increased consumer awareness of the dangers of synthetically prepared chemicals, global restrictions imposed on fungicide application, and an increase in micro-organism resistance towards fungicides.

As a result, the last decade has witnessed a growing interest in natural mycobiocides, with essential oils (EOs) taking centre stage in the search for alternatives to chemical fungicides.

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The complex nature of EOs impedes the development of resistance by the pathogen. The application of EOs also leaves a smaller carbon footprint, because they are biodegradable without being highly unstable. "The application of essential oils as fungicides is also far more acceptable to consumers and is suitable for the organic market," adds Professor Sandra Combrinck, a researcher in the Department of Pharmaceutical Sciences at the Tshwane University of Technology.

The volatile nature of EOs is both a boon and a bane in their fumigant application. The advantage of their volatility and bioactivity in the vapour phase is that it makes EOs suitable for the protection of stored products. EOs as fumigants have good penetrability, for example into wounds on the rind; low concentrations can be applied, which reduces costs; and the sensory properties of the fruit are not affected.

However, EOs are very volatile and this could reduce the efficacy of applications, given that the volatile active components have to be released slowly to ensure consistent protection of the fruit over time.

Encapsulating particles is known to have the potential to slow down the evaporation of volatile substances and thus extend their period of release. In the case of EOs, research has included EO-encapsulating zeolites, nanoparticles, and micelles.

In the fruit industry, disease control involving the use of EOs has been focused on modified atmosphere packaging (MAP) and coatings. In these applications, the successful introduction of EOs depends on the stability and bioavailability of the oils. "In all cases, the inherent volatility of the oils is a complicating factor," says Sandra.

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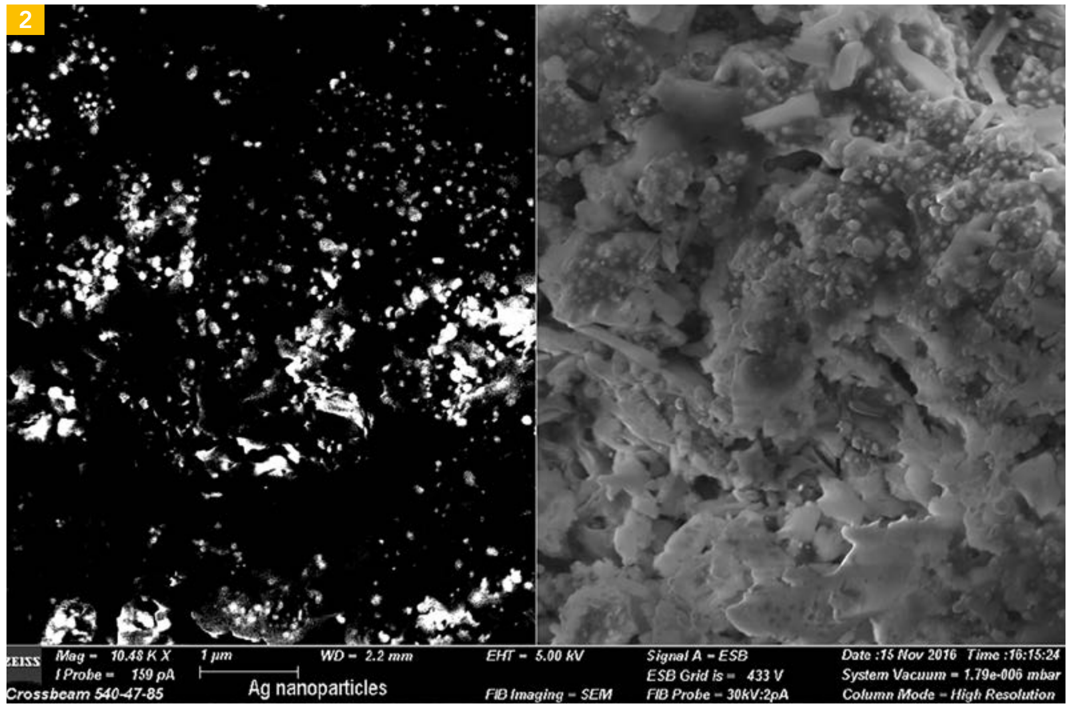

In an industry and PHI Programme-funded study that started in 2015, scientists are now working on combining encapsulation technology and EOs into an innovative approach to control post-harvest decay of citrus.

Project aim and objectives

The aim of the study was to investigate the potential of EOs as post-harvest protection agents, when applied in various encapsulated forms, to inhibit *Penicillium digitatum* (green mould) and *Galactomyces citri-auranti* (sour rot) of citrus.

The specific objectives were to:

- screen a large number of EOs to identify those with high multi-target efficacy;
- establish various *in vitro* antifungal assays and compare the results;
- encapsulate EOs with silver nanoparticles and chitosan, and test them as fumigants and in dips and coatings; and
- design and construct a mobile system for accurate headspace analysis of EOs.

Methodology

In terms of methodology, the following steps were proposed and implemented:

- Identify the most active EOs against both sour rot and green mould.
- Identify the components of the EOs

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PROJECT TITLE
Improved essential oil application through nanotechnology

PRINCIPAL INVESTIGATOR
Prof. Sandra Combrinck

CONTACT DETAILS
+27 (0)12 382 6111
combrincks@tut.ac.za

DURATION
Two years and nine months

PHI PROGRAMME & INDUSTRY CONTRIBUTIONS
R491 275 & R141 275

LEAD INSTITUTION
Tshwane University of Technology (Department of Pharmaceutical Sciences)

BENEFICIARY
The South African citrus and subtropical fruit industries

FOCUS AREA
Post-harvest disease and insect control, including phytosanitary compliance; and post-harvest physiology

HUMAN CAPITAL DEVELOPMENT
One DTech student and one MTech student

PRESENTATIONS AND PAPERS
Six

PUBLICATIONS
Pending

> This project offers a new consumer-acceptable solution that will contribute to safeguarding the existing synthetic chemicals by supporting their functionality, and the demanding markets serviced by our fruit industry.
> Prof. Sandra Combrinck

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by gas chromatography-flame ionisation detection/mass spectrometry (GC-FID/MS) analysis.

- Construct chemomteric models from the GC-FID/MS data to flush out the most active constituents of the EOs.
- Prepare EO-encapsulated nanoparticles and chitosan-EO solutions using the identified EOs and their pure compounds, and determine their *in vitro* antifungal activities.
- Determine the concentrations of the volatile components of the EOs in the headspace using direct sampling of static headspace and Tenax tubes. An appropriate gas chromatograph (GC) sampling system was developed for accurate quantification.
- Conduct headspace analysis of the volatile components using the developed sampling system.

Results

The researchers' analysis identified two oils that were highly active against both sour rot and green mould. In subsequent trials, the one was found to have a substantially lower minimum inhibitory concentration (MIC) values than the other oils tested against the two pathogens. This oil will be further tested by Sainclair Bopima, as part of his M Tech study, in *in vivo* applications; it has potential to be developed into a commercial product.

Three EOs were selected for the modification trials, namely lemongrass, spearmint, and thyme since they are easily available, moderately priced, and active against the targeted post-harvest diseases. Lemongrass alone was the most active against both pathogens.

The MICs determined from the microtiter plate serial dilution assay were higher than those from the toxic medium assay. However, the relative trend in activity remained the same. It is necessary to establish the microtiter assay to investigate synergistic or additive effects between EOs.

The results indicated that adding silver ions to EOs considerably enhanced their activity against the pathogens.

NANOTECHNOLOGY CAN EXTEND SHELF LIFE OF FRESH FRUIT AND VEG

Nanotechnology is an innovative science involving the design and application of small-sized particles measuring one hundred nanometers or less. The miniscule size and large surface area of nanoparticles help to enhance their chemical, mechanical, electrical, optical and catalytic features. Thus, nanotechnology is incorporated into a large variety of consumer and health goods, such as food, food packaging, sunblock, sports clothing, chemical fertilizers and animal feed. Nanoparticles have a tremendous ability to penetrate cells and DNA structures. As chemical reactions occur between particles that are on the surface, a given mass of nanomaterial will be much more reactive than the same mass of material made up of large particles. This means that materials that are inert in their bulk form are reactive when produced in their nanoparticle form.

Source: Food Safety News

Currently, methods of applying these EO-containing particles to pathogen-inoculated fruit are being investigated. It was found that EOs coated with chitosan were more active against the fungi than chitosan or the EO alone.

An understanding of the thermal characteristics of the EOs is important to predict how various active constituents will behave under different temperature conditions, for example in a cold room. The thermogravimetric analysis (TGA) data revealed that the EO-encapsulated silver nanoparticles had a completely different thermal profile to that of the EOs and their active constituents, indicating release of the volatiles at a higher temperature.

Determination of the evaporation rate of the EOs, terpenoids, and the EO-silver nanoparticles is ongoing. "This analysis will enable us to determine the feasibility of using encapsulated EOs for controlled-release purposes," says Sandra.

A headspace sampling system coupled to a GC was developed to determine the concentrations of active EO components used as fumigants to control decay pathogens. The system, which can be converted to a mobile format for use in cold rooms and containers, can monitor the vapour pressure/concentration of EOs/components at different temperatures and volumetric flow rates.

Once the system has been fully validated for analytical ruggedness, it will be used to determine the rate of release of EOs from the encapsulated structures. This research forms part of the doctoral study of Katlego Phala.

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> A nanometre is one billionth of a metre (0,000 000 001m). An average human hair measures 80 000 nanometers in diameter.

1 Sinclair Bopima tests the ability of encapsulated essential oils to inhibit fungal growth on citrus.
2 Dr Wilma Augustyn (left) and Prof. Sandra Combrinck are the supervisors of Katlego Phala and Sinclair Bopima, respectively. Wilma specialises in thermal desorption gas chromatography.
3 *Pennisillium digitatum*, also known as green rot or green mould, is a major source of post-harvest decay in citrus fruit.
4 Katlego Phala uses a gas chromatograph to analyse essential oils.