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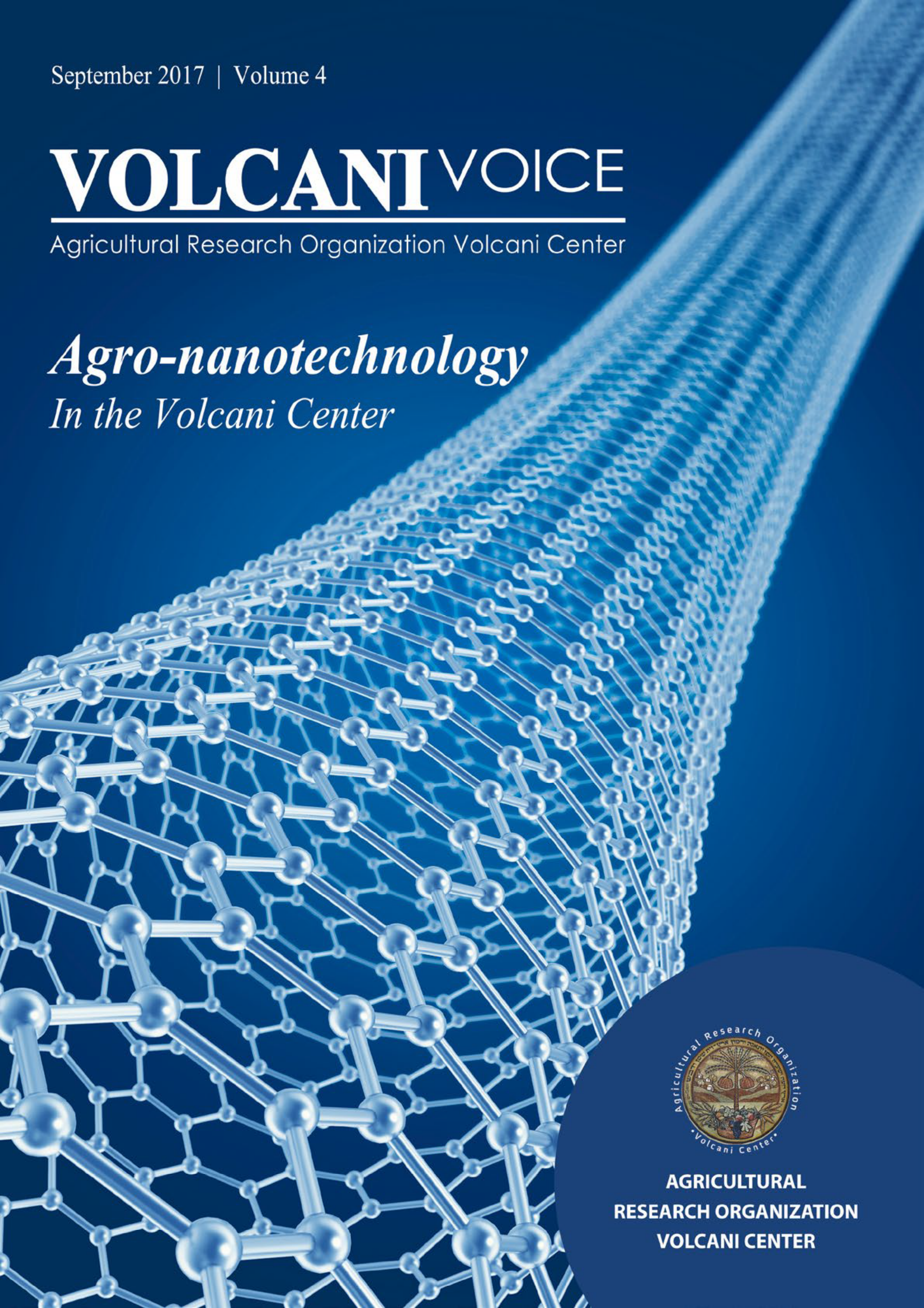

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NANOCURCUMIN for food safety and quality

By V. Rodov[1], I. Shlar[1,2], B. Horev[1], Y. Vinokur[1], M. Shemesh[1], E. Poverenov[1], S. Droby[1], A. Buslovich[1,4], A. Friedlander[1,2], R. Choudhary[3], P. Kohli[3], J.D. Haddock[3], G. Amirian[4], I. Perelshtein[4], A. Gedanken[4]

WHAT IS CURCUMIN?

Curcumin (diferuloylmethane) is a bright-yellow natural polyphenolic phytochemical compound, the major active constituent of turmeric, a spice prepared from dried ground rhizomes of Curcuma longa L. (Zingiberaceae). Turmeric has been an integral part of India's cultural and culinary heritage for thousands of years. Historical evidence says that turmeric was first cultivated in India as early as about 3000 B.C., and for the first time its medicinal potential was documented in 250 B.C. It has been highly valued by Eastern medicinal systems, including Ayurveda and traditional Chinese medicine for its healing properties for wounds, ulcers, skin diseases, inflammations, etc.

Curcumin was first isolated by Vogel in 1842. It was structurally characterized in 1910, synthesized and confirmed in 1913 (Hatcher et al., 2008). In the last 20 years, extensive evidence shows that this phenylpropanoid derivative exhibits a wide range of antimicrobial, anti-inflammatory, antitumor and antiangiogenic activities. Number of scientific publications dealing with curcumin is steadily growing, from 100-150 publications/year in the first half of 1980-ies to 19,400 in 2016 (Figure 1).

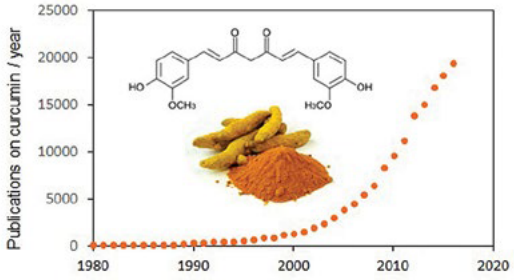

Figure 1. Number of publications on curcumin published annually from 1980 until 2016 (based on Google Scholar). In the insert: turmeric and structural formula of curcumin.

Since first described by Schraufstätter and Bernt in 1949, the antimicrobial properties of curcumin have been demonstrated against a wide range of microorganisms. Curcumin showed by far the highest antibacterial activity among the range of phenolic compounds tested (Figure 2). The combination of high antimicrobial activity with a registered status of food additive and "generally recognized as safe" (GRAS) compound and low human toxicity makes curcumin an attractive candidate for developing a new generation of "green" food preservatives. Such preservatives are expected to control food spoilage and reduce microbiological safety hazards without tainting foods with residues of toxic synthetic chemicals.

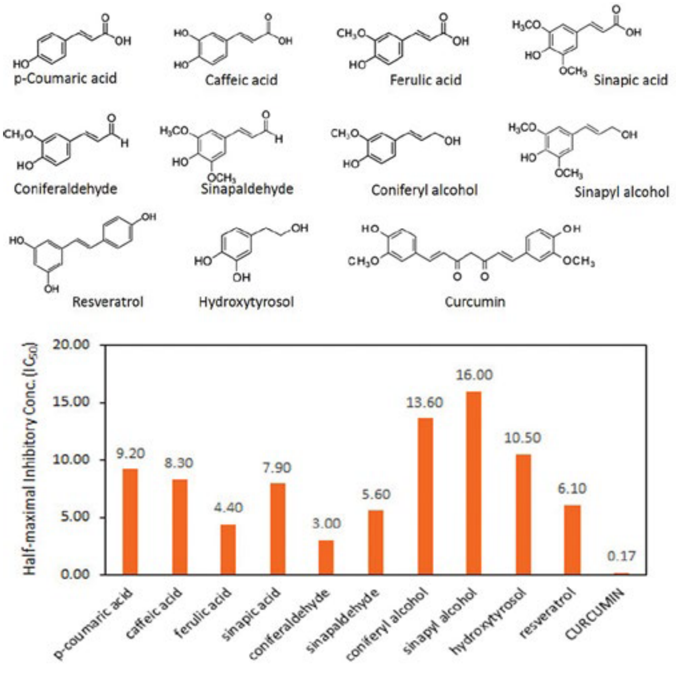

Figure 2. Values of half-maximal inhibitory concentrations (IC50) of various natural phenolic compounds towards Escherichia coli. The compounds were tested as methyl-β-cyclodextrin inclusion complexes. IC50 – a dose that reduces the microbial growth by half. Note that IC50 of curcumin was 20-100 times lower (i.e. the activity higher) than of other compounds tested. Adapted from Dogra et al., 2015, with permission.

WHY “NANO”?

In spite of the many attractive features of curcumin, its poor aqueous solubility creates an obstacle for delivering the bioactivity in food systems and pharmaceutical formulations. Development of appropriate delivery systems is a prerequisite for implementing the potential of curcumin for health and antimicrobial activity Similar to other compounds of limited aqueous solubility, curcumin delivery can benefit from nanotechnological approaches. Development of nanotechnological delivery systems for exploiting the antimicrobial potential of curcumin in food systems was the major objective of the US-Israel collaborative BARD project “Antimicrobial functionalized nanoparticles for enhancing food safety and quality” performed in 2013-2016 by the researchers of the ARO and the Southern Illinois University. The project aimed at the creation of nanocurcumin-based antimicrobial food-contact materials in order to control undesirable phenomena such as cross-contamination and microbial

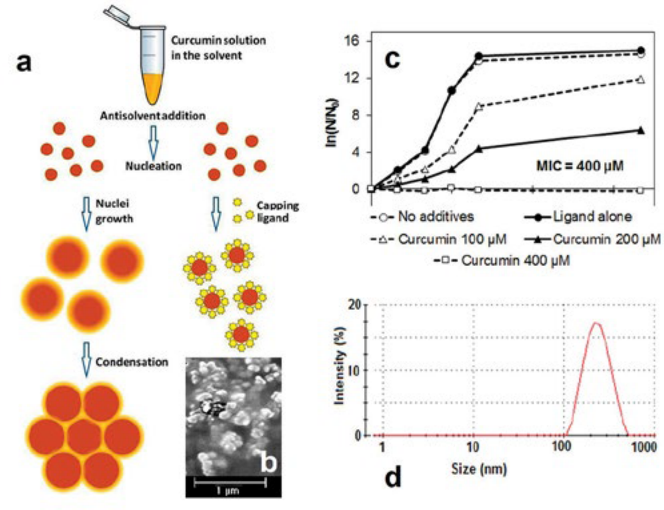

Figure 3. Scheme: production of surface-protected nanoparticles of curcumin by antisolvent precipitation (a). Environmental scanning electron microscopy image of the curcumin particles stabilized with polyquaternium 10 (b). The effect of the nanodispersed curcumin concentration on the kinetics of E. coli growth. Ligand: polyquternium 10 (c). Size distribution of the nanoparticles stabilized with polyquaternium 10 (d). Adopted from Shlar et al. (2015), with permission.

Nanoformulations of curcumin and their activity

Various nanoformulations have been developed in the world for enhancing aqueous solubility and bioavailability of curcumin. The methods of nanoparticle production can be divided into two categories: top-down and bottom-up approaches. In the top-down methods larger units are mechanically broken to get micro or nanoparticles. On the other hand, the bottom-up approach relies on self-assembly of molecules into nanoscale aggregates. Three bottom-up approaches were used in this project.

Surface-protected nanoparticles.

Antisolvent precipitation is one of the popular bottom-up methods based on self-assembly of dissolved molecules into nanoparticles when a poorly water-soluble compound dissolved in organic solvent is admixed into an aqueous medium (antisolvent).. Supersaturation occurs upon the solvent change resulting in nucleation and further particle growth. The process is controlled by mechanical interventions (e.g. ultrasound) and the use of capping ligands (e.g. Polyquaternium 10) in order to control the particle growth and to prevent their agglomeration (Figure 3).

Fig. 3c presents the inhibitory activity of surface-protected curcumin nanoparticles on the growth of E. coli. The minimal inhibitory concentration value (MIC) was found equal 400 µM (0.4 mM of curcumin)

Polydiacetylene nanovesicles

Polydiacetylene (PDA) vesicles are a novel carrier system for hydrophobic materials poorly soluble in water, such as curcumin. Incorporation of a polymerizable diacetylene into the lipid bilayer greatly improves the stability of the vesicles compared with the conventional liposomes. Furthermore, the PDA vesicles can be covalently bound to the surface of solid substrates such as glass.

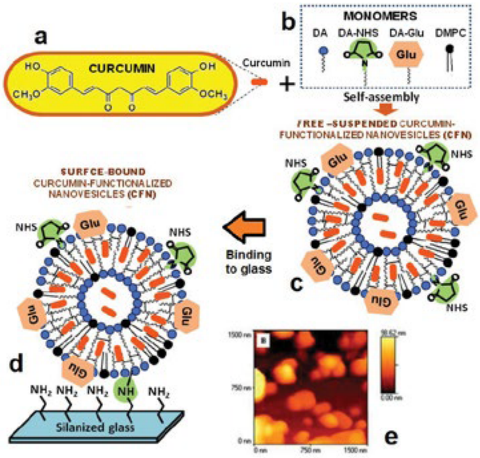

Figure 4. Scheme illustrating self-assembly of curcumin-functionalized nanovesicles and their covalent binding to silanized glass: a- curcumin; b- monomers for self-assembly of the nanovesicle: DA – diacetylene, DA-NHS - N-hydroxysuccinimide (NHS)-tagged DA monomers, DA-Glu – glucose-tagged DA, DMPC - 1,2-dimyristoyl-sn-glycero-3-phosphocholine (phospholipid); c – self-assembly of the nanovesicles; d – binding the nanovesicle to silanized glass; e – atomic force microscope (AFM) image of the nanovesicles. Adopted from Dogra et al., 2015, with permission.

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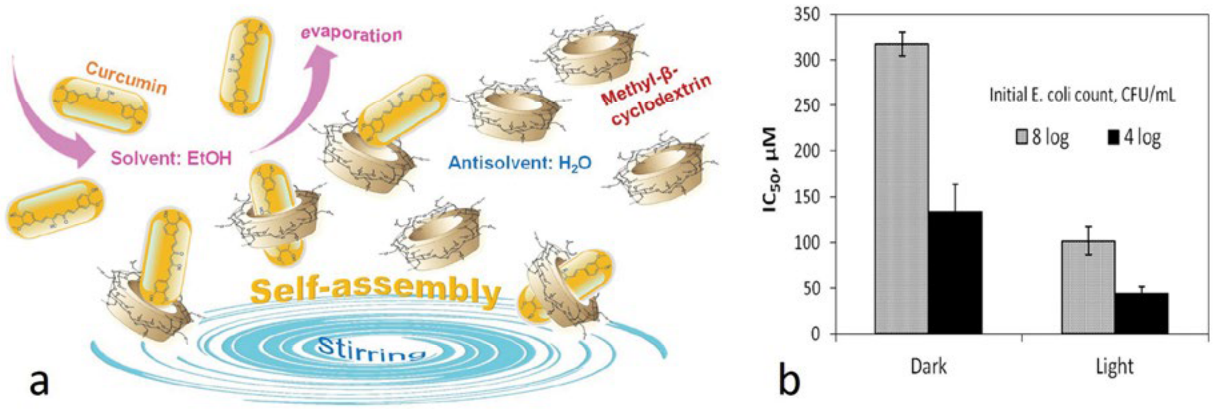

Figure 5. Scheme of self-assembly of curcumin-MBCD inclusion complex (a). Effect of illumination and inoculum size on the activity of MBCD-inclusion complex of curcumin against *E. coli*. Gray bars: the inoculum of ca. 10^8 CFU mL^{-1}; black bars: the inoculum of ca. 104 CFU mL^{-1}. Illumination conditions: blue light (wavelength 430-500 nm), dose 9 J/cm^2. Adopted from Shlar et al., 2017, with permission.

Supramolecular methyl-β-cyclodextrin inclusion complex

Cyclodextrins are cyclic oligosaccharides produced from starch. They have a hydrophilic outer surface and a hydrophobic inner cavity and can therefore form water-soluble inclusion complexes with hydrophobic compounds like curcumin and improve their solubility. Specifically, the methyl-β-cyclodextrin (MBCD) was particularly effective in curcumin solubilization and delivery of its activity to bacterial cells.

The antimicrobial efficacy of curcumin inclusion complexes was a function of the bacterial population size. The diluted suspensions of E. coli (105 CFU mL-1) were more sensitive to curcumin than the dense ones (108 CFU mL-1) with half-maximal inhibitory concentrations (IC50) of 130 and 320 µM, respectively (Fig. 5). Exposure to blue light markedly enhanced the antimicrobial effect of curcumin/MBCD causing approximately threefold reduction of the IC50 values. MIC values of curcumin at the inoculum level of 105 CFU mL-1 went from 500 µM in the dark to 90 µM after illumination.

ANTIMICROBIAL FOOD-CONTACT SURFACES

The decontamination capacity of glass beads covalently coated with curcumin-functionalized nanovesicles was tested with tender coconut water inoculated with E. coli and Listeria monocytogenes. Ten minutes of cold pasteurization by circulating the inoculated product through column filled with CFN-coated glass beads were sufficient for reducing the bacterial counts by 4-5 log CFU/mL and complete elimination of L. monocytogenes (Figure 6).

Sonochemical methods were used for one-step grafting of surface-stabilized curcumin nanoparticles onto flexible and rigid plastic polymers (polyethylene and polyamide) at the laboratory of Prof. A. Gedanken at Bar-Ilan University. One of the advantages of this approach is its simplicity and easy upscaling to commercial level. The attachment of both Gram-negative (Pseudomonas aeruginosa) and Gram-positive (B. subtilis) bacteria to ultrasonically curcumin-impregnated plastics was inhibited by 60-90% compared with regular plastics (Figure 7). The 1-2 log CFU inhibition of biofilm development was observed.

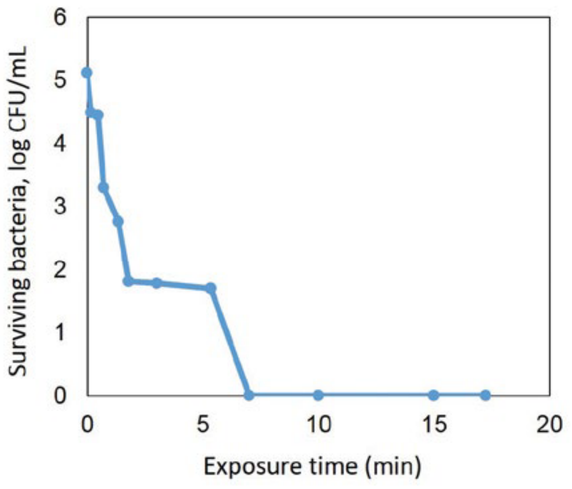

Figure 6. Elimination of L. monocytogenes from tender coconut water by circulating through the column filled with glass beads coated with curcumin-functionalized nanovesicles.

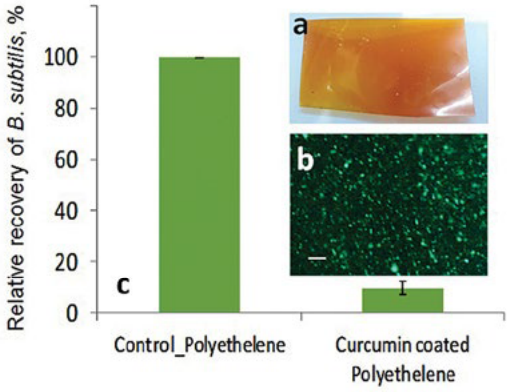

Figure 7. Plastic films sonochemically impregnated with surface-stabilized curcumin particles: a – external appearance; b – fluorescent microscope -

ation of B. subtilis attachment to regular polyethylene vs. curcumin-coated polyethylene.

Contact of fresh-cut melon with polyamide (nylon 6.6) rigid chips inoculated with E. coli or Bac. licheniformis resulted in transfer of bacterial cells onto cut fruit surface (cross-contamination). However, no viable bacteria were detected on the cut fruit if nylon surface was ultrasonically coated with curcumin and after inoculation exposed to blue light photoactivation (Figure 8).

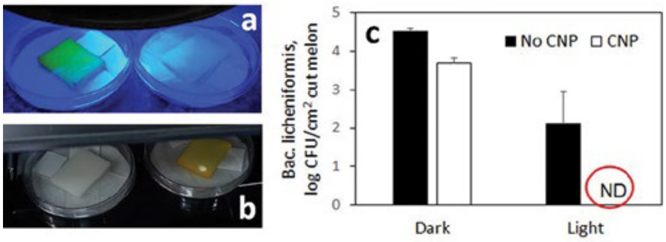

Figure 8. Nylon chips inoculated with B. licheniformis are incubated under blue light (a) or in the dark (b). Yellow chip – coated with curcumin, white chip – control. C- Recovery of B. licheniformis from fresh-cut melon surfaces after contact with contaminated plastic surface. ND – not detected. Note that the only condition that prevented the transfer of bacteria from the plastic surface to cut melon (cross-contamination) was the combination of curcumin coating with blue light illumination.

CONCLUSION

The research has shown that nanotechnological approaches can help in realization of antimicrobial potential of curcumin for improving food safety and preserving quality. Light can enhance the antimicrobial potency of curcumin and contribute to the success of the treatments. ❑

LITERATURE

Dogra, N., Choudhary, R., Kohli, P., Haddock, J.D., Makwana, S., Horev, B., Vinokur, Y., Droby, S. and Rodov, V., 2015. Polydiacetylene nanovesicles as carriers of natural phenylpropanoids for creating antimicrobial food-contact surfaces. Journal of agricultural and food chemistry, 63(9), pp.2557-2565.

Hatcher, H., Planalp, R., Cho, J., Torti, F.M. and Torti, S.V., 2008. Curcumin: from ancient medicine to current clinical trials. Cellular and Molecular Life Sciences, 65(11), pp.1631-1652.

Shlar, I., Poverenov, E., Vinokur, Y., Horev, B., Droby, S. and Rodov, V., 2015. High-throughput screening of nanoparticle-stabilizing ligands: application to preparing antimicrobial curcumin nanoparticles by antisolvent precipitation. Nano-Micro Letters, 7(1), pp.68-79.

Shlar, I., Droby, S. and Rodov, V., 2017. Modes of antibacterial action of curcumin under dark and light conditions: A toxicoproteomics approach. Journal of Proteomics.160(1), pp. 8-20

Dr Victor Rodov

senior Researcher at the Institute of Postharvest and Food Sciences, ARO. Ph.D. in Plant Physiology from the USSR Academy of Sciences, Moscow. Among the research interests: (a) postharvest physiology, biochemistry and technology; (b) functional food products; (c) food packaging; (d) plant secondary metabolites; (e) nanotechnology for maintaining food quality and safety. Teaches at Robert H. Smith Faculty of Agriculture, Food and Environment of the Hebrew University of Jerusalem. Editorial board member of "Postharvest Biology and Technology". Published about 100 research papers including 13 book chapters and invited reviews, and 5 patents.