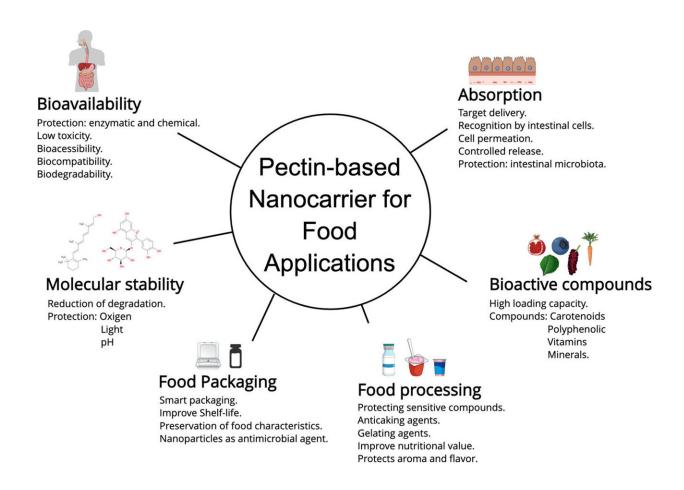


Researchers develop technology to protect food's bioactive compounds during digestion

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Bioactive compounds present mostly in fruit and vegetables perform different bodily functions relating to health and well-being. Their effects



are considered antioxidant, antidiabetic, antiaging and anticancer, among others.

Many studies are looking for ways to optimize absorption of bioactive compounds by the organism and increase their bioavailability—the proportion that enters the bloodstream after absorption. One way is to coat the compounds with another material and package them on the nanometric scale (a nanometer is a billionth of a meter). Nanoencapsulation, as this technique is known, assures slow release of the compounds so that they take longer to digest and can survive the attacks of bacteria in the gut microbiome.

An investigation conducted by a duo of researchers at the University of São Paulo's School of Pharmaceutical Sciences (FCF-USP) in Brazil is one of these studies. Working at the school's Department of Food Science and Experimental Nutrition, they have produced several articles on the subject—the latest of which, published in the *International Journal of Biological Macromolecules*, is a review of the literature on pectin-based nanoencapsulation plus a description of a novel technology developed under the aegis of the Food Research Center (FoRC), a Research, Innovation and Dissemination Center (RIDC).

"We used pectin extracted from residues of citrus fruit albedo and peel, with a degree of purity permitting human ingestion and excluding any kind of hazardous chemical," said João Paulo Fabi, one of the authors and a professor at FCF-USP. Albedo is the layer of white spongy material inside the peels of oranges and lemons, for example.

"In addition to our review of the literature, we describe a novel technology for nanoencapsulation of bioactive compounds using pectin. This entails producing a pectin-lysozyme complex as a protective outer layer for a highly sensitive bioactive compound called anthocyanin," he explained, adding that lysozyme is "a safely edible substance obtained



from egg white and used to enhance the stability of the end-product."

Anthocyanins are water-soluble pigments belonging to the flavonoid family. They are <u>phenolic compounds</u> found in all plants and responsible for the shades of red, blue and purple seen in flowers, fruit, leaves, stalks and roots.

The authors say their methodology can be used to encapsulate other water-soluble bioactive compounds. "We tested anthocyanin because of its challenging sensitivity to many factors, such as light, temperature, pH and gut bacteria," said Thiécla Katiane Osvaldt Rosales, the other author. She is currently a postdoctoral researcher at the Nuclear and Energy Research Institute (IPEN).

Advantages of methodology

According to the researchers, the main advantage of their methodology is that no other compounds are added apart from pectin, lysozyme and anthocyanin. "We used three compounds from sources in nature and mixed them in the laboratory to form a new product, without adding salts, ligands or anything potentially toxic. Furthermore, the nanoparticles are not too small. Very tiny nanoparticles can penetrate barriers and cell membranes, entering the DNA and having toxic effects. The size we obtained is safe," Fabi said.

Rosales outlined the process they developed to produce the nanoparticles. "Pectin and lysozyme are heated separately. The increase in temperature partly alters their structure, and they interact better when heated. They are then rapidly cooled to reach a temperature not harmful to anthocyanin, which is sensitive and fairly unstable. The three substances are blended in an aqueous suspension and agitated for an hour. The result is encapsulated anthocyanin. The suspension is then filtered to separate the non-encapsulated contents," she said.



Special care is taken with factors such as temperature and pH. "We tested the parameters for the purpose of optimization, especially pH. If pH is too high, the anthocyanin breaks down. It can't be too low, either. We found a pH of 5 to be optimal for interaction between the molecules," she explained. "We also tested the duration and intensity of the agitation. We made a point of managing all the details, however minor, because they make a difference in terms of forming stable particles. We've applied for a patent on the methodology."

Results

Finally, the encapsulation was tested for efficacy in a digestion system simulated in the laboratory to mimic the gastric and intestinal phases. "The result was that part of the anthocyanin was released during the digestive process, at the end of gastric digestion, and part remained in the nanostructure, with the possibility of release of this remainder in the gut or absorption together with the nanostructure. We believe this was a good outcome. Partial and gradual release suggests absorption of the compound starts before it enters the gut, with the nanoencapsulated remainder probably being released in the gut or fully absorbed with less structural alteration," Rosales said.

The next step will be animal testing. "We tested the method in vitro and obtained results indicating that the nanoparticles are safe for consumption. We have evidence that cells can absorb them in a non-toxic manner and that the pectin protects the anthocyanin and its properties. We now have to test it in animals, observing the process of oral ingestion, absorption of the anthocyanin using specific markers for absorption, and the route followed in the organism. It's important to verify the extent of absorption and the biological destination," she said.

The nanoparticles are mainly intended for use as a food supplement.

"They can be added to food and dietary supplements, but industrial mass



production would be necessary to include them in a supplement," Fabi said.

It is worth noting that the method does not require expensive equipment or procedures. "In addition, the material used for the nanocapsules, which comes from byproducts of citrus peel, would make the cost even lower for manufacturers. The pectin we used in our study is available commercially and is used by the food industry, mostly for gel formation in jam or as a thickener," Rosales said.

More information: Thiécla Katiane Osvaldt Rosales et al, Pectinbased nanoencapsulation strategy to improve the bioavailability of bioactive compounds, *International Journal of Biological Macromolecules* (2022). DOI: 10.1016/j.ijbiomac.2022.12.292

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