Nature Inspired Solutions for Improving Quality and Safety of Food

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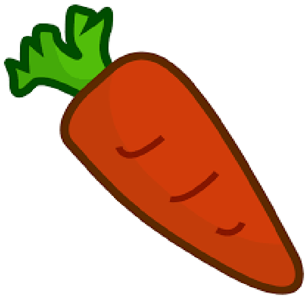
Key Challenges – Food and Nutraceutical Industry

- Limited shelf life and stability
- Limited bioavailability
- Consumer preference for all-natural ingredients
- Unpalatable tastes/odors to consumers and limited integration with mainstream food products

Limited Stability in Current Encapsulation Systems

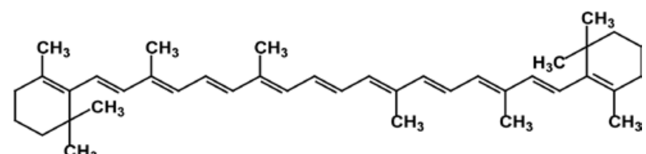
Oxidative Stability of Beta-carotene



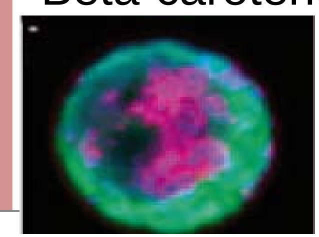
Carrot

Tissue with cells

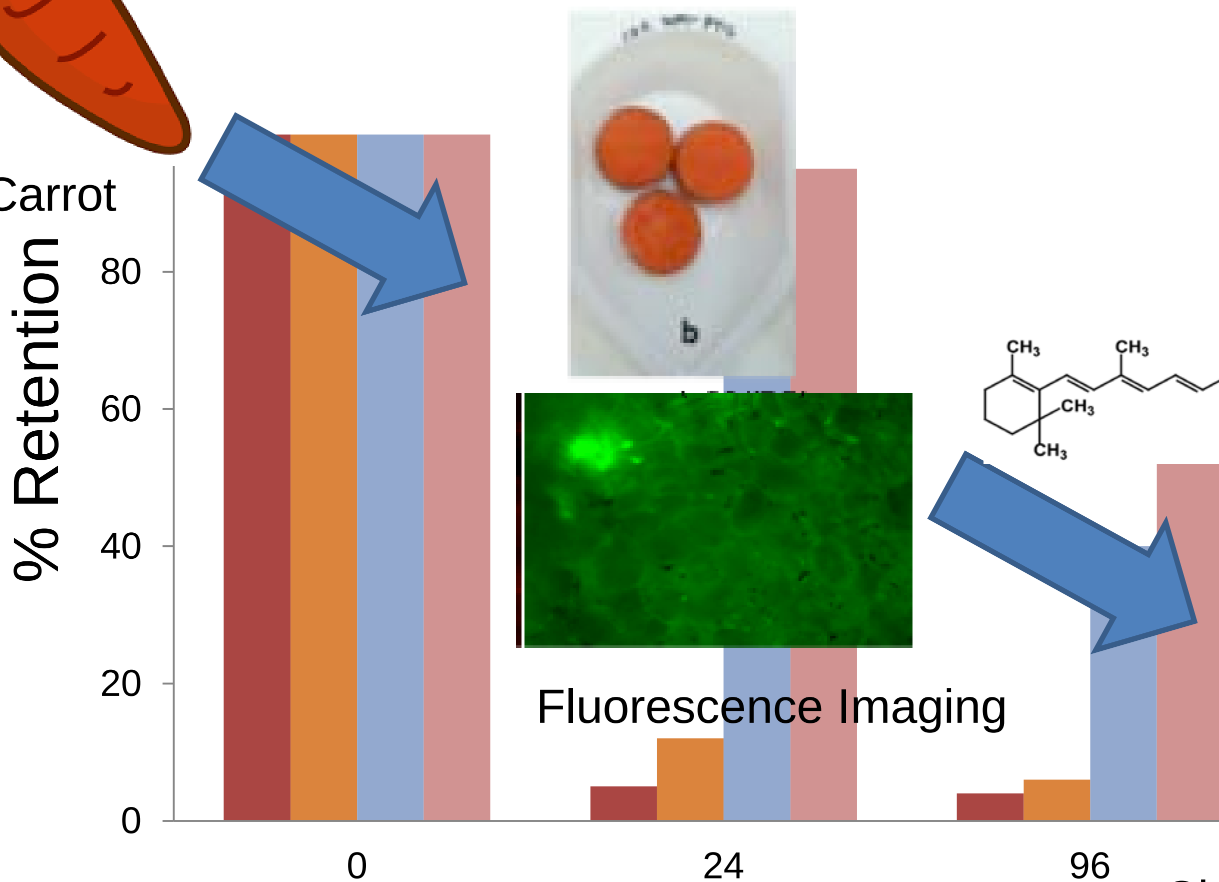
Fluorescence Imaging



Beta-carotene



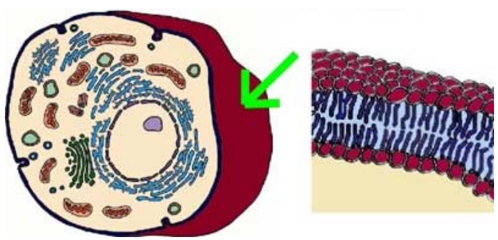
Chemical Imaging



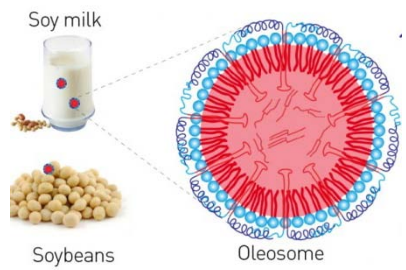
Optical Spectroscopy and Computational Methods in Biology and Medicine, Challenges and Advances in Computational Chemistry and Physics

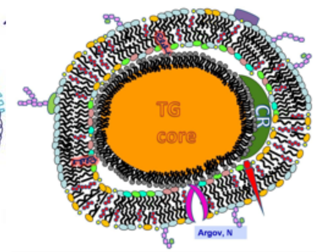
Encapsulation Systems and Nature's Inspiration

Mammalian Cells with Membrane

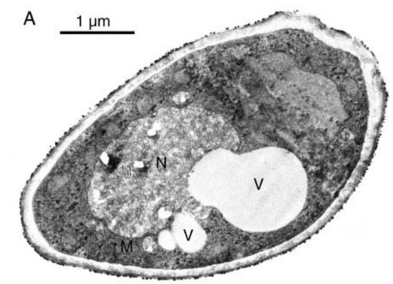


Oleosomes and milk fat globules

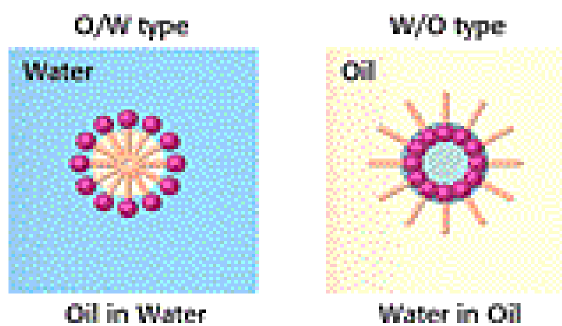




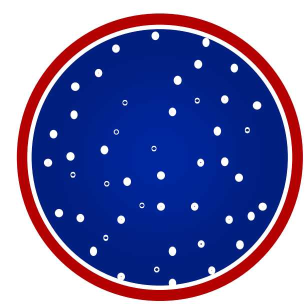
Cell



Liposomes



Emulsions



Microparticles with shell

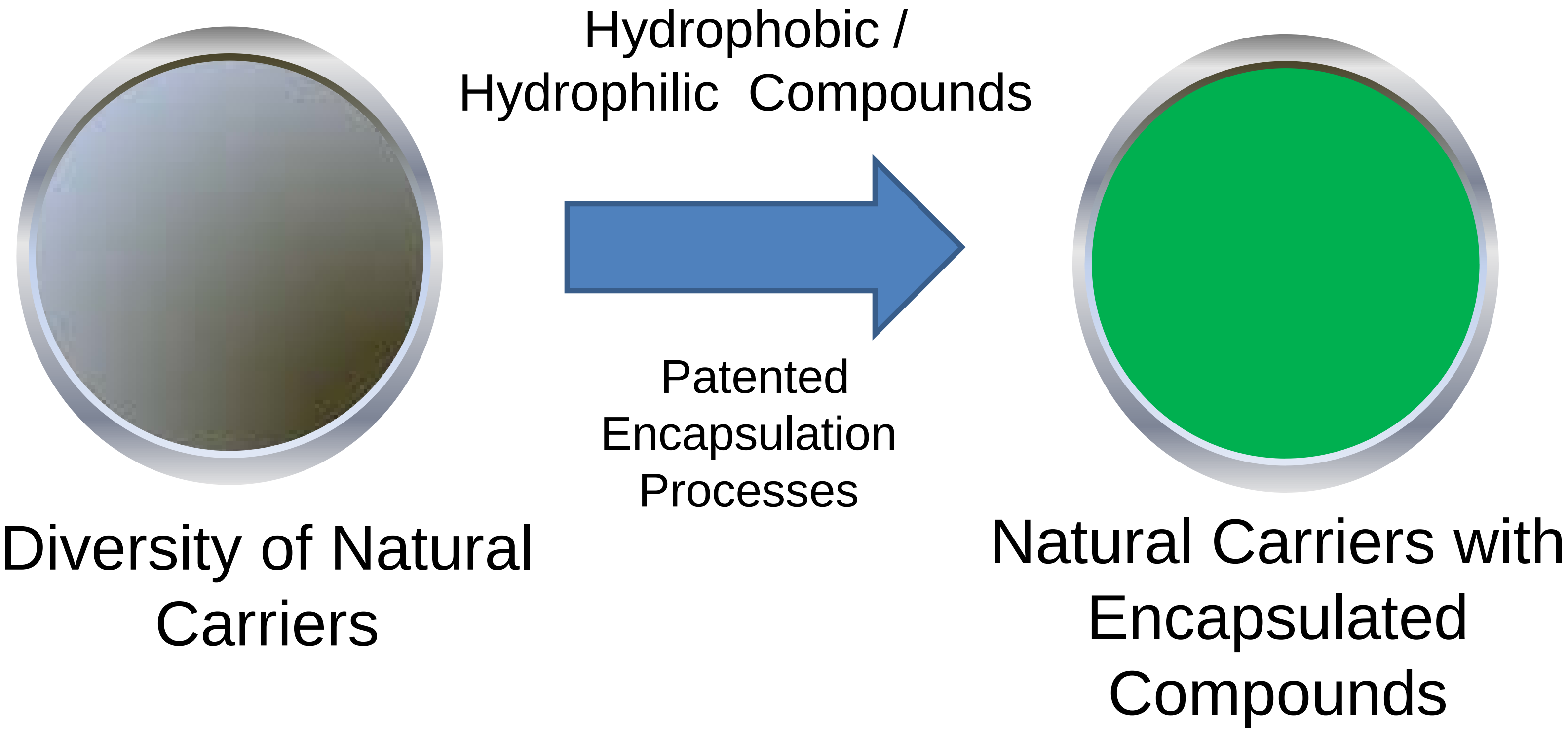
Nature Inspired Encapsulation Approach

Inspired by nature, we developed novel approaches to enable bio-based encapsulation solutions.

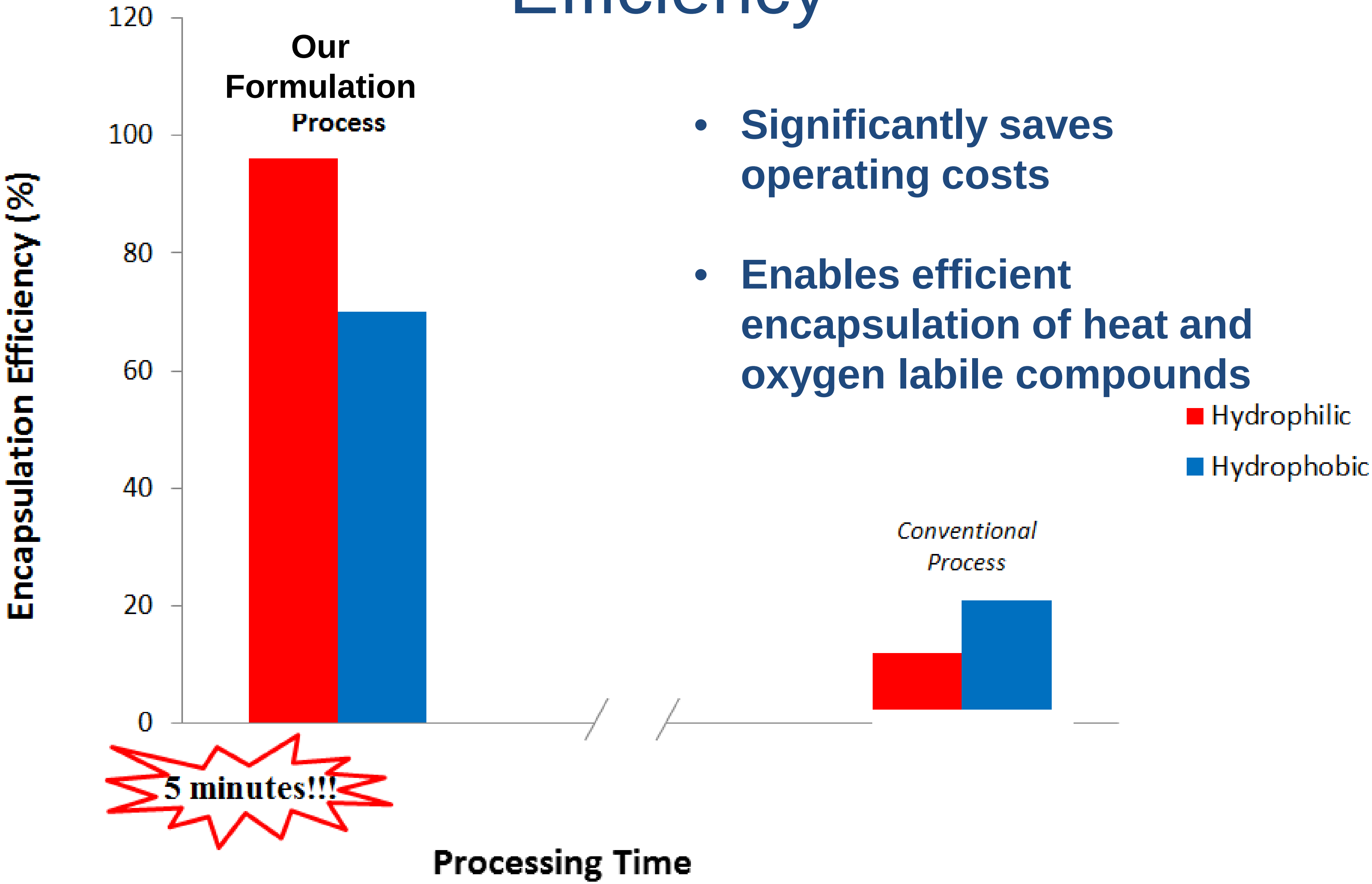
The unique features of our solution include:

- Diversity of bio-based carriers
- Multifold improvement (3-10 fold) in process stability and shelf life of formulations
- Highly cost effective and scalable technology
- Improves delivery and performance of bioactives (potential)

Our Process Innovation



Multifold Enhanced Encapsulation Efficiency



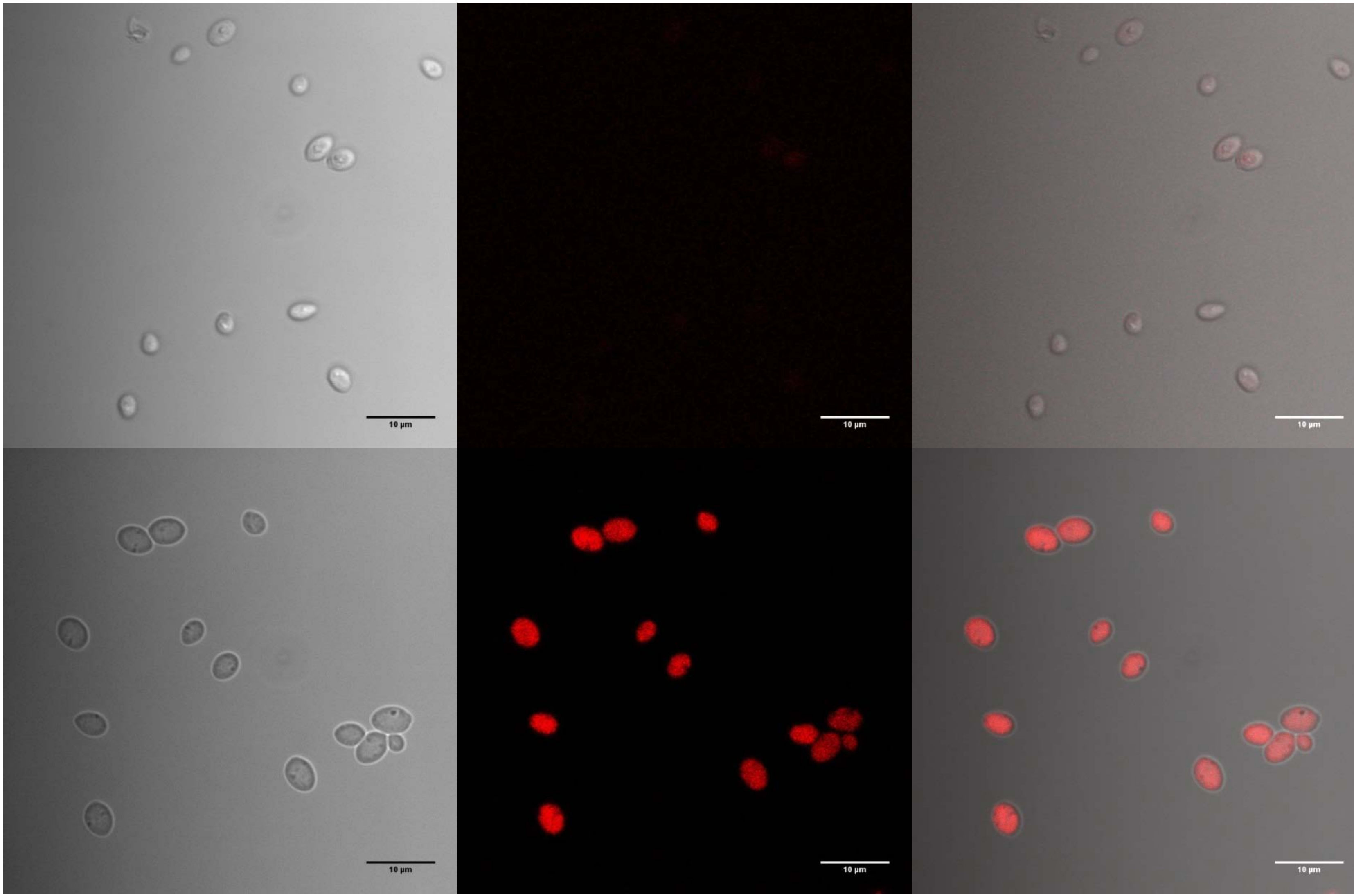
- **Significantly saves operating costs**
- **Enables efficient encapsulation of heat and oxygen labile compounds**

Curcumin Encapsulation in Yeast

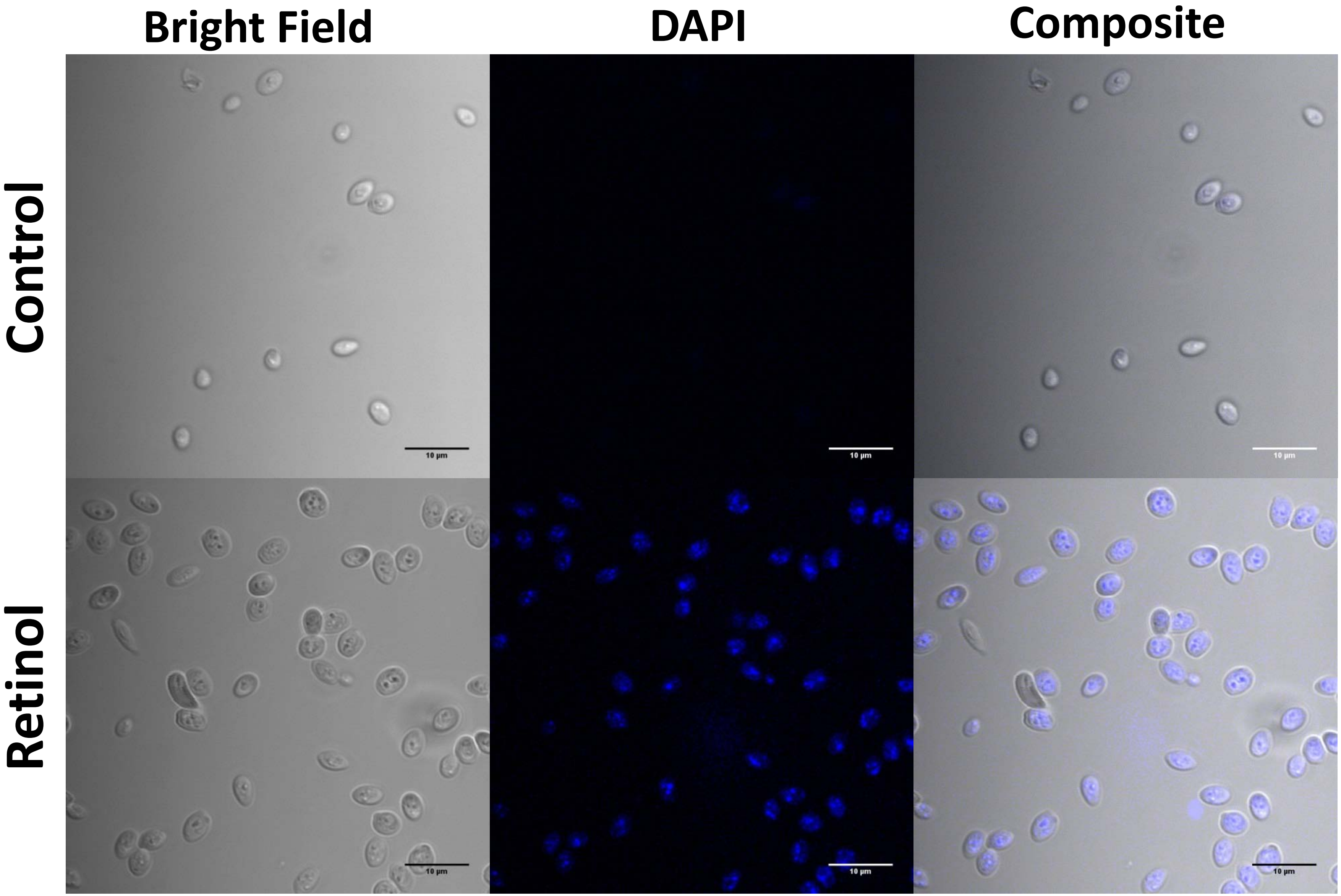
Bright Field | Long Pass | Composite

Control

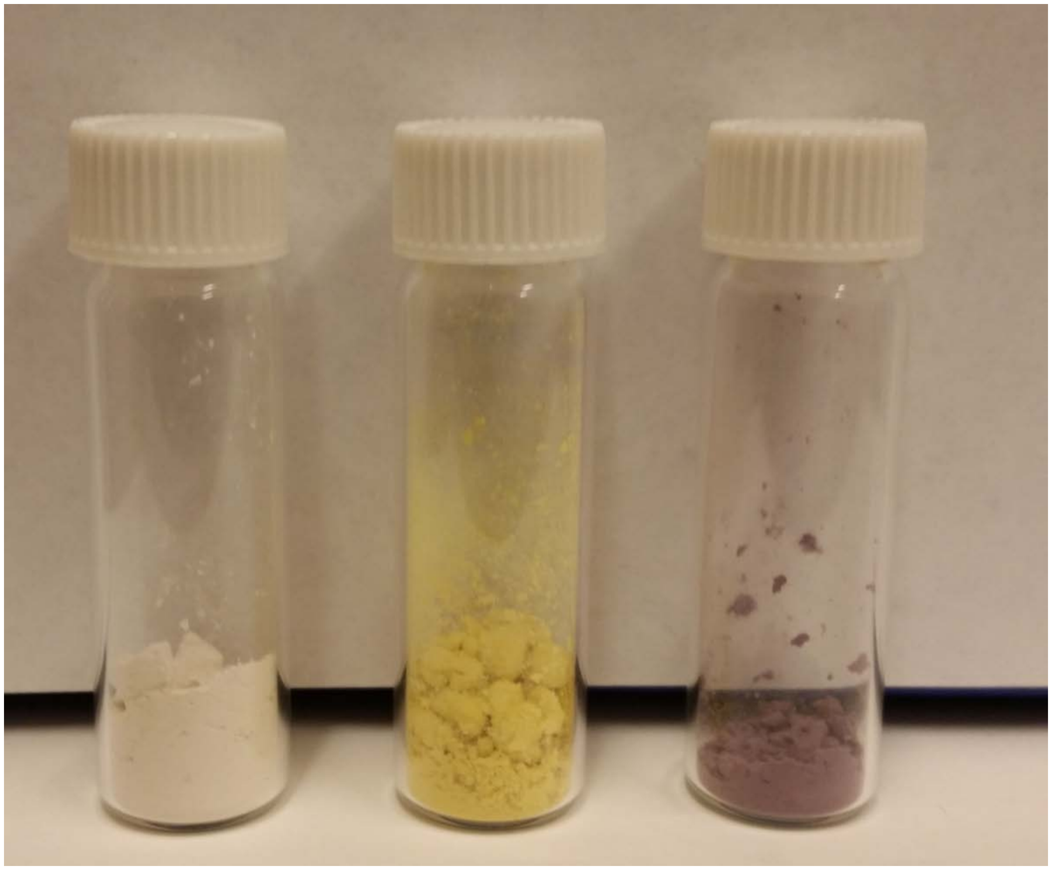
Curcumin



Retinol Encapsulation in Yeast



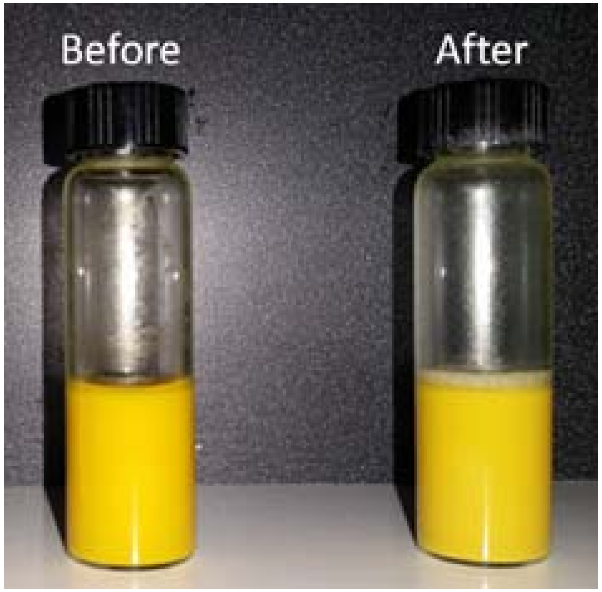
Encapsulate Purified and Crude Extracts



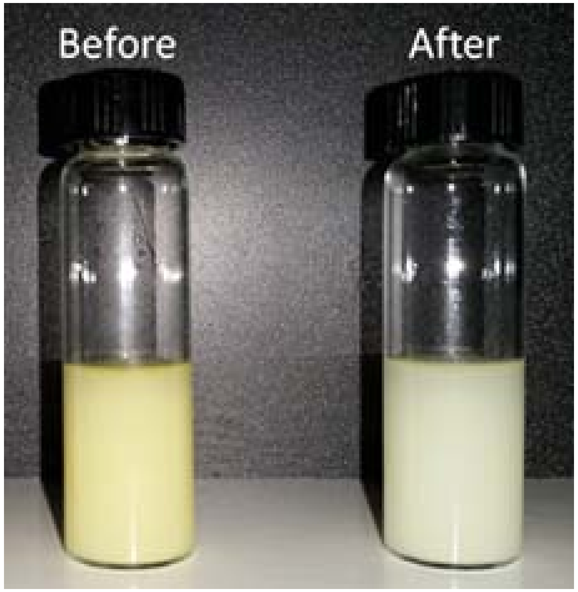
Materials can be in suspension and powders

Yeast **Curcumin** **Grapeskin Extract** **Grapeskin Extract Suspension**

Improved Thermal Processing



Curcumin in Yeast

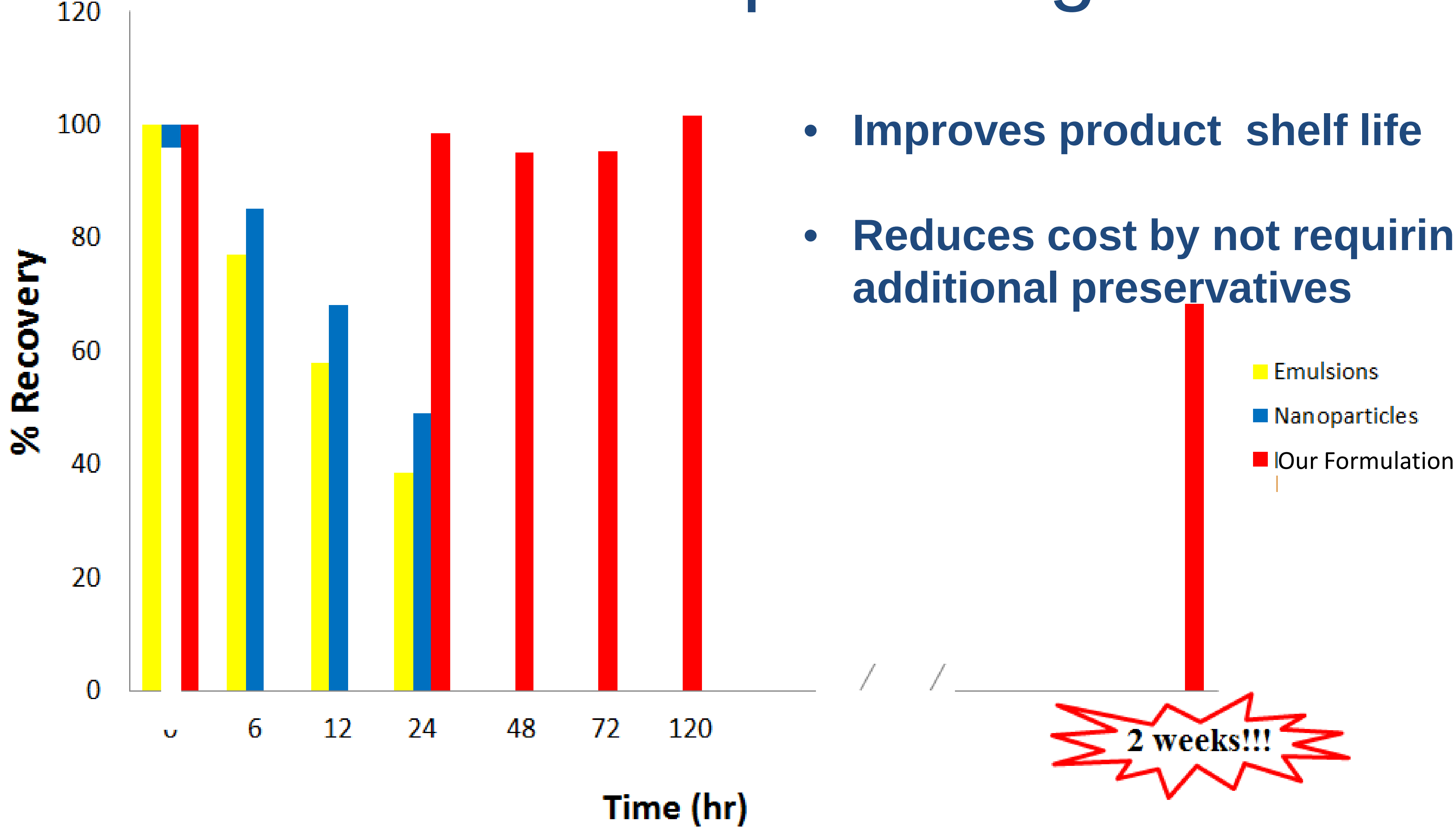


Emulsion Formulation

Enables incorporation of heat stable natural compounds in food and beverage products

Enhanced Shelf Life of Highly Oxidative Susceptible Ingredients

- **Improves product shelf life**
- **Reduces cost by not requiring additional preservatives**



Baking with Curcumin Encapsulated in Yeast

	L	a	b
Control dough	79.11	1.13	11.05
4% Tween20 emulsion dough	72.51	0.15	15.41
5% starch emulsion dough	73.21	-0.10	14.31
0.1 g yeast dough	76.93	-0.22	25.07
Control bread	63.78	1.46	12.62
4% Tween20 emulsion bread	54.76	0.55	16.42
5% starch emulsion bread	54.03	0.30	16.27
0.1 g yeast bread	61.02	-0.04	27.32

Translation of Technology

Milestones

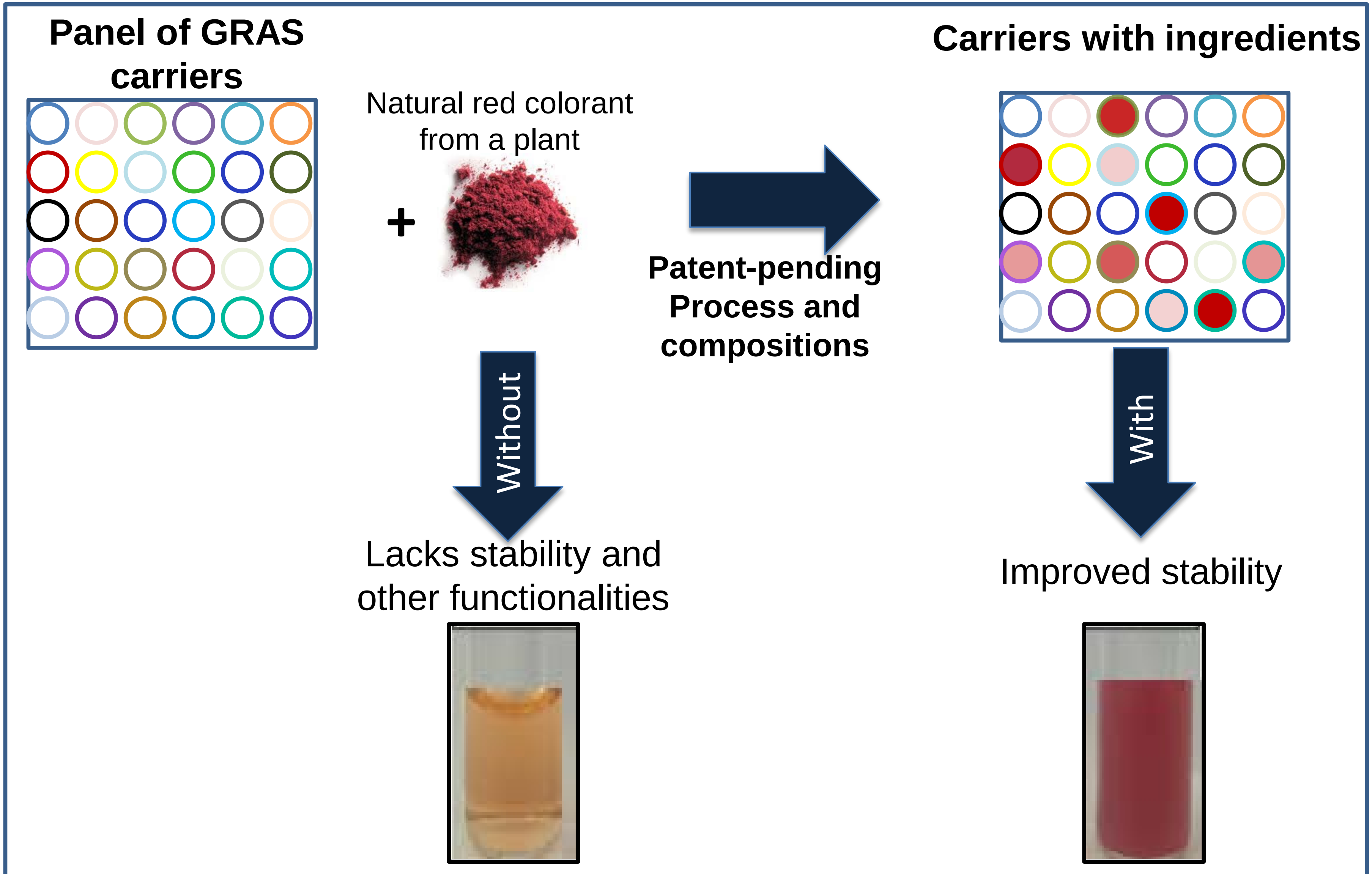
2013 — UC DAVIS FOOD SCIENCE AND TECHNOLOGY

2015 — **Founded InnovaNutra**

2016 — **NSF-SBIR Phase I**
$150,000
R&D Proof-of-concept- natural colors & phytochemicals
2 patents pending

2017 — **NSF Phase II awarded**
$750,000
Process scale-up, industry validation of our formulations

Technology


Panel of GRAS carriers
Natural red colorant from a plant
+
Carriers with ingredients
Patent-pending Process and compositions
Without
With
Lacks stability and other functionalities
Improved stability

Color Stability In a Gum Product

	No color added	Natural red color	InnovaNutra formulation
t = 0			
t = 2 weeks			
t = 4 weeks			

Value Proposition

Shelf-life enhancement without preservatives

> 50%
Shelf-life of finished product

InnovaNutra' s integrated solutions
$460 bn market
(plant-based foods and nutraceuticals)

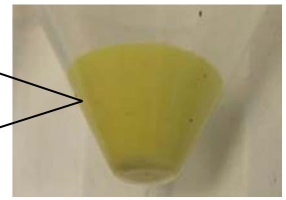
Bitter taste Masking

> 90%
Preservation from gastric degradation

Increase consumer acceptance

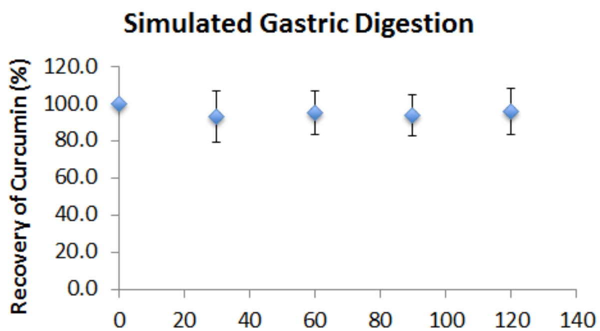
Promote health

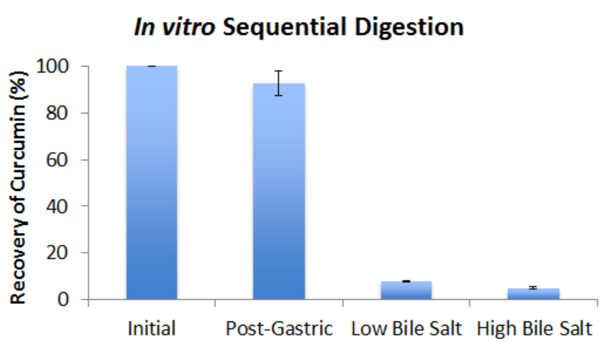
Nutritional Supplement Product



Stabilized **Quercetin**, **Resveratrol** formulations

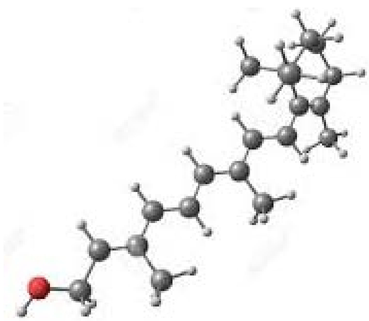
Energy drinks



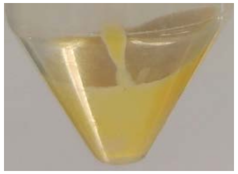


- Non-GMO, no chemical modification
- No preservatives
- Enhanced loading **1 gm = 568 apples**
- Improved pH and oxidative stability (**3-5 fold**)
- Gastric protection and controlled release in intestine
- Taste-masking

Advanced Personal Care Products



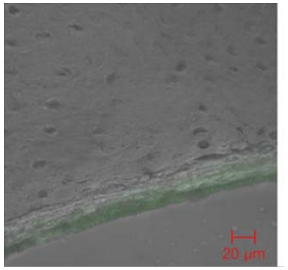
Retinol



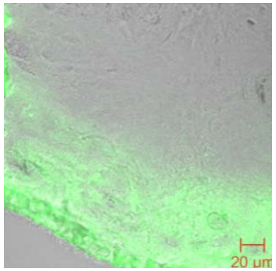
InnovaNutra Retinol Formulation

Skin Creams

Control



After 9 hour



Binding and release on skin

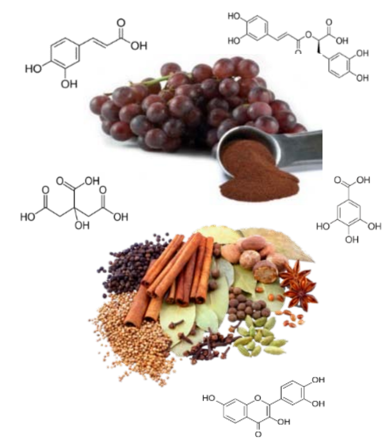
- Improved oxidative stability (**5-fold**)
- Binding to skin and extended release

Natural Antimicrobials

Enhanced Microbial Inactivation by the Combination of
Natural Antimicrobials and **Low Levels of Physical Processes**

Rationale and Motivation

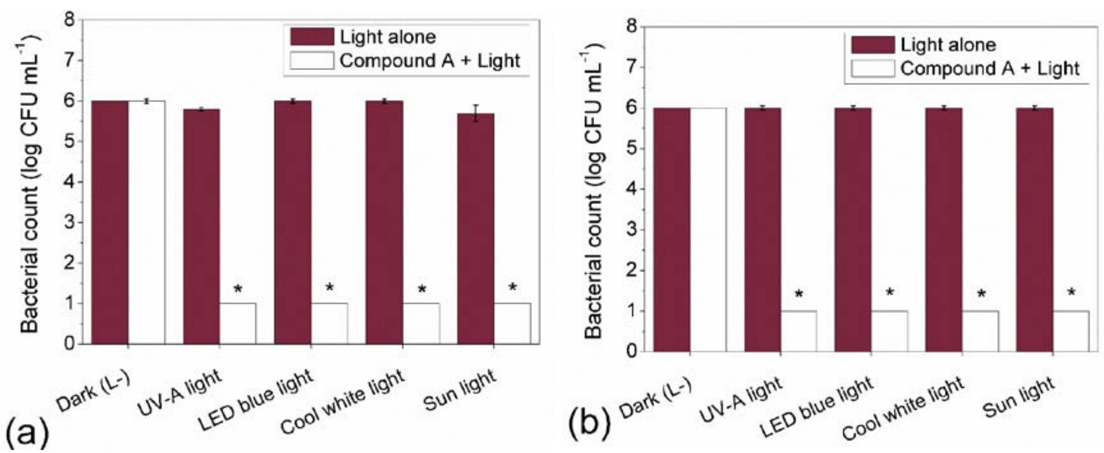
- Increase microbial inactivation while lowering the processing impact on the final quality aspects of the food product
- Reduce sanitizer concentration
- Replace abrasive chemical agents for natural antimicrobial
- Clean-labeling
- Lower energy input



Enhanced Microbial Inactivation by the Combination of Sub-lethal levels of **Antimicrobial** and Low-Intensity **Lights**

Bacteria Inactivation by the Combination of **Light** and **Low Concentrations of Compound A**

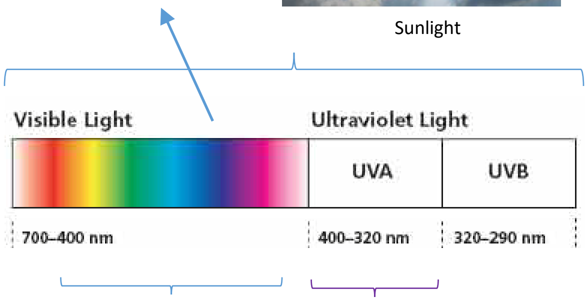
Compound A can be activated by different light sources



Inactivation of (a) *E. coli* O157:H7 and (b) *Listeria innocua*

LED blue light (404 nm)

Sunlight

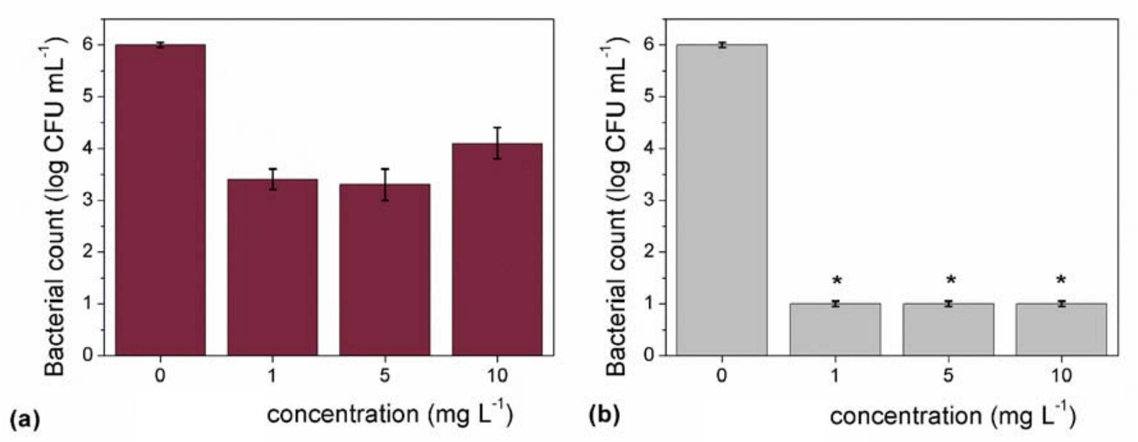


Cool white light

UV-A light

Bacteria Inactivation by the Combination of **UV-A Light** and **Low Concentrations of Compound A**

Low concentrations of Compound A are required

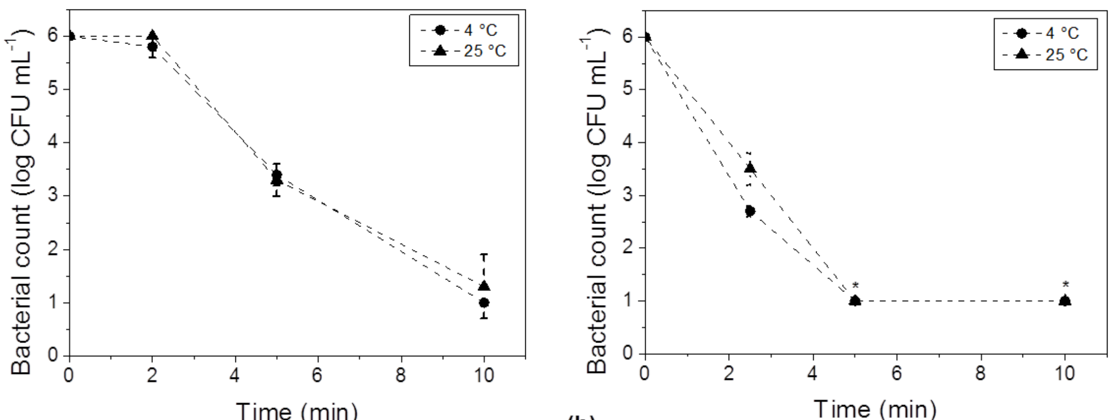


Inactivation of (a) *E. coli* O157:H7 and (b) *Listeria innocua*

More than 5 log CFU/mL reduction in *Listeria innocua* count after sanitation with 1 ppm Compound A + Light

Bacteria Inactivation by the Combination of **UV-A Light** and **Low Concentrations of Compound A**

Sanitation effective at refrigerated temperature



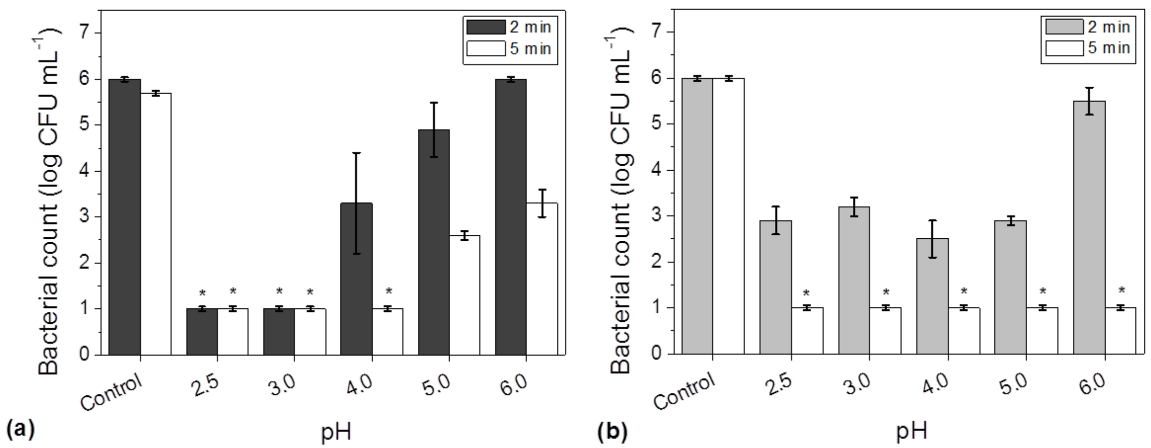
(a) (b)

Inactivation of (a) *E. coli* O157:H7 and (b) *Listeria innocua*

Antimicrobial activity is not reduced at refrigerated temperatures (4 ºC)

Bacteria Inactivation by the Combination of **UV-A Light** and **Low Concentrations of Compound A**

Effective at a broad range of pH



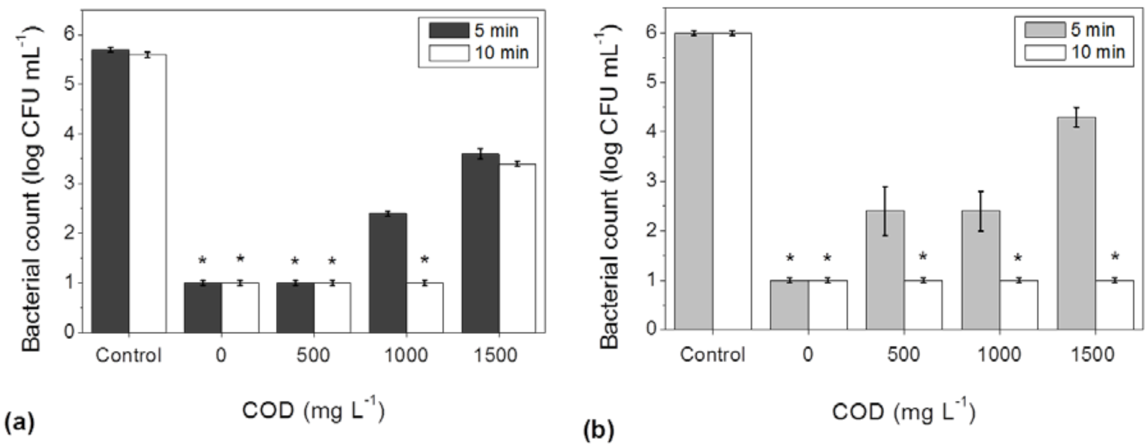
Inactivation of (a) *E. coli* O157:H7 and (b) *Listeria innocua*

Antimicrobial activity significantly enhanced at pH 3

At pH 3 the sanitation time to achieve 5 log *E. coli* O157:H7 inactivation was reduced from 10 min to 2 min

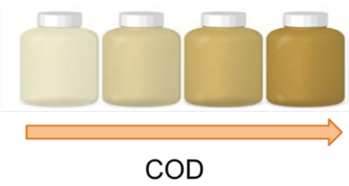
Bacteria Inactivation by the Combination of **UV-A Light** and **Low Concentrations of Compound A**

Effective even at high COD content



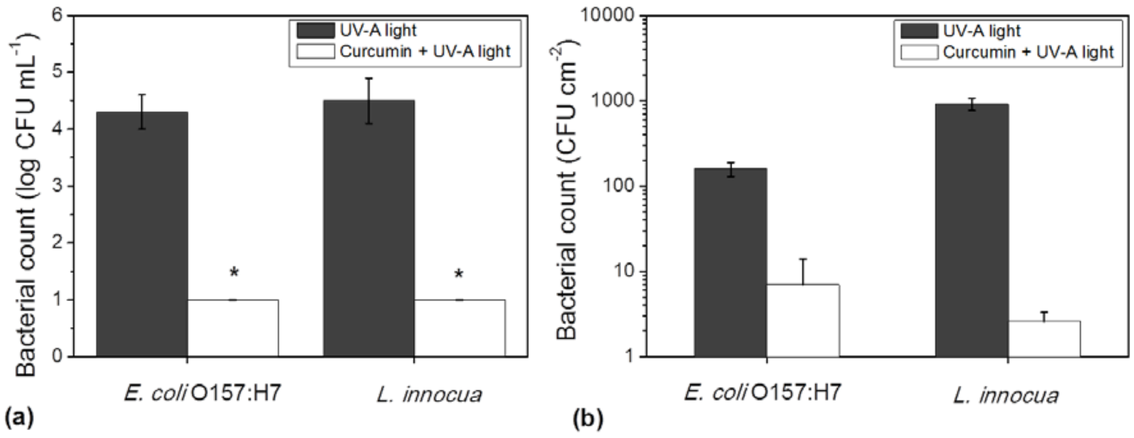
Inactivation of (a) *E. coli* O157:H7 and (b) *Listeria innocua*

More than 5 log CFU/mL bacterial inactivation even at 1000 ppm of COD



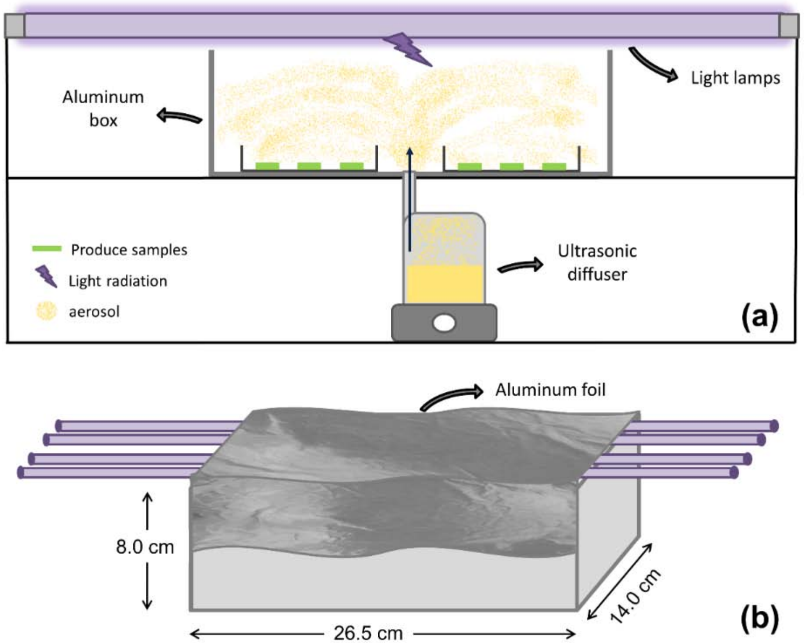
Bacteria Inactivation by the Combination of **UV-A Light** and **Low Concentrations of Compound A**

Inhibition of cross-contamination during spinach washing

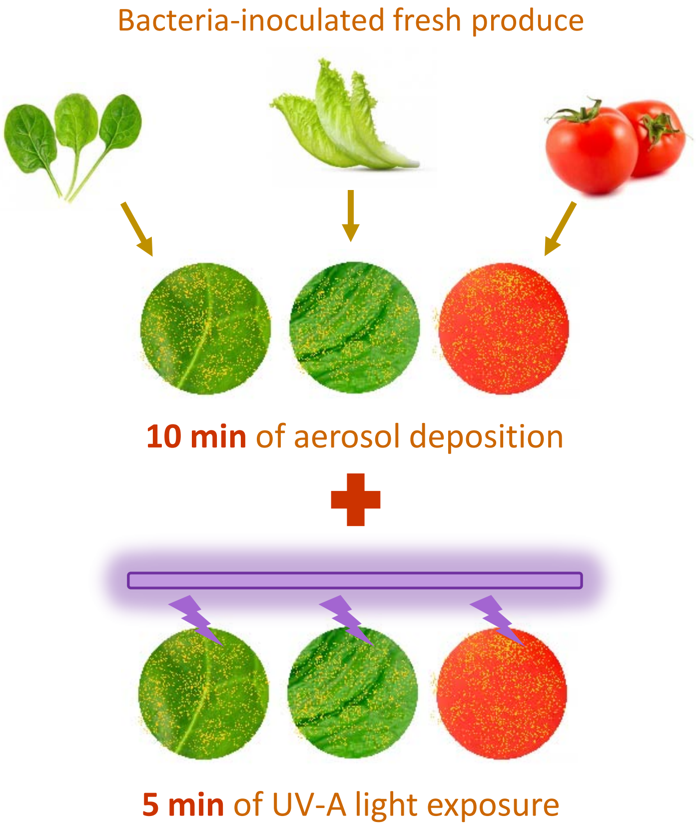


Reduced cross-contamination to spinach while lowering bacteria levels in wash water to below the detection limit

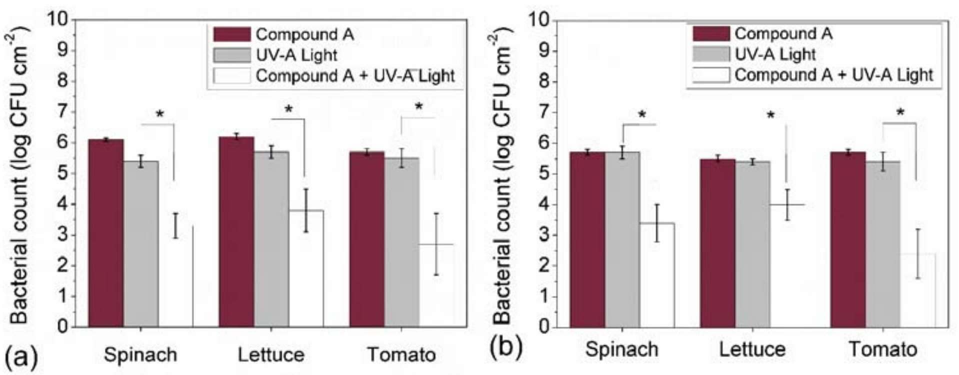
Aerosolization of photo-activable antimicrobial



Set-up used for the aerosolization of antimicrobial and further exposure of produce samples to light.



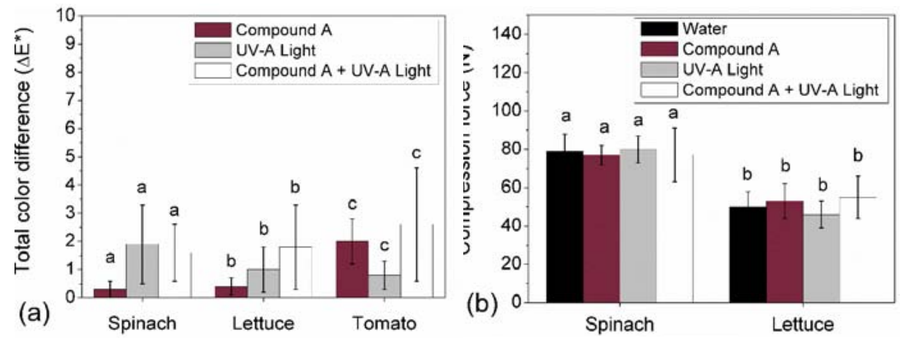
Aerosolization of photo-activable antimicrobial



Bactericidal activity of antimicrobial aerosol in combination with UV-A light against (a) *E. coli* O157:H7 and (b) *L. innocua* inoculated on the surfaces of spinach, lettuce and tomato.

2 to 4 log CFU/cm^2 bacterial inactivation on fresh produce surfaces

Did not significantly affected the total color and texture of the treated fresh produce

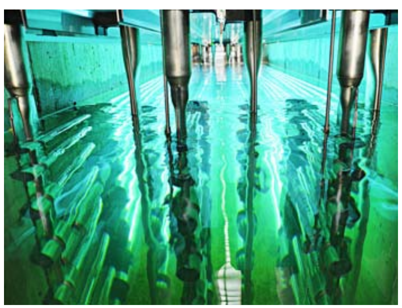


Effect of antimicrobial aerosol and UV-A light on the (a) color and (b) texture of spinach, lettuce and tomato surfaces before and after treatment.

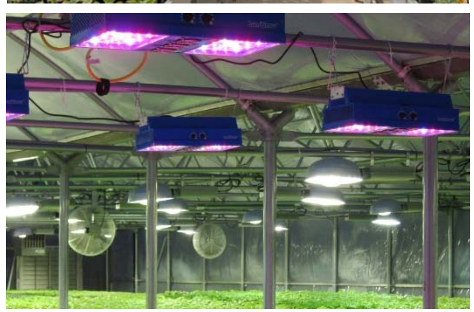
Enhanced Microbial Inactivation by the Combination of **Sub-lethal levels of Antimicrobial** and **Low-Intensity Lights**

Possible applications

Water treatment



Greenhouses



Pre-harvest application

Transportation and Storage

Enhanced Microbial Inactivation by the Combination of **Mild Temperature** and **Sub-lethal Levels of Antimicrobials**

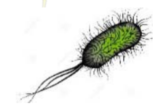
Thermal Inactivation of Bacteria in Juices Supplemented with **Sub-lethal Levels of Antimicrobial H**

Compound H
0.02 – 0.2 % (w/v)

Clarified Apple Juice
(pH = 3.6)

Carrot Juice *with 2 % Lemon juice*
(pH = 4.3)

Coconut Water
(pH = 5.3)



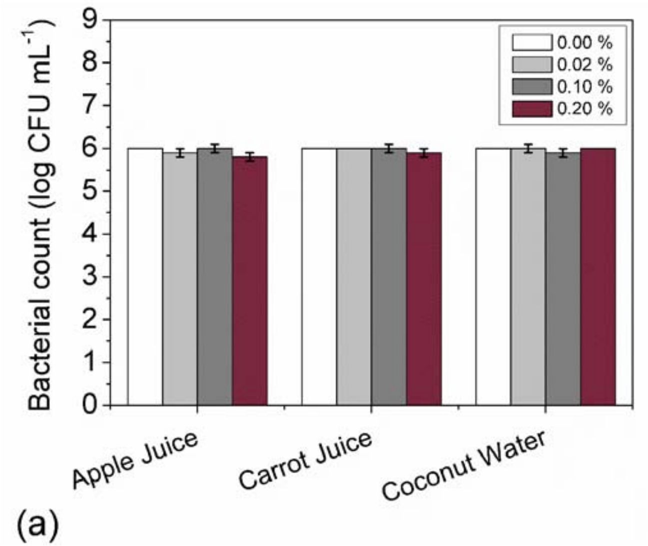
E. coli O157:H7
&
Enterococcus faecium

Mild temperature processing
(55 ºC – 65 ºC)

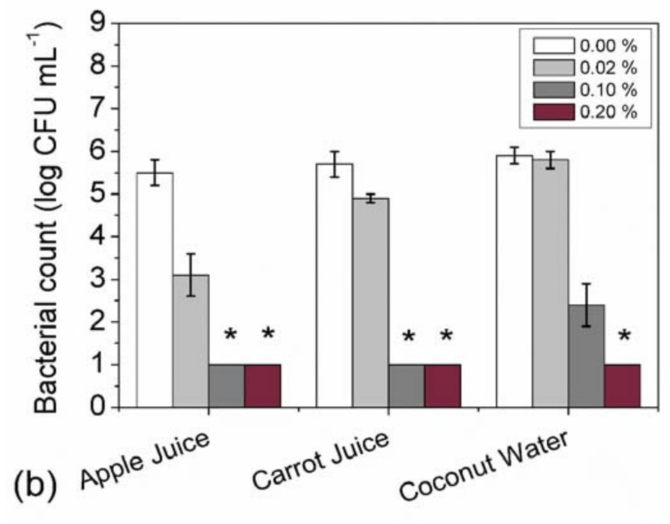
Thermal Inactivation of Bacteria in Juices Supplemented with **Sub-lethal Levels of Antimicrobial H**

Inactivation of *E. coli* O157:H7

25 ºC; 2.5 min



55 ºC; 2.5 min



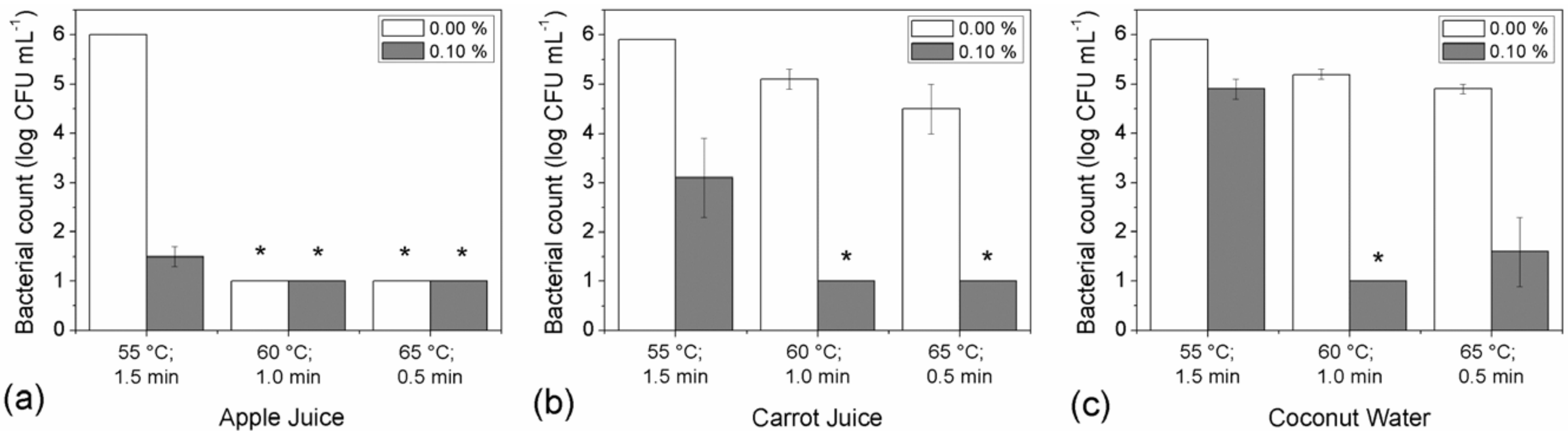
5 log CFU/mL enhanced inactivation

Low concentration of antimicrobial added

Thermal Inactivation of Bacteria in Juices Supplemented with **Sub-lethal Levels of Antimicrobial H**

Inactivation of *E. coli* O157:H7 at different combinations of processing temperature and time:

[55 ºC for 1.5 min - 60 ºC for 1.0 min - 65 ºC for 0.5 min]

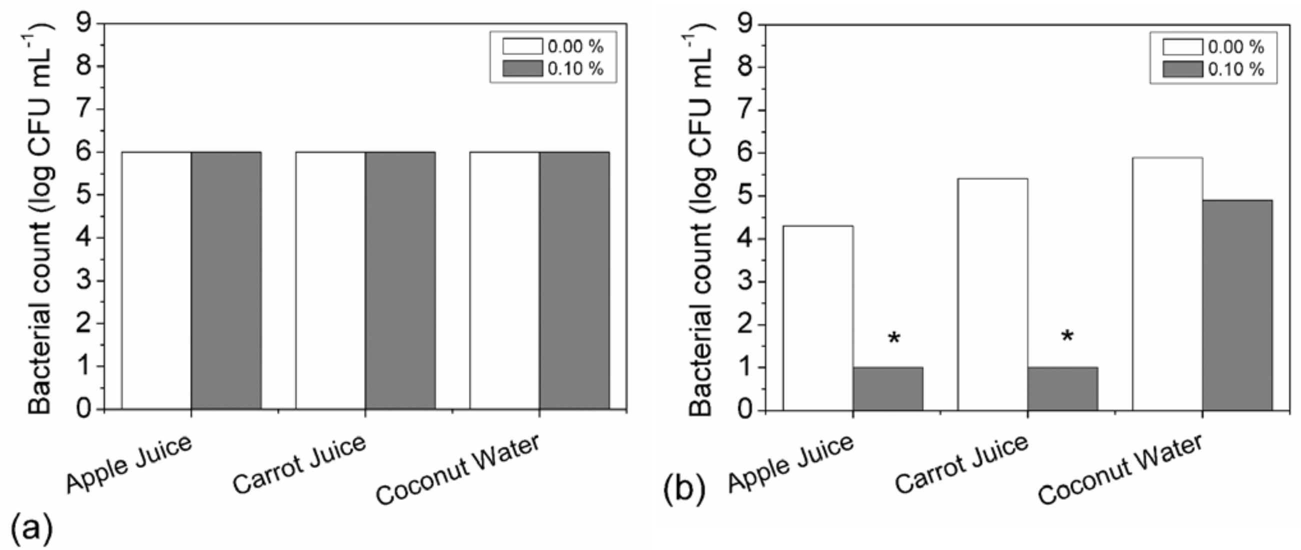


Thermal Inactivation of Bacteria in Juices Supplemented with **Sub-lethal Levels of Antimicrobial H**

Inactivation of *Enterococcus faecium*

25 ºC; 2.5 min

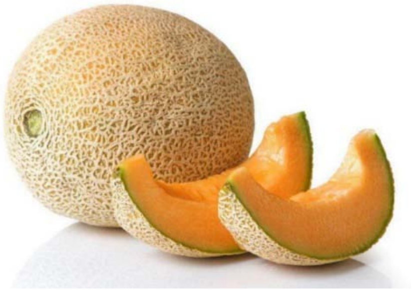
55 ºC; 2.5 min



5 log CFU/mL inactivation in apple and carrot juices

Enhanced Microbial Inactivation by the Combination of **Mild Temperature** and **Low Levels of Antimicrobials**

Do you think this approach could be useful for seed applications or in – field agricultural applications?



Thanks

email: nnitin@ucdavis.edu

Simulated *In Vitro* Digestion of Yeast Microcarriers

Key Outcome: Yeast microcarriers demonstrate minimal release under gastric conditions but release under intestinal conditions

In vitro Gastric Digestion

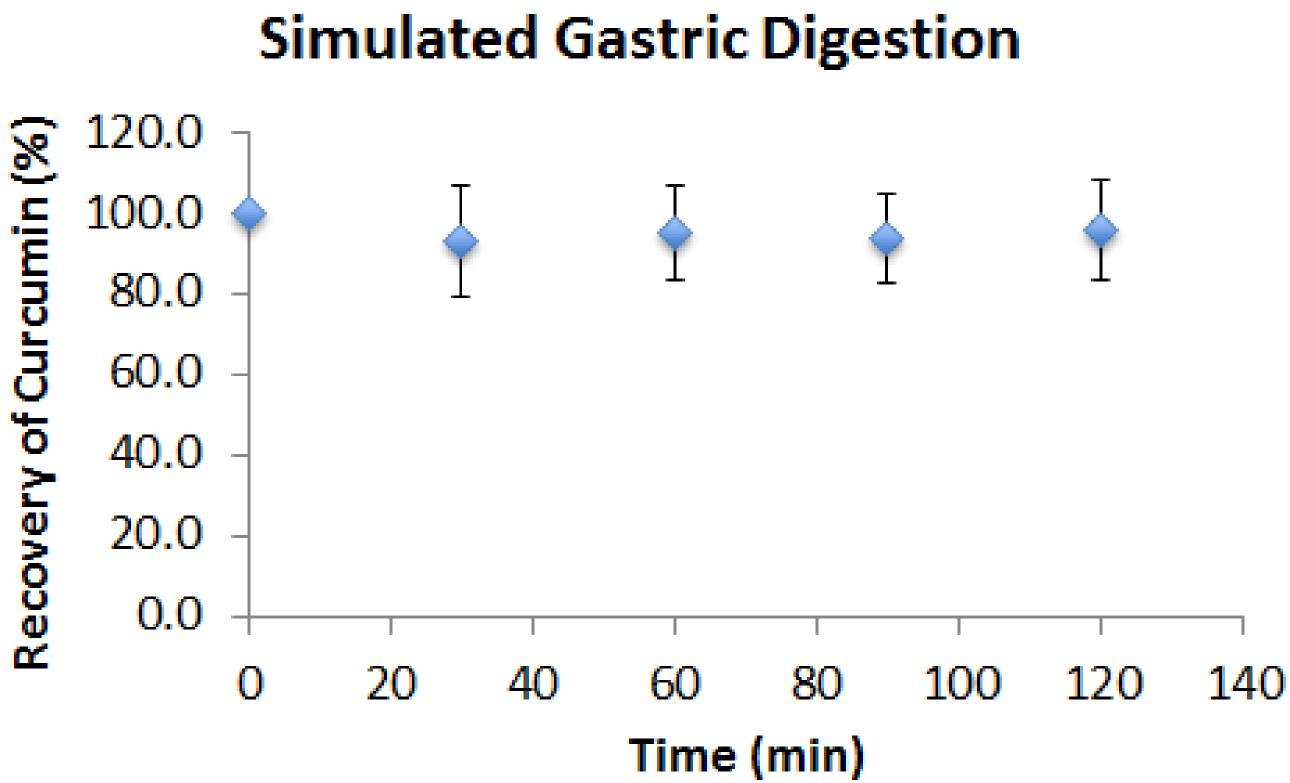


Figure 2: *In vitro* gastric digestion of curcumin from yeast microcarrier. Yeast microcarriers containing curcumin were subjected to gastric conditions, *i.e*. pH = 1.2, pepsin and 37 deg C for 2 hr.

In vitro Intestinal Digestion

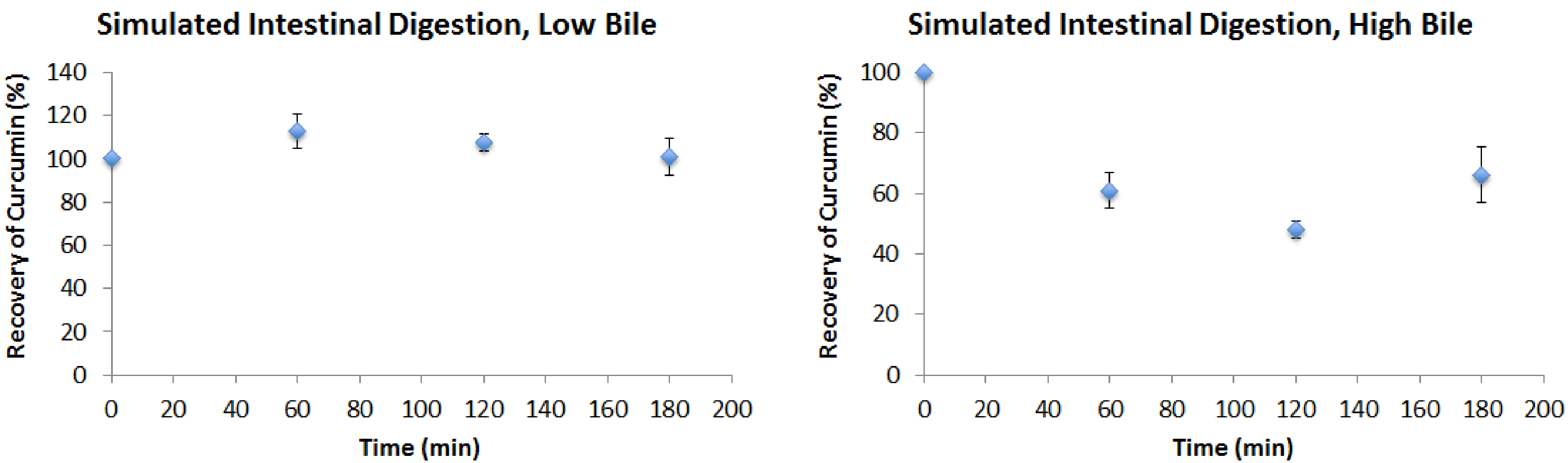


Figure 3: *In vitro* intestinal digestion of curcumin from yeast microcarrier in the presence of low and high bile salt concentrations. Samples were subjected to low bile salt (1.25 mg/mL) or high bile salt (saturated) intestinal conditions at 37 deg C in the presence of lipase for 3 hr.

Sequential Digestion

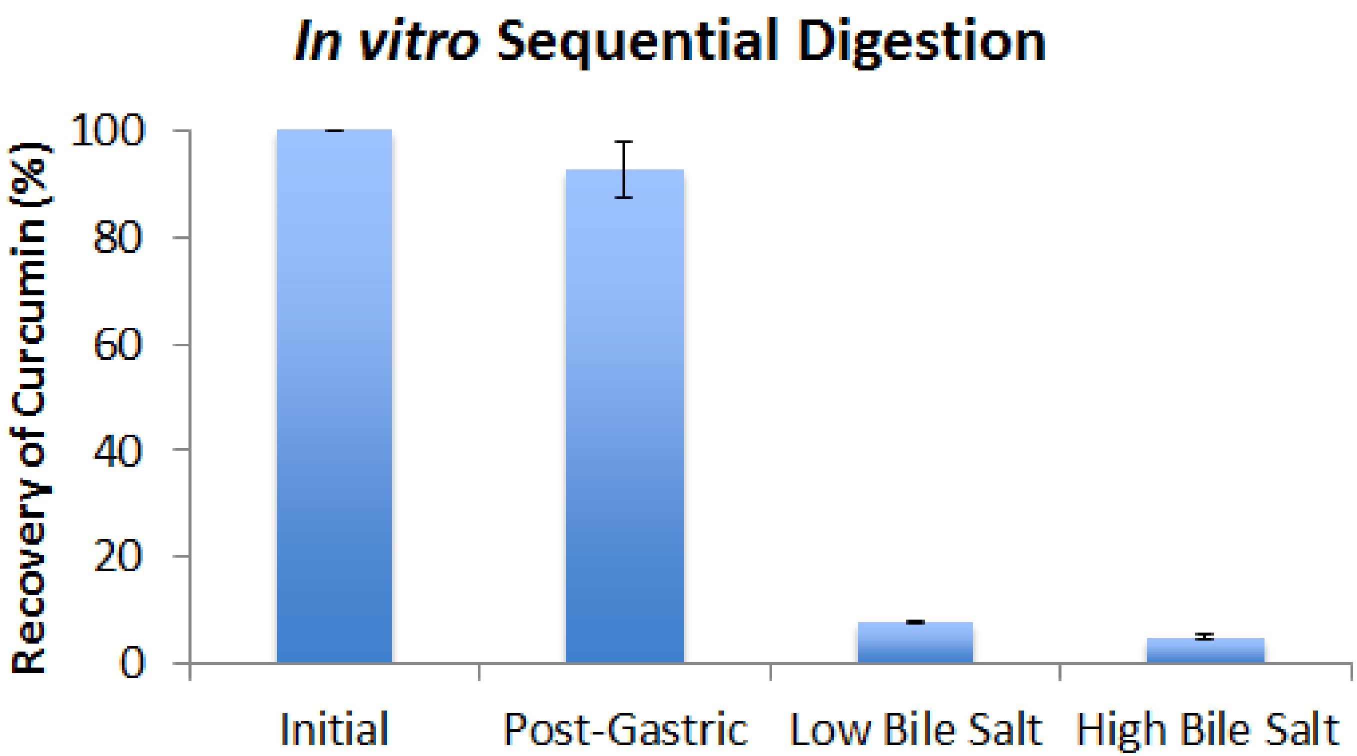


Figure 4: *In vitro* sequential of curcumin from yeast microcarrier. Yeast microcarriers containing curcumin were subjected to gastric conditions, *i.e*. pH = 1.2, pepsin and 37 deg C for 2 hr. Immediately, samples were transferred into either low bile salt (1.25 mg/mL) or high bile salt (saturated) intestinal conditions at 37 deg C in the presence of lipase for 3 hr.

Summary

- Cell based microcarriers provide higher loading and better physicochemical stability than conventional emulsions

- Curcumin encapsulated into yeast is stable and yields a product with a more intense yellow color

- Cells based microcarriers are able to stably retain bioactives through the gastric tract and provide near full release in the intestine under physiological conditions

TEM of Yeast in Simulated Digestion

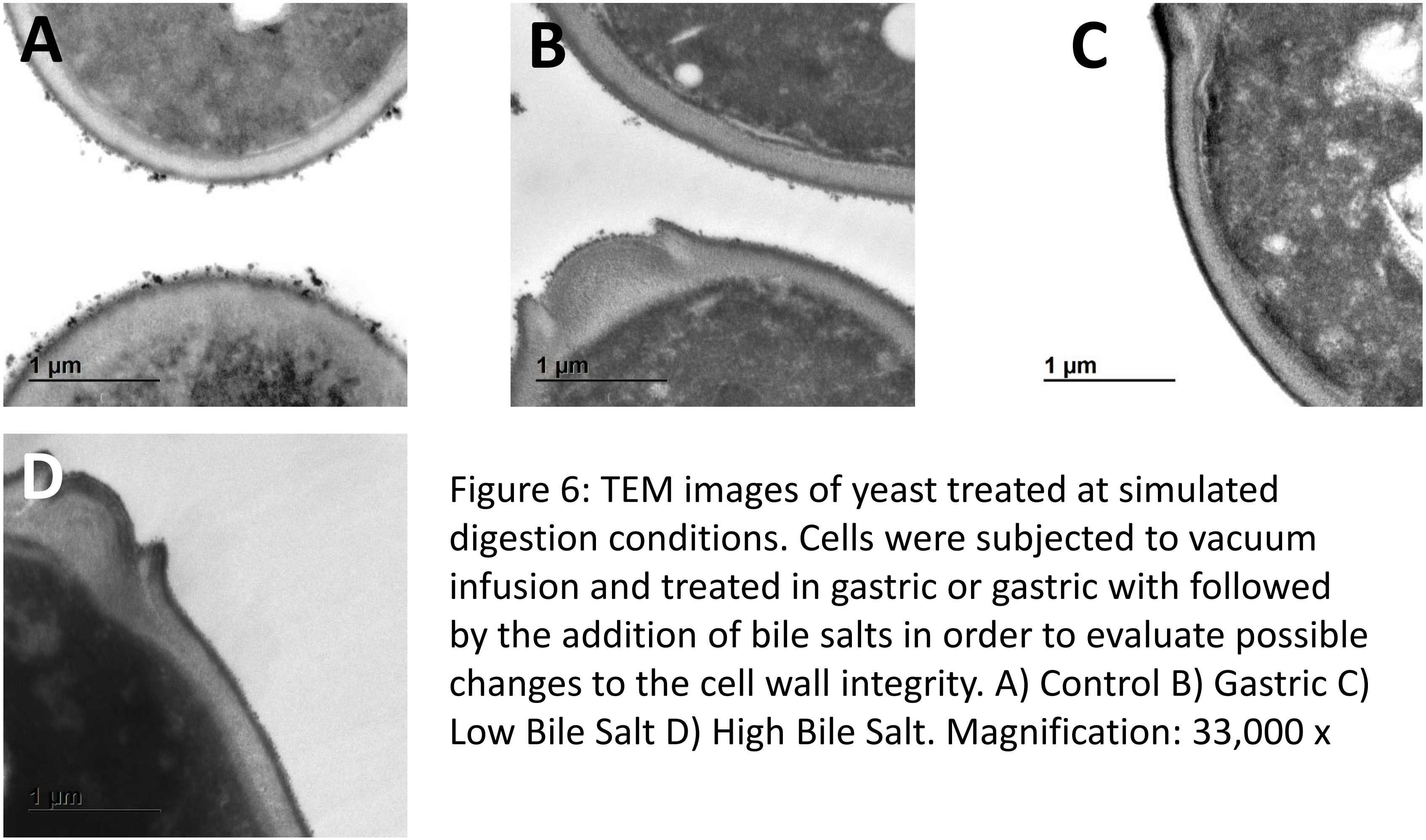
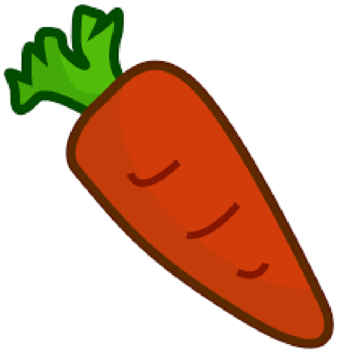
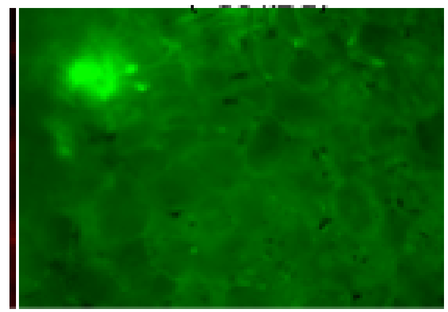


Figure 6: TEM images of yeast treated at simulated digestion conditions. Cells were subjected to vacuum infusion and treated in gastric or gastric with followed by the addition of bile salts in order to evaluate possible changes to the cell wall integrity. A) Control B) Gastric C) Low Bile Salt D) High Bile Salt. Magnification: 33,000 x

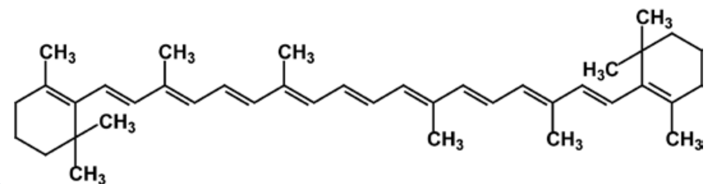


Carrot

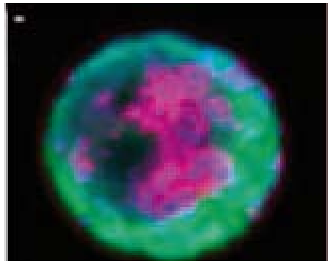
Tissue with cells



Fluorescence Imaging



Beta-carotene



Chemical Imaging

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Baking with Encapsulated Curcumin at a Fixed Concentration

Table 3

Dough and bread with curcumin encapsulated into emulsions and yeast at the same concentrations. In this case, emulsions and yeast encapsulated were utilized such that the final amount of curcumin in the sample was 250 ug. The yellowness of the sample is measured by the "b" value using a Hunter colorimeter.

	L	a	b
Control dough	77.81	1.53	16.02
10% Tween20 emulsion dough	77.03	-3.46	33.03
10% starch emulsion dough	76.94	-2.36	31.51
1 g yeast dough	74.35	-1.35	40.31
Control bread	62.06	1.87	18.28
10% Tween20 emulsion bread	64.15	-1.56	36.93
10% starch emulsion bread	63.02	-0.18	35.55
1 g yeast bread	58.97	0.11	40.13