Request for Report for Projects Awarded in 2013 and 2014 by

Mississippi Center for Food Safety and Post-Harvest Technology

Title: Fabrication and Application of Nano-Vesicular Emulsion Carrier Systems (NVES) for Delivery Of Flavors, Taste-Enhancers, Antioxidants And Antibacterials In Food Systems

Award year: 2015-16

PI: Z. Zee Haque

Co-PI:

Collaborator:

1. Objectives.
    a. Identify and assess natural and edible emulsifiers and conditions capable to stabilizing NVESs
    b. Assess the long-term stability of various NVES under different conditions (e.g., pH, concentration, and physical conditions such as homogenization pressure) will be studied and correlated with protein conformational changes using a combination of proteins with or without polysaccharides under various will allow us to design/formulate.
    c. Investigate the ability of the NVESs to entrap and retain bioactive molecules
2. New Accomplishments toward objectives. Please indicate if all objectives listed were completed.
    a. Various edible proteins have been used to stabilize nanoemulsions. They include whey protein concentrate, bovine serum albumin and β-lactoglobulin. New ones are being tried.
    b. Stable mono-dispersed nano-vesicular emulsion carrier systems are being generated in the desired nano-scale (<100 nm). Stability is being assessed for the different conditions listed in objective B (above).
3. Objectives not accomplished and impediments to meeting objectives.
    a. Investigation of the ability of the NVESs to entrap and retain bioactive molecules has not been accomplished yet.
4. If continuing project, when will new and/or long term objectives be completed?
    a. Long term objectives will be completed by the middle of 2016.
5. Students supported
    a. PhDs (% FTE and name):
        i. Ahmed Saddam Chaloob (20%)
        ii. Basheer Ikdiem (20%)
        iii. Soma Mukherjee (20%)
        iv. Xue Zhang(80%)
    b. M.S. (% FTE and name)
        i. Jingyi Yan (80%)
        ii. Nagham Salah Alawadi (50%)

iii. Wenjie Shao (20%)

c. Undergraduate (number of students)

(b) Leveraged Funds: External Competitive Funding Applied and Awarded based on findings from this project.

a. Applied for:
   i. Funding agency
   ii. Program
   iii. Funding request ($$)

b. Awarded:
   i. Funding agency
   ii. Program
   iii. Funding awarded ($$)

(c) Outputs – In addition to the above, please populate the following sections to be included in a report to be compiled in a FSI Research Accomplishment Booklet. The project report will also be posted in a FSI website to be developed.

Project Summary (Issue/Response)

This research project investigates the formulation and methodology of production of shelf-stable NVESs capable of delivering bioactive materials to food systems and understand the basic protein conformational changes that occur at the interface. The rational being that this will enable us to prevent oxidative degradation and extend shelf life and quality of foods. Whey, the byproduct of cheese manufacture, is a cost-effective source of high quality proteins that are both functional in food systems and nutritious for the human. Whey and other standard edible proteins are being used to stabilize Nano-Vesicular Emulsion Carrier Systems (NVES). These nano-particles (<100 nm) will be used in food systems deliver bioactive nutrients more effectively. Research is also being conducted to identify and enhance the efficacy of natural antioxidants, antibacterial and related bioactive molecules to be used cargo particles in the NVES.

Project Results/Outcomes

In the effort to produce stable NVES, ultra-high-pressure homogenization (UHPH) is being used to generate monodispersed stable peanut oil nano-emulsions within a desired nano-globular range (<100nm) (DNR) stabilized using combinations of whey protein concentrate (WPC), sodium dodecyl sulfate (SDS), Triton X-100 and zwitterionic detergents differing in hydrophobicity. WPC (2.0% w/v), dispersed-phase fraction (φ) of 0.05 and 210 MPa significantly reduced mean globule size (dvs) but grouped frequency distribution of globule sizes was bimodal and larger than DNR. Among binary-emulsifiers sulfobetaine 3-10 (SB3-10) (7.5% w/w WPC) gave particles within DNR (dvs of 73 nm) though still in bimodal distribution.

Circular dichroism analyses showed little disruption of secondary structure of proteins in WPC by SB3-10 (7.5% w/w WPC) whereas Triton X-100 (10% w/w WPC) obliterated it. WPC plus SB3-10 mixture retained some periodic structure even when premixed with Triton X-100 (10% w/w WPC) and remarkably gave a narrow monomodal distribution (volume fraction $\varphi = 1$) well within DNR with highest stability reflected by no creaming in more than 30 days at 22 °C. Unimodal nano distribution of nanoemulsion consisting of a ternary system including WPC, SB3-10 and triton X-100 was conceivably achieved due to the wedging effect of triton X-100 among the bulky proteins in the limited space of nano-globular interface instead of denaturing effect of the detergents alone. These data provide important directions being taken to produce monodispersed stable nano-globular delivery mechanisms with dramatically increased hydrocarbon-aqueous interface that is paramount for efficacy.

In the effort to enhance antioxidative efficacy, Maillard reaction was used to enhances short and long-term radical quenching persistence of sweet whey The research investigated the augmentation of short and long term peroxyl and alkoxyl radical quenching efficacy [antioxidant activity [AA] and persistence [AP], respectively) of Cheddar (CW) and Edam whey (EW) and hydrolytic derivative of casein (CH) that were mutually supplemented with thermally proliferated Maillard reaction products (MRPs) in the presence or absence of lactose (L). Enhanced antioxidant capacity (AC) of the samples, evident by their efficacy to quench 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical cations ($ABTS^{\cdot+}$) was also investigated. Whey and CH (2%, w/v) with and without added lactose (1%, w/v) were dispersed in 0.2M McIlvaine's iso-ionic buffer and subjected to thermal treatment for 0 – 4 hours at 90^{O}C. AA and AP were determined in real time from luminol-induced chemiluminescence (CL) resulting from unquenched radicals produced in vitro by pyrolysis of 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (ABAP). The results exhibited that thermally generated MRPs enhanced antioxidative properties of both types of sweet whey though this effect was more dramatic for CW - conceivably due to its greater intrinsic peptide content. Heating CW for four hours resulted in 65.8, 44.4 and 407.7% augmentations of AA, AP and AC, respectively, compared to unheated sample. Addition of CH and/or L to either types of sweet whey also tended to result in dramatic improvements of their antioxidative properties. Under the same heating regimen, when supplemented with L, CW showed 35, 14 and 25.8% enhancement of AA, AP and AC, respectively, compared to heated CW without added L. Lysine content decreased markedly by 11.6 and 7.7% for CW and EW, respectively, during this heating regimen as Maillard reaction masked the terminal and ε amino groups. These data can potentially lead to the development of powerful new antioxidants to alleviate the detrimental effects of cellular oxidative stress.

Project Impacts/Benefits

Efficacy of flavors, taste-enhancers and protectants, particularly antioxidants, critically depend

on available surface area to allow inter-atomic/molecular interactions. Nano-particulating of interactive globules results in quantum increase in surface area dramatically enhancing this potential. This research will allow development of highly active functional foods.

Project Deliverables

Will be provided by Dr. Dip.