USE OF MILK PROTEINS AS A NATURAL NANO-CAPSULAR VEHICLE FOR ACTIVE COMPONENTS

By

HEBA HASSAN ABD EL-AZIM SALAMA

B.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2002

M.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2007

A thesis submitted in partial fulfillment

of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Science

(Dairy Science & Technology)

Department of Food Science

Faculty of Agriculture

Ain Shams University

2013

Approval Sheet

USE OF MILK PROTEINS AS A NATURAL NANO-CAPSULAR VEHICLE FOR ACTIVE COMPONENTS

By

HEBA HASSAN ABD EL-AZIM SALAMA

B.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2002

M.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2007

This thesis for PhD. degree has been approved by:

Dr. Nabil M. Y. Mehanna ……..

Prof. Emeritus of Dairy Science and Technology, Faculty of Agriculture, Kafr El-Shake University

Dr. Abd El-Monem E. Hagrass ……..

Prof. Emeritus of Dairy Science and Technology, Faculty of Agriculture, Ain Shams University

Dr. Zakaria M. R. Hassan ……..…

Prof. of Dairy Science and Technology, Faculty of Agriculture, Ain Shams University

Dr. Rezk A. Awad ……..…

Prof. of Dairy Science and Technology, Faculty of Agriculture, Ain Shams University

Date of examination: 6 / 3 / 2013

USE OF MILK PROTEINS AS A NATURAL NANO-CAPSULAR VEHICLE FOR ACTIVE COMPONENTS

By

HEBA HASSAN ABD EL-AZIM SALAMA

B.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2002

M.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams University, 2007

Under the supervision of:

Dr. Rezk A. Awad

Prof. of Dairy Science and Technology, Department of Food Science, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Zakaria M. R. Hassan

Prof. of Dairy Science and Technology, Department of Food Science, Faculty of Agriculture, Ain Shams University

Dr. Magdey M. A. El- Sayed

Prof. of Dairy Science and Technology, National Research Centre

ABSTRACT

Heba Hassan Abd El-Azim Salama. Use of milk proteins as a natural nano-capsular vehicle for active components. Unpublished Doctoral of Science Thesis. Food Science Department, Faculty of Agriculture, Ain Shams University, (2013).

Nanoparticles of whey proteins were prepared with the aim of developing a biocompatible carrier for the oral administration of iron and fatty acids as a nutraceuticals. In first part of study, possibility of nanoparticle preparations and its use as a vesicle were characterized for particle size and morphology, zeta potential, loading and association efficincy; encapsulation capacity and in vitro iron release of chitosan-whey protein (CS-WP) nanoparticles. Electron microscopy image analysis for CS-WP nanoparticles showed that the particle size ranged between 13 and 70.6 nm, with an average size of 44.41nm. The formed nanoparticles were appeared spherical in shape with smooth surfaces. Zeta potential values versus pH of the CS-WP were generally changed from positive to negative as the pH raised from 4.5 to 7.5. Zeta potential at pH 4.5 with all iron ratios showed positive charge that increased with loading iron onto the particles. Association efficiency (AE) values were increased by increasing the pH value, while it was decreased by increasing WP concentration. Loading efficiency (LE) was enhanced by increasing the concentration of WP and the maximum LE value was recorded at pH 6.5. The association efficiency (AE) and loading efficiency (LE) of CS-WPC nanoparticles were highly sensitive to pH. No significant differences were found in encapsulation capacity (EC) of iron at different pH values with different protein and iron concentrations, and the values ranged between 99.746 to 99.998 % in all experiments. In vitro, release of iron was very slow from CS-WP nanoparticles after 6 h. of incubation without or with enzyme being less than 1% in all treatments. Bioavailability of iron was improved by application of

nanoencapsulation into CS-WP nanoparticles due to the low relase in simulated gastric conditions.

The cytotoxicity of formulated nanoparticle complexes of different fatty acids (oleic, eliedic, Cis-vaccenic, Trans-vaccenic and linolenic acids) in the presence or absence of different whey proteins fraction and preparation (α-LA, β-lg or WPI) were investigated in second part of study. Nanoparticle complexes formed with each fraction were examined for surface tension, circular dichroism (CD), turbidity, isothermal titration calorimetry (ITC) and Cytotoxic activity. Surface tension values were decreased with adding fatty acid to α-LA, β-lg and WPI. The values obtained were lower in WPI/fatty acid complexes than that of β-lg or α-LA. This would indicate that WPI can bind greater amount of fatty acid than β-lg or α-LA. Cis-fatty acids such as oleic, cis-vaccenic and linolenic caused higher decrease in the surface tension of α-LA, β-lg and WPI nanoparticles than that of trans-fatty acids (eledic and trans-vaccenic acids). The tertiary structure of proteins (α-LA, β-lg or WPI) was lost and changed from fold to unfold after binding with fatty acids. The changes in proteins structure would be correlated to exhibit a cytotoxic activity to tumer cells. All formed protein (α-LA, β-lg or WPI) /fatty acid complexes presented lower turbidity measurements compared to the fatty acid only at same concentration. The turbidity values for nanocomplexes of WPI/fatty acids were lower than that of α-LA or β-lg/fatty acids confirming higher ability in binding fatty acids. The enthalpogram of ITC for β-lg/fatty acid complexes confirmed that there was a reaction occurred between the protein (α-LA, β-lg, WPI) and oleic acid. The enthalpy increased with increasing the protein concentrations. ITC of β-lg complexes showed lower ability to bind oleic acid (0.669 mM/1 mg/ml) compared to α-LA (1.177 mM/ml). All nano complexes formed of α-LA, β-lg or WPI/fatty acids exhibited a cytotoxic ability as a lysis in erythrocytes. The cytotoxic activity of WPI/fatty acid complexes was almost as found with α-LA complexes and slightly higher than of β-lg complexes. Nanocomplexes can be formed of α-LA, β-lg as well as WPI with good cytotoxic effect to tumer cells using cis-vaccenic and

linolenic fatty acids comparable to oleic acid. It was a new interesting observation being that the nanocomplexes formed of WPI with fatty acids has a comparable cytotoxcisty to that of α-LA and β-lg and can be used in tumer therapy.

Keywords: Nanoparticles; Whey proteins; Iron; bioavailability; α-LA; β-lg; Fatty acids; Surface tension; Circular dichroism; Turbidity; Isothermal titration calorimetry (ITC; Cytotoxicity; Loading efficiency; Association efficiency; Encapsulation capacity.

ACKNOWLEDGMENT

Deepest, greatest and sincere thanks to **ALLAH** the most Merciful, Great and Clement God.

I wish to extend my deepest appreciation and sincere gratitude to **Prof. Dr. Rezk A. Awad,** Professor of Dairy Science and Technology, Food Science Department, Faculty of Agriculture, Ain Shams University for the kind attention and greater help provided for the accomplishment of this work and for his efforts, supervising the research, writing the manuscript and encouraging me through this course It is difficult to express in words my deep respect to him.

I wish to find the words that can help to express my gratefulness thanks, deepest gratitude and sincere appreciation to **Prof. Dr. Zakaria M. R. Hassan,** Professor of Dairy Science and Technology, Food Science Department, Faculty of Agriculture, Ain Shams University, for his true efforts throughout the writing the manuscript, and encouraging me through this course.

No words can help me to express my gratefulness thanks and will not be enough words to **Prof. Dr. Magdy El-Sayed and Prof. Dr. Mervet Foda**, Professors of Dairy Chemistry and Technology, Dairy Science Department, National Research Center, for supervising this work, supporting me, plentiful advice and endless efforts provided for me to complete this work.

Thanks are also due to **Prof. Dr. Daniel Otzen**, Professor of Protein Biophysics at Aarhus University, Aarhus, Denmark, for helping and providing with different advises. He learnt me many things which I never have had the opportunity to learn. Thanks also to all of his group members for great help during the period that I spent with them in Denmark to achieve big part of the thesis and plentiful advice.

Great thanks to Academy of Scientific Research and Technology, Egypt for the financial support during this study.

I would like to thank all the stuff members of Food Science and Technology Department at Ain Shams University and members of Dairy Department at National Research Center.

My deepest thanks to my family for valuable cooperation during this investigation.

CONTENTS