THE DEVELOPMENT OF TILAPIA FISH SKIN GELATIN NANOPARTICLES IN ENCAPSULATED BIOPEPTIDES FOR YOGHURT APPLICATION

BY

IQRAH AKBAR

A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Engineering

Kulliyyah of Engineering
International Islamic University Malaysia

FEBRUARY 2020

ABSTRACT

Considerable attention has been directed to nanoparticles based on gelatin biopolymer due to its numerous available active group sites for attaching target molecules and acting as a drug or nutraceutical delivery system. The main aim using such nanoparticles is to improve the therapeutic effects and also to reduce the side effects of formulated drugs due the natural and cheap availability of gelatin. Gelatin obtained from fish skin and scales can provide a potential alternative source with almost the same rheological properties as mammalian gelatin and is a beneficial way to use fish waste such as skin, bones and fin which is generally discarded. Nanoencapsulation of bioactive compounds within fish skin gelatin nanoparticles can improve the bioavailability, delivery properties, and solubility of the nutraceutical within the blood stream. Many of such bioactive peptides (biopeptides) are potent antioxidants and as oxidative stress is the main cause of the onset of various chronic diseases encapsulation of antioxidant biopeptides within a fish gelatin nanocarrier system could be a potential remedy to prevent or delay the onset of such diseases and for better health prospects. The purpose of this study was to prepare a safe and cost efficient novel food delivery nanoparticle system encapsulating a desirable antioxidant biopeptide. A high quality protein isolate was chosen from four species; sunflower corn, palm kernel cake and moringa oriefera by alkaline extraction and isoelectric precipitation, and sunflower protein isolate (0.322±0.82 mg/ml protein content) was used as starting material for the generation of an extensive enzymatic protein hydrolysates using sequentially an endo-protease (Alcalase) and an exo-protease (Flavourzyme) and the protein hydrolysates, (biopeptide) with a degree of hydrolysis of 53.6 %, was white and non bitter. The antioxidant activity of the sunflower protein hydrolysates (biopeptide) was quite high at DPPH of 89±1.01% and FRAP assay of 968±0.68 µm/L. Gelatin was extracted from Tilapia fish at an average yield of 10% wt/wt of fish skin and scales. The proximal composition of the gelatin was similar to that of the commercial gelatins, with slightly higher moisture content. The tilapia skin gelatin had whitish yellow color and a similar pH to commercially available gelatin. Gelatin nanoparticles were prepared by a two step desolvation process encapsulating the sunflower protein hydrolysates (biopeptide) and the average diameter of the biopeptide loaded gelatin nanoparticle is between 228.3±0.11 and 1305±0.6 nm. Protein loading efficiency is 76±1.1 % at an optimal pH of 2, glutaraldehyde concentration of 2 ml and biopeptide concentration of 0.1 mg/ml and exhibits DPPH at 92% and FRAP assay of 978±0.65 µm/L. To understand the absorption and protein release of the GNPs, the biopeptide loaded gelatin nanoparticles were subjected to simulated gastrointestinal conditions mimicking the human stomach and intestine and showed a peptide release of 0.1464 and 0.277 mg/mL upon pepsin and pancreatin digestion respectively. This system also demonstrates the capability of preventing the denaturation of protein encapsulated in the GNPs. Cell adhesion studies with human fibroblasts have shown that gelatin nanoparticles do not affect the number of cells adhered to glass as compared to control cells with no particles. Standard cell viability assay demonstrated that cells incubated with gelatin nanoparticles remained more than 100% viable at concentration of 25µg/ml. Upon addition of GNPs loaded with antioxidant biopeptide to frozen yoghurt, negligible difference was measured for pH, hardness and cohesiveness; however, syneresis and adhesiveness saw a slight variation. The antioxidant activity of yoghurt fortified with the antioxidant biopeptide showed an increase with increasing concentration of GNPs added and would deliver the desired food nanoparticle delivery system.

خلاصة البحث

هناك توجه عام واهتمام كبير بالجسيمات النانوية القائمة على البوليمر الحيوي الجيلاتيني وذلك لوجود العديد من مواقع المجموعات النشطة المتاحة لإرفاق الجزيئات المستهدفة والعمل كنظام توصيل دوائي أو غذائي. يهدف هذا النظام إلى تحسين التأثيرات العلاجية وكذلك الحد من الآثار الجانبية للأدوية المصنعة مثل الجيلاتين والذي هو بوليمر حيوي طبيعي غير سام وقابل للتحلل البيولوجي ومتاح بسهولة، وهو رخيص التكلفة أيضاً لذا يستخدم الجيلاتين في التركيبات الاساسية. بإمكان جيلاتين الجلد في الاسماك التي يعيش في المياه الدافئة أن يوفر مصدراً بديلاً محتملاً للجيلاتين مع نفس الخصائص البيولوجية تقريباً للجيلاتين في الثدييات، وهي طريقة مفيدة لاستخدام المخلفات السمكية مثل الجلد والعظام والزعانف والمركبات الحيوية النشطة داخل الأسماك والتي يتم التخلص منها عموماً. يمكن للتغطية النانوية للمركبات النشطة بايولوجيا في جلد السمك تحسين التوافر البيولوجي، وخصائص التكيف، وقابلية التغذية في نطاق مجرى الدم. العديد من هذه الببتيدات الحيوية النشطة بيولوجيا هي مضادات أكسدة قوية، وبما أن الإجهاد التأكسدي هو السبب الرئيسي لظهور العديد من الأمراض المزمنة، فإن تغليف الببتيدات الحيوية المضادة للأكسدة ضمن نظام ناقلات النانو للجزيئات في جيلاتين السمك يمكن أن يكون علاجًا ممكناً لمنع أو تأخير ظهور مثل هذه الأمراض والحصول على نتائج صحية أفضل. الغرض من هذه الدراسة هو إعداد نظام جسيمات متناهية الصغر آمن وفعال من حيث التكلفة لتوصيل الأغذية يحتوي على مادة ثنائية الببتيد المضاد للأكسدة. تم اختيار عزل بروتين عالي الجودة من أربعة أنواع؛ تم استخدام ذرة عباد الشمس، ونواة نخيل الزيت، ونبات المورينجا عن طريق الاستخلاص القلوي والترسيب الإيزو كهربي، وعزل بروتين عباد الشمس (0.322 ملغ / مل من محتوى البروتين) كمواد البدء لتوليد بروتينات إنزيمية واسعة تتحلل باستخدام البروتينات الداخلية المتسلسلة (ألكالسي) وإكسو البروتيني (Flavourzyme) وبروتين هيدروليات، والببتيد الحيوي مع درجة من التحلل المائي بنسبة 53.6 ٪ ، كانت بيضاء وغير مريرة. كان نشاط

مضادات الأكسدة من تحليلات بروتين عباد الشمس (الببتيد الحيوي) عالياً جداً عند DPPH من 89 ± 1.01 ٪ ومقايسة FRAP البالغة 968±0.68 ميكرومتر/ لتر.
م استخراج الجيلاتين من أسماك البلطي بمعدل غلة 10٪ بالوزن من جلد السمك وحراشفه بخصائصه الفيزيائية والكيميائية.كان التركيب القريب من الجيلاتين مشابهاً للجيلاتين التجاري، مع محتوى رطوبة أعلى قليلاً. ولونه مائلاً للصفرة ودرجة حموضة مماثلة للجيلاتين المتوفر تجاريا. تم تحضير الجسيمات النانوية الجيلاتينية بواسطة عملية انحلال ذات مرحلتين لتغليف بروتينات عباد الشمس (الببتيد الحيوي) ومتوسط قطر جسيمات الجيلاتين النانوية المحملة بالببتيد ما بين 228.3±0.11 و 1305 ميكرومتر. وكفاءة تحميل البروتين هي 76±1.1 ٪ في درجة الحموضة المثلى 2 ، وتركيز الغلوتارلديهايد (glutaraldehyde) من 2 مل وتركيز الببتيد الحيوي من 0.1 ملغ / مل ويعرض DPPH عند 92 ٪ ومقايسة FRAP البالغة 978±0.65 ميكرومتر / لتر. لفهم الامتصاص وإطلاق البروتينات من GNPs، تعرضت جسيمات الجيلاتين النانوية المحملة بالببتيد الحيوي لمحاكاة لظروف الجهاز الهضمي تحاكي المعدة والأمعاء البشرية وأظهرت إطلاق الببتيد من 0.1464 و 0.277 ملغم / مل عند هضم البيبسين والبنكريستين على التوالي. هذا النظام يوضح أيضا القدرة على منع تشويه البروتين المغلف في GNPs. وقد أظهرت دراسات التصاق الخلايا مع الخلايا الليفية البشرية أن جزيئات الجيلاتين النانوية لا تؤثر على عدد الخلايا الملتصقة بالزجاج مقارنة بخلايا التحكم التي لا تحتوي على جزيئات. أثبت اختبار قابلية الخلية القياسية أن الخلايا المحتضنة مع الجسيمات النانوية تظل أكثر من 100 ٪ قابلة للحياة بتركيز 25 ميكروجرام / مل. بعد إضافة الـ GNPs المحملة بالببتيد الحيوي المضاد للأكسدة إلى الزبادي المجمد، تم قياس الفرق الهامشي في درجة الحموضة والصلابة والتماسك. ومع ذلك شهدت التآزر و الالتصاق اختلاف طفيف. وأظهر نشاط مضادات الأكسدة في الزبادي المدعم بالببتيد الحيوي المضاد للأكسدة زيادة مع زيادة تركيز GNPs المضاف وسيوفر نظام لتوصيل الجسيمات النانوية الغذائية المطلوبة.

APPROVAL PAGE

The thesis of Iqrah Akbar has been approved by the following:

Irwandi Jaswir
Supervisor

Parveen Jamal
Co-Supervisor

Nassereldeen Ahmed Kabbashi
Internal Examiner

Noriham Abdullah
External Examiner

Hadi Nur
External Examiner

Roslina Othman
Chairman

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Iqrah Akbar

Signature .... Date ....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

THE DEVELOPMENT OF TILAPIA FISH SKIN GELATIN NANOPARTICLES IN ENCAPSULATED BIOPEPTIDES FOR YOGHURT APPLICATION

I declare that the copyright holders of this thesis are jointly owned by the student and IIUM.

No part of this unpublished research may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without prior written permission of the copyright holder except as provided below

1. Any material contained in or derived from this unpublished research may be used by others in their writing with due acknowledgement.

2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purposes.

3. The IIUM library will have the right to make, store in a retrieved system and supply copies of this unpublished research if requested by other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Iqrah Akbar

……..…………………….. …………………………..
Signature Date

ACKNOWLEDGEMENTS

First of all, I would like to thank Allah SWT for giving me a chance to pursue education and finish my doctoral report. I would like to thank my main supervisor, Dr. Irwandi Jaswir who has helped me throughout the period of my studies. I could not have finished my work without his constant support and guidance.

I would also like to sincerely thank my beloved co-supervisor, Dr. Parveen Jamal, who did not only help me through all the ups and downs of my study period but also continuously mentored me to aim higher and achieve my goal.

I would like to thank my sister, Isra Akbar, for helping me in finishing my studies and motivating me throughout my journey of this degree. I would not have finished my PhD without her help and effort. I would also like to thank my parents for being there for me.

.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

SAE	Society of Automotive Engineers
µL	Microliter
ACE	Angiotensin-Converting Enzyme
ANOVA	Analysis of Variance
Apps	Applications
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
AU	Anson Units
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CO_2	Carbon Dioxide
CPP	Calcium-Binding Phosphopeptides
Da	Dalton
DPPH	2,2-diphenyl-1-picrylhydrazyl
DOE	Design of Experiment
DSM	Defatted Sunflower Meal
EDTA	Ethylenediaminetetraacetic acid
et al	(et alia): and others
FAO	Food and Agricultural Organization of the United Nations
FCCCD	Face Centred Central CompositeDesign
FDA	Food and Drug Administration
FITC	Fluorescein
FRAP	Ferric Reducing Antioxidant Assay
G	Gram
g/mL	gram/liter
GNP	Gelatin Nanoparticles
GRAS	Generally Recognized as Safe
h	Hours
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric Acid
HCl	Hydrochloric Acid
HGC	Human Chorionic Gonadotropin
HMW	High Molecular Weight
HPLC	High Performance Liquid Chromatography
IEP	IsoElectric Point
KH_2PO_4	Potassium Phosphate
LAB	Lacto Bacillus Bacteria
LAPU	Leucine Aminopeptidase Unit
LDA	Laser Doppler Anemometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
Na_2SO_3	Sodium Sulphate
NPBRO	Nano-emulsion containing Purple Rice Bran Oil
PBS	Phosphate Buffer Solution
PEG	Poly ethylene Glycol

PCS	Photon Correlation Spectroscopy
PG	Propyl Gallate
RSM	Response Surface Methodology
SFM	Sunflower Meal
TCA	TriChloroAcetic Acid
TFA	TriFlouroAcetic Acid
TBHQ	Tert-Butyl Hydroquinone
WPC	Whey Protein Concentrate

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Gelatin is a hydrocolloid polymer and is a derivative of collagen extracted from skin, bone, and connective tissues of various animal kinds. On partial hydrolysis of collagen, it becomes a denaturalized protein and has found various applications as an important alternative source of protein for applications not only in the food and pharmaceutical industry but also in usage in materials and cosmetic industries (Jelloui et al., 2011) depending upon its rheological properties. While various physico-chemical properties of gelatin such as the molecular composition, the color, taste and odor of gelatin, solubility, transparency are determining factors for its application, viscous properties, strength and stability to heat in terms of gelling and melting temperatures, which are the important criteria for establishing its overall commercial applicability. Therefore, gelatin is extremely versatile in its application in the food industry as a packaging material due to its biodegradability, foaming and fining agent, emulsifier, colloids stabilizer and a nano-encapsulating agent (Gómez-Guillén et al., 2011). Moreover, its shows promising biomedical utility including its usage as a protein stabilizer in numerous formulations such as vaccines, sponges (Gelfoam®) as well as plasma expanders. Gelatin has been established as a safe food supplement and has been documented as “Generally Recognized as Safe” (GRAS) by the US Food and Drug Administration (FDA).

Pig skin at 46 %, bovine hide at 29.4 % and pork and cattle bones at 23.1 % respectively account for the world’s major gelatin production whereas fish gelatin, in the year 2010, reported 1.5 % of total gelatin production. However, the current production of gelatin has been doubled since 2002, suggesting that the need for alternatives for mammalian gelatin is on the rise (Gómez-Guillén et al., 2009). The gelatin obtained from mammalian sources has raised serious socio-cultural concerns of not being halal and acceptable by various religious groups all over the world as well as the sanitary aspects regarding the animals such as the epidemic of mad cow disease in cows. Hence, an interest has risen in better utilization of the by-products of fish industry as gelatin sources and therefore, the past decade has seen much attention has been directed to exploration and exploitation of various fish species for their gelatin content and various gelatin extraction processes have been optimized to achieve this goal (Gómez-Guillén et al., 2002; Karim & Bhat, 2009). Worldwide, 78 % of the fish catch is consumed by humans as a main course, more than 21% of fish industry by products account for non-food usage in terms of skin, scales and internal organs (Vannuccini, 2004). As stated by Kelleher (2005), fish industry and processing generates a large biomass of fish waste in the form of skin, bones and fins which accounts for 7.3 million tons/year and is discarded into the environment. This has led to the establishment of fish waste as a potent halal gelatin source which confirms to many socio-cultural norms. Malaysia being a tropical country surrounded by ocean on all sides has many varieties of fish and the fish industry is blooming, thereby releasing large quantities of fish waste which can in turn be used for gelatin production.

Gelatin has shown such acceptance as it is cheap and is readily available. Moreover, its biocompatibility, biodegradability and low antigenicity are added advantages for its wide applications (Elzhogby, 2013). Gelatin is a derivative of collagen, which is the most abundant protein source in animals, thus becoming highly available; it is natural and does not produce any harmful by-products on degradation.

However, a major drawback associated with gelatin is in terms of its molecular weight which can cause irregularity and instability of gelatin derived nanoparticles. Nonetheless, a lot of successful research has been done on gelatin nanoparticles and its variants and have been employed in a range of biomedical applications such as encapsulation of bioactive compounds, targeted drug delivery and sustained release inside the human body (Dwivedi et al., 2012; Garg et al., 2012; Gaihre et al., 2009; Li et al., 2013).

Addition of various bioactive compounds showing promising health benefits such as vitamins, minerals, antioxidants, antimicrobial, biopeptides, probiotics, enzymes, polyphenols and even targeted drugs have become a growing trend in the contemporary food industry, thereby improving the functional and nutritional value of food. Nanoencapsulation of bioactive compounds is widely employed for achieving stabilized food composition and the nanocarrier food systems comprise of lipid or natural biodegradable polymer-based capsules such as albumin, gelatin, alginate, collagen, chitosan, and α-lactalbumin are most often utilized for encapsulation (Reis et al., 2006; Graveland-Bikker & De Kruif, 2006). Since the delivery of any bioactive compound to various sites within the body is directly affected by the particle size (Kawashima, 2001; Hughes, 2005) nanoparticles improve the bioavailability, delivery properties, and solubility of the nutraceuticals due to more surface area per unit volume and thus their biological activity and allow them to enter the bloodstream from the gut more easily. Nanoencapsulation also protect the bioactive compounds in the digestion stream from oral and rapid intestinal degradation until their release at targeted sites (Gouin, 2004).

Epidemiological studies have established bioactive peptides derived from major protein sources such as meat, milk, egg, soybeans, fish, nuts, legumes with numerous health benefits such as antihypertensive, antioxidant, anti-inflammatory, antimicrobial, immuno-modulatory and other biologically relevant activities(Liu, 2004; Pan et al., 2009; Sacks et al., 2006). Many of such bioactive peptides (biopeptides) are potent antioxidants and as oxidative stress is the main cause of the onset of various chronic diseases such as various forms of cancer, tumor, hypertension, artheritis, antioxidant biopeptides could be a potential remedy to prevent or delay the onset of such diseases. Nanoencapsulated antioxidant biopeptides are highly permeable through the human intestines where fast degradation and better uptake of peptides into the blood stream takes place and therefore incorporation into food systems can provide with many health benefits.

1.2 STATEMENT OF THE PROBLEM

Food and pharmaceutical industry worldwide are experiencing an exponential demand for gelatin due to its wide attributes as a natural, nontoxic, biodegradable, readily available and cheap polymer. Gelatin polymer has also gained wide popularity for production of nanoparticles with numerous available active sites for attaching targeting molecules and drug or nutraceutical delivery systems aiming to improve the therapeutic effects, targeted delivery and reduce the side effects. While mammalian sources such as pigs and cows are the main gelatin sources, alternatives are required to meet the socio-cultural and health concerns associated with it. Moreover, recent outbreaks such as mad cow disease in cows and the nonhalal status of pigs, there has been a certain push to further the demand of non-mammalian gelatin sources. Therefore, fish skin gelatin extracted can be a potential alternative exhibiting the same rheological and chemical properties as mammalian gelatin (Irwandi et al., 2009). Fish industry generates a lot of by-products (7.3 million tons/year, DoF Annual Fisheries

Statistics, 2014) in the form of skin, bone and fins which can provide an economically beneficial way to produce gelatin. Consequently, research is required to investigate an increased utilization of collagenous fish waste for the production of gelatin nanoparticles and its application in the functional food industry.

Upon enzymatic degradation proteins break into biopeptides which can currently centered focus of research in the functional food industry as biopeptides exhibit various biological roles such as antioxidants, antimicrobials, antifungal, and even as potent drugs and thereby can prevent or delay the onset of cellular damage due to free radical oxidation (Nimalaratne et al., 2015). These biopeptide fractions can be incorporated, suspended and dispersed or encapsulated into different forms such as emulsions, liposomes, nutraceuticals, and other edible biopolymers to gaining their optimum functionality, bioavailability, stability and targeted effectiveness (Amar-Yuli et al., 2010; Livney, 2010; Patel & Velikov, 2011; Elzoghby et al., 2012). While the major protein sources in the industry are meat, milk or fish sources, various oil producing seeds such as sunflower, corn, canola, palm kernel etc have exceptionally high protein content left behind after the oil extraction. The meals after oil extraction can be successfully hydrolyzed to yield numerous peptide fractions with antioxidant ability and can be a commercial way of consuming the left over from the oil industry. Therefore, in addition to solving environmental issues, the more complete utilization of the oil meal as a source for inexpensive protein for human needs could also respond to continuously increasing worldwide demand for proteins (González-Pérez et al., 2005; González-Pérez & Vereijken, 2007). The use of fish gelatin nanoparticles to encapsulate antioxidant biopeptide would give the food and pharmaceutical industry an ingredient with greater functional flexibility and permit the industry to more easily maintain kosher/halal status, and give the consumer new opportunities for functionally developed foods and provide more religiously acceptable foods.

1.3 RESEARCH PHILOSOPHY

There is an exponential need for halal sources of gelatin especially fish, not just in the religious sectors but worldwide due to the various diseases and non halal status associated with mammalian gelatin. Fish gelatin has shown promising results in nanoencapsulation of various drugs and peptide molecules due to their targeted delivery, delayed release and minimum side effects. Antioxidant biopeptides derived from the byproducts of oil industry into gelatin nanoparticles could create a new dimension in the food nutraceutical and pharmaceutical industry. Therefore, the philosophy of this research is geared towards establishing fish skin and scales as a potential halal gelatin alternative based on its rheological properties for application as nanocarriers in the food industry. Biopeptides were obtained from defatted oil cakes left behind as byproducts of the oil industry. With the demand for protein on the rise, it proved to be a viable yet cheap alternative to meet the current demands. The protein from the defatted oil cakes was enzymatically hydrolyzed to split into various peptide fractions and the antioxidant peptide sequence was purified. Various enzymes have the ability to clip the parent protein molecule into desired biopeptides fractions. The hydrolyzed protein isolate showing the highest antioxidant activity was encapsulated in the gelatin nanoparticle so produced and the loaded nanoparticles were studied for various factors. It was essential to analyze the morphology of the particles, their cellular uptake and digestion in the gastro intestinal tract. An investigation of the cytotoxicity of the gelatin nanoparticles loaded with antioxidant biopeptides and the viability of the cells was tested to confirm its safe use in the food industry. As the antioxidant loaded gelatin nanoparticles were rendered safe for human consumption based on the cytotoxicity tests, their food application was tested by adding them to a yoghurt sample.

1.4 SIGNIFICANCE OF THE RESEARCH

Industries, such as those related to food, agricultural, pharmaceuticals, cosmetics and photography have increased their efforts for obtaining gelatin based nanocarries such as polyphenols, vitamins, carotenoids, proteins, drugs and vitamins from sources other than bovine or porcine origin due to their major constraints and socio cultural aspects. With the ever growing demand of gelatin due to biocompatibility and availability, production and utilization of fish based gelatin not only satisfies the need of the industry but also serves as a means to utilize the discarded byproducts of the fish industry (Silva et al., 2014). One of the major applications of this fish based gelatin is in nanotechnology for encapsulating bioactive compounds and pharmaceutical agents and their targeted release in the body.

In recent years, there has been great interest in finding new antioxidants from natural sources for use in food and medicinal materials to replace synthetic antioxidants. Vitamin C, α-tocopherol and phenolic compounds, which are present naturally in vegetables, fruits and seeds, possess the ability to reduce oxidative damage associated with many diseases, including cancer, cardiovascular diseases, atherosclerosis, etc. Hydrolyzed proteins from many animal and plant sources have been found to possess antioxidant activity, and many sources of bioactive peptides have been exploited including the defatted oil meal cakes. Bioactive peptide is usually inactive when exists as a part of the parent protein but can be released during food processing and enzymatic hydrolysis. The size and composition of a peptide determines its antioxidant and free radical scavenging properties (Nimalaratne, 2014). Thereby, formulating such biopeptides into a nanoparticulate system can improve its bioavailabilty into the cellular components (Ribeiro et al., 2010). Keeping that in consideration, the current study set up a new nanocarrier system containing antioxidant biopeptides which can pose many therapeutic uses. Since nanoparticle formation by desolvation is a self-charge neutralization process, the effective size of the nanoparticles is bound to be dependent on the pH of the preparatory solution (Saxena et al., 2005). Among the suitable compounds, the gelification properties of gelatin as well as the strong dependence of gelatin ionization with pH made this compound an interesting candidate to be used to the effective intracellular delivery of active biomacromolecules (Nahar et al., 2008). An optimum desolvation process was established for maximum biopeptide uploading dependent on three factors namely pH (2-12) (Nahar et al., 2008; Saxena et al., 2005), glutaraldehyde concentration which helps in crosslinking the nanoparticles and stability and biopeptide concentration(0.1-1.0 mg/ml) (Park et al., 2004; Jiang et al., 2015; Vanvervoot & Lugwig, 2004; Ofokansi et al., 2010). The cellular uptake of the nanoparticles and their cytotoxicity was determined. Once the cytotoxicity was determined, yoghurt sample was prepared which was fortified with gelatin nanoparticles to establish the food application of antioxidant loaded gelatin nanoparticles (GNPs) produced. With the acceptance of biopeptides obtained from oil industrial byproducts as antioxidant agents, and encapsulation in GNPs, it has presented a new dimension to the current usage of gelatin nanoparticles obtained from fish.