M.Sc. PROJECT AND DISSERTATI ON

ON

ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPONENT FROM PEANUT

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

SCHOOL OF AGRICULTURE

LOVELY PROFESSIONAL UNIVERSITY

INDIA

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CERTIFICATE

This is to certify that **Thokchom joshila Devi**(Registration no. 11712486) has personally completed M.Sc. pre dissertation entitiled **"ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPONENT FROM PEANUT"** under my guidance and supervision. To the best of my knowledge, the present work is the result of hid original investigation and study. No part of dissertation has ever been submitted for any other purpose at the university.

The project report is appropriate for the submission and the partial fulfilment of the conditions for evaluation leading to the award of Master of Food technology and science.

Date: May, 2018

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Declaration

I hereby declare that the work presented in the pre- dissertation report entitled "ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPONENT FROM PEANUT" is my own and original. The work has been carried out by me at School of Agriculture, Lovely professional university, Phagwara, Punjab, India; under the guidance of Er. Poorva Sharma, Assistant professor at school of Agriculture, Lovely professional university, Phagwara, Punjab, India for the award of the degree of Master of Food Science and Technology.

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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

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PROJECT AND DISSERTATION PLAN PROPOSAL

OF The proposed Research Project for the degree of

MASTER'S OF SCIENCE

IN

Food sciences and technology

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INTRODUCTION

Peptide is a compound containing of two or more amino acids connected in a chain, the carboxyl group of each acid being linked to the amino group of the next by a bond of the type -OC-NH-. The most significant characteristics of a peptide, besides its length determined by number of amino acids in the chain, is it arrangement (Draelos, 2010). The arrangement is the accurate order in which the numerous amino acids are connected together. Peptides implement several significant biologic roles. The word hormone comes from Greek and means messenger. Hormones can be classified as peptide hormones or steroid hormones. Both steroids and peptides performance in like way. Some trouble, either internal or external, tips to the release of a small quantity of peptide in a cell, blood, gland, or in some other organ (Sederma SAS, Le Perray en Yvelines, 2010). The peptide then travels in the body until it cooperates with a target receptor any on the cellular surface or inside the cell nucleus after having entered the cell wall. Bioactive peptides are the organic substances formed by amino acids linked by peptide bonds and they are protein synthesized in the cell in the form of large pre-propeptides. Bioactive peptides are released through enzymatic proteolysis (gastrointestinal digestion, in vitro hydrolysis by proteolytic enzymes) of proteins and also through food processing (cooking, fermentation, ripening). Bioactive peptides are recognized for their skill to prevent protein-protein interactions due to their small size and specificity. Nature leftovers the major source of bioactive peptides since plants, animals, fungi, microbes and their products contain many proteins in them(Eric Banan-Mwine Daliri, Deog H. Oh and Byong H. Lee,2016). Generally bioactive peptides having 3-20 amino acids and both animal and plant protein containing potential bioactive sequences. They are extranutrinional constituents that typically occur in small quantities in foods. Many bioactive compounds have been discovered and evaluate their effects on health, phenolic compounds, including their subcategory, flavonoids are present in all plants and have been studied complete in cereals, legumes, nuts, olive oil, fruits and vegetables, tea and red wine. Proteins could be fermented by bacteria. The bacteria proteolytic enzymes hydrolyze the proteins to statement peptides into the hydrolysate. The hydrolysates are then verified in vitro for a biological activity. If the hydrolysates show moral bioactivity, they are then confirmed finished in vivo testing. The biologically active hydrolysate can then be established into a functional food. The bioactive peptides in the hydrolysates can also be separated and purified into nutraceuticals for non-pharmacological therapy (Koyama, M.; Hattori, S.; Amano, Y.;

Watanabe, M.; Nakamura, K2014). Bioactive peptides are identified for their high tissue affinity, specificity and efficiency in helping the health. For this purpose, the examination for food-derived bioactive peptides has enlarged exponentially (Eric Banan-Mwine Daliri, Deog H. Oh and Byong H. Lee,2016).

Peanut *(Arachis hypogaea*) is widely harvested, planted and consumed all around the world. Oil is also major constituents in peanut and extracted oil from peanut is highly nutritional valuable due to its high protein content. About two thirds of the world production is crushed for oil, and the residual one third is consumed as nutrition (I. D. Akcali, A. Ince, and E. Guzel, 2006). The fundamental properties of peanuts underneath thought will be their geometrical shape and the linked dimensions, specific mass and friction coefficients of hulled peanuts, kernels and shells. Specific mass and friction coefficient depend mainly on the geometrical shape and the related dimensions. It is required to know specific mass for a number of good details. For the storage, handling and the processing of the product specific mass is required (I. D. Akcali, A. Ince, and E. Guzel,2006). . Peanuts are also known as groundnuts and it is a legume crop that grows in the tropical and sub-tropical regions in the world. Consuming peanut is good for our health and beneficial for the skin and hair because of the essential nutrients such as vitamins, minerals, antioxidants, essential fatty acids etc. present in them. But in another way consuming too much of peanut is also not good they can causes many side effects to our body. One of the most concerning toxins related to the peanut is Aflatoxin, which is a carcinogenic that increases the risk of liver cancer and it also reduces the growth rate in children

SCOPE OF THE STUDY

People now-a- days are so busy to their work due to which they don’t get proper balance diet food for the proper growth and maintenance of the body. The lack of proper nutrition and balance diet food, people are facing so many health problems like malnutrition, heart diseases, obesity etc. the scope of this study to isolation and characterization of the bioactive components and the peanut itself high in nutritive components such as protein, vitamins, minerals, fiber which can helps in to prevent malnutrition problem and also acts as flavoring agent to the product.

REVIEW OF LITERATURE

Functional/physiological properties of bioactive peptides

Antimicrobial activity

Peptides of microorganism, animals and plants have antimicrobial property. Antimicrobial peptides show inhibitory effect against microbe-caused food weakening and attack of a wide range of pathogens containing bacteria, fungi, and virus. The action mode and effectiveness of these biologically active peptides and antimicrobial agents vary be subject to on their structural features.

Animal derived antimicrobial peptides show inhibiting against a much larger spectrum of microorganisms than those formed by bacteria. Antimicrobial peptides have certain common feature. Most antimicrobial peptide are composed of less than 50 amino acids with 50% hydrophobic amino acid. The look of loe pH and high salt-tolerant microorganism has been a big challenge for foodborne pathogen control. Bioactive peptides with antimicrobial property as such as those formed by lactic acid bacteria (LAB) in fermented foods and many food constituent peptide are good applicants as food additives. A large group of antimicrobial peptides belong to bacteriocins which are ribosomally created and post-translationally changed peptide.

Bacteriocins are classified into numerous classes and sub-classes: class I (Lantibiotics), class IIa (Listeria active or pediocin-like bacteriocins), class IIb (2-peptide bacteriocins),class IIc (sec-dependent bacteriocins), class IId (other class II bacteriocins), class III (thermosensitive proteins), and class IV (complex bacteriocins that require lipid or carbohydrate moiety).

Antimicrobial activity of bacteriocins against pathogenic microorganisms has been elluciated at cellular and molecular levels. The inhibition has been related with the interaction of peptides with membrane and other cellular mechanisms. Eukaryotic organisms such s fungi, animals and plants also harvest bioactive peptides with antimicrobial activity.

Antioxidant activity

Protein, protein hydrolysates, singular peptides and amino acids consume antioxidant properties. Proteins from a variety of plant, animals and microbial sources such as gluten, egg albumin, casein, soy protein and yeast protein also demonstration antioxidant activity. In some cases, peptide fraction or protein hydrolysates showed greater antioxidant activity than whole protein or amino acid mixtures that shows the major role of peptides in antioxidant action of protein. Matoba shows the formation of peptide was more critical than maintaining protein structure even heating fixed not the antimicrobial efficiency of protein.

Protein digests have varied antioxidant activities depending on the peptide structure i.e, size of the peptides and this amino acid sequence which are influenced by the source of protein and conditions and conditions of the hydrolysis process involved.

Soy protein are obtained from native or heated soy protein by different enzymes, such as pepsin, papain, chymotrypsin. Alcalase, protamex and flavourzyme resulted in different degree of hydrolysis ranging from 17 to 20.6% and antioxidant activity ranging from 28 to 65% measured as inhibition against formation of thiobarbituric acid reactive substances (TBARS) in a liposome – oxidizing system. (Chen et.al) identified six antioxidant peptides from the proteolytic digest of soybean and peptides were composed of 5.16 amino acid residues with hydrophobic amino acid V or L at the N- terminal position and P, H or Y in the sequence.

(Saito et al, 2003) constructed 2 tripeptide libraries and investigated their antioxidant capacity by different mean.

(Cheison et al, 2007) prepared protein hydrolysates from whey using both a single and a 2-stage enzymatic membrane reactor.

(Hernandez –Ledesma et al, 2007) evaluated the radical scavenging activity of several β-Lactoglobulin derived peptides and suggested the position of amino acids.

Anticancer activity

Proteins, peptides, and amino acids have been implicated in preventing the development of the different types of cancer. Bowman Birk protease inhibitor (BBI), a water soluble protein insulated from legumes and many monocotyledonous seeds, has shown anticarcinogenic activity in vitro

and animal models. Soybean Kunitz trypsine inhibitor was reported to suppress ovarian cancer cell invasion by blocking urokinase upregulation. From bovine milk, bovine lactoferrin and lactoferrician were able to inhibit lung matastasis. Lectins from mistletoe extract induced powerful anticancer effects in mice inoculated with tumor cells.

Numerous peptide in different sizes from various sources have been indicated to render an anticancer effect. Lunasin, a novel chemopreventive peptide from soybean has been found to suppress chemical carcinogen and viral oncogene-induced transformation of mammalian cells and inhibit skin carcinogens in mice. It exhibits an inhibition effects against core histone acetylation in mammalian cells suggesting its involvement in chromatin modification a process implicated in cell cycle control and suppression of carcinogenesis. It selectively kills cells being transformed or newly transformed by binding to exposed deacetylated core histones, disrupting the histone acetylation-deacetylation dynamics and leading to the death cells. The affinity of lunasin for hypoacetylated chromatin is attributed to its polyaspartyl structure at the carboxyl end.

Bioactive peptides from soybean with cancer preventive effects have also been reported (Azuma et al.) and (Kanamoto et al.) demonstrated that a high MW fraction (HMF) of proteinase treated soybean isolate suppressed colon and liver tumorgenesis in experimental animals.

A glycopeptide isolated from soybean hydrolysate containing mainly D, E, P, G and L has been shown to be cytotoxic against P388D1 mouse lymphoma cells. (Kim et al.) purified an anticancer peptide from the hydrophobic peptide fraction of thermoase-treated soy protein hydrolysate.

Cholesterol Lowering Effect

The most significant risk factors donating to the development of cardiovascular diseases is hyperlipidemia, specially hypercholesterolemia. In prevention and treatment of hypercholesterolemia, numerous synthetic drug and natural extracts with cholesterol- lowering effect have been explored and a large body of literature indicates that the proteins from soybean can reduce blood cholesterol level in experimental animal models as well as in human subjects. An early clinical study clearly revealed that the substitution of animal proteins with soy protein resulted in a 22-25% decrease in LDL cholesterol and a 20-22% decrease in total cholesterol in hypercholesterolemic patients. The U.S. Food and Drug Administration (FDA) recommended a

daily intake of 25g of soybean protein foe lower level of serum cholesterol and reduction in the risk of cardiovascular disease.

According to (Adams et al.2002, Ali et al.2004, and Zhan and Ho 2005), the hypocholesterolemic effect of soybean was first attributed to the presence of isoflavones. Since ethanol-washed isoflavone-free soy protein showed less cholesterol-lowering capacity than that containing isoflavones.

(Fukui et al.2002) his experiment indicated that isoflavones alone did not exhibit a cholesterol-lowering effect. Hence it was speculated that the iaoflavone protein interaction may contribute to the hypocholesterolemic effect of soy proteins. It has been demonstrated that soy peptides may be responsible at least in one part, for the hypocholesterolemic property of soy protein, based on the observation that soy protein hydrolysate showed a stronger serum cholesterol lowering effect than intact soy protein. (Sirtori et al.1993) have also reported that 7S globulin, a major storage protein in soybean, decrease plasma cholesterol concentration by 35% in rats.

Zhong et al.2007 investigated that soy protein hydrolysates prepared with different enzymes at various degrees of hydrolysis(DH) and found that soy protein hydrolysate produced with Alcalase at DH 18% had the highest hypocholesterolemic activity in mice and decreasing the total serum cholesterol and LDL level by 24 and 34%.

BIOACTIVE COMPOUNDS

PHENOLIC

Phenolic compounds, normally denoted to polyphenols and are present in all plants and in our diet. Many phenolic compounds partake antioxidant properties. Examples of phenolic compounds are tannins, flavonoids and phenolic acids with a C6 ring structure. Most common polyphonic compounds existing in plant food are flavonoids.(Sampson et al,2002) recently reported an analysis of specific flavonoids in fruits and vegetables grown in the United States and the Netherlands. The vast majority of plant phenolics are simple phenols and flavonoids. Flavonoids can be classified into 13 classes comprising greater than 5000 compounds. Flavonols, flavones and their glycosides are the common in flavonoids. The primary phenols in cereals and legumes are flavonoids, phenolic acids, and tannins. The major polyphenols in wine include phenolic acids, anthocyanins, tannins, and other flavonoids. The most abundant phenolic compound in fruits is

flavonols. Nuts having high contain of tannins. Olive oil contains both phenolic acids and hydrolyzable tannins.

PHYTOESTROGENS

Phytoestrogens or estrogenic compounds in plants, are divide into 3 main classes; isoflavonones, coumestanes and lignans. They all are diphenolic compounds by structurally that are similar to estrogen and as might be probable fix to the estrogen receptor. They both act as partial estrogen agonists and antagonists, having similar opposing actions compared with the estrogen. In peas and beans at low concentration, coumestrol is found in Lucerne, alfalfia and clovers. The primary dietary source of lignans is flaxseed oil, but it can be found in varying concentrations in soybeans, seaweed, whole grains, fruits and vegetables. Lignans are the most ubiquitous phytoestrogens because they exist as minor constituents of many plants. The most extensively studied with respect to CVD are isoflavonoids. Source of isoflavonoids are soy foods, and many studies with human and nonhuman primates have been conducted evaluating the effects of soy food and consistency of the soy foods on numerous CVD risk factors(Anthony MS, Clarson TB, Hughes CL Jr, Morgan TM,1996). Soy foods have been shown to have favorable effects on plasma lipids and lipoproteins. A meta-analysis of 38 clinical studies reported that total cholesterol was decreased by 9%, LDL cholesterol by 13% and triglycerides by 11% when an average of 47 g of soybean protein was consumed, with a greater response observed in subjects having a higher baseline cholesterol level. (Crouse et al, 1999)

PEANUT

Peanut (*Arachis hypogaea*) is a legume crop grown mainly for its edible seeds. The peanut also known as the groundnut and it is widely planted, harvested and consumed all around the world. The peanut belongs to the family Fabaceae; this is also known as the Leguminosae and commonly known as the bean or pea. Peanut have the capacity for nitrogen fixing bacteria in root nodules so peanut require less nitrogen containing fertilizer and improve the soil fertility, making them valuable in crop rotation(Suchoszek-Lukaniuk et al. 2011). Peanuts have publicized as a functional food with numerous functional components like Coenzyme Q10 which defends the heart through the period of absence of oxygen example high altitude sand clogged arteries(Shalini S. Arya1 & Akshata R. Salve1 & S. Chauhan,2007).

FAT

According to the American peanut council (APC), peanut contains about 50 % monounsaturated fatty acids (MUFAs), 14%saturatedfattyacids and 33%Paraformaldehyde (PFAs) which is good for heart (Feldman 1999). Peanut products are more beneficial for heart when compared to other low fat diets. Monounsaturated fat peanut diets helps in lowering body cholesterol by 11 % and bad low density lipoprotein cholesterol by 14 %. There is strong evidence supporting an association between monounsaturated fat as well as overall nut intake helps in the cardiovascular diseases (Matilsky et al. 2009).

Protein

Peanuts contains all the essential 20 amino acids in significant amount and they are good source of the amino acid arginine (USDA 2014). According to Protein Digestibility Corrected Amino Acid Score (PDCAAS) legume proteins such as soy proteins and peanut proteins are nutritionally equivalent to eggs and meat for human body growth (FAO 2002). It can be used as an ingredient for protein fortification according to amino acid profile of the peanut (Yu et al. 2006). Proteins in peanuts is plant based, it contains components like fiber, bioactive components that provides positive health benefits. The peanut proteins good emulsifying activity, excellent water retention, foaming capacity, emulsifying stability, high solubility and can be widely used in the food industry (Wu et al. 2009).

Peanut digestibility

Peanuts contains are highly digestible components. The protein of peanut is more digestible as compare to animal protein (Singh and Singh, 1991). It contains amino acid i.e. lysine, methionine, threonine (Venkatachalam and Sathe, 2006). Fat digestibility depends on the structure of different fatty acids. Since peanuts are legumes ,they contain antinutritional components such as phytic acid which decreases the bioavailability of other nutrients, but they contain in less amount in peanuts as compare to other legumes such as soybean (Schlemmer, 2009). The fiber in peanuts is mainly insoluble and contains less amount of soluble fiber (Higgs 2003).

Fibre

According to Food and Drug Administration they are good source of fiber. Sucrose and starch constitute the major while reducing sugars constitute the minor proportion of the peanut carbohydrates (Tharanathan et al. 1975). And due to this they have low glycemic index (GI) and glycemic load (GL) (Foster and Powell 2002).

Scientific Classification

Kingdom:	Plantae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Tribe:	Dalbergieae
Genus:	Arachis
Species:	*A. hypogaea*

Binomial name

Arachis hypogaea

Parts

Parts of the peanut include:

1. Shell – outer covering, in contact with dirt
2. Cotyledon – main edible part
3. Seed coat – brown paper-like covering of the edible part
4. Radicle – embryonic root at the bottom of the cotyledon
5. Plumule – embryonic shoot emerging from the top of the radicle

Benefits of peanut milks:

Peanut milk be responsible for some nutritional profits we won't get from cow's milk. But it does not have real milk, this drink is made by mixing peanuts with water and adding sweeteners or flavors such as cinnamon.

I. Cholesterol-reducing Nutrients- in spite of its nutritional benefits, peanut milk should be consume in control because one cup takes about 214calories, with 162 of the total calories coming from fats. Maximum of the lipids in peanut milk contain of healthy unsaturated fats that reduces blood levels of bad cholesterol and increase good cholesterol. Peanut milk recollects the nut's natural soluble fiber, which also helps reduces cholesterol.

II. Benefits from Magnesium- Magnesium helps hundreds of metabolic procedures in body, where it reliefs produce energy and safeguards muscles, nerves and heart keep in work. It also helps reduce blood pressure, and for strong bones it is required.

III. Antioxidant Activity From Vitamin E- When we abstract fats, they're put inside an outer cover made from fats and proteins. These lipoproteins carrying cholesterol and other fats from sensitive molecules commonly known as free radicals. By deactivating free radicals earlier they cause harm vit.E helps preserve the physical integrity of lipoproteins.

IV. Maintaining Metabolism With Vitamin B-6: Our body be subject to the vitamin B-6 to active other than 100 enzymes that trigger metabolic reactions. Some of these B-6-dependent enzymes relief kind hemoglobin for red blood cells and create amino acids and neurotramsitters. Vitamin B-6 also reliefs convert an amino acid – homocystein, which is useful substance.

OBJECTIVES

- To optimize the extraction of milk from peanut.
- To analyze the physico-chemical properties of extracted from peanut.
- To determine the proteolytic activity of probiotic strain.
- To check the antimicrobial activity of the fermented milk.

MATERIALS AND METHODS

CHEMICALS

All chemicals used for the present study were of analytical grade. The routine chemicals were procured from Hi media, SRL, Sigma and CDH and are as follows:
Lactobacillus MRS broth, Lactobacillus MRS agar (Hi media), Cysteine hydrochloric acid (SRL), L- leucine (Hi-Media), Peptone (Difco), o-phthaldialdehyde (Hi-Media), β-mercapto-ethanol (Sigma-aldrich), Sodium tetraborate (Hi-Media), Methanol (SRL), *n*-butanol (SRL), OPA solution Acrylamide (Sigma), Bis-acrylamide (Sigma), Glycerol (SRL), Coomassie brilliant blue (SRL), Bromophenol blue (SRL), Glacial acetic acid (Qualigens), Potato dextrose agar (Hi-Media), D-Glucose (SRL), Sodium phosphate monobasic anhydrous (SRL), Sodium phosphate dibasic dihydrate (SRL), Hydrochloric acid (Rankem).

PLASTIC WARES AND GLASS WARES

Micro-centrifuge tubes ,storage bottles,etc. will purchas from Tarsons. Petri dishes were procured from oxygen. Measuring cylinders, conical flasks, beakers, glass bottles, test tubes etc. were procured from M/s. Borosil Prior to use, all the glass wares were cleaned by washing with a mild detergent followed by rinsing with tap water and finally with distilled water and autoclaved at 121 ^{o}C, 15 psi for 15 minutes. Micropipettes (Eppendorf), syringe filters (Millex GV), syringes (Dispo Van), vials, glass pipettes, parafilm and aluminum foil etc. were also used.

RAW MATERIALS

Peanut will be use as substrate for preparation of bioactive were procured from local market of Jalandhar.

Bacterial strains and Growth condition

Probiotic strains *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus delbrueckii* were procured from Microbial Type Culture Collection (MTCC), Chandigarh. All LAB strains were maintained as frozen stock at -80°C in de Man Rogosa Sharpe (MRS) broth with 15% glycerol. Cultures were propagated twice in MRS broth and incubated at 37°C prior to use.

Proteolytic activity of assay

Qualitative assay for proteolytic activity

Proteolytic activity of all probiotic strains will be examine by the method given by Beganovic et al., 2013. Agar well diffusion method by using peanut milk agar will use for the assay. Actively grown culture of probiotic strains will place in the well of skim milk agar plate at the centre of plate and incubate at 37°C for 4h. After 4h incubation absence or presence of clear zone around the agar well inoculated with a particular strain will record.

Strain compatibility

Strain compatibility was tested as suggested by Pescuma et al. (2010).

Preparation of fermentation medium

For the preparation of fermentation medium peanut will use. Microbial fermentation method was used for the production of bioactive peptide. During fermentation, have to check the pH fermentation medium, titrable acidity, proteolytic and hydrolytic assessment at 2h interval.

pH and titrable acidity

The titrable acidity was determined by measuring the produced lactic acid by titrating 20 ml sample with 0.1 N NaOH to pH 8.2 in the presence of phenolphthalein as an indicator. The amount of NaOH used (milliliter) was multiplied by two, and titratable acidity was thus obtained in Soxhlet-Henkel degrees (ºSH) while the volumetric productivity was expressed in g $l^{-1}h^{-1}$, calculated by multiplying the ºSH by 0.225 and dividing by the fermenting time. (Bulatovic et al., 2012, Varga, 2006).

Moisture content (AOAC, 2010)

Peanut milk (2ml) will dry in a clean, dry and pre-weighed petri dish and keep in with lid open at 100-105°C for 1-3 hours in hot air oven till constant weight. After cooling in desiccator, loss in weight was calculated as moisture of sample and expressed as per cent moisture.

$$\text{Moisture \%} = \frac{\text{Weight of fresh sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of fresh sample (g)}} \text{x} 100$$

Alcohol test

Alcohol test will perform as per the method given by Kuthu *et al.,* 2013. 10ml of sample will take and mix with 5ml of alcohol and checked whether milk will clot or not. This test will perform to check the heat stability of sample

Fat test

Fat will estimate by using Gerber method by using Butyrometer.

Clot on boiling (COB)

COB test will perform as per the method given by Kuthu *et al.,* 2013. 5ml of sample will take in test tube and heat for few minutes. This test will perform to check the coagulation in the milk sample.

TSS (total soluble solid)

Total solids will determine by subtracting the moisture %.

Protein test

Protein content will be determine by using Kjeldahl method.

Quantitative assay for proteolytic activity

Proteolytic activity of probiotic bacteria in the fermented sample was determined by using the *o*-phthaldialdehyde (OPA) test. The increase in optical density at 340 nm relative to the control was determined by using the spectrophotometer (Pescuma et al., 2010).

Principle

α – Amino group released by hydrolysis of protein react with *o*-phthaldialdehyde and β-mercaptoethanol to form an adduct that absorbs strongly at 340 nm. The absorptivity is similar for all α – amino groups.

Preparation of OPA solution

For the preparation of 50 ml OPA solution 2.5 ml of 20% (w/v) SDS (Sodium dodecyl sulphate), 25 ml of 100 mmol/l sodium tetraborate, 40 mg of OPA dissolved in 1 ml of methanol, 100 µl of 2-mercaptoethanol was mixed properly and final volume was made up with distilled water.

Standard curve of L-leucine

For the preparation of standard curve of L-leucine, a stock solution of leucine containing 0.02 g leucine in 10 ml was prepared. Different concentrations of L-leucine covering the range of 2 - 10 µl of stock were prepared in addition to a blank that did not contain L-leucine. Each sample volume was made up to 50 µl by using distilled water and mixed with 1.0 mL of OPA solution. The samples were incubated at room temperature for 5 minutes. The absorbance was determined at 340 nm using spectrophotometer.

Preparation of sample for proteolysis assessment

For the preparation of sample for proteolysis assessment, fermented peanut protein concentrate was incubated with 0.75 mol/l trichloroacetic acid (1:3) at 4ºC for 30 minutes and centrifuged (5000 rpm, 10 min). Supernatant was used to check the proteolytic activity.

Determination of proteolytic activity

Fifty microlitre of obtained supernatant of sample remove was mixed with 1 ml of OPA solution and incubated at room temperature for 5 min. Optical density was taken at 340 nm. Proteolytic activity was expressed as µg leucine released per ml by using the standard curve of L-leucine.

Hydrolytic assessment

Hydrolytic assessment was measured by SDS-PAGE as suggested by Schagger and Von Jagow (1987) with few modifications. Fermented and non-fermented peanut was first treated with SDS (10%) for 10 min at 90ºC temperature, centrifuged at 10,000 rpm for 10 min. Supernatant was collected and 2 µl of each sample was prepared separately in denaturing 4X buffer and heated at

100°C for 5min before electrophoresis. Gel was run in a Tris-glycine buffer at a constant current of 25 mA. After electrophoresis, proteins were stained with Coomassie Brilliant Blue R-250.

Fractionation of peptides

Bioactive peptides were fractionated by using vivaspin centrifugal concentrator by using different molecular weight membrane.

Bioactivity analysis

Various screening assays were used to determine whether the peptide extracts had bioactivity. Three types of bioactivity were analysed: antimicrobial, antioxidant and ACE-inhibitory activities. Antimicrobial activity was measured by using agar well diffusion method against pathogenic bacteria. Antioxidant activity was measured against a free radical DPPH and ACE-inhibitory activity was determined by measuring the amount of hippuric acid produced after the peptide extract was exposed to the ACE and its substrate HHL. All experiments were conducted in triplicate (Pritchard et al., 2010).

REFERENCE

- Bazzano LA, He J, Ogden LG, et al. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am J Clin Nutr. 2002;76:93– 99.
- Bezkorovainy A., Topouzian N. Bifidobacteriumbifidus var. Pennsylvanicus growth promoting activity of human milk casein and its derivatives. Int. J. Biochem. 1981;13:585-590.
- Bounous G., Gold P. The biological activity of undenatured dietary whey protiens: Role of glutathione. Clin. Invest. Med. 1991;14:296-309.
- Brown K. D., Blakeley D. M. Partial purification and characterization of a growth factor present in goat's colostrum. Biochem. J. 1984;219:609-617.
- Calder P.C. Glutamine and the immune system. Clin. Nutr. 1994;13:2-8.
- Chabance B., Marteau P., Rambaud J. C., Migliore-Samour D., Jolles P., Boynard M., Perrotin P., Buillet R., Fiat A. M. Casein peptided release and passage to the blood in humans during digestion of milk or yogurt. Biochimie. 1998;80:155-165.
- Clare D. A., Catignani G. L., Swaisgood H. E. Biodefense properties of milk: the role of antimicrobial protiens and peptides. Curr. Pharm. Des. 2003;9:1239-1255.
- Clare D. A., Swaisgood H. E. Bioactive milk peptides: A prospectus. J. Dairy Sci. 2000;83:1187-1195.
- Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. Washington, DC: Institute of Medicine of the National Academies, National Academies Press, 2002
- Gobbetti, M., Stepaniak, L., De Angelis, M., Corsetti, A., & Di Cagno, R. (2002) Crit. Rev. Food Sci. Nutr. 42, 223–239
- Gobbetti, M., Stepaniak, L., De Angelis, M., Corsetti, A., & Di Cagno, R. (2002) Crit. Rev. Food Sci. Nutr. 42, 223–23
- Hernandez-Ledesma, B., Quiros, A., Amigo, L., & Recio, I. (2007) Int. Dairy J. 17, 42–49

- Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. 1993;342:1007–1011.
- Jauhiainen, T., Ronnback, M., Vapaatalo, H., Wuolle, K., Kautiainen, H., & Korpela, R. (2007) Int. Dairy J. 17, 1209–1211
- Jauhiainen, T., Ronnback, M., Vapaatalo, H., Wuolle, K., Kautiainen, H., & Korpela, R. (2007) Int. Dairy J. 17, 1209–1211
- Jeong, H.J., Lam, Y., & de Lumen, B.O. (2002) J. Agric. Food Chem. 50, 5903–5908
- Kim, S.Y., Je, J.Y., & Kim, S.K. (2007) J. Nutr. Biochem. 18, 31–38
- Kong, B., & Xiong, Y.L. (2006) J. Agric. Food Chem. 54, 6059–6068
- Liu S, Lee I-M, Ajani U. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: The Physicians' Health Study. Int J Epidemiol. 2001;30:130– 135.
- Lovati, M.R., Manzoni, C., Corsini, A., Granata,A., Frattini, R., Fumagalli, R., & Sirtori, C.R. (1992) J. Nutr. 122, 1971–1978
- Mao, X.Y., Ni, J.R., Sun, W.L, Hao, P.P., & Fan, L. (2007) Food Chem. 103, 1282–1287
- Matsumoto, H., Ito, K., Yamagishi, M., Nakamura, Y., & Tokunaga, Y. (2004) J. Jpn. Soc. Food Sci. Technol. 51, 546–553
- Miguel, M., Recio, I., Ramos, M., Delgado, M.A., & Aleixandre, M.A. (2006) J. Dairy Sci. 89, 3352–3359
- Mizushima, S., Ohshige, K., Watanabe, J., Kimura, M., Kadowaki, T., Nakamura, Y., Tochikubo, O., & Ueshima, H. (2004) Am. J. Hypertens.17, 701–706
- Muir, A.D. (2005) Agro. Food Ind. Hi-Tech. 16, 15–17
- Nakano, D., Ogura, K., Miyakoshi, M., Ishii, F., Kawanishi, H., Kurumazuka, D., Chol-Jun-Kwak, Ikemura, K., Takaoka, M., Morigushi, S., Iino, T., Kusumoto, A., Asami, S., Shibata, H., Kiso, Y., & Matsumura, Y. (2006) Biosci. Biotechnol. Biochem. 70, 1118–1126
- Papadimitriou, C.G., Vafopoulou-Mastrojiannaki, A., Silva, S.V., Gomes, A.M., Malcata, F.X., & Alichanidis, E. (2007) Food Chem. 105, 647–656
- Pihlanto-Leppälä, A. (2000) Trends Food Sci. Technol. 11, 347–356

- Qi, W., Su, R., & He, Z. (2007) J. Sci. Food Agric. 87, 461–469
- Sakanaka, S., & Tachibana, Y. (2006) Food Chem. 95, 243–249
- Silva, S.V., Pihlanto, A., & Malcata, F.X. (2006) J. Dairy Sci. 89, 3336–3344
- Silva, S.V., Pihlanto, A., & Malcata, F.X. (2006) J. Dairy Sci. 89, 3336–3344
- Tachibana, N., Matsumoto, I., Fukui, K., Arai, S., Kato, H., Abe, K., & Takamatsu, K. (2005) J. Agric. Food Chem. 53, 4253–4257
- Vermeirssen, V., Van Camp, J., & Versraete, W. (2004) Br. J. Nutr. 92, 357–366
- Zhan, S., & Ho, S.C. (2005) Am. J. Clin. Nutr.81, 397–408
- LintnerK.(2007)Peptides, amino acids and proteins in skin care? Cosmet Toiletries 122, 26–34.
- Rizvi SI, Maurya PK. (2007) Markers of oxidative stress in erythrocytes during aging in humans. Ann N Y Acad Sci 1100, 373–82.
- CraftBD, HargroveJL, GreenspanP, HartleDK, AmarowiczR, PeggR B(2010)Recent Advances in food and flavor chemistry. Food flavor and encapsulation, health benefits, analytical methods, and molecular biology of functional foods, Cambridge, UK: R Soc Chem 283– 296