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DETERMINATION OF THE BIOPRESERVATIVE ACTION OF CHITOSAN NANOPARTICLES ALONG WITH PEA PROTEIN HYDROLYSATE

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Abstract :

The increasing concern of consumers about food quality and safety and the rejection of chemical additives has promoted the breakthrough of biopreservative field. Chitosan, a polysaccharide of glucosamine and NAG linked by β(1->4) bonds, has been studied extensively. In present work, chitosan was transformed into nanoparticles and was fortified with pea protein hydrolysate. The current work can be employed in the field of food safety. This may be preferred over other preservation strategies based on itsnatural, inexpensive, sustainable source, environment friendly process and non toxic nature.

Keywords : Biopreservative, chitosan, chitosan nanoparticles, pea protein hydrolysate, food safety

1. Introduction :

Chitosan is the acquired polysaccharide from deacetylated chitin, which is the major constituent of crustacean exoskeleton. Both ofchitin and chitosan can also exist in fungi mycelia and some insect's exoskeleton. Chitosan has numerous astonishing bioactivitiesincluding antioxidation, metal chelation and inhibition of cancerous cells and microbial pathogens. Chitosan was stated as effectualantibacterial materials with approved GRAS nature. (2)

Nanoparticles synthesized from natural or synthetic polymers, are very effectual with their particles' size range from 10-1000 nm. Polymers NPs show astonishing chemical and physical features, resulting from their effects. Chitosan nanoparticles were proved asnatural materials with excellent physicochemical, biological and structural properties, along with their eco – friendly and bioactivenature. (2)

Chitosan nanoparticles have the properties such as surface and interface effect, along with original chitosan bioactive characteristics.Chitosan nanoparticles can be prepared by multiple methods, but the frequently used is ionotropic gelation using STPP.

Protein plays a critical role in providing the body with essential amino acids for basic nutrition and energy. Additionally, proteins can be a source of physiologically active compounds and bioactive peptides. These peptides remain dormant within the parent protein, however when treated with enzyme or acid or microbial fermentation can activate vast bioactivities. When compared to whole protein, protein hydrolysate that contain a complex mixture of peptides are more digestible and bioavailable. Except for a few, such as soy and rice, plants and pulses have been largely unexplored as sources of protein hydrolysates. While individual synthetically produced peptides may face regulatory and economic hurdles as food preservatives, protein hydrolysates help to overcome these limitations and deliver bioactive peptides in a natural, cost effective way which is more favorable in the industrial setting. (4)

In this study, the aim is to add knowledge around the untapped potential of plants as sources of bioactive hydrolysates. Accordingly, the synthesis of chitosan nanoparticles and their fortification with pea protein hydrolysate was planned for this study.

2. Materials and methods:

2.1. Chitosan extraction :

Shrimp shells were collected and dried. First, the shells were treated with 2 N NaOH, washed with water, then treated with 2N HCl(Demineralization), the flask kept on RT shaker for 24 hrs. Then, washed with D/W and acid discarded. Then, it was treated

with 2.5% NaOH at 100°C for 30 min. (Deproteinization). The deproteinization step was repeated. Then, it was treated with 15% NaOHat 100°C for 30 min. This step yields chitin. Finally, deacetylation was carried out using 84 % NaOH at 100 °C for 1.5 hr.

2.2. Pea protein isolate extraction :

Yellow and green dried peas were collected and grounded into fine powder. 4 g pea in 60 ml D/W was adjusted to pH 9.5. The flaskwas kept at RT shaker for 2 hrs. Then it was centrifuged at 1500 rpm for 20 min. The supernatant was precipitated with HCl (pH=4.5). Then, it was centrifuged at 1500 rpm for 20 min. The pellet was collected and washed with water (pH =7.0)

2.3. Pea protein hydrolysates preparation :

9% (w/w) pea protein isolate was adjusted to pH 8.0. Then, trypsin was added according to the E/S ratio = 0.1% with constant stirring at 55°C. Then, the aliquots were taken at 1,5,10 and 15 min respectively, centrifuged at 2500 rpm. Enzyme inactivation carried out at 90 °C for 10 min.

2.4. Nanoparticles synthesis :

STPP was added to chitosan (1mg/ml in 1% acetic acid solution) (pH= 5.2) dropwise while vigorous stirring. The appearance of opaque solution confirms the presence of chitosan nanoparticles. For, chitosan nanoparticles loaded with PPH, chitosan was mixed with PPH and then STPP was added.

2.5. Antibacterial evaluation of natural products :

Antibacterial activity of chitosan and pea protein hydrolysate was studied against *S. aureus* and *E. coli.*

3. Results and discussion :

3.1. Chitosan extraction :

Chitosan was extracted from shrimp shells and the extraction was confirmed when it was dissolved in 1% acetic acid.

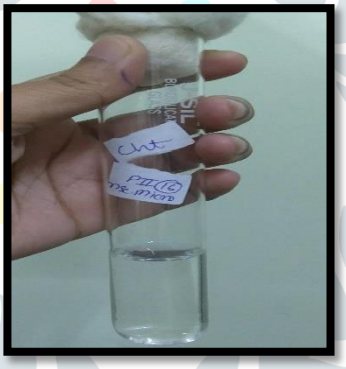

Fig. 1: Chitosan dissolved in 1% acetic acid

3.2. Estimation of protein content in pea protein isolate :

Yellow and green peas were used to prepare pea protein isolate, then the concentration of protein was determined by Folin – Lowry method.

Table 1: Concentration of protein

Sample	Concentration of protein
Green pea protein isolate	590 microgram /ml
Yellow pea protein isolate	1540 microgram /ml

3.3. Nanoparticles synthesis :

Cross linking of chitosan depends on the availability of cationic sites and negatively charged species. During cross linking, it shouldbe noted thatchitosan is stable at acidic pH while pea protein has minimum solubility at lower pH (4-6). Hence, tween 80 should beused as a surfactant. The prepared nanoparticles showed absorption maxima at 400 nm.

3.4. Antibacterial evaluation of chitosan and pea protein hydrolysate:: Antibacterial evaluation was carried out by disk diffusion method

Table 2: Antibacterial activity of chitosan :

Sample	Test organism	Zone of inhibition
Chitosan	*Escherichia coli*	12 mm
	Staphylococcus aureus	10.5 mm

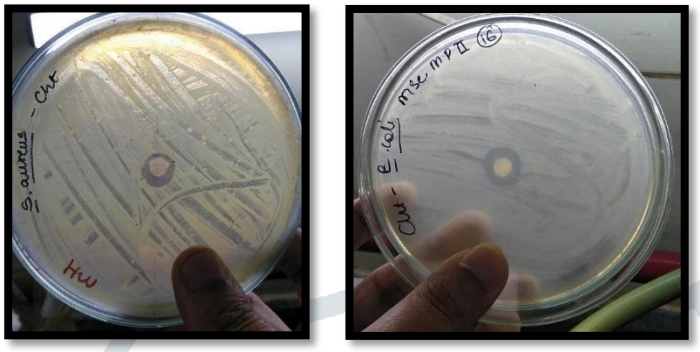

Fig. 2: Antibacterial activity of chitosan against *E. coli* and *S. aureus*

Table 3: Antibacterial activity of pea protein hydrolysate :

Sample	Test organism	Aliquots(min.)	Zone of inhibition(mm)
Green pea protein hydrolysate	*Escherichia coli*	1 min.	10 mm
		5 min.	13.5 mm
		10 min.	15.5 mm
		15 min.	18 mm
	Staphylococcus aureus	1 min.	23 mm
		5 min.	23 mm
		10 min.	22 mm
		15 min.	24.5 mm
Yellow pea protein hydrolysate	*Escherichia coli*	1 min.	13 mm
		5 min.	12.5 mm
		10 min.	11.5 mm
		15 min.	18 mm
	Staphylococcus aureus	1 min.	24.5 mm
		5 min.	28 mm
		10 min.	23.5 mm
		15 min.	21.5 mm

Since, 15 min. aliquots showed better results, it was selected for further study.

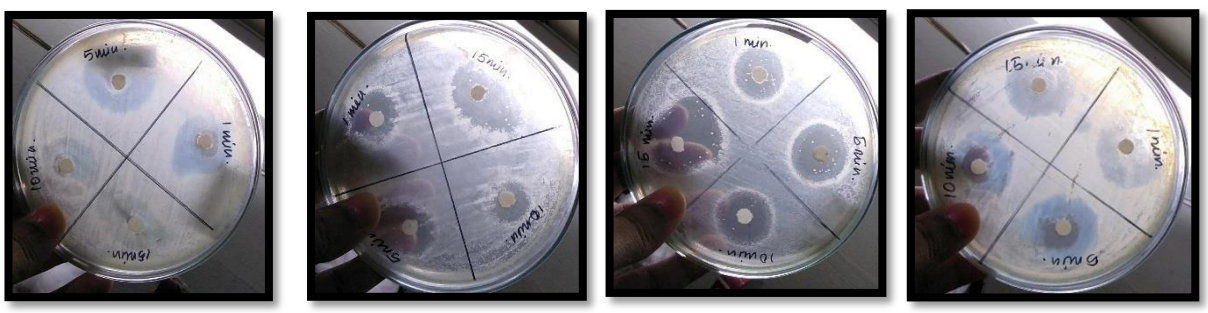

Fig. 3 : Antibacterial activity of pea protein hydrolysate against *E.coli* and *S. aureus*

4. Conclusion :

The aim of this study was to evaluate the biopreservative action of chitosan nanoparticles along with pea protein hydrolysate.

The first component, chitosan is obtained through partial deacetylation of chitin, creating a polysaccharide composed of glucosamine and NAG units linked by β-(1->4) bonds. The presence of amino groups in its structure confers a cationic nature.

The second component is the pea protein hydrolysate. Pulses are sustainable legume crops and their edible dry seeds are rich in proteins and dietary fiber with low fat content. Additionally, pulses contain several bioactive compounds such as polyphenols and saponins. (1)The pea, as the second most important leguminous crop, is rich in proteins (20-25%). The pea protein has been utilizedas emerging plant protein ingredient in plant based foods to replace animal based protein due to its high nutritional value, non GM,sustainable and functional benefits.(5) Protein plays a critical role in providing the body with essential amino acids for basic nutritionand energy. Additionally, proteins can be a source of physiologically active compounds and encrypted bioactive peptides which arecontained with their amino acid sequence.(4)

Protein hydrolysate contain network of peptides which often function synergistically to exert a wide range of bioactivities. It is wellknown that some of the peptides within the pea protein hydrolysate exert their activities through electrostatic attraction, irreversiblepermeabilization and lysis. One of the constraint of using hydrolysate in food product is the possibility of changes in the appearanceand color of the products. But, the problem can be solved using charcoal powder. Protein hydrolysate are likely to be better toleratedand present less adverse impacts on taste when applied to food in comparison to other natural preservative, such as essential oils. Natural oils have also been reported as potentially being able to alter the gut microbiome. This is not a concern with peptides and protein hydrolysate as they are subjected to natural digestion and absorption in the GI tract. (4)

Protein hydrolysate, such as PSH, represent an environmentally friendly, plant based means to preserve food using the naturally occurring bioactive components found within food proteins.

Both the components of the study showed considerable good results individually. When chitosan nanoparticles will be fortified withpea protein hydrolysate, it will enhance the antibacterial activity of chitosan and it can be used in food industry for preservation offood. This could also be used to prepare packaging material, since chitosan has film forming ability when treated with glycerol. Formation of films from protein blends has the potential to produce synergistic properties in the newly formed films.

Both the green as well as yellow pea protein hydrolysate showed higher antibacterial activity against *Staphylococcus aureus* whilechitosan was more effective against *Escherichia coli*. Thus, a combination of both can achieve a greater antibacterial activity, whichcan be employed in food safety.

The use of natural compounds with biocidal activity to fight the growth of bacteria responsible for foodborne illness is one of the main challenges that research in food sector addresses.(3) Hence, the finding in this study can be employed to eliminate pathogenic food – borne bacterial pathogens.

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