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Enhancement and Stabilization of Protein Content through Natural Elicitation and Biopolymer Encapsulation of *Vigna radiata* and *Pisum sativum* sprouts.

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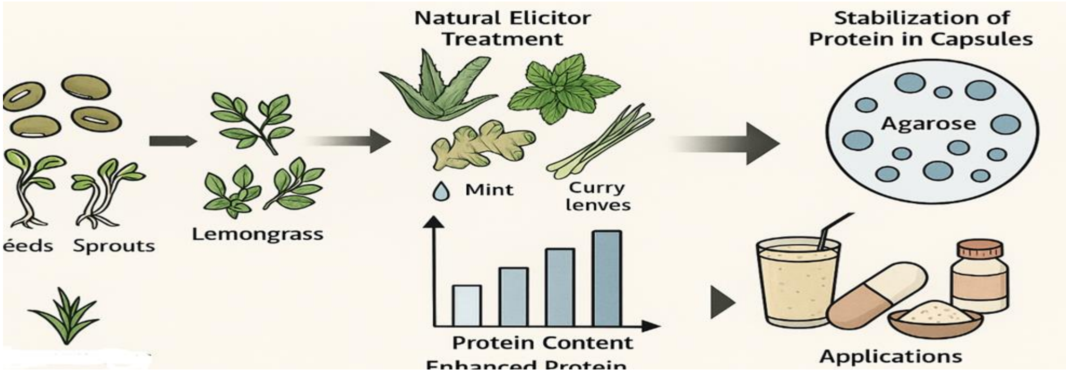
ABSTRACT:

The rising demand for sustainable. Plant based protein alternatives has highlighted the nutritional potential of legume-derived proteins. However, broader adoption remains limited due to undesirable sensory traits, instability and short shelf life. This study explores a dual approach to enhance protein content and improve stability in Vigna radiata (mung bean) and Pisum sativum (Pea) sprouts through natural elicitation and agarose biopolymer encapsulation. Sprouts were treated with different combinations of natural elicitors, including aloe vera, ginger, mint, lemongrass and curry leaves. A 1:1:1:1:1 blend was found most effective in enhancing protein concentration, as measured by the Lowry method. In comparison to untreated controls, pea sprouts showed a 419.65% increase in protein content (from 3.419 to 17.767 μg/mL), while mung sprouts exhibited a 41.70% increase (from 10.814 to 15.323μg/mL). Biochemical assays validated protein quality. The Biuret test confirmed retention of native protein structure with minimal denaturation. The Bradford assay indicated higher levels of neutral proteins, and the Xanthoproteic test confirmed the presence of aromatic amino acids. Proteins were then encapsulated using agarose, which effectively protected them from degradation and facilitated controlled release of bioactive compounds. This encapsulation not only stabilized protein structure but also enhanced shelf life, addressing one of the major limitations of plant-based proteins. Overall, this study demonstrates a synergistic, clean- label and scalable strategy to improve the nutritional and functional properties of legume sprouts. The combined use of natural elicitation and biopolymer encapsulation holds great promise for application in functional foods and nutraceuticals, supporting the transition toward sustainable and health promoting protein sources.



Introduction

Plant proteins are known as important alternatives to animal proteins due to their low impact on the environment, production and health services. Beans like *Pisum Sativum* (Pea) and *Vigna Radiata* (Mung Bean) are rich in protein, essential amino acids, and biological activities, and are widely used in many parts of the world (Boye et al., 2010). Challenges related to limited protein content, reduced stability and short durability have hindered the general use of functional foods (Stone et al., 2015). Awareness of nature presents promising and environmentally friendly techniques to improve vegetable protein biosynthesis and secondary metabolites. Activities such as aloe vera, ginger, mint and Lemongrass are known to activate the metabolic roads related to defense. This improves the accumulation of protein and other beneficial phytochemicals (Baenas et al., 2014; Ramakrishna & Ravishankar, 2011). Not only are they effective, but they also have clean labels and clean trends in organic foods, making them ideal for developing natural products. In addition, the application of packaging to biopolymer-based facilities is based on biopolymers, especially those derived from agarose, which can significantly improve the functional stability of biological activities. Agarose is a biodegradable polysaccharide that exhibits excellent gel formation, providing support and structural protection against heat, oxidation, and enzymatic degradation (McClements, 2018). This ensures protein integrity and allows controlled release. This is extremely important for stable food formulas in the set. This research is important because these two creative strategies have led to overcoming the current limits of the plant protein system and capsules. The emphasis is placed on peas and Mung Bean, and the goal is to create a nutritional research culture, accessible and culturally accepted. The double approach is to improve the protein content and stability and contribute to the development of sustainable protein supplements. In addition, it supports nutritional security and sustainable environmental sustainability, and supports market requirements for clean vegetable labels (Joshi & Kumar, 2015).

Despite the potential advantages of plant protein, its use is often limited by factors such as incomplete records of essential amino acids, decline in digestion and sensitivity to degeneration during storage and treatment. This study aims to investigate the impact of various herbal elicitor combinations—Aloe vera, Ginger, Mint, Lemongrass, and Curry leaves—on the protein content of Pisum sativum and Vigna radiata sprouts, to enhance their nutritional profile (Xie et al., 2022). It also seeks to assess the effectiveness of agarose-based encapsulation in preserving protein integrity and extending the shelf life of both treated and untreated sprouts. Furthermore, the study aims to evaluate and compare the efficacy of different elicitor combinations to identify the most potent formulation for protein enhancement, thereby contributing to the development of functional plant-based protein sources.

Materials and Methods

Plant Material and Elicitor Preparation

Seeds of Pea *(Pisum sativum)* and Mung Bean *(Vigna radiata)* were randomly selected as experimental models for the present investigation as shown in Fig 01.

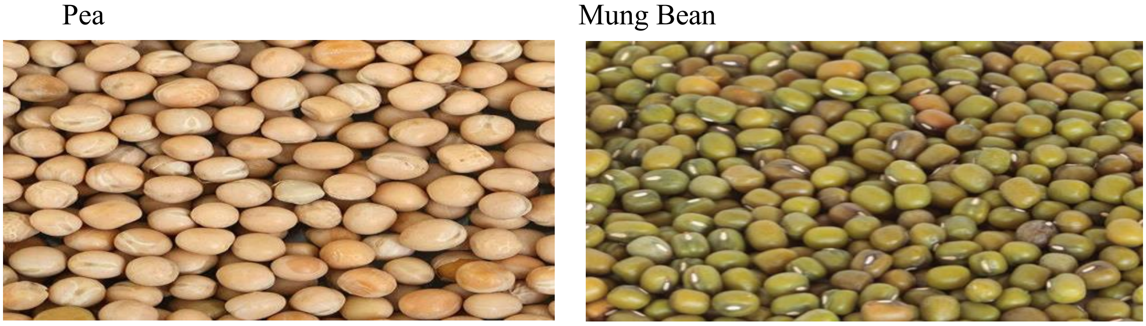


Fig 01: Pea (*Pisum sativum) and* Mung Bean (*Vigna radiata)*

Four different plant materials were selected as elicitors, namely Aloe vera (A) *(Aloe Barbadians)*, Ginger (G) *(Zingiber officinale)*, Curry leaves (C) *(Murraya koenigii)*, Lemongrass (L) *(Cymbopogon citratus)*, and Mint (M) (Mentha spp.), respectively. The elicitor blends were prepared by homogenizing 400 g of plant material with chilled, sterilized water in a chilled mortar pestle. Then the homogenized material was filtered through four layers of muslin cloth. The filtrate collected was made up to 1 liter using chilled, sterilized water (Toro et al., 2022). Table 01 represents the components and their ratio of elicitors.

The seeds were completely washed beneath running tap water, followed by flushing with RO water to remove surface contaminants. The cleaned seeds were poured into a composite watery arrangement of normal elicitors for 6 days at 25°C as shown in Fig 02 (Thappa et al.,2023).

Table 01: Combination of Elicitor

Pea	Mung Bean	Elicitor	Pea	Mung Bean	Elicitor
U1	M1	Water	U6	M6	A+L+C (1:2:1)
U2	M2	A+G+M (1:1:2)	U7	M7	A+L+C (1:1:2)
U3	M3	A+G+M (1:2:1)	U8	M8	A+G (1:2)
U4	M4	A+G+M (2:1:1)	U9	M9	A+G (2:1)
U5	M5	A+L+C (2:1:1)	U10	M10	A+L+C+M (1:1:1:1)

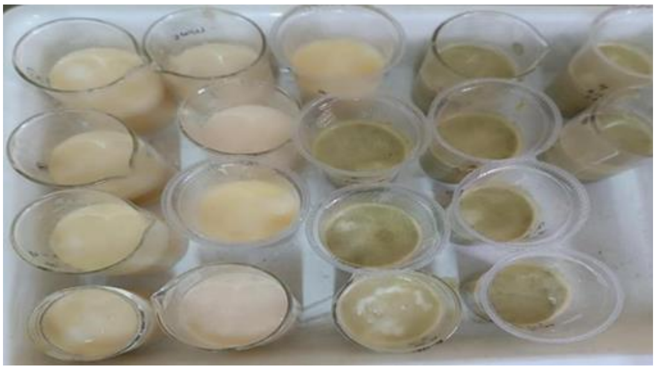


Fig. 02: Seeds soaked in different combinations of elicitors

Protein Extraction

The overnight soaked sprouts were removed from the elicitor combinations, and 1 g of the sprouts was homogenized using 4 mL chilled extraction buffer (pH 7.0). The homogenized mixture was filtered and centrifuged at 12000 rpm at 4°C for 20 minutes. The supernatant containing solubilized protein content was carefully collected and stored at -20 °C until further use in qualitative and quantitative protein assays (www.iitg.ac.in).

Qualitative Protein Estimation

Qualitative assessment of the presence of proteins was performed using three classic colorimetric assays: the Biuret test, the Bradford assay, and the Xanthoproteic test.

Biuret Test: The standard solution was prepared by dissolving 500 mg of BSA in 100 mL of distilled water to obtain a final concentration of 5 mg/ml. The Biuret reagent, consisting of an alkaline copper sulfate solution, was freshly prepared before testing to ensure reactivity and accuracy. Equal volumes of Biuret reagent and protein solutions, including both standards and sample extracts, were pipetted into clean test tubes. The mixtures were incubated at 25°C for 15 minutes to allow development of the violet color, indicating the presence of proteins (hbmahesh.weebly.com).

Bradford Method: The Bradford reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol, followed by the addition of 100 mL of 85% phosphoric acid. The final volume was made up to 1 liter with distilled water, and the solution was filtered before use. A volume of 0.25 mL from each sample was combined with 2.5 mL of Bradford reagent in test tubes. After 5 minutes of incubation at ambient temperature, absorbance was recorded at 595 nm. A blank containing only the reagent and distilled water served as the reference. The method's responsiveness enabled clear differentiation of protein presence across differently treated sprout samples (www.iitg.ac.in).

Xanthoproteic Test: 0.5 mL of protein extract was transferred into a clean, dry test tube, followed by the addition of 0.5 mL of concentrated nitric acid. The mixture was gently heated using a test tube holder over a flame until gas evolution ceased. After allowing the solution to cool to room temperature, the development of a yellow coloration was observed, indicating the nitration of aromatic side chains. A few drops of 10 M sodium hydroxide—prepared by dissolving 40 g of NaOH in 100 mL of distilled water—were added to the cooled solution. The subsequent color shift from yellow to orange served as a positive indication for the presence of aromatic amino acids within the protein sample (https://microbenotes.com/xanthoproteic-test/).

Quantitative Protein Estimation

Quantifiable estimation of extracted protein was done by the Lowry method using bovine serum albumin (BSA) as a standard protein. Standard bovine serum albumin (BSA) solution (30 mg%) was prepared by dissolving 30 mg of BSA in 100 mL of distilled water. Lowry Reagent I was freshly prepared by mixing 100 mL of 2% sodium carbonate solution in 0.1 N NaOH with 1 mL of 1% $CuSO_4 \cdot 5H_2O$ and 1 mL of 2% sodium potassium tartrate (calin sodium). The Folin-Ciocalteu reagent (commercially available) was diluted with distilled water to a concentration of 1N to serve as Lowry Reagent II. A series of standard BSA solutions ranging from 0.1 to 1.0 mL in 0.1 mL increments was pipetted into ten labeled test tubes (S1 to S10). Each volume was adjusted to 1 mL with distilled water to ensure uniformity across all samples. In parallel, sample extracts were prepared and treated in the same manner, using appropriate volumes and adjusting with distilled water to maintain a consistent final volume of 1 ml. To each tube, 5 ml of freshly prepared Lowry Reagent I was added, and the contents were mixed thoroughly. The reaction mixtures were then allowed to stand at room temperature for 10 minutes. Following this incubation, 0.5 mL of diluted Folin-Ciocalteu reagent (1N) was added rapidly to each tube. Immediate mixing was carried out to ensure proper reaction kinetics, and the tubes were then incubated at room temperature for 30 minutes. After incubation, absorbance was measured at 680 nm using a spectrophotometer, with the blank serving as the reference. A standard calibration curve was plotted using the absorbance values of the BSA standards. Protein concentrations in unknown samples were determined by extrapolating their absorbance values from the standard curve (egyankosh.ac.in).

Biopolymer Encapsulation

Biopolymer encapsulation using agarose offers a reliable technique for immobilizing protein derived from sprouted seeds, creating a controlled environment that preserves its integrity and functionality.

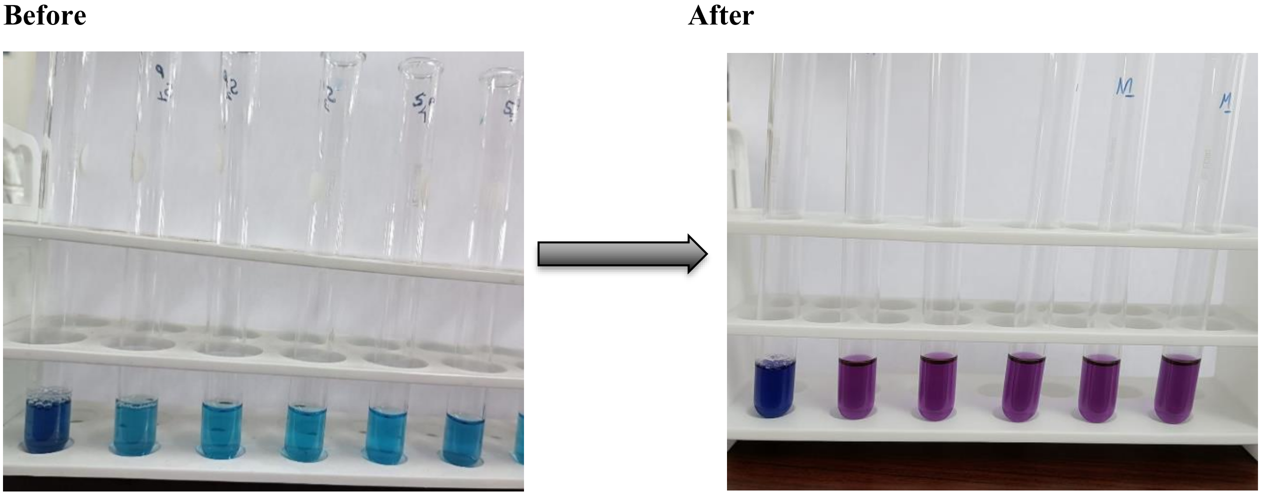
To initiate the encapsulation process, a 5% (w/v) agarose solution was prepared by dissolving 5 g of agarose powder in 100 ml of distilled water with continuous heating and stirring until a clear, homogeneous solution was obtained. This solution was then subjected to autoclaving at 121 °C for 20 minutes to ensure sterility. Following sterilization, the agarose was allowed to cool to approximately 35 °C, reaching a semi-liquid state suitable for blending with biological material. Concurrently, sprouted seeds were homogenized under aseptic conditions to isolate 2 g of fresh wet biomass. This biomass was thoroughly mixed with 8 g of the cooled agarose solution to yield a uniform suspension. The resulting mixture was carefully introduced dropwise into 100 mL of sterile vegetable oil maintained at 35 °C. Under gentle stirring, spherical beads began to form due to phase separation between the aqueous and lipid phases. The oil suspension was then transferred into an ice-water bath maintained at 10–15 °C to accelerate the gelation of the agarose droplets. Once solidified, the beads were isolated by low-speed centrifugation at 100 rpm for 5 minutes. Residual oil was eliminated by washing the beads repeatedly with sterile distilled water, with centrifugation steps performed after each wash to ensure complete purification (Liu et al., 2017).

The encapsulated beads were then examined under sterile conditions to assess morphological consistency and spherical integrity. Final preparations were stored under refrigeration for subsequent experimental use. This encapsulation method provides a robust and reproducible platform for immobilizing plant-based cells, facilitating future studies involving biosynthetic pathways and cellular response under varied conditions.

Result and Discussion

Qualitative Determination of Protein

The Biuret assay demonstrated the presence of peptide bonds in both treated and untreated sprouts. However, elicitor-treated samples showed more intense violet coloration, indicating a higher abundance of intact proteins. This suggests that elicitation not only increases protein synthesis but also preserves the structural integrity of synthesized proteins, possibly by reducing proteolytic activity. Figure 03 shows the before and after change in colour and intensity of extracted protein from pea and bean mung under the Biuret test.


Before

After

Fig 03: Result of the Biuret test

The Bradford method is based on the observation that when proteins bind to Coomassie brilliant blue, the absorbance maximum of the dye shifts from 465 to 595nm. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible colour change. The dye reagent primarily reacts with arginine, followed by histidine, lysine, tyrosine, tryptophan, and phenylalanine. The observation revealed that the above amino acid content in the mung bean after elicitor treatment increased comparatively, while no such enhancement was observed for the pea sprouts. Figure 04 are the graphical representation of the concentration of protein estimated by Bradford method with their exponential variance.

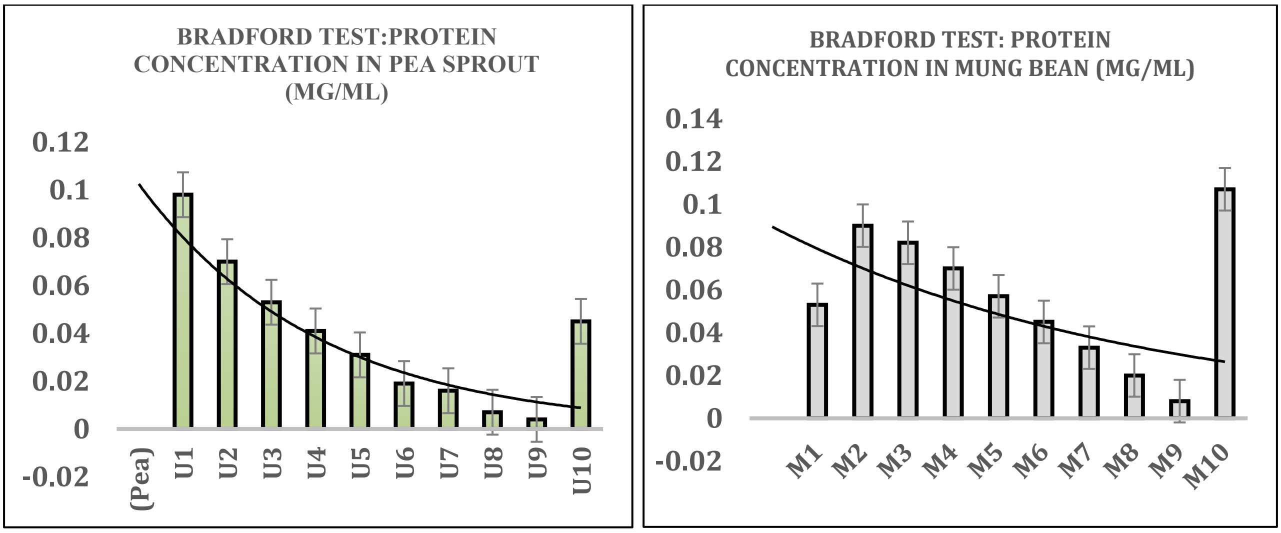


Fig 04: Bradford Test for Protein estimation in Pea and Mung bean sprouts

The Xanthoproteic test results (Fig 05) show a clear increase in yellow coloration after elicitor treatment, indicating higher levels of aromatic amino acids like tyrosine and tryptophan in both pea and mung bean sprouts. Control samples were pale or colorless, while treated samples showed deeper yellow shades, reflecting enhanced synthesis of these essential amino acids. This suggests that natural elicitors effectively stimulate the production of bioactive compounds crucial for nutrition and functional food development.

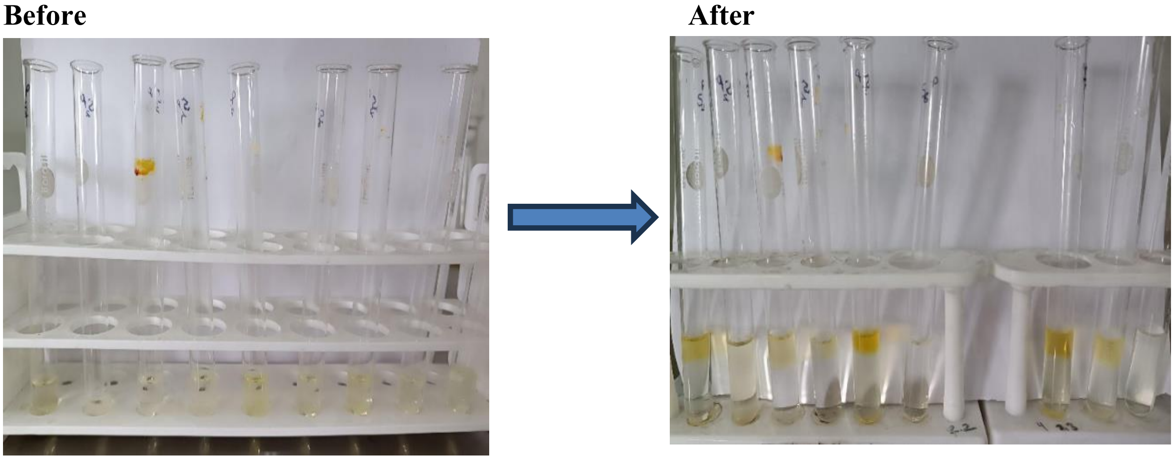


Fig 05: Results of Xanthoproteic test

Quantitative Estimation of Protein

The protein content in Peas varies significantly depending on the elicitor treatment applied. The untreated control sample (U1), which was treated with water, showed a relatively low concentration of 3.42 µg/ml. However, upon treatment with different combinations of natural elicitors, there was a notable increase in protein accumulation. Among the treated samples, the highest concentration was recorded in U10 (A+L+C+M, 1:1:1:1) at 17.77 µg/mL, followed by U8 and U9, which had concentrations of 13.85 µg/mL and 12.14 µg/mL, respectively.

The inspired tests for the most part extended between 6.09 to 17.77 µg/mL, showing a 3- to 5-fold increment compared to the untreated control. This proposes that Pea grows, in spite of having a lower normal protein pattern, is exceedingly responsive to elicitation procedures aimed at boosting protein biosynthesis. This upgrade may be ascribed to elicitor-induced stretch, fortifying metabolic and defense-related pathways, particularly those including amino corrosive synthesis and protein aggregation.

Mung Bean sprout contains a higher protein concentration compared to Pea grows. The untreated control (M1) showed a concentration of 10.81 µg/mL, which is significantly higher than the control estimate of Pea.

Elicitor-treated Mung Bean tests too appeared increments in protein substance, in spite of the fact that the relative changes were more direct. The most elevated concentration was observed in M10 (A+L+C+M, 1:1:1:1) at 15.32 µg/ml, followed by M4 (A+G+M, 2:1:1) at 12.85 µg/mL, and M3 (A+G+M, 1:2:1) at 12.25 µg/ml. Not at all like Pea, the increment in protein content in Mung Bean was within a 1.1- to 1.4-fold extent, which still affirms the positive but less articulated effect of elicitation.

Mung bean (10.81 µg/mL) had over three times the protein concentration of Pea (3.42 µg/mL) under control conditions. The highest protein concentration accomplished in treated Mung bean was 15.32 µg/mL (M10), while treated Pea came to 17.77 µg/mL (U10). In spite of Mung Bean having a better beginning point, Pea showed a more sensational increment in protein content upon elicitor application.

Pea grows displayed a better overlay increment in reaction to elicitors, proposing a more significant inducibility of metabolic pathways related to protein biosynthesis. On the other hand, Mung Bean, which has higher basal protein levels, appeared less responsive but still showed noteworthy advancements upon treatment. This analysis confirms that both species benefit from elicitor treatments, but pea sprouts hold more potential for elicitor-based protein enhancement, making them a strong candidate for functional food development strategies. The graph presented as Fig 06 show the percentage change in the protein concentration of both the sprouts under different elicitor treatments.

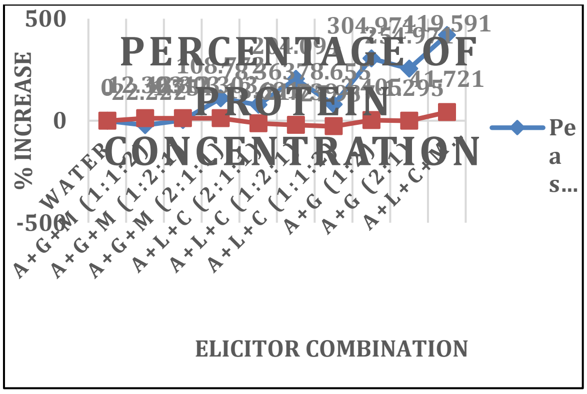


Fig 06: Percentage in Protein Concentration of Pea and Mung Bean under Elicitation Treatment

Agarose Gel Encapsulation of Protein

The encapsulation of protein extracts using agarose resulted in irregularly shaped beads due to the rapid solidification of the agarose solution during the pouring process. This quick gelation limited the formation of uniform spherical beads, leading to uneven morphology. Despite the irregular shapes, the beads effectively entrapped the protein content, indicating the method's functionality for preserving bioactive compounds. Optimizing temperature control and pouring techniques in future trials could improve bead uniformity and enhance encapsulation efficiency. Fig 07 and Fig 08 signify agarose beads for pea and Mung bean, respectively.

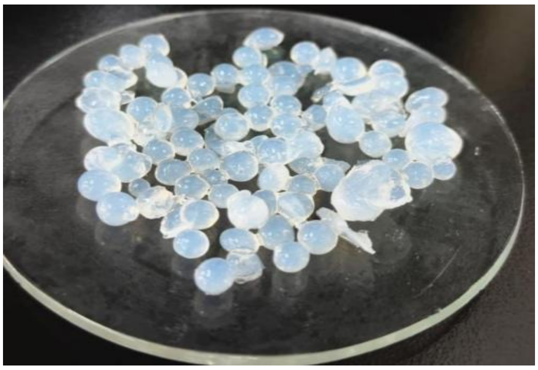


Fig 07: Agarose Beads of Pea

Fig 08: Agarose Beads of Mung Bean

Conclusion

This study investigated the use of natural plant-based elicitors—aloe vera, ginger, mint, lemongrass, and curry leaves—to enhance the protein content of *Pisum sativum* (pea) and *Vigna radiata* (mung bean) sprouts. The elicitor treatment significantly influenced protein biosynthesis, demonstrating that specific combinations of natural extracts can serve as effective biochemical triggers to stimulate metabolic pathways associated with protein production. The results confirm the potential of eco-friendly and cost-effective elicitation techniques for improving the nutritional profile of legume sprouts, particularly in functional food applications. The comparative analysis of different elicitor combinations revealed notable variation in protein yield, suggesting that the synergy between bioactive compounds in the elicitor blends plays a crucial role in modulating protein expression. These findings underline the importance of selecting appropriate elicitor combinations to maximize biological response. Additionally, qualitative and quantitative biochemical assays—including the Lowry, Bradford, Biuret, and Xanthoproteic tests—provided clear evidence of protein presence and structural integrity in the treated samples, thereby validating the effectiveness of the elicitation process. To further enhance the stability and potential application of the protein-enriched sprout material, an encapsulation strategy using agarose gel was implemented. The encapsulation process successfully immobilized the bioactive sprout components within the gel matrix, preserving their functional properties and extending their usability in food systems. This approach demonstrates promising potential for the development of encapsulated functional ingredients, which may offer advantages in targeted delivery, shelf-life extension, and nutritional consistency in commercial formulations. In conclusion, this research provides a comprehensive insight into the integration of natural elicitation and biopolymer encapsulation to improve plant-based protein content and stability. It supports the broader vision of sustainable food innovation and offers a valuable foundation for future studies aimed at developing nutritionally enhanced, functional, and health-promoting food products.

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