



Hemp Protein as a Sustainable Emulsifier in Food Systems

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Abstract

The present study examined the feasibility of hemp protein as a sustainable vegan-based emulsifier in food systems through the development of a protein-rich vegan mayonnaise. Hemp seeds were selected, since it has high quality protein, balanced amino acid profile and nutritional value. The primary objective was to evaluate the emulsifying efficiency and stability performance of hemp protein in comparison with a conventional (egg based) mayonnaise. Hemp based mayonnaise and the control sample were analyzed for physicochemical and microbiological parameters. The control sample (CS) exhibited a pH of 3.73 ± 0.06 , whereas the hemp sample (HS) showed a higher pH of 5.77 ± 0.06 , indicating a moderate difference in acidity profile. Both the samples exhibited high emulsion stability (approximately 98%), indicating good structural integrity. Further, analysis of proximate composition revealed that the hemp-based mayonnaise had higher protein ($6.68 \pm 0.04\%$) and ash ($2.07 \pm 0.02\%$) contents, along with lower fat ($10.03 \pm 0.08\%$), compared to the egg-based mayonnaise, which contained protein ($2.63 \pm 0.05\%$), ash ($1.67 \pm 0.03\%$), and fat ($48.00 \pm 0.12\%$). These results highlight the nutritional advantage of the hemp-based mayonnaise over the conventional egg-based formulation. Microbiological evaluation confirmed that both samples were **within acceptable safety limits**, with the hemp-based mayonnaise showing slightly lower microbial counts and absence of Salmonella, likely due to the absence of egg.

Keywords: Emulsifier, Emulsion stability, Hemp protein, Microbial safety, Sustainability and Vegan mayonnaise.

1. Introduction

Mayonnaise is highly relished acidic condiments having oil-in water colloidal structure that contains approximately 65-80 % of oil and is composed by amalgamating egg yolk and oil with salt, sugar, mustard and vinegar for pH adjustment (Lee et al., 2024). It is an important in sauces because of its taste and flavour profile. The traditional mayonnaise is prepared utilizing egg yolk containing more than 60% of oil and fat, which is high in cholesterol, is implicated to cause various diseases, such as cardiovascular diseases, obesity, and even diabetes. Addition of egg yolk as an emulsifying agent can lead to salmonella contamination makes the traditional mayonnaise unsafe for consumption (Pei et al., 2023). Completely replacing egg yolk with other emulsifiers, whey protein, casein and meat protein is widely investigated by several researchers. These ingredients provide several advantages, like less

cholesterol and fat content, and can be helpful in increasing microbiological stability. More than that, vegetable protein isolate can be used for food emulsion stability because of lower interfacial tension between hydrophobic and hydrophilic components, mostly vegetable proteins from soya, sunflower, pea, tomato seed, wheat, white lupine and faba are already tested for oil-in-water (o/w) emulsion (Nikzade et al., 2012) . An excellent alternative to egg yolk as an emulsifier can be plant-based hemp seed. Hemp seed contains high proteins (25 to 30 %), lipids, essential amino acids, high nutritional traits and emulsifying properties. There is a boom in new product development with hemp seed protein; however, there is limited researches based on hemp seed. Hemp protein shows poor solubility, less emulsifying stability and water-holding capacity because of its complex structure or its presence of a significant amount of non-digestible fiber content

(approximately 22.2 % of cellulose) in hemp seed (Rawal et al., 2023). Canolas are highly cultivated as nutritionally significant oilseed crops which contains enormous amount of monounsaturated fatty acids, particularly oleic acid, along with the antioxidants such as tocopherols and phytosterols. Canola oil contributes its beneficial effects on glycemic control, for blood pressure regulation, cardiovascular health and potential anticancer activity. Such health-promoting properties make canola oil a valuable dietary ingredient and a functional component in therapeutic nutrition. Moreover, canola oil also shows good emulsifying capacity, mild flavour and oxidative stability (Loganes et al., 2016). Keeping in view of the above information, the present study aims to develop a hemp-based vegan mayonnaise as an alternative to the conventional egg-based mayonnaise, in order to meet growing demand for hemp-derived and vegan products[1].

2. Materials and Methods

2.1. Materials

The particulars such as egg, canola oil, lemon, mustard and salt were procured from a local supermarket in Bangalore, Karnataka, India. Shelled hemp seeds were procured from the online shopping site. All reagents used for the analysis were analytical grade. The formulation of developed hemp-based mayonnaise (HS) contains the following ingredients: 325 g canola oil (85% of the formulation), 125 g hemp seed (12.5%), 30 ml of RO water, 12-13 ml lemon juice, 5g mustard, and 4.5 g salt. For the control sample (CS), the formulation used was identical, except that the hemp seeds were replaced with three egg yolks. For the development of samples, the hemp seeds were soaked in warm water at 50°C for 15 minutes to activate the hemp seeds. After soaking, the hemp seeds were ground into a fine paste then Water, lemon juice, mustard and salt were mixed with the paste, and the mixer was stirred continuously while gradually adding canola oil to form a sustainable emulsion. The control sample was prepared similarly, substituting hemp seed paste with egg yolks. Both emulsions were homogenized using a blender at 700 W until a uniform consistency was achieved. The final sample was stored at 4°C under refrigeration, and all analysis was conducted after 24

h to ensure stability.

2.2. Proximate Analysis of Control and Hemp-Based Mayonnaise

2.2.1. Moisture content

Two gram of sample was kept in a pre-dried moisture dishes. The initial mass of the sample was recorded as W_1 and the petridish was then placed in the hot air oven maintained at 100 °C for 12 h. After drying, the mass of the dried sample was recorded as W_2 (Tasie & Gebreyes, 2020). The moisture content of the sample was calculated by using the following equation.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

2.2.2. Crude Fat

Soxhlet extraction method was used to estimate the crude fat content of the control and hemp-based sample. The empty beaker weight (W_1) was taken and all the beakers were loaded into the system. 2 g of each sample (W) was placed in a cellulose extraction thimble and extracted with petroleum ether for 6 to 8 hours. After extraction, the solvent was evaporated, and the remaining lipid residues were weighed (W_2). ("AOAC 2023; Method No. 986.14)

$$\text{Crude fat (\%)} = \frac{W_2 - W_1}{W} \times 100$$

2.2.3. Crude Protein

Kjeldahl method was used to determine the crude protein content of the sample. The distilled samples were titrated with 0.1 N NaOH up to the endpoint was reached. After which, protein content was calculated (Jeong and Oh, 2025).

2.2.4. Crude Fiber

Fibra-Plus apparatus was used to estimate the crude fiber content of the sample by sequential acid and alkali-hydrolysis method (AOAC, 2023: method No. 978.10).

2.2.5. Ash content

Gravimetric metric method was used to determine the ash content of the sample. Accurately five gram of the sample was weighed into a crucible[2]. The crucible was placed in a muffle furnace and heated at 550 °C for about 6 h till all the material was completely charred. It was then cooled in a desiccator and weighed. The percentage of ash was calculated by using the following expression. (AOAC 2023;



Method No. 984.18)

$$\text{Total ash (\%)} = \frac{\text{weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

2.2.6. Carbohydrates

The available carbohydrates content was estimated by difference method (AOAC, 2023).

2.3. Quality parameters of Control and Hemp-Based Mayonnaise

2.3.1 pH

Digital pH meter was used to measure the pH of the samples. One gram of sample was dissolved in 9 ml of distilled water in a beaker, and the electrode of the pH meter was immersed in the test solution. The pH value and temperature of sample were recorded simultaneously. All the measurements were replicated thrice.[4] (Ana Maria Blejan and Nour, 2023)

2.3.2. Titratable acidity

Titration method was used to determine the Titratable acidity of the samples. Ten gram of sample was diluted with 90 ml of distilled water and titrated against 0.1 N NaOH solutions until the endpoint was reached. Titratable acidity was calculated based on the volume of NaOH consumed and expressed with respect to the sample weight. The analysis was performed following (AOAC 2023; Method No. 942.15)[5].

2.3.3 Centrifugal stability of mayonnaise

Three gram of mayonnaise samples (W_0) were heated in a water bath at 60°C for 60 min, followed by centrifugation at 8000 rpm for 10 minutes. After the centrifugation, the supernatant was meticulously removed, and the remaining mayonnaise was weighed (W_1). The stability of emulsion was calculated using the following expression:

$$\text{Emulsion stability (\%)} = \frac{W_1}{W_0} \times 100$$

2.4 Microbiological analysis

Total plate bacterial count: The total plate bacterial count was determined using (PCA) plate count agar by using pour plate technique[3]. Plates were incubated at 30°C for 72 hr and all the analysis were conducted in triplicates.

2.4.1. Yeast and mold count

Yeast and mold detected using (PDA) potato

dextrose agar. All plates were incubated at 25 °C for duration of 72 hrs.

2.4.2. Pathogen detection

E.coli and Coliform were detected using eosin methylene blue agar (EMB), following the standard procedure. Plates were incubated at 30 °C for duration of 72 hrs.

2.4.3. Salmonella detection

Salmonella was detected using MacConkey agar. Samples are plated on MacConkey agar. Plates were incubated at 37 °C for 24 hours

2.5 Sensory Evaluation

Sensory evaluation of the mayonnaise samples (CS and HS) was carried out by 9-point hedonic scale with score ranging from 1 =dislike extremely to 9 =like extremely for the attributes such as Appearance, taste, mouthfeel, texture and overall acceptability. A panel of 15 semi-trained panelists (10 female, 5 male) were selected to evaluate the sample in a random order at room temperature. Sensory data was analyzed in MS Excel using the fuzzy logical similarity analysis method (Pavani *et al.*, 2023)

2.6 Statistical Analysis

All experiments were performed in triplicate, including physicochemical, microbial and sensory analyses. The results are expressed as mean \pm standard deviation (SD). Data were analysed using Microsoft Excel. For comparing the CS and HS were made by calculating the mean differences and standard deviation. Significant differences between samples were assessed using t-tests (Excel's built-in Data Analysis tool) or by observing whether the means \pm SD overlap. For sensory evaluation, the average scores for each attribute were calculated, and the highest mean scores were considered as the most acceptable[6].

3. Results and Discussion

3.1. Proximate composition of control and hemp-based mayonnaise

The proximate composition *viz.*, moisture content, crude fat, crude fiber, total ash/ mineral content and carbohydrates of control and hemp-based mayonnaise samples was determined using different standard analytical methods and are represented in the Table 1[10]. The average moisture content of the hemp-based mayonnaise was recorded as 87.48 \pm

0.30 %, whereas the control sample showed moisture content of 86.13 ± 1.58 %. The results indicate that the developed hemp-based mayonnaise exhibited slightly higher moisture content associated to the control sample[9]. The crude protein content of the CS and HS was found to be 2.63 ± 0.05 % and 6.68 ± 0.04 %, respectively, indicating an appreciable increase in protein content in the hemp-based mayonnaise due to the incorporation of hemp ingredients[11]. The ash/mineral content of the control sample was determined to be 1.67 ± 0.03 %, while the hemp-based mayonnaise exhibited a slightly higher ash content of 2.07 ± 0.02 %, suggesting the presence of higher mineral content in the developed product[7]. Similarly, the crude fat content of the control sample was found to be 48.00 ± 0.12 %, whereas the hemp-based mayonnaise showed a significantly lower fat content of 10.03 ± 0.08 %, indicating that the developed product can be considered a comparatively lower-fat alternative. Similar findings have been reported by Jeong & Oh, 2025[8]. The crude fibre content was observed to be 0.15 ± 0.02 % in the control sample and 1.20 ± 0.05 % in the hemp-based mayonnaise, which may be attributed to the dietary fibre present in hemp components[12]. The carbohydrate content was found to be 47.55 ± 0.82 in controlled sample and 80.02 ± 0.43 in hemp-based mayonnaise, indicating a significant higher carbohydrate level in the hemp-based mayonnaise. Overall, the incorporation of hemp ingredients resulted in a product with higher protein, fiber, ash and carbohydrate content, along with reduced fat content, compared to the conventional mayonnaise formulation[13][14].

Crude fibre	0.15 ± 0.02	1.20 ± 0.05
Ash content	1.67 ± 0.03	2.07 ± 0.02
Crude protein	2.63 ± 0.05	6.68 ± 0.04
Carbohydrates	47.55 ± 0.82	80.02 ± 0.43

3.2. Physiochemical properties of Control and Hemp-Based Mayonnaise

Quality parameters such as pH, Titratable acidity and centrifugal stability of control and hemp based mayonnaise were determined and discussed hereunder. The control sample exhibited a pH of 3.73 ± 0.06 , whereas the hemp-based mayonnaise showed a higher pH of 5.77 ± 0.06 . This increase in pH may be attributed to the compositional characteristics of hemp seeds, particularly the presence of plant proteins and minerals that influence the buffering capacity of the system. The obtained results are in line with other plant-based mayonnaise formulations, where the replacement of egg components with plant-derived ingredients resulted in relatively higher pH values (Jeong and Oh, 2025; He *et al.* 2021; Raikos *et al.*, 2020). The titratable acidity of the control sample was demonstrated to be 0.82 ± 0.04 % (as acetic acid), while the hemp-based mayonnaise showed a slightly lower value of 0.76 ± 0.03 %. The higher acidity in the control sample may be due to the conventional formulation of egg-based mayonnaise, which typically includes vinegar and other acidic ingredients contributing to its distinctive flavor and preservative properties. The control sample revealed a centrifugal stability of 97.82 ± 0.41 %, while the hemp-based mayonnaise exhibited 98.34 ± 0.32 % which is slightly higher. The stability values indicate that the hemp-based formulation was capable of forming a stable oil-in-water emulsion comparable to conventional mayonnaise. This stability may be attributed to the functional properties of hemp seed components, particularly proteins and polysaccharides, which can act as natural emulsifying agents and help maintain emulsion stability. A similar observation has been reported by Jeong & Oh, 2025.

Table 1 Proximate composition of Control and Hemp-Based Mayonnaise

Parameters	Control sample (%)	Hemp sample (%)
Moisture content	86.13 ± 1.58	87.48 ± 0.30
Crude fat	48.00 ± 0.12	10.03 ± 0.08

Table 2 Physiochemical properties of Control and Hemp-Based Mayonnaise

Parameters	Control sample (%)	Hemp sample (%)
pH	0.03 ± 0.01	0.052 ± 0.001
Titrateable Acidity (% acetic acid)	0.82 ± 0.04	0.76 ± 0.03
Centrifugal stability	98.34 ± 0.32%	98.34 ± 0.32

3.3. Sensory characters of mayonnaise

The sensory characteristics of control (egg-based) mayonnaise and hemp-based mayonnaise were evaluated based on 9-point Hedonic scale and is presented in Table 3. The appearance (5.2 ± 0.8), taste (5.1 ± 0.9), mouth feel (5.0 ± 0.7), texture (5.3 ± 0.8) and overall acceptability (5.2 ± 0.8) of the control sample was found to be lower, whereas the hemp-based mayonnaise recorded a slightly higher score of 5.6 ± 0.7 (appearance), 5.7 ± 0.8 (taste), 5.5 ± 0.7 (mouth feel), 5.8 ± 0.6 (texture) and 5.7 ± 0.7 (overall acceptability) indicating that the developed formulation possessed a pleasant visual quality, better flavor acceptance, smoother and more desirable mouth coating effect, stable and creamy emulsion structure. The radar chart (Fig. 1) further illustrates the comparative sensory profile of both samples, where the hemp-based mayonnaise consistently shows higher scores across all evaluated attributes demonstrating that the developed hemp-based mayonnaise provides a well-balanced sensory profile and good consumer acceptance[15]. Shown in Table 3 Mean Sensory score of Control and Hemp-Based Mayonnaise

Table 3 Mean Sensory score of Control and Hemp-Based Mayonnaise

Attribute	Control Sample(CS)	Hemp Sample(HS)
Appearance	5.2 ± 0.8	5.6 ± 0.7

Taste	5.1 ± 0.9	5.7 ± 0.8
Mouthfeel	5.0 ± 0.7	5.5 ± 0.7
Texture	5.3 ± 0.8	5.8 ± 0.6
Overall Acceptability	5.2 ± 0.8	5.7 ± 0.7

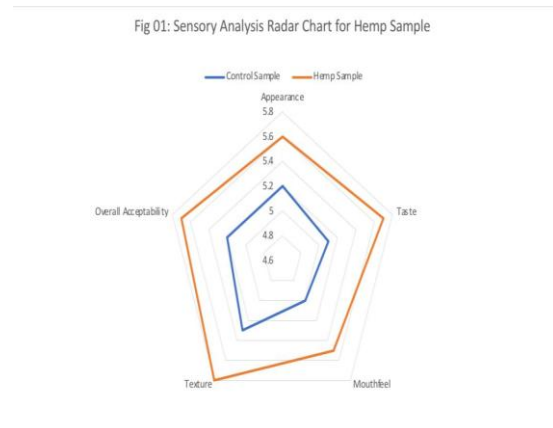


Figure 1 Sensory Evaluation of Control and Hemp-Based Mayonnaise Using a 9-Point Hedonic Scale

3.4. Microbiological quality of Control and Hemp-Based Mayonnaise

The microbiological quality of control and hemp based mayonnaise is presented in Table 4. The total plate count of the control sample was recorded to be 2.3 log CFU/g, whereas, hemp-based mayonnaise counted 2.0 log CFU/g, slightly lower than the control sample, indicating good microbial stability in both samples[16]. Yeast and mold also showed a low counts ranging from 1.4 to 1.6 log CFU/g, which is within the acceptable limit for mayonnaise products and Coliform counts were minimal and E-coli was absent in both the samples. Salmonella spp[17]. was detected in traceable amounts in the egg-based control sample, whereas no specific Salmonella growth was observed in the hemp-based mayonnaise sample. The absence of Salmonella in the hemp-based formulation may be attributed to the absence of egg components, which are commonly associated with Salmonella contamination. Overall, the microbiological counts obtained in this study were within acceptable limits and it indicate that both mayonnaise samples were microbiologically safe for

consumption[18][19]. Shown as Figure 1 Sensory Evaluation of Control and Hemp-Based Mayonnaise Using a 9-Point Hedonic Scale Shown as Table 4 Microbiological quality of Control and Hemp-Based Mayonnaise

Table 4 Microbiological quality of Control and Hemp-Based Mayonnaise

Microorganism	Control Sample (CS)	Hemp Sample (HS)
Total Plate Count (log CFU/g)	2.3 ± 0.05	2.0 ± 0.04
Yeast and Mold (log CFU/g)	1.6 ± 0.03	1.4 ± 0.02
Coliforms (log CFU/g)	1.2 ± 0.02	1.0 ± 0.02
E. coli	Not detected	Not detected
Salmonella spp.	Detected in trace amounts	Not detected

Conclusion

The present study illustrated the successful development of a **hemp-based mayonnaise as an alternative to conventional egg-based mayonnaise** with improved nutritional and functional properties. The proximate analysis revealed that the hemp-based formulation contained **higher protein (6.68 ± 0.04 %)**, **fibre (1.20 ± 0.05 %)**, **ash (2.07 ± 0.02 %)** and **carbohydrate (80.02 ± 0.43)** along with **lower fat content (10.03 ± 0.08 %)** compared to the control sample, indicating enhanced nutritional value. Physicochemical evaluation exhibited that the hemp-based mayonnaise had a **higher pH (5.77 ± 0.06)** and comparable titratable acidity and centrifugal stability, substantiating the ability of hemp components to form a **stable oil-in-water emulsion** similar to conventional mayonnaise. Further, sensory analysis demonstrated that the hemp-based mayonnaise received **slightly higher consumer acceptability** and a well-balanced sensory profile. Microbiological evaluation confirmed that both the samples were **within acceptable safety limits**, with the hemp-based mayonnaise showing slightly lower microbial

counts and absence of *Salmonella*, likely due to the absence of egg. Overall, the inventions indicate that hemp seeds can be adequately utilized in the formulation of **nutritious, stable and microbiologically safe vegan mayonnaise**, offering a promising plant-based alternative to traditional egg-based mayonnaise.

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