

Hemp seeds (*Cannabis sativa L.*) Incorporated Chutney: A Step Toward Functional Foods

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Abstract – Chutney, a traditional Indian condiment, is valued for its rich flavors and nutritional qualities. With rising demand for functional foods, this study explores the incorporation of roasted hemp seed powder into green chutney to enhance its nutritional and preservative properties. Hemp seeds are a rich source of protein, essential fatty acids, and antioxidants, making them suitable for improving traditional recipes. Control and experimental chutneys were prepared by replacing groundnuts with roasted hemp seed powder at levels of 5%, 10%, and 15%. Sensory evaluation was conducted using a 9-point hedonic scale by a semi-trained panel. Physicochemical parameters, including protein, total phenol, and flavonoid content, were analyzed using standard methods. Antioxidant activity was assessed through FRAP, DPPH-RSA, ABTS-RSA and RPA assay. Microbial quality was evaluated by determining total viable counts over 14 days of storage under refrigeration (4 °C) and freezing (−18 °C). The 5% hemp seed chutney received the highest overall acceptability in sensory evaluation, comparable to the control sample. Yeast and mold count were present in the control sample but absent in the hemp seed-enriched samples. Physicochemical analysis revealed increased protein, phenolic, and flavonoid contents with higher hemp seed concentrations. Antioxidant activity also improved proportionally with hemp seed addition. Microbial studies showed a reduction in total viable counts over the storage period, with chutney containing 15% hemp seeds exhibiting the lowest microbial load, particularly under frozen conditions. The findings suggest that roasted hemp seed powder can be successfully incorporated into chutney formulations to enhance nutritional value, antioxidant capacity, and shelf-life, offering a promising functional food option.

Keywords: Green chutney / nutraceutical properties / antioxidant capacity / shelf life / sensory evaluation

Résumé – Graines de chanvre (*Cannabis sativa L.*) incorporées dans le chutney : une étape vers les aliments fonctionnels. Le chutney, un condiment traditionnel indien, est apprécié pour ses saveurs riches et ses qualités nutritionnelles. Avec la demande croissante d'aliments fonctionnels, cette étude explore l'incorporation de poudre de graines de chanvre grillées dans un chutney vert pour en améliorer les propriétés nutritionnelles et la conservation. Les graines de chanvre sont une riche source de protéines, d'acides gras essentiels et d'antioxydants, ce qui les rend adaptées à l'amélioration des recettes traditionnelles. Des chutneys témoins et expérimentaux ont été préparés en remplaçant les arachides par de la poudre de graines de chanvre grillées à des taux de 5 %, 10 % et 15 %. Une évaluation sensorielle a été réalisée à l'aide d'une échelle hédonique à 9 points par un panel semi-entraîné. Les paramètres physico-chimiques, y compris les teneurs en protéines, en phénols totaux et en flavonoïdes, ont été analysés par des méthodes standards. L'activité anti-oxydante a été évaluée par les tests FRAP, DPPH-RSA, ABTS-RSA et RPA. La qualité microbiologique a été évaluée en déterminant les populations viables totales sur 14 jours de stockage sous réfrigération (4 °C) et congélation (−18 °C). Le chutney à 5 % de graines de chanvre a reçu la meilleure acceptabilité globale lors de l'évaluation sensorielle, comparable à l'échantillon témoin. Des populations de levures et de moisissures étaient présentes dans l'échantillon témoin mais absentes dans les échantillons enrichis en graines de chanvre. L'analyse physico-chimique a révélé une augmentation des teneurs en protéines, en phénols et en flavonoïdes avec des concentrations plus élevées de graines de chanvre. L'activité anti-oxydante s'est également améliorée proportionnellement avec l'ajout de graines de chanvre. Les études microbiologiques ont montré une réduction des dénombrements viables totaux sur la

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période de stockage, le chutney contenant 15 % de graines de chanvre présentant la charge microbienne la plus faible, en particulier dans des conditions de congélation. Les résultats suggèrent que la poudre de graines de chanvre grillées peut être incorporée avec succès dans les formulations de chutney pour améliorer la valeur nutritionnelle, la capacité antioxydante et la durée de conservation, offrant une option prometteuse d'aliment fonctionnel.

Mots-clés : Chutney vert / propriétés nutraceutiques / capacité anti-oxydante / durée de conservation / évaluation sensorielle

Highlights

Incorporation of roasted hemp seed powder (5–15%) into green chutney improved protein content, antioxidant potential (FRAP, DPPH, ABTS, RPA), and microbial stability, suggesting its efficacy as a nutritionally enhanced, shelf-stable functional condiment.

1 Introduction

Nutraceuticals are defined as “specially designed preparations” that are made to meet certain nutritional needs and/or provide preventive healthcare. In addition to a supplement diet, nutraceuticals are formulated nutrients that aid in the treatment and prevention of certain ailments. The phrase “nutraceutical” coined in 1989 by Dr. Stephen De Felice, is derived from the words “nutrition” and “pharmaceutical.” These are foods, or portions of foods, that are good for treating and/or preventing diseases, among other health advantages. The field of nutrition science has expanded its knowledge to include human health, the prevention and treatment of chronic illnesses, and the early detection of nutrient shortages (Puri *et al.*, 2022).

The word “chutney” refers to a broad range of hot relishes and condiments used in Indian cooking, which are typically consumed as a side dish or as snacks, depending on personal preference (Yogeswari *et al.*, 2016). In different regions of the nation and among different religious communities, taste of chutney varies significantly. For Hindus, the diverse tastes and textures hold particular significance (Meena *et al.*, 2017). In India, a wide range of pickles and chutneys made from vegetables, lentils, and spices are eaten in big quantities with rice and morning foods like *vada*, *chapatti*, *idly*, and *dosa*. Numerous culinary adjuncts, such as traditional chutneys, quick chutneys, and chutney powders, have been developed and standardized in literature. These developments have been made possible by the different raw materials that are accessible at different times of the year (Rao *et al.*, 2013).

An essential component of Indian cooking, chutneys are created from chopped, cooked fruits or vegetables, or a combination of the two, combined with vinegar, spices, and other seasonings, and then reduced to a smooth pulp. Chutneys can be preserved by several methods, including by infusing citrus juice with salt, vinegar, or oil. It could taste bitter, sour, sweet, or a mix of the three. While some have variations in taste like hot and spicy, others are sweet and tangy. Often it has coarse to fine texture and can be either moist or dry. Chutney is becoming increasingly common in Western cuisine these days (Kowsalya *et al.*, 2018).

Around the world, industrial hemp (*Cannabis sativa* L.) is a crop that is both economically valuable and adaptable, gaining interest from researchers and entrepreneurs. As the hemp plant was mentioned in the Vedas, an ancient sacred text, and because it has long been a significant component of Indian culture, it has a deep cultural connection to India. In addition to the awareness of hemp’s superior fibre content and nutritional profile, the substantial economic, environmental, and social benefits offered by the hemp plant creates a demand for sustainable agricultural practices. The Uttarakhand state government has granted the Indian Industrial Hemp Association (IIHA) the licence in recent years because of hemp’s myriad industrial potential applications, including biofuels, construction, medicine, and textiles. The Narcotic Drugs and Psychotropic Substances Act (1985) classifies cannabis as a controlled substance, yet it also allows for its industrial mass growth. Moreover, the state government may grant licences for cultivation of hemp plant with low tetrahydro-cannabinol (THC) concentrations (below 0.3%) under section 10 of the Act (www.indiacode.nic.in). A member of the *cannabaceae* family, hemp is an annual herbaceous plant that grows to a tall height with slender leaves, a robust stem, and clusters of tiny greenish-yellow flowers (www.britannica.com/plant/hemp). Industrial hemp differs from marijuana in that it has a lower THC level, which does not promise psychoactive effects. The structure of the plant makes it possible to remove nutrient-rich seeds and robust bast fibre from its outer bark. (Biswakarma *et al.*, 2023).

Hemp seeds typically have stripes of a darker brown colour and generally measure 2.5–3.5 mm in length, although their color and size can vary depending on the variety and growing condition. Hemp seeds have been utilised in the food business and medicine due to their high nutritional content. They can be eaten raw, boiled, or roasted. Hemp seeds include a 20–30% carbohydrate content, 10–15% insoluble fibre, 25–35% oil, and 20–25% protein. Hemp seed protein is primarily composed of albumin and legumin, as per an earlier report. It has a higher essential amino acid content than soybean and is suitable for young children (ages 2–3 to 5 years old). Hemp seeds in particular are a good source of arginine, which is a nutrient that may be added to the diet to boost cardiovascular health (Chen *et al.*, 2023).

To our knowledge till date no study has been reported on the addition of roasted hemp seed powder in green chutney preparation. The present research was planned to incorporate hemp seeds in chutney preparation and studying its physical properties as well as nutraceutical and antioxidant value.

The present research was carried out to study the effect of different storage temperature on sensorial attributes, physico-chemical, microbial, antioxidant and nutritional characteristics of hemp seeds incorporated traditional green chutney.

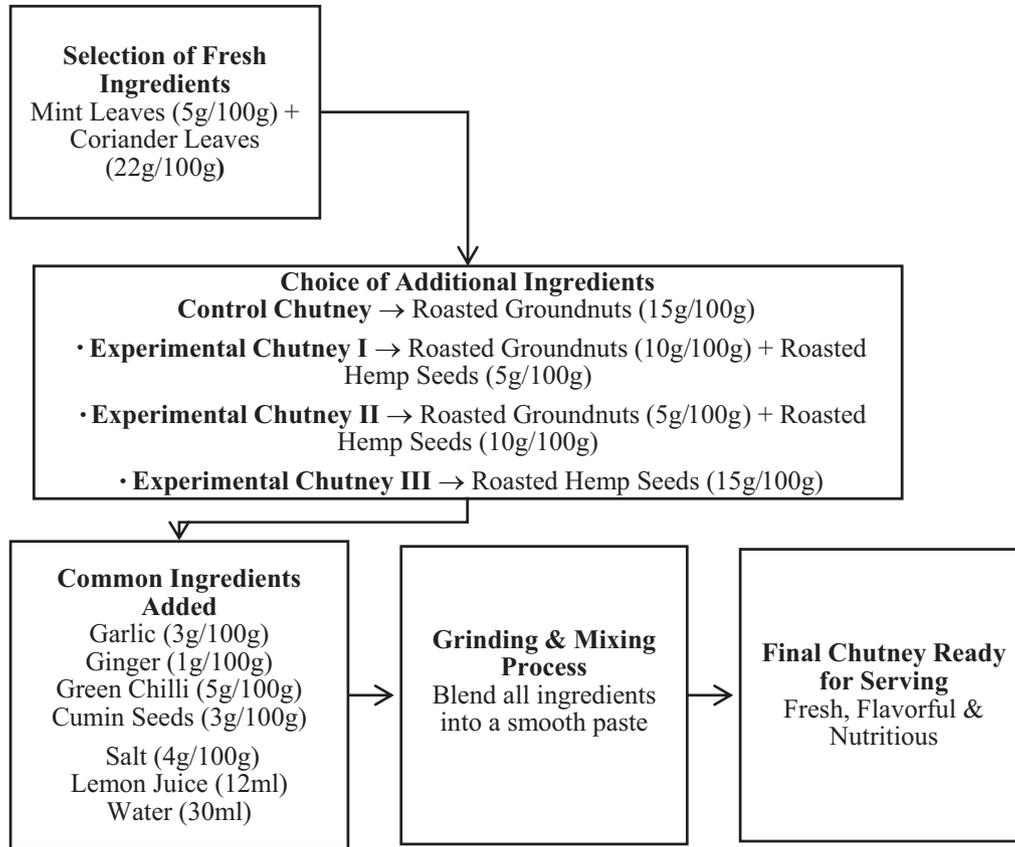


Fig. 1. Preparation of control and experimental chutney.

2 Materials and methods

2.1 Development of control and experimental chutney

2.1.1 Procurement of raw materials

Hemp seeds were procured from the local market of Haldwani, Uttarakhand. The seeds were cleaned to remove unwanted extraneous matter. Coriander leaves, mint leaves, green chillies, lemon, ground nuts, and spices were procured from the local market of Anand, Gujarat.

2.2 Preparation of control and experimental chutney

See [Figure 1](#).

2.3 Studying organoleptic characteristics of chutney

A panel of 10 semi-trained evaluators conducted an organoleptic assessment of both control and experimental chutney, focusing on color, odor, taste, mouthfeel, and overall acceptability. They used a 9-point Hedonic Rating Scale, where scores from 9 to 1 indicated preferences ranging from “liked extremely” to “disliked extremely”. The evaluation included an analysis of color, odor, taste, mouthfeel, and overall acceptability for both types of chutney.

2.4 Proximate composition of chutney

Moisture: Moisture content was determined by using [AOAC method \(1990\)](#)

Fat: Estimation of Fat using Soxhlet method by Pelican SOCS-PLUS

Approximately 2–3 g of moisture free control and experimental chutney samples were accurately weighed then transferred into a cellulose extraction thimble (W3). The thimble was covered with a small plug of defatted cotton to prevent sample loss and inserted into the thimble holder. The holder was placed inside a pre-weighed, clean, and moisture-free beaker (W1). Subsequently, 100 mL of petroleum ether (40–60 °C) was added to each beaker.

The extraction process was initiated by placing the beakers into the Pelican SOCS-PLUS system. Water circulation through the condenser was ensured for efficient solvent condensation. The program was run for approximately 3 h to allow complete extraction of lipids.

After completion, the beakers were removed, and the thimbles were discarded. The residual petroleum ether in the beakers was evaporated by placing the beakers in a hot air oven at 60 °C until constant weight was achieved. The beakers were then cooled in a desiccator and weighed (W2).

[Table 1](#) formulation adapt from Prabhavathi, S. N., & Prakash, J. (2017). Sensory attributes of fresh herb chutneys

Table 1. Composition of control and experimental chutney (per 100 g).

Ingredients	Control Chutney	Experimental Chutney I	Experimental Chutney II	Experimental Chutney III
Mint leaves	5 g	5 g	5 g	5 g
Coriander leaves	22 g	22 g	22 g	22 g
Roasted groundnuts	15 g	10 g	5 g	–
Roasted Hemp seeds	–	5 g	10 g	15 g
Garlic	3 g	3 g	3 g	3 g
Ginger	1 g	1 g	1 g	1 g
Green Chilli	5 g	5 g	5 g	5 g
Cumin Seeds	3 g	3 g	3 g	3 g
Salt	4 g	4 g	4 g	4 g
Lemon Juice	12 ml	12 ml	12 ml	12 ml
Water	30 ml	30 ml	30 ml	30 ml

prepared using a flavor enhancer. EC nutrition, 10(1), 26-36. With slight modification for control chutney and for experimental chutney we have modified as per the overall acceptability test done by carrying out several trials on preliminary basis.

Calculation:

The crude fat content was calculated using the following formula:

$$\text{Crude fat} \left(\frac{\text{g}}{100\text{g}} \right) = \frac{W2 - W1}{W3} \times 100,$$

where $W1$ = Initial weight of the empty beaker (g); $W2$ = Final weight of the beaker after extraction and drying (g); $W3$ = Weight of the original sample (g).

Note: Ensure all glassware and thimbles are completely dry before use to avoid error due to moisture content.

Protein: Estimation of Protein using Kjeldahl method by Pelican Kel-Plus Nitrogen Estimation System Supra-LX (VA)

Protein content of fat free control and experimental chutney were determined by Kjeldahl apparatus (Kel-Plus Supra-LX (VA)) using approximately 0.2–0.5 g of the sample was taken in the digestion tube. 4 g of the digestion mixture and 10 mL of conc. H₂SO₄ were added to the tube. The whole mixture was then subjected to digestion process for 90 min. This process was done with continuous circulation of 15% NaOH and D/W.

Segment 1 was set at 250 °C for 15 min. Segment 2 was at 250 °C for 5 min. Segment 3 and 4 were set at 300 °C for 5 min. Segment 5 and 6 were set 350 °C for 5 min. Segment 7 was set at 420 °C for 10 min. Segment 8 was set as 420 °C for 60 min. Output of these entire segments was 100%.

After the digestion process, the samples were cooled for at least 10 min. The volume of the digestion mixture was made up to 40 mL with D/W. The content was distilled for 7 min and the distillate was collected in a flask. The distillation process required 4% boric acid containing known amount of methyl red indicator and 40% NaOH solution. This was then titrated against 0.1 N HCl. Protein content was determined by using the formula.

Calculation:

$$\% \text{Nitrogen} = \frac{14.01 \times \text{Normality of Acid} \times \text{Titre value} \times 100}{\text{Sample weight} \times 1000},$$

$$\% \text{Protein} = \% \text{Nitrogen} \times 6.25$$

where, Titer value = sample titer value – blank titer value.

2.5 Chemical analysis of chutney**2.5.1 Methanolic extraction**

Control and experimental chutney powder were extracted using 80% acidified methanol (pH 2.0) thrice for 1.5 h at 37 °C using an orbital shaker at 150 rpm. The extract was centrifuged, the supernatant was collected, and the volume was made upto 50 mL. The extracts were stored at –20 °C till further analysis.

2.6 Assessment of phenolic content and antioxidant capacity**2.6.1 Total Phenolic Content (TPC)**

Total phenolics were estimated using the Folin–Ciocalteu method by Singleton & Rossi, (1965). A known aliquot of sample extract was mixed with FC reagent and 7.5% Na₂CO₃, incubated at 37 °C for 30 min, and absorbance was read at 750 nm using a visible spectrophotometer. Gallic acid (5–20 µg/mL) was used as standard. Results were expressed as mg gallic acid equivalents (GAE) per 100 g sample. The TPC was determined using following formula:

$$\text{Total Phenol} \left(\frac{\text{mg GAE}}{100\text{g}} \right) = \frac{\text{Standard Concentration}}{\text{Standard O.D.}}$$

$$\times \frac{\text{Sample O.D.}}{\text{Aliquot Taken}} \times \frac{\text{Volume made up}}{\text{Sample taken}} \times \frac{100}{1000} \times \text{Dilution factor}$$

2.6.2 Total flavonoid content (TFC)

Flavonoids were determined following Singleton *et al.* (1999). The extract was reacted with NaNO₂, AlCl₃, and NaOH, and the absorbance was measured at 510 nm with the help of a

Table 2. Sensory score of control and experimental chutney samples.

Samples	Colour	Odour	Taste	Mouth feel	Overall Acceptability
Control	8.05 ^a	7.95 ^a	8.00 ^a	8.10 ^a	8.15 ^b
Sample	±0.94	±1.23	±1.07	±1.25	±0.98
Experimental	8.00 ^a	7.80 ^a	7.70 ^a	7.65 ^a	8.15 ^b
Sample I	±0.79	±1.19	±1.45	±1.66	±0.87
Experimental	7.55 ^a	7.55 ^a	7.60 ^a	7.20 ^a	7.60 ^a
Sample II	±1.14	±1.35	±1.14	±1.60	±0.99
Experimental	7.65 ^a	7.50 ^a	7.45 ^a	7.25 ^a	7.35 ^a
Sample III	±0.81	±0.82	±1.19	±1.11	±0.98
F value	1.425	0.657	0.720	1.713	3.502*

Values are Mean ± SD scores of a hedonic rating test by a panel of 10 members x 2 replications.

Mean values bearing the same superscripts within the column do not differ significantly ($p \leq 0.05$).

*Indicates significant difference at $p \leq 0.05$.

visible spectrophotometer. Rutin (25–100 µg/mL) served as standard. Results were expressed as mg rutin equivalents (RE) per 100 g sample. TFC was estimated by applying the given equation.

$$\text{Total Flavonoid} \left(\frac{\text{mg RE}}{100\text{g}} \right) = \frac{\text{Standard Concentration}}{\text{Standard O.D.}} \times \frac{\text{Sample O.D.}}{\text{Aliquote Taken}} \times \frac{\text{Volume made up}}{\text{Sample taken}} \times \frac{100}{1000} \times \text{Dilution factor.}$$

2.6.3 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was performed as per [Benzie and Strain \(1996\)](#). The extract was mixed with FRAP reagent, incubated at 37 °C for 10 min, and absorbance was measured at 593 nm by means of a visible spectrophotometer. Trolox was used as standard, and results were reported in mg trolox equivalents (TE) per 100 g. The following formula was used to evaluate the FRAP:

$$\text{TAC} \left(\frac{\text{mgTE}}{100\text{g}} \right) = \frac{\text{Standard Concentration}}{\text{Standard O.D.}} \times \frac{\text{Sample O.D.}}{\text{Aliquote Taken}} \times \frac{\text{Volume made up}}{\text{Sample taken}} \times \frac{100}{1000} \times \text{Dilution factor.}$$

2.6.4 DPPH radical scavenging activity (DPPH-RSA)

Antioxidant activity was measured using the DPPH method ([Brand-Williams et al., 1995](#)). The extract was mixed with DPPH solution, incubated at 37 °C for 20 min, and absorbance was recorded at 517 nm utilizing a visible spectrophotometer. Trolox (5–20 µg/mL) was used as standard, and results were expressed in mg TE/100 g. The DPPH was calculated using the following formula:

$$\text{Percent inhibition} = \frac{(Ac - Ae)}{Ac} \times 100,$$

where Ac = absorbance of control; Ae = absorbance of extract.

$$\text{TAC} \left(\frac{\text{mgTE}}{100\text{g}} \right) = \frac{\text{Standard \% Inhibition}}{\text{Standard O.D.}} \times \frac{\text{Sample \% Inhibition}}{\text{Aliquote Taken}} \times \frac{\text{Volume made up}}{\text{Sample taken}} \times \frac{100}{1000} \times \text{Dilution factor.}$$

2.6.5 ABTS Radical Scavenging Activity (ABTS-RSA)

The ABTS assay was performed following [Re et al. \(1999\)](#). The extract was reacted with ABTS reagent, and absorbance was read at 734 nm through the use of a visible spectrophotometer. Trolox (1–4 µg/mL) was used as standard. Results were expressed in mg TE/100 g. The calculation of ABTS was carried out using this formula:

$$\text{Percent inhibition} = \frac{(Ac - Ae)}{Ac} \times 100,$$

where, Ac = absorbance of control; Ae = absorbance of extract.

$$\text{TAC} \left(\frac{\text{mgTE}}{100\text{g}} \right) = \frac{\text{Standard \% Inhibition}}{\text{Standard O.D.}} \times \frac{\text{Sample \% Inhibition}}{\text{Aliquote Taken}} \times \frac{\text{Volume made up}}{\text{Sample taken}} \times \frac{100}{1000} \times \text{Dilution factor.}$$

2.6.6 Reducing Power Assay (RPA)

Reducing power was evaluated according to [Oyaizu \(1986\)](#). The extract was treated with phosphate buffer, potassium ferricyanide, and ferric chloride, and absorbance at 700 nm was measured using a visible spectrophotometer. Trolox (10–40 µg/mL) was used as standard. Results were

Table 3. Moisture, Fat and Protein content of control and experimental chutney samples

Samples	Moisture (gm%)	Fat (gm%)	Protein (gm%)
Control	68.09 ^a	32.31 ^d	25.86 ^a
Sample	±0.25	±0.54	±0.16
Experimental	71.27 ^c	26.85 ^c	29.90 ^b
Sample I	±0.30	±0.54	±0.20
Experimental	69.78 ^b	24.43 ^b	33.01 ^c
Sample II	±0.45	±0.40	±0.08
Experimental	71.49 ^c	20.87 ^a	37.23 ^d
Sample III	±0.09	±0.20	±0.33
F Value	79.03 ^{**}	349.69 ^{**}	1478.59 [*]

Values are Mean ± SD of three observations.

Mean values bearing the same superscripts within the column do not differ significantly $p \leq 0.05$.

*Indicates significant difference at $p \leq 0.05$.

expressed in mg TE/100 g. This equation was employed to determine the RPA:

$$\text{TAC} \left(\frac{\text{mgTE}}{100\text{g}} \right) = \frac{\text{Standard Concentration}}{\text{Standard O.D.}} \times \frac{\text{Sample O.D}}{\text{Aliquote Taken}} \\ \times \frac{\text{Volume made up}}{\text{Sample taken}} \times \frac{100}{1000} \times \text{Dilution factor.}$$

2.7 Color measurement

Color measurement of control and experimental samples were conducted using the Hunter L^* , a^* , b^* color scale, which is structured in a cubic form. The ' L^* ' axis ranges vertically from top to bottom, with 100 representing black at its maximum. The ' a^* ' and ' b^* ' axes have no defined numerical limits: positive ' a^* ' denotes red, negative ' a^* ' indicates green, positive ' b^* ' signifies yellow, and negative ' b^* ' signifies blue.

2.8 Storage studies

Chutney samples were packed in glass jars and stored at refrigerated (4 °C) and freezing (-18 °C) temperatures for a duration of 14 days. Both fresh and stored samples were analysed for total viable count (TVC) and yeast and mold count (Patel and Patel, 2016) to assess the impact of storage conditions.

3 Results

Table 2 shows the Sensory scores of control and experimental chutney samples. The results for colour indicate that there were no significant differences between the control (8.05), Experimental sample I (8.00), Experimental sample II (7.55), and Experimental sample III (7.65). Similar trends were observed for odour, taste, and mouthfeel, with no significant differences between the samples. However, for overall acceptability the control sample (8.15) and Experimental sample I (Containing 5% of roasted hemp seed powder) received significantly higher

scores (8.15) compared to Experimental sample II (7.60) and Experimental sample III (7.35), indicating that the control and Experimental sample I were more favourably accepted by the panel.

The study investigated the moisture, fat, and protein content of control and experimental chutney samples, revealing significant differences across the various samples (Tab. 3). The control sample exhibited significantly lower ($p \leq 0.05$) moisture content of 68.09%, while Experimental sample I and Experimental sample III demonstrated significantly higher ($p \leq 0.05$) moisture levels of 71.27% and 71.49%, respectively. In contrast, the control sample contained significantly highest fat content at 32.31%, whereas Experimental sample III recorded the significantly lowest fat content at 20.87%. Protein content varied significantly, with the control sample showing the least protein at 25.86%. Experimental sample II had the significantly highest protein content at 33.01%, followed closely by Experimental sample III at 37.23%. Statistical analysis indicated significant differences among the samples for all measured parameters, with p -values less than 0.05.

The analysis of total phenols, total flavonoids, and antioxidant capacities across various chutney samples reveals significant differences in their phytochemical compositions (Tab. 4). The total phenolic content ranged from 145.74 to 182.47 mg GAE/100 g. The control sample exhibited the highest total phenol content of 170.62 mg GAE/100 g, while Experimental sample I had a notably lower level of 145.74 mg GAE/100 g. In contrast, Experimental sample II and III demonstrated increased phenolic content, with values of 182.47 mg GAE/100 g and 181.96 mg GAE/100 g, respectively.

Regarding total flavonoids, the control sample had a content of 228.29 mg RE/100 g, while Experimental sample I showed a significant marginal increase to 231.78 mg RE/100 g. Experimental sample II and sample III exhibited significantly higher flavonoid levels, reaching 281.54 mg RE/100 g and 308.24 mg RE/100 g, respectively, indicating the beneficial effects of the additional ingredients in these formulations (Tab. 4).

The ferric Tripyridal Triazine (TPTZ) complex is reduced to its ferrous, coloured form in the presence of an antioxidant,

Table 4. Total phenols, total flavonoids and total antioxidant capacity of control and experimental chutney samples.

Samples	Total Phenol (mg GAE/100 g)	Total Flavonoids (mg RE/100 g)	FRAP (mg TE/100 g)	DPPH (mg TE/100 g)	ABTS (mg TE/100 g)	RPA (mg TE/100 g)
Control Sample	170.62 ^d ±2.59	228.29 ^a ±1.48	93.21 ^a ±1.36	149.03 ^a ±127	36.37 ^a ±0.250	285.00 ^b ±3.83
Experimental Sample 1	145.74 ^b ±1.22	231.78 ^b ±0.89	146.13 ^c ±1.10	154.18 ^b ±0.51	38.19 ^b ±0.33	271.00 ^a ±7.57
Experimental Sample 2	182.47 ^a ±1.18	281.54 ^c ±1.97	111.78 ^b ±2.70	157.37 ^c ±0.38	44.61 ^c ±0.34	313.00 ^c ±3.83
Experimental Sample 3	181.96 ^c ±2.59	308.24 ^d ±1.50	179.50 ^d ±3.41	160.62 ^d ±0.24	52.25 ^d ±0.59	387.97 ^d ±7.02

Values are Mean ± SD of four observations.

Mean values bearing the same superscripts within the column do not differ significantly ($p \leq 0.05$).

*Indicates significant difference at $p \leq 0.05$.

Table 5. L^* a^* b^* values of control and experimental chutney samples.

Sample	L^*	a^*	b^*
Control Sample	44.55 ^c ±0.08	-4.91 ^a ±0.04	39.46 ^d ±0.27
Experimental Sample I	45.25 ^d ±0.03	-3.60 ^b ±0.02	38.88 ^c ±0.05
Experimental Sample II	41.68 ^b ±0.05	-2.78 ^c ±0.01	34.70 ^a ±0.02
Experimental Sample III	38.47 ^a ±0.03	-2.15 ^d ±1.07	37.61 ^b ±1.92
F Value	8540.86**	6387.51**	623.05**

Mean values bearing the same superscripts within the column do not differ significantly ($p \leq 0.05$).

*Indicates significant difference at ($p \leq 0.05$).

which is the basis of the Ferric Reducing Antioxidant Potential (FRAP) approach. Antioxidants having a lower reduction potential than the Fe^{3+}/Fe^{2+} combination are directly measured by the FRAP assay (Hevorsen *et al.*, 2006). The Ferric Reducing Antioxidant Power (FRAP) values indicated a marked increase in antioxidant capacity, with the control sample at 93.21 mg TE/100 g, while Experimental samples I, II, and III displayed significantly higher ($p \leq 0.05$) values of 146.13 mg TE/100 g, 111.78 mg TE/100 g, and 179.50 mg TE/100 g, respectively. Similarly, the DPPH scavenging assay results showed that all experimental samples outperformed the control, with Experimental sample III achieving the significant highest ($p \leq 0.05$) scavenging activity at 160.62 mg TE/100 g (Tab. 4).

Table 4 shows ABTS-RSA of control and experimental sample varied from 36.37 to 52.25 mg TE/100 g. A significantly higher ($p \leq 0.05$) value of ABTS-RSA was recorded in Experimental sample III (52.25 mg TE/100 g) followed by Experimental sample II and I (44.61 mg TE/100 g and 38.19 mg TE/100 g) respectively while the least value was shown by control sample (36.37 mg TE/100 g).

The Reducing Power Assay (RPA) results indicate a notable increase in antioxidant activity across the chutney samples. The control sample exhibited an RPA value of 285.00 mg TE/100 g.

In contrast, Experimental sample I showed a significant lowest ($p \leq 0.05$) value of 271.00 mg TE/100 g, suggesting a minor reduction in reducing power. However, Experimental sample II and III displayed significantly higher ($p \leq 0.05$) RPA values of 313.00 mg TE/100 g and 387.97 mg TE/100 g, respectively (Tab. 4). This substantial increase in RPA for the experimental samples indicates enhanced electron-donating ability and potential antioxidant efficacy, to be specific in sample III.

Table 5 shows the color analysis of control and experimental samples was conducted using L^* , a^* , and b^* values, representing lightness, redness/greenness, and yellowness/blueness, respectively. The control sample exhibited an L^* value of 44.55, which was significantly different ($p \leq 0.05$) from all experimental groups. Experimental sample I had the highest L^* value at 45.25, followed by Experimental sample II with a value of 41.68, and Experimental sample III with the lowest lightness at 38.47. This may be attributed to the fact of increasing level of roasted hemp seed powder from Experimental I to Experimental III samples that might have decreased the colour of chutney samples. Regarding the a^* values, which indicate the shift from green to red, the control sample had an a^* value of -4.91, representing the significantly greatest ($p \leq 0.05$) green intensity. Experimental sample I showed a reduced greenness with an a^* value of -3.60, Experimental sample II recorded -2.78, and Experimental

Table 6. Total plate count of fresh and stored (at refrigerated temperature) control and experimental chutney samples

Sample	0 Day	7 Day	14 Day	F value
Control	6.44 ^{bC}	5.79 ^{cB}	4.51 ^{bA}	904.71*
Sample	±0.09	±0.03	±0.04	
Experimental Sample I	6.36 ^{bC}	5.68 ^{bB}	4.52 ^{bA}	9006.28*
	±0.00	±0.02	±0.02	
Experimental Sample II	6.27 ^{bC}	5.76 ^{cB}	4.52 ^{bA}	569.89*
	±0.12	±0.02	±0.03	
Experimental Sample III	5.68 ^{aC}	5.12 ^{aB}	4.42 ^{aA}	25.26*
	±0.43	±0.04	±0.03	
F Value	8.84*	423.44*	7.63*	

Values are Mean ± SD of three observations.

Mean values with different superscripts (a, b, c) within the columns differ significantly ($p \leq 0.05$).

Mean values with different superscripts (A, B, C) within the row differ significantly ($p \leq 0.05$).

*Indicates significant ($p \leq 0.05$) difference.

Control – 15 g groundnut seeds.

Experimental I – 10 g groundnut seeds + 5 g roasted hemp seeds.

Experimental II – 5 g groundnut seeds + 10 g roasted hemp seeds.

Experimental I – 15 g roasted hemp seeds.

Table 7. Total plate count of fresh and stored (at freezing temperature) control and experimental chutney samples.

Sample	0 Day	7 Day	14 Day	F value
Control	6.44 ^{bC}	5.39 ^{dB}	4.41 ^{bA}	815.91*
Sample	±0.09	±0.07	±0.00	
Experimental Sample I	6.36 ^{bC}	5.06 ^{cB}	4.42 ^{bA}	497.79*
	±0.00	±0.15	±0.01	
Experimental Sample II	6.27 ^{bC}	4.86 ^{bB}	4.37 ^{bA}	546.74*
	±0.12	±0.07	±0.01	
Experimental Sample III	5.68 ^{aC}	3.77 ^{aA}	4.29 ^{aB}	61.18*
	±0.43	±0.00	±0.06	
F Value	8.840*	230.70*	10.42*	

Values are Mean ± SD of three observations.

Mean values with different superscripts (a, b, c) within the columns differ significantly ($p \leq 0.05$).

Mean values with different superscripts (A, B, C) within the row differ significantly ($p \leq 0.05$).

*Indicates significant ($p \leq 0.05$) difference.

Control – 15 g groundnut seeds.

Experimental I – 10 g groundnut seeds + 5 g roasted hemp seeds.

Experimental II – 5 g groundnut seeds + 10 g roasted hemp seeds.

Experimental I – 15 g roasted hemp seeds.

sample III had the significantly lowest ($p \leq 0.05$) greenness (−2.15). For the b* values, representing yellowness, the control sample (39.46) had the significantly highest ($p \leq 0.05$) yellowness, which significantly differed from all experimental samples. Experimental sample I had a slightly lower b* value of 38.88, followed by Experimental sample III (37.61) and Experimental II (34.70) exhibited the significantly lowest ($p \leq 0.05$) yellowness.

The control sample exhibited a detectable count of yeast and mold count (YMC), whereas no YMC was observed in the experimental sample.

Table 6 illustrates the TPC of control and experimental samples on comparing fresh and stored samples of chutney on 0 day was significantly higher ($p \leq 0.05$) in the control sample

(6.44) indicating the highest microbial load followed by experimental sample I (6.36), sample II (6.27) and III (5.68). On 7th day the TPC ranged from 5.79 to 5.12. The highest was again observed in the control sample which was significantly different from ($p \leq 0.05$) experimental sample I (5.68) and III (5.12). After 14 days of storage the TPC count was found to almost the same in Control sample (4.51), Experimental sample I (4.52), II (4.52). The lowest value was recorded in Experimental sample III (4.42) which had significantly lowest value ($p \leq 0.05$) among all the sample.

The Total Plate Count (TPC) of fresh and stored chutney samples decreased significantly over 14 days of storage at freezing temperatures, reflecting the inhibitory effect of freezing on microbial growth. On Day 0, the Control Sample

exhibited the highest TPC (6.44), followed by Experimental Samples I (6.36), II (6.27), and III (5.68). By Day 7, microbial counts reduced in all samples, with the Control Sample still having the highest TPC (5.39), while Sample III showed the lowest (3.77), indicating stronger microbial inhibition. At Day 14, TPC levels ranged from 4.41 to 4.42 for control chutney and Experimental chutney I. Sample III consistently had the lowest count (4.29) (Tab. 7).

4 Discussion

The sensory evaluation results clearly demonstrated that the incorporation of 5% hemp seed powder into chutney preparations resulted in a product comparable to the control sample, as reflected in the overall acceptability scores. These findings are consistent with Mummaleti and Beera (2019), who found no significant difference in the sensory acceptability of chutney powders with and without hemp seed powder. Specifically, the sensory attributes, such as color, taste, flavor, appearance, and overall acceptability, showed no noticeable changes between the experimental and control samples, indicating that the inclusion of hemp seeds did not adversely affect the organoleptic qualities of the chutney.

In terms of nutritional value, Experimental Sample III, containing 15% hemp seed powder, exhibited significantly higher protein content compared to all other samples. Hemp seed powder, known to contain approximately 34% protein (Ertas & Aslan, 2020), substantially contributed to this increase in protein content, offering a viable plant-based protein source. This aligns with the findings of Settaluri *et al.* (2012), who observed that groundnut seeds in control chutney contributed a considerable amount to protein content, yet hemp seeds provided an even more concentrated source of protein. This protein enhancement is particularly valuable, given the growing demand for plant-based protein alternatives in the food industry. The high protein content of hemp seeds, along with their favorable lipid profile (rich in polyunsaturated fatty acids), makes them a promising addition to functional food products.

Furthermore, the incorporation of hemp seeds resulted in a notable increase in the total phenolic content (TPC) of the chutney, which is indicative of enhanced antioxidant properties. Our results showed significantly higher TPC levels than those reported by Khedkar *et al.* (2022), who observed TPC concentrations of 120.56 mg/g GAE in curry leaf chutney powders. The higher TPC in hemp seed-enriched chutneys suggests a beneficial role of hemp seeds in promoting the health benefits associated with phenolic compounds, such as their potential to reduce oxidative stress and inflammation. This finding aligns with the work of KC *et al.* (2020), who observed increased antioxidant properties in chutneys as the content of bioactive ingredients, such as amala pulp, was raised. These findings underline the potential of hemp seeds not only as a nutritional enhancement but also as a functional ingredient that could contribute to the health-promoting properties of chutneys.

In terms of shelf life and microbial stability, the results indicated that refrigeration significantly slowed down microbial growth, but did not completely halt it. These observations are consistent with those of Yadla and Sachdev (2013), who reported that fresh tomato salsa had a shelf life of one week at ambient temperature and up to two months when

refrigerated. Our study further corroborates these findings, with experimental sample III consistently exhibiting the lowest microbial counts, which could be attributed to the antimicrobial properties of hemp seeds. According to Shah and Sengupta (2014), dry fruit chutney has an excellent shelf life of ten to fifteen days if stored in an airtight container in the refrigerator. Patel *et al.*, (2020) reported that on day 0, the counts of fresh lassi samples were 7.49 log CFU/mL for control and 7.46 log CFU/mL for experimental lassi, according to SPC (Standard Plate Count) analysis. During the first seven days of storage, the SPC did not significantly increase. After that, the storage period resulted in a non-significant ($p \leq 0.05$) decrease in the SPC as the storage period progressed, as demonstrated by the Storage Control Experimental Period. This trend was consistent across both the control and experimental samples. Hemp seeds contain bioactive compounds, such as polyphenols and fatty acids, known for their antimicrobial effects. These compounds likely contributed to the reduction in microbial growth observed in the experimental samples, indicating that hemp seeds can enhance the preservation and safety of chutney (Tanase *et al.*, 2024).

Moreover, the samples stored at freezing condition showed that, although freezing slowed down microbial activity, it did not completely cease the growth. Freezing is known to suppress microbial growth, but certain microorganisms can still survive under these conditions, albeit at a slower rate (Fatima *et al.*, 2015). The antimicrobial properties of hemp seeds remained effective even in frozen conditions, as evidenced by the lowest microbial counts in Experimental Sample III. This suggests that hemp seeds could be beneficial for improving the storage stability of chutney, particularly when stored under freezing conditions. The enhanced preservation properties of hemp seed-enriched chutney have implications for extending the product's shelf life, making it a more viable option for long-term storage and distribution.

The overall findings of this study underscore the potential of hemp seeds to not only improve the nutritional quality of chutney, but also enhance its functional properties, including its antioxidant capacity and antimicrobial activity. These attributes make hemp seeds a promising ingredient for the development of nutritionally enriched and shelf-stable chutney products. Given the increasing consumer demand for functional foods, the inclusion of hemp seeds offers an innovative approach to improving the health benefits and preservation qualities of traditional chutney preparations.

5 Conclusion

The incorporation of roasted hemp seed powder into green chutney significantly enhances its nutritional composition, elevating its antioxidant capacity and improving microbial stability, thereby extending the product's shelf life. Sensory evaluations indicate that the chutney remains organoleptically acceptable, with the formulation containing 5% hemp seed powder demonstrating the highest level of consumer acceptability, as confirmed by a combination of Lab* values. The inclusion of 15% hemp seed powder resulted in a substantial increase in the protein and flavonoid content, as well as a marked enhancement in total antioxidant capacity, which was assessed using multiple assays including FRAP,

DPPH, ABTS, and RPA. Chutney samples formulated with 15% hemp seed powder exhibited the greatest reduction in total plate count (TPC) during storage, which suggests the presence of bioactive compounds in the hemp seeds that contribute to the preservation of the product. Notably, yeast and mold colonies (YMC) were observed in the control sample, whereas the experimental samples containing hemp seed powder showed no such microbial growth. The improved protein and antioxidant content suggest potential health benefits, making this hemp-infused chutney a promising functional food product. Further research could explore additional preservation techniques and commercial viability.

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Author contribution statement

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