

# Formulation And Quality Assessment Of Curry Leaves-Based Chutney: A Focus On Nutrition And Storage Stability

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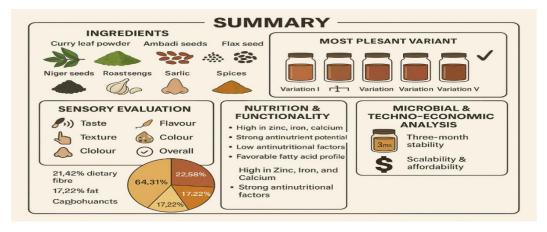
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## **ABSTRACT**

The purpose of this study was to create and assess a functional chutney designed especially for those with hyperlipidaemia. Nutrient-dense components including curry leaf powder, Ambadi seeds, flax seeds, Niger seeds, roasted Bengal gram, garlic, and certain spices were added to the chutney. To determine the most pleasant variant, five formulations were made by adjusting the amounts of curry leaf powder and Ambadi seeds. Taste, texture, flavour, colour, appearance, and general acceptance were all evaluated by a semi-trained panel. The variation with the greatest sensory ratings, Variation IV, which contained 30g of curry leaf powder and Ambadi seeds apiece, was confirmed by consumer acceptability testing. According to colour studies, a deeper, more enticing crimson hue was a result of a greater Ambadi seed concentration. According to a nutritional study, the best-performing variety has 64.31% carbs, 17.22% fat, 21.42% dietary fibre, and 22.58% protein per 100g. It also supplied significant levels of zinc, iron, and calcium. Because of its high phenolic, flavonoid, betacarotene, and chlorophyll content, the chutney demonstrated strong antioxidant potential. Low amounts of tannins, oxalates, and phytates were found in the antinutritional factors analysis; these levels were within safe bounds and did not impair the bioavailability of minerals. A positive balance was found by fatty acid profile, with polyunsaturated and monounsaturated fatty acids outnumbering saturated fats. The product's stability and safety for up to three months under optimal storage conditions were validated by microbial analysis. A techno-economic analysis emphasised the chutney's scalability and affordability. In general, the created chutney provides a wholesome, economical, and palatable dietary solution for the treatment of hyperlipidaemia.

Keywords: Functional food, Hyperlipidemia, Sensory evaluation.



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#### 1. INTRODUCTION

In Indian cooking, curry leaves (*Murraya koenigii.L.*), a popular fragrant herb, are used to season and improve the flavours of a variety of foods. Curry leaves are still underutilised in processed or value-added food forms, despite their widespread use in cooking. In addition to powerful bioactive substances including flavonoids, carbazole alkaloids, tannins, and phenolic acids, they are naturally abundant in iron, calcium, dietary fibre, and other vital minerals. These phytochemicals have a number of health-promoting qualities, such as antibacterial, hepatoprotective, hypolipidemic, antidiabetic, and antioxidant effects.

Non-communicable diseases (NCDs) include obesity, type 2 diabetes, cardiovascular disease, and hyperlipidaemia have become much more common worldwide in recent years. Interest in creating and consuming functional foods that offer health advantages beyond basic nourishment has increased as a result of this trend. Functional foods boost general health, lower the risk of chronic illnesses, and encourage metabolic equilibrium. A sustainable and culturally acceptable dietary approach is the incorporation of traditional, readily available, and nutrient-dense foods, such as curry leaves, into functional food forms.

Curry leaves are frequently thrown away in large amounts, leading to post-harvest losses, although being consumed in fresh form in everyday cooking. Making value-added goods out of curry leaves not only increases their shelf life but also boosts their use and lowers food waste. Among its many forms, chutneys are popular in traditional Indian cuisine, easy to make, and culturally recognisable. The creation of a curry leaf chutney offers the chance to produce a food product that is organoleptically acceptable, shelf-stable, and health-promoting.

The nutritional profile, microbiological safety, and storage characteristics of this value-added curry leaf chutney, however, have not been well studied. To guarantee customer safety and product efficacy, a comprehensive inquiry is necessary into the stability of bioactive substances and nutrients during storage, as well as the microbiological quality under various packaging and ambient circumstances. As a result, the current study was conducted with the goal of creating a value-added curry leaves chutney and assessing its nutritional composition, antioxidant potential, microbiological stability, and shelf life under different storage circumstances. This project seeks to demonstrate the practicality of using curry leaves in a functional food format that promotes health, lowers food waste, and increases dietary intake of important bioactive in an accessible manner.

# 2. MATERIALS AND METHODS

### Preparation of Curry Leaves Powder

Fresh curry leaves were sourced from the Parbhani market, cleaned thoroughly, and blanched in boiling distilled water with 0.1% magnesium oxide for 15–20 seconds. After blanching, the leaves were dried in a mechanical dryer and ground into powder, then stored in airtight pouches.

# Chutney Formulation

Five variations of functional chutney were prepared using curry leaves powder, Ambadi seeds, flax seeds, Niger seeds, roasted Bengal gram, garlic, cumin, chili powder, and salt. Variations differed in the proportions of curry leaves and Ambadi seeds.

# Consumer and Sensory Assessment

A panel of 20 semi-trained participants used a 9-point Hedonic Scale to assess sensory perception. Consumer acceptance was evaluated across a range of demographics, including hyperlipidemic people, who were told about the product's health advantages and gave it ratings based on sensory characteristics.

### Techno-Economic Analysis and Colour

Hunter Colorimeters were used to measure hue, chroma, a\*, b\*, and L\* colour values. The affordability and viability of chutney as a health-focused product were confirmed by a techno-economic study that calculated the cost of producing 1 kg of the product is 35.7 rupees.

### Nutrient Analysis of Curry Leaves Chutney

Proximate Composition: Moisture, protein, fat, fiber, carbohydrates using standard methods (AOAC). Mineral Content: Iron and calcium using Atomic Absorption Spectrophotometer. Vitamins: Niacin, thiamine, riboflavin, and beta-carotene using HPLC. Total Antioxidant Activity: DPPH method. Oxalates and Phytates: Using standard methods (Chinma & Igyor, 2007; Wheeler & Ferral, 1971) Energy value was calculated based on the content of proteins, fats, and carbohydrates.

### Shelf-Life Study of the Developed Curry leaves Chutney

The best accepted variation of curry leaves chutney was selected for shelf-life study. It was packed in HDPE, LDPE and Laminated aluminum foil pouch and stored at room temperature for a period of 3 months. The samples were drawn fortnightly for assessing the quality in terms of moisture and organoleptic evaluation. Peroxide value, free fatty acids, were estimated at the beginning and at the end of the storage period.

### Microbial Evaluation

Total plate count, yeast count and mold count were estimated before storage and after completion of storage period in all the packages by serial dilution agar technique given by (Dubey and Maheshwari in 2000)

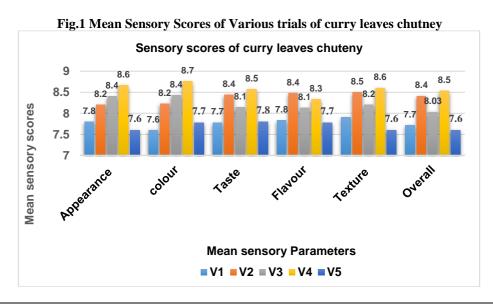
## 1. RESULTS AND DISCUSSION

Table 1. Mean sensory Scores of various trials of curry leaves chutney

Variation	Appearance	colour	Taste	Flavour	Texture	Overall
						acceptability
V1	7.80	7.60	7.78	7.83	7.91	7.72
V2	8.20	8.23	8.44	8.48	8.50	8.41
V3	8.40	8.43	8.14	8.13	8.20	8.03
V4	8.67	8.76	8.57	8.33	8.60	8.53
V5	7.60	7.78	7.80	7.78	7.60	7.60
SE±	0.075	0.069	0.072	0.079	0.061	0.070
CD@5%	0.245	0.225	0.237	0.257	0.200	0.228

Table 1. presents the mean sensory scores of various trials of curry leaves chutney. A nine-point hedonic scale was used to evaluate the five curry leaf chutney varieties' sensory qualities. The sensory analysis's findings are shown in the table. In every sensory attribute appearance, colour, taste, flavour, texture, and general acceptability Variation IV was the most favoured of the five. Colour (8.76), taste (8.57), flavour (8.33), and overall acceptability. The present study aimed to develop and evaluate a functional curry leaves-based chutney tailored for hyperlipidaemic individuals.

The chutney incorporated nutrient-dense ingredients, including curry leaves powder, Ambadi seeds, flax seeds, Niger seeds, roasted Bengal gram, garlic, and selected spices. Five variations were formulated by altering the proportions of curry leaves powder and Ambadi seeds to identify the most acceptable combination. Sensory evaluation by a semi-trained panel assessed attributes such as appearance, colour, taste, flavour, texture, and overall acceptability. Variation IV, containing 30g of curry leaves powder and 30g of Ambadi seeds, emerged as the most preferred formulation. Consumer testing further validated its high acceptability.



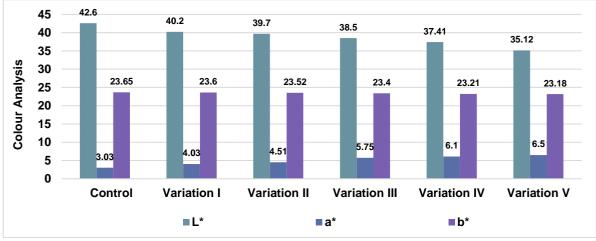
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**Table.2 Colour Analysis of Curry leaves Chutney** 

Sample	L*	a*	b*
Control	42.60	3.03	23.65
Variation I	40.20	4.03	23.60
Variation II	39.70	4.51	23.52
Variation III	38.50	5.75	23.40
Variation IV	37.41	6.10	23.21
Variation V	35.12	6.50	23.18
SE ±	1.06	0.55	0.08
CD @5%	2.36	1.22	0.18

L\* (lightness), a\* (redness) & b\* (yellowness)\*Each value is mean of three determinationsV1 (45:15:14:10:10), V2 (40:20:13:10:10), V3 (35:25:12:10:10), V4 (30:30:11:10:10) & V5 (25:35:10:10:10) (curry leaves powder: Ambadi seeds: Bengal gram roasted: Niger seed: flax seed) According to Table 2. the control sample had the greatest L\* (lightness) value. This value decreased from V1 to V4 as the amount of ambadi seeds rise, most likely as a result of their darker pigmentation. Ambadi seeds' reddish colour caused the a\* (redness) values to gradually increase. There were slight decreases in b\* (yellowness) readings, which may have been caused by the combined pigment impact of niger, flax, and ambadi seeds. Significant variations in a\* between particular pairings (V1–V2 and V3–V4) as well as in L\* and b\* between samples were validated by statistical analysis.

Fig 2. Colour analysis of developed curry leaves chutney



L\* (lightness), a\* (redness) & b\* (yellowness)

Table. 3 Nutrient content of curry leaves chutney / 100 g

Nutrients	Amount	Amount (per 10g)
Moisture (g)	5	0.5
Protein (g)	22.58	2.258
Fat (g)	17.22	1.722
Crude Fiber(g)	21.42	2.142

Ash (%)	9.24	0.924
Ascorbic Acid (mg)	16.17	1.617
Carbohydrate (g)	64.31	6.431
Energy (Kcal)	349	34.9
Thiamin (mg)	0.225	0.0225
Riboflavin (mg)	1.031	0.1031
Niacin (mg)	1.85	0.185

Table 3. exhibits a robust nutritional profile was shown by the created curry leaf chutney, which qualified it as a functional meal for those with hyperlipidaemia. It helps decrease blood cholesterol and promotes satiety due to its high protein (22.58%) and fibre (21.42%) content. Additionally, the chutney has high levels of calcium (2480 mg) and iron (121.69 mg), which support healthy bones and prevent anaemia. It is perfect for diets that are health-conscious and calorie-conscious because to its desirable nutrient profile, moderate energy (349 kcal/100g), and fat levels. The addition of roasted Bengal gram, curry leaves, Ambadi seeds, and flax seeds increases the plant's medicinal properties even further. In general, the chutney provides a nutrient-dense, economical, and easy way to manage hyperlipidaemia.

**Table 4. Nutritional Composition of Curry Leaves Chutney** 

Component Per 100g Per 10g						
Fiber Content	1 et 100g	Tel log				
	64.26	C 126				
Total Dietary Fiber (g)	64.26	6.426				
Insoluble Fiber (g)	48.20	4.82				
Soluble Fiber (g)	16.06	1.606				
Mineral Composition						
Calcium (mg)	409.06	40.906				
Iron (mg/kg)	5.74	0.574				
Zinc (mg)	2.03	0.203				
Sodium (mg)	27.59	2.759				
Potassium (mg)	609.68	60.968				
Magnesium (mg)	183.38	18.338				
Phosphorus (mg)	303.47	30.347				
Nutraceutical & Antioxidant	S					
Total Antioxidant Activity	385.19	38.519				
(%)						
Beta-Carotene (µg)	30.73	3.073				
Phenols (mg)	363.75	36.375				
Flavonoids (mg)	7326	732.6				
Chlorophyll (mg)	9.10	0.91				
Antinutrients						
Phytates (mg)	210.45	21.045				
Oxalates (mg)	1.85	0.185				
Tannins (mg)	0.27	0.027				

The nutritional analysis of curry leaves chutney reveals its significant potential as a functional food with high dietary and therapeutic value. As shown in the table 4. the chutney contains a substantial amount of total dietary fiber (64.26 g/100 g), with insoluble fiber (48.20 g) being predominant over soluble fiber (16.06 g). This high fiber content supports gastrointestinal health, improves bowel movement regularity, and contributes to satiety and weight management. Soluble fiber, in particular, plays a critical role in regulating postprandial blood glucose levels and serum cholesterol, thereby offering protective effects against cardiovascular diseases (Thanuja et al., 2024; Sagar et al., 2016).

The chutney also demonstrated a rich mineral profile, especially with high levels of potassium (609.68 mg), calcium (409.06 mg), phosphorus (303.47 mg), and magnesium (183.38 mg) per 100 g. These minerals are essential for maintaining electrolyte balance, neuromuscular function, bone mineralization, and enzymatic activities. A high potassium-to-sodium ratio is particularly favourable in dietary management of hypertension and cardiovascular health (Deshmukh & Patel, 2023). Additionally, iron (5.74 mg/kg) and zinc (2.03 mg) contribute to oxygen transport, immune modulation, and cellular

metabolism (Sharma et al., 2022).

From a nutraceutical perspective, the chutney displayed exceptional antioxidant potential, with total antioxidant activity recorded at 385.19%. The presence of flavonoids (7326 mg), phenols (363.75 mg), chlorophyll (9.10 mg), and beta-carotene (30.73 µg) in high concentrations underlines its capability to combat oxidative stress, neutralize free radicals, and protect against inflammation and degenerative diseases. These bioactive compounds are widely recognized for their anti-inflammatory, cardioprotective, and chemo preventive properties (Verma & Singh, 2022; Krishnan & Ramesh, 2023).

Although the chutney contains antinutritional factors such as phytates (210.45 mg), oxalates (1.85 mg), and tannins (0.27 mg) per 100 g, their concentrations are within acceptable limits and are not expected to significantly interfere with mineral bioavailability. The low levels of these compounds indicate minimal adverse effects while maintaining the nutritional integrity of the product.

Fernandes & Roy (2024) Reported that low levels of these antinutrients in functional foods may help reduce oxidative stress and protect against chronic diseases. Kumar & Bhatnagar (2023) Concluded that moderate tannin intake can promote gut health without significantly impairing nutrient status.

## **Fatty Acid Profile of Curry leaves Chutney**

The chutney contains 52.93% saturated fat, 32% polyunsaturated fat (PUFA), and 15.06% monounsaturated fat (MUFA). Among PUFAs, omega-6 is predominant (31.38%), with a minor proportion of omega-3 (0.32%). While saturated fats are linked to heart risks, the presence of MUFA and PUFA enhances the chutney's nutritional value.

Table 5. Fatty acid profile of Curry leaves chutney /100g

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Nutrients	Percentage (%)	Amount(g)					
Saturated fat	52.93	5.293					
Polyunsaturated fatty acids (PUFA)	32.00	3.2					
Monounsaturated fatty acids (MUFA)	15.06	1.506					
Omega 3 fatty acids	0.32	0.032					
Omega 6 fatty acids	31.38	3.138					

The information is shown in Table 5. 52.93 per cent of the curry leaf chutney's fatty acid composition is saturated fat, a comparatively high percentage associated with heart health hazards. But it also includes healthy unsaturated fats, such as 15.06 per cent monounsaturated (MUFA) and 32 per cent polyunsaturated (PUFA1). A healthy ratio of omega-6 to omega-3 is essential, as evidenced by the fact that 31.38 per cent of the PUFAs are omega-6 (3.138 g/100g) and 0.32per cent are omega-3 (0.032 g/100g). Unsaturated fats, particularly PUFAs, promote the health of the immune system, neurological system, and heart. These necessary fats can lower the incidence of chronic diseases, enhance lipid profiles, and reduce inflammation, as mentioned by Kumar et al. (2021).

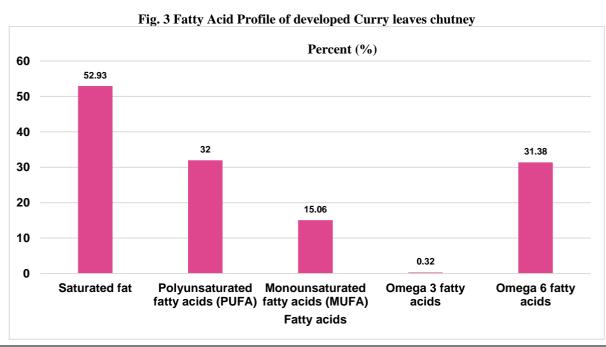


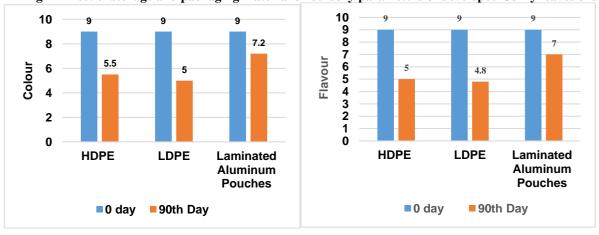
Table 6. Effect of storage and packaging material on sensory parameters of Curry leaves Chutney (25°C room temperature)

temperature)								
<b>Storage Duration</b>	HDPE	LDPE	Laminated aluminium					
(Days)	$(Mean \pm SD)$	$(Mean \pm SD)$	Pouches (LAP) (Mean ± SD)					
0	$9.00 \pm 0.00$	$9.00 \pm 0.00$	$9.00 \pm 0.00$					
15	$8.44 \pm 0.10$	$8.24 \pm 0.12$	$8.84 \pm 0.08$					
30	$7.84 \pm 0.12$	$7.52 \pm 0.15$	$8.42 \pm 0.10$					
45	$7.24 \pm 0.15$	$6.90 \pm 0.18$	$8.10 \pm 0.12$					
60	$6.74 \pm 0.18$	$6.28 \pm 0.20$	$7.70 \pm 0.15$					
75	$6.14 \pm 0.22$	$5.80 \pm 0.25$	$7.34 \pm 0.18$					
90	$5.28 \pm 0.25$	$4.90 \pm 0.28$	$7.00 \pm 0.20$					

All packing materials, including HDPE (High-Density Polyethylene), LDPE (Low-Density Polyethylene), and laminated aluminium pouches (LAF), had sensory ratings of 9.00 at the start of storage (Day 0), suggesting no early variation in product acceptability. All packing styles did, however, show a progressive drop in sensory ratings over time, with differences in the pace of degradation. The sensory ratings decreased somewhat on Day 15, with LAF continuing to score higher (8.84  $\pm$  0.08) than HDPE (8.44  $\pm$  0.10) and LDPE (8.24  $\pm$  0.12). The sensory ratings were drastically decreased by day 60, especially in LDPE (6.28  $\pm$  0.20), which was followed by HDPE (6.74  $\pm$  0.18). LAF, on the other hand, demonstrated a comparatively higher score (7.70  $\pm$  0.15), indicating superior retention of sensory attributes such flavour, texture, scent, and general acceptance. (Table.6).

The final sensory ratings after 90 days were  $7.00 \pm 0.20$  (LAF),  $4.90 \pm 0.28$  (LDPE), and  $5.28 \pm 0.25$  (HDPE). This suggests that LAF preserved better sensory qualities than HDPE and LDPE, which showed significant drops in product quality. After reviewing cutting-edge food packaging technologies, Singh et al. (2024) came to the conclusion that composite materials, including aluminium laminates, retain customer acceptance for longer even under ambient circumstances by reducing sensory losses and prolonging shelf life. comparison to single-layered plastic materials, Patel and Verma (2023) showed that laminated pouches preserved superior textural qualities and overall acceptability in spice-based food preparations throughout extended storage. Light, air, and moisture are three major elements that hasten the degradation of sensory capabilities, and the laminated structure served as an efficient barrier against them. According to Thakur and Choudhary (2023), multilayered aluminum-based packaging offered superior defence against microbiological growth and oxidative rancidity, two factors that are essential for preserving the freshness of semi-moist items like chutneys.

Fig. 4 Effect of storage and packaging material on sensory parameters of developed Curry leaves chutney



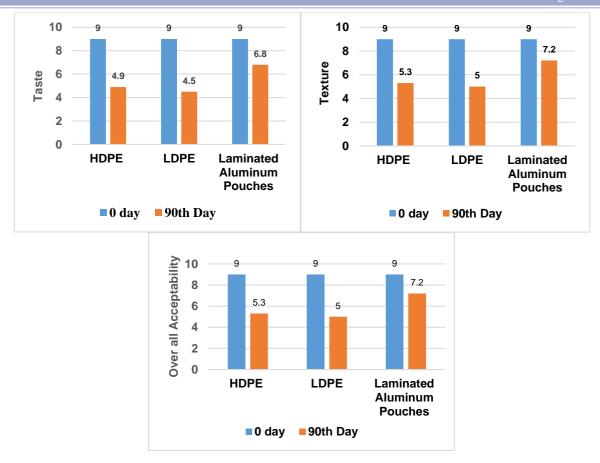


Table 7. Effect of storage and packaging material on biochemical parameters of Curry leaves Chutney

Storage Duration (Days)	Packaging Material	Moisture Content (%)	Peroxide Value (mEq O <sub>2</sub> /kg fat	Free Fatty Acids (% Oleic Acid)
0	HDPE	$5.0 \pm 0.2$	0.85±0.005	0.22±0.02
	LDPE	$5.0 \pm 0.2$	0.85±0.005	0.22±0.02
	Laminated Aluminium Pouch	5.0 ± 0.2	0.85±0.005	0.22±0.02
90	HDPE	4.3±0.2	3.00. ±0.12	0.55±0.03
	LDPE	4.2±0.2	3.45±0.15	0.60±0.04
	Laminated Aluminium Pouch	4.5±0.2	2.40±0.11	0.45±0.03

Table 7. demonstrates that choosing the right packing material is essential to preserving the food items' biochemical integrity and increasing their shelf life. In this study, curry leaf chutney kept in laminated aluminium pouches outperformed HDPE and LDPE in terms of stability during a 90-day period. Better protection against both oxidative and hydrolytic rancidity was shown by lower moisture loss  $(4.5 \pm 0.2\%)$  and smaller increases in peroxide value  $(2.40 \pm 0.11 \text{ mEq O}_2/\text{kg})$  fat) and free fatty acids  $(0.45 \pm 0.03\%)$ .

These results are consistent with earlier studies by Rathod and Kapoor (2022), who highlighted the significance of moisture retention in food preservation, and Singh et al. (2023), who demonstrated enhanced oxidative stability in low-oxygen-permeable materials. The usefulness of laminated aluminium pouches in offering superior barrier qualities was further

validated by Patel et al. (2023), confirming the present findings. Thus, it is advised to use laminated packaging to maintain the quality of foods like chutney that contain fat while they are being stored.

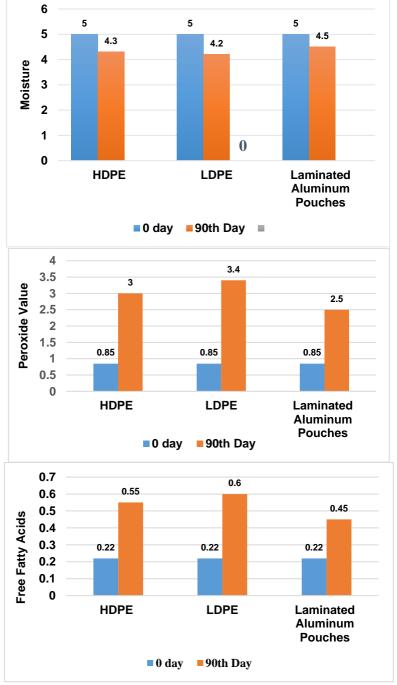


Fig. 5 Effect on storage and packaging material on biochemical parameters

**Microbial Evaluation of Curry Leaves Chutney** 

Microbial spoilage is often the major factor limiting the shelf life of product. Spoilage from microbial growth causes economic loss for manufacturer and consumer. These losses can be minimized to some extent by adopting desirable packaging material during storage.

Table 8. Effect of Packaging Material on Yeast & Mould Count (log CFU/g) of Curry Leaves Chutney at 0 and 90 Days of Storage

Microbial Parameter	Packaging Material	0 Days (Mean ± SD)	90 Days (Mean ± SD)	t-test (p- value	ANOVA (p-value)	Significant Difference
Yeast & Mould Count (log CFU/g)	HDPE	$1.20\pm0.05$	$3.00 \pm 0.12$	0.6501	0.5114	No
	LDPE	$1.22 \pm 0.04$	$3.45 \pm 0.15$			
	Laminated Aluminium pouches	$1.15 \pm 0.06$	$2.40 \pm 0.11$			
After (90 <sup>th</sup> day)	HDPE	$2.50 \pm 0.10$	$5.80 \pm 0.20$	0.6790	0.4947	No
	LDPE	$2.55 \pm 0.12$	$6.20 \pm 0.25$			
	Laminated Aluminium pouches	$2.45 \pm 0.08$	$5.40 \pm 0.18$			

Note: CFU/g = Colony Forming Units per gram; SD = Standard Deviation; p-value indicates the probability of observing the data given that the null hypothesis is true.

Table 8. The microbiological stability of curry leaf chutney over a 90-day storage period revealed an overall rise in the numbers of yeast, mould, and total plates in all evaluated packing materials, including laminated aluminium pouches, HDPE, and LDPE. Despite the fact that laminated aluminium pouches had the lowest microbial counts and LDPE samples the highest, statistical analysis showed no discernible variations across the packaging materials. This implies that although laminated aluminium packing could have some benefit in preventing the growth of microorganisms, other important aspects include initial contamination and storage conditions. According to earlier research (Desai et al., 2023; Singh & Sharma, 2023; Bhat & Mehta, 2022), laminated aluminium pouches have better barrier qualities that reduce microbial proliferation, especially by restricting moisture retention that encourages fungal development. These findings are consistent with those findings. To definitively prove these patterns, more research with bigger sample numbers is required.

#### 3. CONCLUSIONS

The curry leaves-based chutney that was produced, especially Variation IV with equal portions curry leaves powder and Ambadi seeds, was found to be the most palatable formulation because it balanced flavour, texture, and appearance all of which are important considerations for customer loyalty. According to nutritional analysis, it is appropriate for those with hyperlipidaemia because of its high protein, dietary fibre, calcium, and iron content. Its high fibre content promotes healthy digestion and lowers cholesterol, while bioactive substances including flavonoids, phenols, beta-carotene, and chlorophyll provide antioxidant protection that may lower the risk of cardiovascular disease and oxidative stress. In addition to low amounts of antinutrients, the chutney contains healthy unsaturated fats, such as omega-6 fatty acids, which ensure safety and preserve mineral bioavailability. Its price and viability as a functional food were demonstrated by techno-economic analysis, and shelf-life and microbiological experiments verified that the product remained stable for up to three months when kept in laminated aluminium pouches. For the management of hyperlipidaemia and the improvement of heart health, this chutney is an all-around wholesome, palatable, and useful nutritional intervention.

#### 4. ACKNOWLEDGEMENTS

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

## **AUTHORS CONTRIBUTION**

Ms. Dipali Sakharam Sangekar was primarily responsible for the conceptualization of the research, formulation and

standardization of the curry leaves-based chutney, conducting nutritional and storage stability analyses, interpreting the data, and preparing the original draft of the manuscript. Dr. Vijaya Shivajirao Pawar provided overall supervision, methodological guidance, and technical support related to food processing and storage studies, and contributed to the critical review and editing of the manuscript. Mr. Eknath Ashroba Langote assisted in sample collection, data compilation, and statistical analysis, and participated in reviewing and editing the manuscript. Dr. Godawari Shivajirao Pawar contributed to the selection of ingredients, particularly from a botanical and nutritional standpoint, validated plant-based nutritional data, and provided inputs during manuscript review. All authors have read and approved the final version of the manuscript.

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