



Formulation and optimisation of a spicy sauce from raw tamarind: Impact on physicochemical, nutritional and sensory properties

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ABSTRACT

Tamarind is a nutritious and drought tolerant wild fruit that is underutilized in most of the countries including India. An experimental study was carried out to develop and optimize a spicy sauce derived from raw tamarind pods of the DTS-1 variety, with subsequent evaluation of its physicochemical, nutritional, sensory, and microbial parameters. The study was conducted to formulate and optimize nine formulations with varying levels of sugar, salt, vinegar, and pectin, which were evaluated during 90 days of ambient storage. Results indicated significant differences among treatments regarding color, taste, odour, consistency, total soluble solids, titratable acidity, ascorbic acid, total phenols, and microbial load. Treatment T₇ (1.5 g salt, 25 g sugar, 1 ml vinegar, 0.5 g pectin) showed excellent sensory acceptability, color retention, and microbial safety. The study concludes that the optimized raw tamarind spicy sauce possesses potential characteristics for commercial production, providing a value-added product with improved shelf life and consumer preferences.

Key words: DTS-1, spicy sauce, bacterial count, pectin, sensory score.

INTRODUCTION

Tamarindus indica L., commonly known as tamarind, is a tropical tree species native to Africa and widely naturalized in over 50 countries because of its adaptability and multipurpose uses. Among the various parts of this species, the raw, unripe fruit remains markedly under exploited, despite its ready availability in tropical and subtropical regions. While raw tamarind is traditionally used in local cuisines as a souring agent or consumed with salt and spices, its nutritional and functional potential has not been widely utilized or commercialized, with most existing applications focusing on ripe pulp products (EISiddig, 6).

Raw tamarind fruit is characterized by a firm, fibrous pulp and strong acidic taste, primarily due to high concentrations of organic acids such as tartaric and citric acids and comparatively lower sugars than found in mature fruit (EISiddig, 6; Abdi and Serrem, 1). Nutritional analyses indicate that unripe tamarind pulp contains meaningful levels of carbohydrates, dietary fibre, essential minerals such as calcium, phosphorus, and iron, and vitamins including B₂ and

vitamin C, making it a valuable nutrient source even at the raw stage (Hossain *et al.*, 8). Moreover, studies comparing raw and mature stages of tamarind fruit report that bioactive phenolics and antioxidant capacity are present across maturity levels, indicating that raw tamarind pulp possesses appreciable antioxidant potential and may serve as a functional food ingredient (Hossain *et al.*, 8).

Beyond basic nutrition, raw tamarind contains bioactive compounds such as phenolics and flavonoids, which contribute to a range of health promoting effects, including antioxidant, antimicrobial, lipid lowering, antidiabetic, and digestive activities (Isha and Milind, 11). Laboratory investigations show that crude extracts of tamarind pulp irrespective of maturity exhibit inhibitory activity against carbohydrate metabolizing enzymes such as α amylase and α glucosidase and enhance glucose uptake in cell based models, suggesting antidiabetic potential that may also be relevant in raw fruit preparations (Ghaly *et al.*, 7; Krishna *et al.*, 12).

Despite these promising nutritional and functional properties, raw tamarind remains underutilized in product development, and standardized methods for processing it into value added foods are limited (Pandian *et al.*, 14). This represents a clear research gap, as harnessing raw tamarind for culinary and commercial applications could preserve its unique qualities while diversifying its use beyond traditional consumption.

In this context, the present study aimed to develop a spicy sauce using raw tamarind pulp and

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evaluate its physicochemical, nutritional, and sensory characteristics, with the objective of promoting the use of this under exploited raw fruit and enhancing its commercial potential.

MATERIALS AND METHODS

The present investigation was carried out in Bagalkot taluk, located in the Northern Dry Zone (Zone-3) of Karnataka, India, at 16°10' N latitude and 72°42' E longitude and an altitude of 542 meters above mean sea level. The region experiences semi-arid climatic conditions with an average annual rainfall of 318 mm, predominantly received from May to November. The maximum temperature reached 41.4 °C in May and the minimum dropped to 13.4 °C in February. Green, mature, unripe pods of the DTS-1 variety of tamarind were procured in December 2022 from Kitturu Rani Chennamma College of Horticulture, Arabhavi, Belagavi district, Karnataka, while all other chemicals and ingredients, including analytical-grade salt, sugar, vinegar, pectin, and preservatives, were purchased from reliable local sources. Nine treatments of tamarind spicy sauce were prepared (Table 1), categorized as low (T₁–T₃), medium (T₄–T₆), and high (T₇–T₉) sugar levels, and optimized with varying proportions of salt, vinegar, and pectin. A spice infusion bag was applied uniformly across all treatments, and sodium benzoate at 0.025% was added as a preservative. Prepared sauces were filled into sterilized, airtight glass bottles and stored at ambient temperature (25 ± 2 °C) until analysis.

Physicochemical properties were assessed using standard procedures. Sample weights were determined using an electronic balance and recorded in grams, while volumes were measured by placing 5 g of each sample in a graduated cylinder and

recording the displaced volume in milliliters. Bulk density was calculated by placing 40 g of sample in a 100 mL standard cylinder and tapping until a constant volume was obtained, with density expressed as the ratio of weight to volume (Crowe and Murray, 5). Flow characteristics or viscosity of the sauce were measured using a Bostwick Consistometer, recording the time required for the sample to flow under gravity. Total soluble solids (TSS) were measured using a hand-held refractometer and expressed in °Brix following Tumpa *et al.* (19). Titratable acidity was estimated by potentiometric titration using 0.1 N NaOH with phenolphthalein as an indicator; 2 mL of sample were diluted to 100 mL with distilled water and 5 mL aliquots titrated to a pale pink endpoint, with results expressed as percent tartaric acid equivalent (Ranganna, 15). Sample pH was determined with a calibrated digital pH meter using standard buffer solutions of pH 4.0, 7.0, and 9.2 (Zahangir *et al.*, 20). Ascorbic acid content was analyzed by titration with 2,6-dichlorophenol indophenol dye following Ranganna (15), where 5 mL aliquots of the homogenized sample diluted in 4% oxalic acid were titrated and results expressed as mg/100 mL. Total phenolic content was estimated using the Folin–Ciocalteu method with 80% methanol extracts and gallic acid as standard, incubating the reaction mixture at 60 °C for 10 minutes, cooling rapidly, and measuring absorbance at 650 nm; results were expressed as mg gallic acid equivalents per 100 mL (Madaan *et al.*, 2011). Colour was quantified using a Digital Hunter Colorimeter (D25) in the CIELAB system, where L* represented lightness, a* red/green, and b* yellow/blue (Chouhan *et al.*, 4). Non-enzymatic browning was assessed by mixing 10 mL of sample with 10 mL distilled water and 30 mL of 60% ethanol, centrifuging, and measuring the absorbance of the supernatant at 440 nm with 60% ethanol as a blank (Sumitha *et al.*, 18). Total microbial load was estimated using serial dilution and spread plate methods, with bacterial, fungal, and mold counts expressed as colony-forming units per gram (cfu/g) (Ranganna, 15). Sensory evaluation was conducted by a panel of ten semi-trained members, assessing colour, taste, flavour, texture/consistency, and overall acceptability on a nine-point hedonic scale, where 9 indicated “like extremely” and 1 indicated “dislike extremely” (Begum *et al.*, 3). All experimental data were analyzed using a completely randomized design (CRD), and Duncan’s multiple range test was applied for post hoc comparisons at p = 0.01, with critical difference values calculated wherever significant F values were observed.

Table 1: Treatment details for optimization of ingredients for raw tamarind spicy sauce.

Treatment	Raw tamarind pulp (g)	Salt (g)	Sugar (g)	Vinegar (ml)	Pectin (g)
T ₁	100	1.5	15	1	0.5
T ₂	100	2.0	15	1.5	0.75
T ₃	100	3.0	15	2	1.0
T ₄	100	1.5	20	1	0.5
T ₅	100	2.0	20	1.5	0.75
T ₆	100	3.0	20	2	1.0
T ₇	100	1.5	25	1	0.5
T ₈	100	2.0	25	1.5	0.75
T ₉	100	3.0	25	2	1.0

RESULTS AND DISCUSSION

Significant variations were observed among treatments for the physicochemical properties of raw tamarind spicy sauce. Treatment T₉ (3 g salt, 25 g sugar, 2 ml vinegar, and 1 g pectin) recorded the highest weight (95.27 g) and volume (84.85 ml), followed by T₈, whereas T₁ showed the lowest weight (87.15 g) and volume (72.74 ml). The greater weight and volume in T₉ may be due to higher quantities of ingredients, particularly sugar and pectin, which increased product yield. Density exhibited an inverse relationship with volume, T₁ recorded the highest density (1.19 g/ml), comparable to T₂ and T₃, whereas T₉ and T₈ had the lowest density (1.12 g/ml), similar to T₇, reflecting expansion of the product matrix in high-sugar formulations.

Consistency varied among treatments. T₁ recorded the highest value (6.15 min), similar to T₂ and T₃, whereas T₉ showed the lowest (5.51 min), comparable to T₈ and T₇. The lower viscosity in high-sugar formulations may be attributed to the plasticizing effect of sugar, which reduces intermolecular forces and enhances flowability (Table 2).

TSS increased with storage, from 30.00 °Brix initially to 34.36 °Brix at 90 days after storage (DAS) (Table 3). T₈ and T₉ recorded the highest initial TSS values, while T₁ had the lowest, reflecting reduced sugar content. At 90 DAS, T₉ showed the maximum TSS (34.70 °Brix), likely due to polysaccharide breakdown and moisture loss, consistent with Shakir *et al.* (16). Titratable acidity increased gradually during storage (Table 3), with T₁ recording the highest (2.08%) and T₇ the lowest (1.73%) initially. At 90 DAS,

Table 2: Effect of different treatment on weight, volume, density and consistency of raw tamarind spicy sauce.

Treatment	Weight (g)	Volume (ml)	Density (g/ml)	Consistency (min)
T ₁	87.15 ^g	72.74 ^f	1.19 ^a	6.15 ^a
T ₂	87.67 ^{fg}	73.46 ^e	1.19 ^a	6.13 ^a
T ₃	87.95 ^f	74.15 ^e	1.18 ^a	6.12 ^a
T ₄	90.61 ^e	78.43 ^d	1.15 ^b	5.83 ^b
T ₅	90.81 ^e	79.34 ^c	1.14 ^b	5.82 ^b
T ₆	91.18 ^d	79.91 ^c	1.14 ^{bc}	5.80 ^b
T ₇	94.52 ^c	83.41 ^b	1.13 ^{cd}	5.52 ^c
T ₈	94.82 ^b	84.33 ^a	1.12 ^d	5.52 ^c
T ₉	95.27 ^a	84.85 ^a	1.12 ^d	5.51 ^c
Mean	91.11	78.96	1.15	5.82
S.Em±	0.05	0.16	0.00	0.01
C.D. @ 1%	0.21	0.65	0.01	0.04

T₁ remained the highest (2.19%) and T₇ the lowest (1.86%), with T₈ and T₉ slightly higher. The increase may be associated with pectin hydrolysis and ascorbic acid degradation (Iftikhar *et al.*, 9). pH declined correspondingly (Fig. 1), with T₁ highest (4.33) and T₈ lowest (3.95) initially, decreasing further at 90 DAS (T₁: 3.83, T₉: 3.59), reflecting acid accumulation (Singh and Sharma, 17).

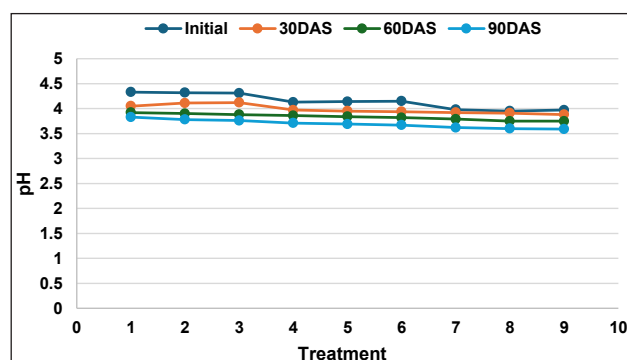
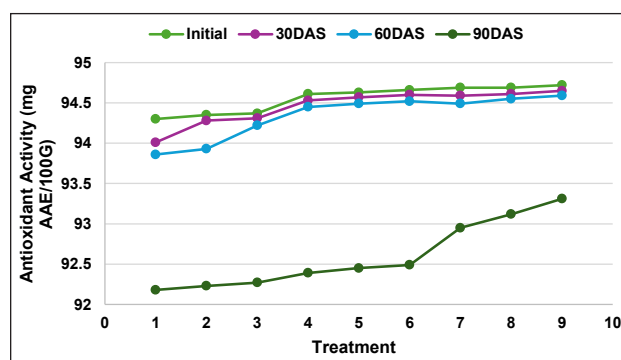
Ascorbic acid content decreased slightly during storage (Table 4). T₉ had the highest initial content (2.76 mg/100 g), comparable to T₈, while T₃ had the lowest, similar to T₁ and T₂. At 90 DAS, T₉ retained

Table 3: Effect of different treatment on TSS (°Brix) and titratable acidity of raw tamarind spicy sauce during ambient storage.

Treatment	TSS (°Brix)				Titratable acidity			
	Initial	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS
T ₁	30.26 ^c	31.16 ^c	31.56 ^c	32.10 ^d	2.08 ^b	2.08 ^a	2.15 ^a	2.19 ^a
T ₂	30.43 ^c	31.21 ^c	31.71 ^c	32.20 ^d	2.05 ^b	2.07 ^a	2.16 ^a	2.22 ^a
T ₃	30.46 ^c	31.47 ^c	31.87 ^c	32.40 ^d	2.04 ^a	2.11 ^a	2.18 ^a	2.23 ^a
T ₄	31.83 ^b	32.66 ^b	33.16 ^b	33.60 ^c	1.83 ^c	1.88 ^b	2.00 ^b	2.06 ^b
T ₅	31.93 ^b	32.76 ^b	33.26 ^b	33.80 ^c	1.80 ^d	1.88 ^b	2.04 ^b	2.08 ^b
T ₆	31.66 ^b	32.86 ^b	33.36 ^b	33.90 ^c	1.82 ^{cd}	1.92 ^b	2.05 ^b	2.10 ^b
T ₇	33.30 ^a	34.06 ^a	34.16 ^a	34.30 ^b	1.73 ^f	1.78 ^c	1.82 ^c	1.86 ^c
T ₈	33.43 ^a	34.16 ^a	34.28 ^a	34.55 ^a	1.74 ^f	1.77 ^c	1.83 ^c	1.88 ^c
T ₉	33.35 ^a	34.36 ^a	34.46 ^a	34.70 ^a	1.77 ^e	1.80 ^c	1.86 ^c	1.90 ^c
Mean	31.85	32.75	33.09	33.51	1.88	1.92	2.01	2.06
S.Em±	0.14	0.17	0.05	0.08	0.00	0.003	0.003	0.003
C.D. @ 1%	0.60	0.90	0.23	0.35	0.01	0.03	0.03	0.05

Table 4: Effect of different treatment on ascorbic acid and total phenolic content of raw tamarind spicy sauce during ambient storage.

Treatment	Ascorbic acid				Total phenolic content			
	Initial	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS
T ₁	2.65 ^{ef}	2.62 ^c	2.55 ^c	2.50 ^b	129.30 ^c	128.99 ^c	127.79 ^c	127.64 ^c
T ₂	2.66 ^f	2.60 ^c	2.53 ^c	2.49 ^b	129.50 ^c	129.40 ^c	128.21 ^c	127.34 ^c
T ₃	2.63 ^c	2.58 ^c	2.53 ^c	2.47 ^b	129.60 ^c	128.40 ^c	127.89 ^c	127.58 ^c
T ₄	2.69 ^d	2.63 ^{bc}	2.60 ^b	2.52 ^b	135.40 ^b	135.01 ^b	133.63 ^b	133.10 ^b
T ₅	2.68 ^{ed}	2.61 ^b	2.56 ^d	2.52 ^b	135.60 ^b	134.12 ^b	133.89 ^b	133.45 ^b
T ₆	2.70 ^c	2.64 ^b	2.60 ^c	2.53 ^b	135.80 ^b	135.51 ^b	134.04 ^b	133.86 ^b
T ₇	2.73 ^b	2.70 ^a	2.65 ^b	2.60 ^a	139.30 ^a	139.12 ^a	137.39 ^a	136.61 ^a
T ₈	2.74 ^{ab}	2.70 ^a	2.65 ^b	2.58 ^a	139.40 ^a	138.98 ^a	138.41 ^a	137.71 ^a
T ₉	2.76 ^a	2.73 ^a	2.68 ^a	2.63 ^a	139.70 ^a	139.62 ^a	138.05 ^a	137.73 ^a
Mean	2.69	2.64	2.59	2.53	134.84	134.35	133.25	132.77
S.Em±	0.007	0.01	0.006	0.01	0.35	0.35	0.35	0.35
C.D. @ 1%	0.02	0.05	0.02	0.06	1.45	1.44	1.43	1.43

**Fig. 1.** Effect of different treatment on pH of raw tamarind spicy sauce ambient during storage.**Fig. 2.** Effect of treatment on antioxidant activity of raw tamarind spicy sauce during ambient storage.

the highest content (2.63 mg/100 g), whereas T₃ remained the lowest (2.47 mg/100 g). Sugar may have protected ascorbic acid from oxidation (Apaiah and Barringer, 2). Total phenolic content also declined slightly (Table 4), with T₉ initially the highest (139.70 mg GAE/100 g) and T₁, T₂, and T₃ the lowest. At 90 DAS, T₉ maintained the highest content (137.73 mg GAE/100 g), and T₂ the lowest (127.34 mg GAE/100 g). Antioxidant activity followed a similar pattern (Fig. 2), with T₉ consistently exhibiting the highest activity and T₁ the lowest, showing minor reduction over storage due to phenolic and ascorbic acid degradation.

Colour characteristics were influenced by treatment and storage (Table 5). L* values were highest in T₃ (30.32) and lowest in T₇ (27.30), indicating darker products in high-sugar treatments. a* values

were highest in T₉ (5.03) and lowest in T₁ (4.20), whereas b* values declined over storage, lowest in T₇ (7.92). Non-enzymatic browning increased slightly during storage (Table 6), with T₉ showing the highest index (0.048) and T₁ the lowest (0.038), likely due to Maillard reactions in high-sugar formulations.

Sensory evaluation indicated that T₇ (1.5 g salt, 25 g sugar, 1 ml vinegar, 0.5 g pectin) consistently received the highest scores for colour, taste, flavour, consistency and overall acceptability (Fig. 3) at both initial and 90 DAS, whereas T₁ scored the lowest. The higher sugar content in T₇ balanced the natural tartness of tamarind, enhancing acceptability. Minimal declines in sensory scores during storage suggested good product stability.

Microbial counts increased gradually with storage (Table 7). Initial bacterial loads ranged from 2.64

Table 5: Effect of treatment on color values of raw tamarind spicy sauce during ambient storage.

Treatment	L*				a*				b*			
	Initial	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS
T ₁	30.30 ^a	29.97 ^a	28.89 ^a	28.80 ^a	4.20 ^c	4.30 ^c	4.39 ^c	4.43 ^c	12.79 ^a	12.57 ^a	12.45 ^a	12.33 ^a
T ₂	30.31 ^a	30.01 ^a	28.95 ^a	28.91 ^a	4.23 ^c	4.29 ^c	4.39 ^c	4.45 ^c	12.80 ^a	12.71 ^a	12.50 ^a	12.38 ^a
T ₃	30.32 ^a	30.03 ^a	28.98 ^a	28.94 ^a	4.25 ^c	4.29 ^c	4.34 ^c	4.49 ^b	12.81 ^a	12.75 ^a	12.65 ^a	12.53 ^a
T ₄	28.40 ^b	28.36 ^b	27.30 ^b	27.01 ^b	4.31 ^b	4.37 ^b	4.41 ^b	4.49 ^b	9.00 ^b	8.83 ^b	8.72 ^b	8.63 ^b
T ₅	28.41 ^b	28.38 ^b	27.31 ^b	26.99 ^b	4.31 ^b	4.37 ^b	4.42 ^b	4.47 ^b	9.15 ^b	8.84 ^b	8.54 ^b	8.46 ^b
T ₆	28.42 ^b	28.36 ^b	27.13 ^b	27.09 ^b	4.32 ^b	4.39 ^b	4.46 ^b	4.62 ^b	9.19 ^b	8.85 ^b	8.79 ^b	8.70 ^b
T ₇	27.30 ^c	27.30 ^c	25.99 ^c	25.91 ^c	5.01 ^a	5.11 ^a	5.23 ^a	5.31 ^a	7.92 ^c	7.82 ^c	6.90 ^c	6.83 ^d
T ₈	27.35 ^c	27.30 ^c	26.02 ^c	25.94 ^c	5.02 ^a	5.12 ^a	5.21 ^a	5.25 ^a	7.93 ^c	7.84 ^c	7.79 ^c	7.71 ^c
T ₉	27.38 ^c	27.31 ^c	26.02 ^c	25.95 ^c	5.03 ^a	5.14 ^a	5.21 ^a	5.68 ^a	7.95 ^c	7.89 ^c	7.31 ^c	7.24 ^c
Mean	28.68	28.54	27.41	27.37	4.52	4.59	4.67	4.79	9.94	9.78	9.52	9.42
S.Em±	0.07	0.07	0.07	0.07	0.01	0.01	0.01	0.01	0.02	0.07	0.01	0.20
C.D. @ 1%	0.30	0.29	0.29	0.29	0.04	0.04	0.05	0.05	0.10	0.29	0.16	0.81

Table 6: Effect of treatment on non-enzymatic browning (OD values) of raw tamarind spicy sauce during ambient storage.

Treatment	Non-enzymatic browning			
	Initial	30 DAS	60 DAS	90 DAS
T ₁	0.038	0.038	0.047	0.047
T ₂	0.039	0.039	0.050	0.050
T ₃	0.040	0.038	0.052	0.052
T ₄	0.041	0.041	0.049	0.049
T ₅	0.042	0.042	0.052	0.052
T ₆	0.043	0.043	0.054	0.053
T ₇	0.044	0.044	0.054	0.053
T ₈	0.046	0.046	0.056	0.055
T ₉	0.048	0.048	0.057	0.056
Mean	0.042	0.042	0.052	0.052
S.Em±	0.00	0.00	0.00	0.00
C.D. @ 1%	NS	NS	NS	NS

to 3.90 log₁₀ cfu/ml and fungal counts from 1.30 to 1.62 log₁₀ cfu/ml. T₉ consistently had the lowest microbial loads, whereas T₁ had the highest. At 90 DAS, T₉ continued to show the lowest counts and T₁ the highest. Reduced microbial counts in high-sugar and high-salt formulations may be due to lower water activity. All microbial counts remained within FSSAI limits (FSSAI, 10).

The study successfully developed a raw tamarind spicy sauce with desirable physicochemical and sensory attributes by optimizing salt, sugar, vinegar, and pectin levels. Among the formulations, T₇ showed

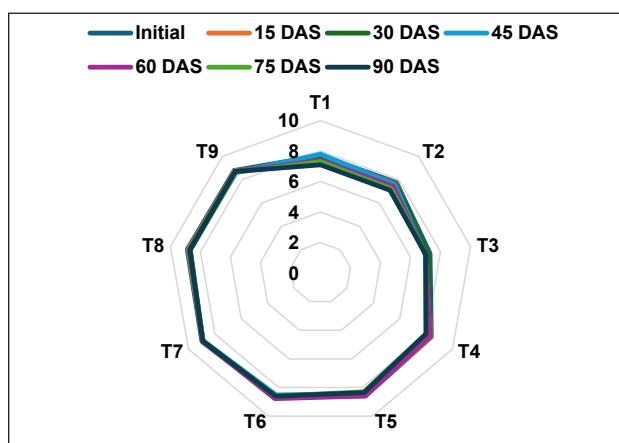


Fig. 3. Effect of different treatment on overall acceptability (9-point hedonic scale) of raw Tamarind spicy sauce during ambient storage.

the highest overall acceptability, with excellent color, taste, aroma, consistency, and microbial stability up to 90 days of storage. Higher sugar levels influenced viscosity and total soluble solids, while careful use of preservatives ensured safety. The optimized sauce demonstrates the potential of raw tamarind pods for value-added product development and offers a practical option for commercial production in agro-based industries.

AUTHOR’S CONTRIBUTION

Conceptualization of research (K.V.); Designing of the experiments (K.V. and B.G.); Contribution of experimental materials (V.M.G.); Execution of lab experiments (K.V. and P.S.); Analysis of data

Table 7: Effect of different treatment on microbial load of raw tamarind spicy sauce during ambient storage.

Treatment	Total bacterial count (cfu/ml)				Total fungal count (cfu/ml)			
	Initial	30 DAS	60 DAS	90 DAS	Initial	30 DAS	60 DAS	90 DAS
T ₁	3.90 ^a	4.00 ^a	4.20 ^a	4.50 ^a	1.56 ^a	1.62 ^a	1.91 ^a	2.12 ^a
T ₂	3.40 ^a	3.90 ^a	4.19 ^b	4.20 ^c	1.54 ^{ab}	1.61 ^a	1.92 ^a	2.06 ^b
T ₃	3.30 ^{ab}	4.00 ^a	4.23 ^a	4.30 ^b	1.53 ^b	1.59 ^a	1.90 ^a	2.00 ^c
T ₄	3.40 ^{ab}	3.70 ^b	3.89 ^c	3.91 ^d	1.43 ^c	1.56 ^b	1.78 ^c	1.94 ^d
T ₅	3.20 ^b	3.40 ^c	3.67 ^d	3.87 ^e	1.42 ^c	1.54 ^b	1.72 ^c	1.95 ^d
T ₆	3.10 ^{bc}	3.50 ^c	3.59 ^e	3.79 ^f	1.39 ^{de}	1.53 ^b	1.70 ^c	1.93 ^d
T ₇	2.90 ^c	3.20 ^d	3.35 ^f	3.50 ^g	1.38 ^e	1.44 ^{bc}	1.54 ^d	1.71 ^e
T ₈	2.75 ^{cd}	3.00 ^e	3.33 ^f	3.43 ^h	1.32 ^f	1.46 ^c	1.58 ^d	1.69 ^e
T ₉	2.64 ^d	2.85 ^f	3.13 ^f	3.21 [□]	1.30 ^f	1.40 ^d	1.59 ^d	1.67 ^e
Mean	3.18	3.49	3.73	3.86	1.43	1.53	1.74	1.89
S.Em±	0.01	0.02	0.01	0.01	0.00	0.01	0.02	0.010
C.D. @ 1%	0.06	0.10	0.07	0.07	0.02	0.03	0.03	0.04

and interpretation (K.V. and S.S.); Preparation of manuscript (K.V. and S.S.).

DECLARATION

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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