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RETORT-PROCESSED SEERAGA SAMBA RICE BIRYANI AMBIENT STORAGE QUALITY

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ABSTRACT
Seeraga Samba (*Oryza sativa* var. *indica*), a premium aromatic short-grain rice cultivar from Tamil Nadu, India, has not previously been investigated for its suitability in retort-processed ready-to-eat (RTE) foods. This study aimed to optimize the retort processing conditions for Seeraga Samba vegetable biryani and evaluate its physicochemical, microbiological, and sensory stability during ambient storage. Response Surface Methodology (RSM) using a Central Composite Design (CCD) was employed to optimize processing temperature (110–121.1°C) and holding time (5–15 min), with colour (Lovibond L*), moisture content, pH, and microbial count as response variables. The optimized process conditions of 121.1°C for 10 min achieved a composite desirability of 0.900 and a thermal lethality (F_0) value of 10.3 ± 0.4 min, ensuring commercial sterility. The optimized product was packaged in multilayer retort pouches (PET/Al/PA/CPP) and stored at ambient temperature (28 ± 2°C) for 90 days. During storage, carbohydrate, protein, fat, moisture, ash, and crude fibre contents ranged from 24.14–25.47%, 2.48–2.80%, 1.62–2.37%, 68.56–70.76%, 0.94–1.05%, and 0.53–0.86%, respectively. The pH decreased from 6.3 to 5.9, while the Lovibond L* value declined from 52.1 ± 0.8 to 50.1 ± 0.6, indicating only slight physicochemical changes during storage. No microbial growth was detected throughout the storage period, confirming the commercial sterility of the product. Sensory evaluation showed that the overall acceptability remained above the acceptable limit, with a score of 6.7 ± 0.3 on a 9-point hedonic scale after 90 days. The findings demonstrate that retort processing is an effective preservation technique for producing a safe, shelf-stable, and acceptable Seeraga Samba vegetable biryani with a minimum ambient shelf life of 90 days, thereby providing a scientific basis for the commercial utilization and value addition of this traditional rice cultivar in the growing RTE food sector.

KEYWORDS: Ambient storage, Biryani, Retort processing, Seeraga Samba, Thermal sterilization.

1.INTRODUCTION
Rice (*O. sativa L.)* is the major cereal crop of most parts of India, being one of the largest producers and consumers in the world. In the Indian context, another unique gene, badh2, that leads to the production of 2 acetyl 1 pyrroline, is present in Seeraga Samba (O. sativa var. indica), a short grain rice grown in the Cauvery delta of Tamil Nadu, which gives it a distinctive fragrance (Bindusree et al., 2017). It has a low glycaemic index, is rich in minerals and has a firm cooked texture that does not stick to spices or other foods with (Balasubramanian et al., 2019). Its characteristics make it the ideal ingredient for traditional Thalappakatti (Dindigul style) biryani which is gaining popularity among consumers and is now in the process of being accorded a Geographical Indication.
Urbanization, dual income family and lifestyle changes are the key factors behind the growth of the Indian ready to eat (RTE) food market, which is expected to grow from USD 0.89 billion in 2024 to USD 2.10 billion by 2030 (CAGR 15.30%) (Patgiri, 2022). In this segment, there exists the growing preference for thermally-stabilized traditional rice based meals that need not be transported in cold chain. Retort processing – thermal sterilization with hermetic (commercial sterility) conditions ($F_0 \geq 3$ min at 121.1°C) is a proven technology for the production of shelf stable ready-to-eat foodstuffs packaged in multilayer flexible pouches which has advantages compared to metal cans in terms of heat transfer, weight and quality conservation (Jimenez et al., 2024). Previous studies have successfully applied retort processing to Indian ethnic foods, including mutton kheema (F_0 = 6.09, 60day ambient stability) (Lavanya et al., 2016) and chicken biryani (120 day microbial safety) (Thomas, 2022). However, all such work has used long grain Basmati rice varieties. The scientific gap is clear: Seeraga Samba's distinct starch architecture, short grain morphology, and volatile profile impose fundamentally different thermal behaviour and spice matrix interactions during high temperature sterilization. No published data exist on its retort processing behaviour, nor on the consequent physicochemical, microbiological, nutritional, and sensory changes during extended ambient storage.

This study makes an attempt to shed light on this and is the first systematic study on retort processed Seeraga Samba vegetable biryani. Optimization of retort temperature and holding time by response surface methodology to achieve commercial sterility with the maximum quality retention and assessment of the ambient storage stability (90 days) for the product based on microbiological safety, physicochemical parameters (moisture, pH, colour, lipid oxidation) and sensory acceptability. This study not only provides a scientific novelty to the farmers of Cauvery delta area but also provides them another avenue to value add the farmers' indigenous rice variety and meet the market demand for shelf stable traditional Indian cuisine.

2. MATERIALS AND METHODS

2.1 Materials

Agricultural cooperative organizations were identified in Cauvery delta region of Tamilnadu, India and offered Seeraga Samba rice (O. sativa var. indica). The fresh vegetables such as cauliflower (Brassica oleracea var. botrytis), green peas (Pisum sativum), potatoes (Solanum tuberosum), and carrots (Daucus carota) were purchased in a cool condition from the local wholesale vegetables market in Coimbatore, India and were taken into the laboratory. A certified local supplier (Coimbatore, India) provided the spices (bay leaf, cinnamon, cloves, cardamom, star anise, black pepper, cumin, coriander powder, red chili powder, turmeric), cooking oil, ghee, onion, tomato, ginger-garlic paste, green chili, curd, mint leaves, coriander leaves, and salt.

All chemical and reagent were used analytical grade. Petroleum ether (b.p. 40-60°C), orthophosphoric acid, 2-thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were obtained from Merck Life Science Pvt. Ltd. (Mumbai, India). Malondialdehyde (MDA) standard, peptone water, plate count agar (PCA), reinforced clostridial medium (RCM) and potato dextrose agar (PDA) were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Distilled deionized water was used for the trials.

2.2 Methods

2.2.1 Formulation and Standardization of Vegetable Biryani

The vegetable biryani formulation was developed through preliminary trials to establish a recipe with maximum sensory acceptability. Seeraga Samba rice was pre-soaked and cooked in a 1:1.5 rice-to-water ratio until approximately 50% cooked, a stage that facilitates subsequent starch gelatinization during retort processing. Fresh vegetables (cauliflower, green peas, potatoes, and carrots) were washed, peeled where required, and uniformly cut into 2–3 cm cubes to ensure consistent heat penetration during thermal processing (Jimenez et al., 2024).

For gravy preparation, onions, ginger-garlic paste, tomatoes, green chilies, and curd were sautéed with whole spices (bay leaf, cinnamon, cloves, cardamom, star anise, black pepper, cumin) tempered in hot ghee and oil. The partially cooked rice was layered over the gravy base using the pakki method, followed by incorporation of the prepared vegetables. Fresh mint and coriander leaves were added during the final stage of cooking to preserve their volatile aroma compounds. The formulation was optimized through sensory evaluation by a panel of expert tasters, who assessed the product for colour, aroma, taste, and texture to determine the optimum ingredient combination (Fig. 1).

2.3 Optimization of Retort Processing Conditions

Retort processing parameters were optimised by using RSM based on Central Composite Design (CCD). Independent parameters were retort temperature (X: 110-121.1C) and holding time (X: 5-15 min). The CCD consists of five levels (−α, −1, 0, +1, +α) with α = 1.414 for rotatability, which included a total of 13 experimental runs such as four factorial points, four axial points and five centre points replications. Dependent response parameters considered were colour (Lovibond L value), moisture content (%), pH and microbial load (log CFU/g). The experimental data were fitted to a second-order polynomial regression model (Myers et al., 2009):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (1)$$

where Y is the predicted response, β_0 is the intercept, β_1 and β_2 are linear coefficients, β_{11} and β_{22} are quadratic coefficients, β_{12} is the interaction coefficient, X_1 and X_2 are coded independent variables, and ε is the residual error. Model adequacy was evaluated using coefficient of determination (R^2), adjusted R^2, lack-of-fit test, and analysis of variance (ANOVA) at $p < 0.05$ (Myers et al., 2009). Numerical optimization was performed using the desirability function approach in Design-Expert® software version 13.0 (Stat-Ease Inc., Minneapolis, MN, USA). Optimized conditions were validated in triplicate experiments; a deviation <10% between predicted and experimental values was considered acceptable (Jimenez et al., 2024; Stumbo, 1973).

2.4 Retort Processing and Heat Penetration Study

Standardized vegetable biryani (200 ± 5 g per pouch) was hot-filled (>80°C) into four-layer retort pouches (PET/Al/PA/CPP; dimensions 15 × 20 cm; thickness 110 μm) to minimize microbial load and reduce headspace condensation. Pouches were hermetically sealed using an impulse sealer at 180°C for 3 s (Jimenez et al., 2024). Retorting was conducted in a laboratory-scale steam-air overpressure retort at the optimized temperature and holding time determined from the RSM model. Overpressure (1.2–1.5 bar) was maintained throughout the process to prevent pouch deformation. After the holding period, pouches were cooled with chilled water while maintaining overpressure until the core temperature fell below 40°C (Jimenez et al., 2024).

The sterilization value (F_0) was calculated using the General Method (Stumbo, 1973; Holdsworth & Simpson, 2015) by integrating lethality (L) over the entire process duration:

$$F_0=10^{T-Tref/z}dt$$

where T is the product temperature at the cold point (°C), Tref=121.1°C, z = 10°C (for Clostridium botulinum spores), and t is process time (min). Lethality at each interval was calculated as $L=10^{T-121.1/10}$, with cumulative F_0 summed across come-up time (CUT), holding, and come-down time (CDT) phases (Jimenez et al., 2024).
The experimental CCD design encompassed a range of processing conditions, with F_0 values varying accordingly. At the lowest set point (110°C for 5 min), the cumulative F_0 was 2.1 min, which falls below the minimum botulinum cook requirement ($F_0 \geq 3$ min) for low-acid foods (Jimenez et al., 2024). Conversely, at the optimized condition (121.1°C for 10 min), the F_0 reached 10.3 ± 0.4 min, substantially exceeding the ≥3 min botulinum requirement. A safety margin target of $F_0 \geq 6$ min was established for the optimized condition to ensure commercial sterility with an adequate margin of safety, consistent with recommendations for low-acid shelf-stable products (Lavanya et al., 2016; Thomas, 2022). Retorted pouches were incubated at 37°C for 7 days and at 55°C for an additional 7 days to confirm commercial sterility (Lavanya et al., 2016; Thomas, 2022).

2.5 Storage Study Design

The prepared seeraga samba biryani was filled into pouches and stored at room temperature (28 ± 2°C) for 90 days. Samples were taken on day 0 (beginning), day 30, day 60, and day 90 and analysed for physicochemical, nutritional, microbiological, and sensorial parameters. Three pouches were analysed for each sampling point (n = 3).

2.6 Physicochemical Analysis

Moisture Content

The moisture content was determined using a hot-air oven drying method (AOAC 930.04) (AOAC International, 2016). Samples were dried in a hot-air oven at 105 °C for 24 h, and the results were expressed as a percentage on a wet basis.

pH Measurement

The pH was measured using a digital pH meter (accuracy ± 0.01 pH units; Lab India, Mumbai, India) following the AOAC Official 981.42 method. The sample was prepared as a 1:9 (w/v) homogenate of sample in distilled water. The instrument was calibrated with standard buffers (pH 4.0 and pH 7.0) before use.

Colour Measurement

The colour of the samples was measured using a digital tintometer (model LC-100; Lovibond Tintometer Group, Salisbury, UK). The colour measurement was carried out according to the method described by Lovibond Tintometer Group (2020) using standard whiteness, and the results were expressed in terms of Lovibond lightness (L).

Lipid Oxidation (TBARS)

Lipid oxidation was estimated by quantifying thiobarbituric acid reactive substances (TBARS) according to the procedure described by Witte et al. (1970). Sample homogenate (20 g) was blended with 50 mL of cold extracting solution (20% trichloroacetic acid in 2 M orthophosphoric acid). After blending for 2 min, the mixture was diluted to 100 mL with distilled water and filtered through Whatman filter paper No. 1. The filtrate (5 mL) was then mixed with 5 mL of 0.005 M TBA solution and heated in a boiling water bath for 15 min, followed by cooling to room temperature. The absorbance was read at 532 nm against a blank using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The TBARS value, expressed as mg of malondialdehyde per kg of sample, was calculated based on a standard curve prepared from a series of known malondialdehyde concentrations (Witte et al., 1970).

2.7 Nutritional Analysis

Proximate composition was determined at each sampling period using the following AOAC methods (AOAC International, 2016):
Moisture content: Determined by oven drying at 105°C (method 930.06). Crude protein: Calculated by total Kjeldahl method using the factor N x6.25(AOAC method 920.87). Crude Fat: Determined by soxhlet extraction with petroleum ether 40°C – 60°C for 6 h (AOAC 920.39). Ash: Determined by ignition in a muffle furnace at 550°C for 5 h (AOAC 942.05). Carbohydrate: Calculated from the difference using the equation below. Carbohydrate = 100% – (% Moisture + % Crude protein + % Fat + % Ash)

2.8 Microbiological Analysis

Microbiological analyses were all carried out under an aseptic condition, according to APHA guidelines (Chauhan & Jindal, 2020). Sample (10 g) was homogenized for 2 min with 90 mL of sterile 0.1% (w/v) peptone water in a stomacher (Seward Stomacher 400, UK) to create a 10^{-1} dilution and then serially diluted by 10-fold steps. Aerobic plate count (APC) was done by pour-plating on PCA (Hi-Media, Mumbai, India) at 37°C for 48 h (Chauhan & Jindal, 2020). The aerobic and anaerobic plate counts were performed on RCM (Hi-Media, Mumbai, India) and incubated aerobically and anaerobically (Gas-Pak system) for 72 h at 37°C (Chauhan & Jindal, 2020). Spread plating method was used to determine yeast and mould count on PDA (Hi-Media, Mumbai, India) which was made acidified with tartaric acid and incubated at 25°C for 5 days (Beuchat & Cousin, 2001). The data were presented as log CFU/g.

2.9 Sensory Evaluation

The 20 semi-trained panelists (faculty members, research scholars, and staff) evaluated the samples (appearance/colour, aroma, taste/flavour, texture, and overall acceptability) using a 9-point hedonic scale (1-9). The panelists were trained during two pre-training sessions (1-2 h each) on the description of each characteristic, procedures, and the 9-point hedonic scale (Meilgaard et al., 1999). The panelists received coded commercial products with random three-digit numbers and were asked to evaluate the samples without knowing their identity and the storage period. The cut-off limit for acceptability was set at 6.0 ("like slightly") (Bindusree et al., 2017).

2.10 Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) and response surface methodology (RSM) analysis and, finally, means were compared using Duncan's Multiple Range Test (DMRT) at $p < 0.05$ level of significance. Analysis of variance (ANOVA), RSM Modelling, and numerical optimization were carried out using Design-Expert® version 13.0 (Stat-Ease Inc., Minneapolis, MN, USA). Other statistical analyses were achieved using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). The results were expressed as the mean ± standard deviation (SD) of triplicate measurements (n = 3).

3. RESULTS AND DISCUSSION

3.1. Optimization of Retort Processing Conditions Using RSM

3.1.1. Model Fitting and ANOVA

The CCD experimental design produced 13 trials using two independent variables including the retort temperature (X_1: 110–121.1 °C) and holding time (X_2: 5–15 min). A second-order polynomial equation was formulated for the predicted response of colour (Lovibond L), moisture, pH, and microbial load. The analysis of variance (ANOVA) indicated a significant model ($p < 0.05$) with non-significant lack of fit ($p > 0.05$) for all responses. The high coefficient of determination (R2) ranging from 0.82 to 0.96 was achieved, which is comparable to R2 values reported for other retort processes optimized using the RSM (Jimenez et al., 2024; Stumbo, 1973). Similarly, a good agreement between the adjusted R2 and predicted R2 for the model was evident as their values differed by less than 0.2, suggesting that no over-fitting of the data occurred during the analyses (Myers et al., 2009; Solomon, 2021).

The holding time (X_2) was revealed to be the most significant process parameter ($p < 0.05$) for the Lovibond L, moisture, and pH of the samples. However, the retort temperature (X_1) showed a major impact on the microbial load and F0 value of the processed product. Additionally, the interaction effect (X_1X_2) was considered statistically significant ($p < 0.05$) in most cases for the F0 value and overall acceptability of the meals. Thus, the combined effect of the retort temperature and holding time should be considered to achieve optimal microorganism inactivation and desirable sensory attributes of the meals without compromising the product quality. These results are consistent with the findings of Song et al. (2013), who processed retorted short-rib patties using the RSM.

3.1.2. Effect of Processing Conditions on Quality Responses

3.1.2.1. Colour (Lovibond L)

A significant decrease ($p < 0.05$) in Lovibond L values was observed for the sample during prolonged retort processing (Fig. 1, Table. 1), indicating progressive darkening of the product. The darkening of the vegetable biryani was explained by Maillard reactions between reducing sugars (formed by starch hydrolysis in rice) and free amino acids in the spice matrix, as well as caramelization of sugars at high temperature (Jimenez et al., 2024).

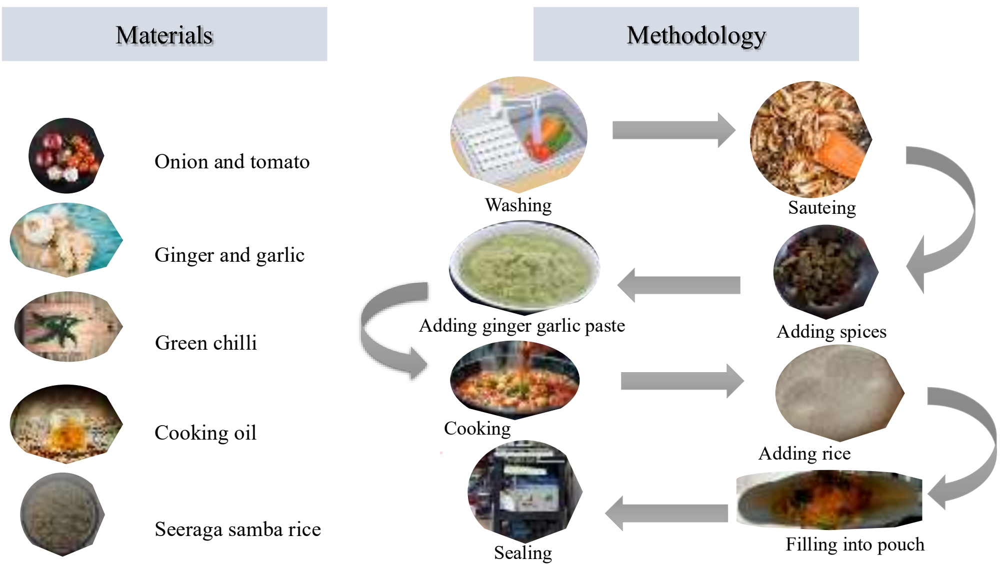

Fig. 1. Material and Methodology used for preparing biryani base Masala

Table 1. Central Composite Design (CCD) Experimental Runs and Responses of Retort-Processed Seeraga Samba Vegetable Biryani

Run	Temperature (°C)	Time (min)	Colour (Lovibond L)	pH	Moisture (%)	Microbial Load (log CFU/g)
1	110.00	10	56.3 ± 0.3	6.72 ± 0.02	62.5 ± 0.4	3.0 ± 0.10
2	121.10	15	53.6 ± 0.4	6.47 ± 0.02	58.6 ± 0.5	1.4 ± 0.08
3	115.55	10	54.2 ± 0.3	6.61 ± 0.02	61.2 ± 0.4	2.5 ± 0.10
4	115.55	5	54.9 ± 0.3	6.65 ± 0.03	61.8 ± 0.4	2.7 ± 0.10
5	115.55	10	54.2 ± 0.3	6.50 ± 0.02	61.2 ± 0.4	2.5 ± 0.10
6	110.00	5	57.2 ± 0.4	6.80 ± 0.03	62.9 ± 0.5	3.2 ± 0.12
7	121.10	10	52.1 ± 0.3	6.30 ± 0.02	58.1 ± 0.4	1.0 ± 0.05
8	110.00	15	55.2 ± 0.3	6.69 ± 0.02	62.1 ± 0.4	2.9 ± 0.10
9	121.10	5	53.9 ± 0.4	6.51 ± 0.02	59.7 ± 0.4	1.7 ± 0.08
10	115.55	10	55.3 ± 0.3	6.61 ± 0.02	60.2 ± 0.4	2.5 ± 0.10
11	115.55	15	54.0 ± 0.3	6.58 ± 0.02	60.5 ± 0.4	1.9 ± 0.08
12	115.55	10	54.2 ± 0.3	6.51 ± 0.02	60.2 ± 0.4	2.5 ± 0.10
13	115.55	10	54.2 ± 0.3	6.61 ± 0.02	61.2 ± 0.4	2.5 ± 0.10

Comparable darkening trends have been reported in other retort-processed rice products. Khunprama et al. (2022) observed significant browning in retort-processed fried rice at 116°C (F_0 = 5 min), which they attributed to Maillard reactions intensified by elevated retort temperatures. Although Khunprama et al. expressed their results as an increase in colour values (using a browning index or similar scale where larger numbers correspond to darker colour), whereas the present study uses Lovibond L (where lower values indicate darkening), both findings reflect the same underlying phenomenon of thermal-induced browning. The colour changes were more pronounced at temperatures above 118°C and holding times exceeding 12 min, possibly because short-grain Seeraga Samba rice has a higher amylopectin content compared to long-grain Basmati rice, thus generating more reducing sugars that participate in Maillard reactions.

$$L* = 54.68 - 1.36A - 0.41B - 0.89A2 - 0.39B2 + 0.31AB \qquad (2)$$

Where, A-Temperature and B-Time

3.1.2.2. Moisture Content

The moisture content was found to decrease significantly ($p < 0.05$) with an increase in retort temperature and holding time (Fig. 2, Table. 2). The reduction in moisture content could be related to the moisture transferred from the product to the headspace and then evaporated through the film during the come-up period and holding period (Jimenez et al., 2024). Higher moisture loss at higher retort temperatures agrees with the findings of Thomas (2022), who recorded a similar trend in moisture content of retorted chicken biryani stored under ambient conditions.

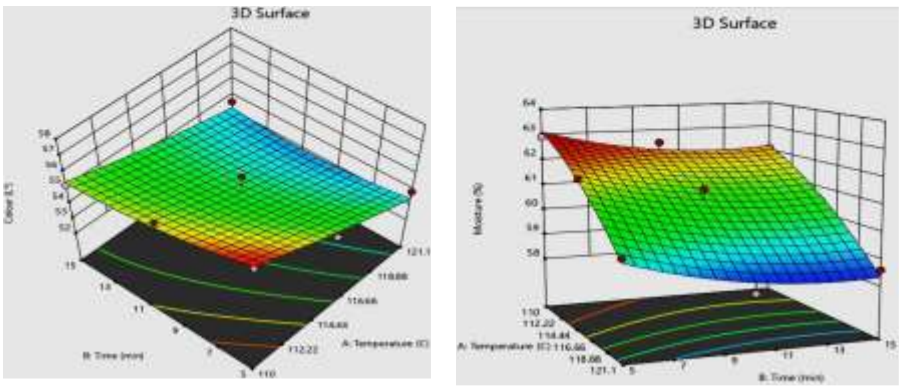

Fig. 2. Effect of treatment time and temperature on colour value and Moisture

Table 2. Physicochemical, Colour, and Proximate Composition of Retort-Processed Seeraga Samba Vegetable Biryani

S.No	Parameter	Day 0	Day 30	Day 60	Day 90
1	Carbohydrate (%)	24.80 ± 0.22	24.26 ± 0.21	25.47 ± 0.18	24.14 ± 0.20
2	Protein (%)	2.90 ± 0.05	2.48 ± 0.04	2.66 ± 0.05	2.80 ± 0.04
3	Fat (%)	2.05 ± 0.04	1.62 ± 0.03	2.37 ± 0.04	1.81 ± 0.03
4	Moisture (%)	70.20 ± 0.30	70.76 ± 0.31	68.56 ± 0.28	70.20 ± 0.26
5	Calorific Value (kcal/100 g)	126.50 ± 1.15	121.54 ± 1.12	133.85 ± 1.25	124.05 ± 1.08
6	Ash (%)	1.10 ± 0.03	0.94 ± 0.02	0.94 ± 0.02	1.05 ± 0.03
7	Crude Fibre (%)	0.54 ± 0.01	0.86 ± 0.02	0.65 ± 0.02	0.53 ± 0.01
8	Lovibond L*	53.4 ± 0.9	52.1 ± 0.8	51.2 ± 0.7	50.1 ± 0.6
9	pH	6.50 ± 0.02	6.30 ± 0.02	6.10 ± 0.03	5.90 ± 0.02

Contrary to the present findings, Lavanya et al. (2016) recorded an increase in moisture content of retorted mutton kheema biryani. This might be due to the presence of higher amounts of starch and hydrophilic components such as rice in biryani. Starch present in rice tends to absorb more water upon gelatinization and might influence the moisture content of the biryani.

$$\text{Moisture}=61.09-1.45A-0.52B-0.68A2-0.32B2+0.23AB \qquad (3)$$

Where, A-Temperature and B-Time

3.1.2.3. pH

A significant drop in pH ($p < 0.05$) was recorded with an increase in the retort process due to the production of organic acids such as lactic, acetic, and short-chain fatty acids by starch and protein hydrolysis, along with the dissolution of carbon dioxide in the moisture during thermal processing (Khunprama et al., 2022). However, it was evident that the pH values remained within the permissible limits (5.8-6.5) for low-acid foods to inhibit the growth of Clostridium botulinum (Jimenez et al., 2024).

The drop in pH was slower for Seeraga Samba than that reported for chicken biryani by Thomas (2022). This might be explained by the buffer capacity of the starch matrix of rice and the presence of vegetables that might resist rapid pH changes during thermal processing (Fig 3; Table 3).

$$\text{pH}=6.61-0.13A-0.06B-0.05A2-0.04B2+0.03AB \qquad (4)$$

Where, A-Temperature and B-Time

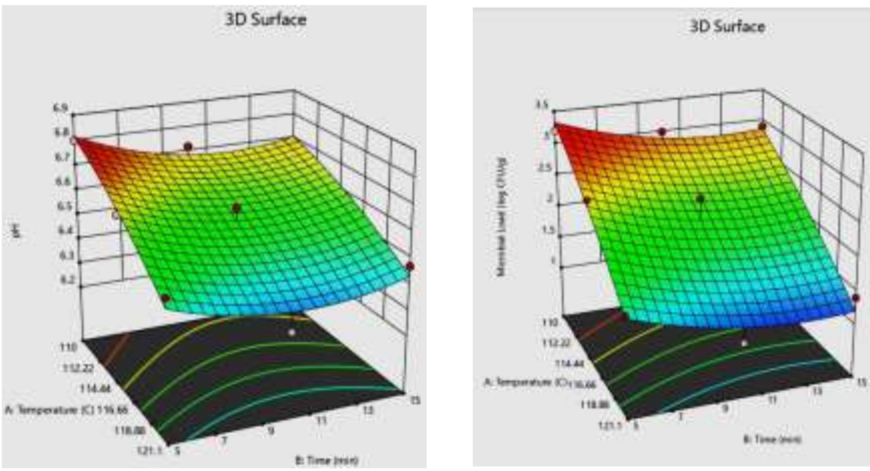

Fig. 3. Effect of treatment time and temperature on pH and Microbial load

Table 3. Microbial Quality of Retort-Processed Seeraga Samba Vegetable Biryani

Storage Period	Aerobic Plate Count (log CFU/g)	Anaerobic Count (log CFU/g)	Yeast & Mould Count (log CFU/g)
Day 0	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)

Day 30	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)
Day 60	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)
Day 90	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)	ND (< 1 log CFU/g)

3.1.2.4. Microbial Load

Microbial load (log CFU/g) decreased significantly ($p < 0.05$) with increasing retort temperature and holding time (Fig 3; Table 6). The pre-retort microbial load of the vegetable biryani was 3.0 ± 0.2 log CFU/g. At 115.55°C for 10 minutes (Run 3), the microbial load was reduced to 2.5 ± 0.1 log CFU/g (Table 1), which is insufficient to achieve commercial sterility. However, under the optimized conditions (121.1°C for 10 min), the microbial load was reduced to <1 log CFU/g (below the detection limit of 1.0 log CFU/g), thereby achieving commercial sterility.

Thomas (2022) similarly reported that retort processing of chicken biryani at optimized conditions reduced the microbial load below detectable limits, with no bacterial growth detected after 120 days of storage. The present study's findings are consistent with those of Thomas (2022), confirming that high-temperature short-time (HTST) retort processing is effective for producing commercially sterile rice-based ethnic foods.

The exponential relationship between temperature and microbial destruction (Stumbo, 1973) indicates that processing at relatively high temperatures can substantially reduce processing time while achieving commercial sterilization. This is particularly relevant for Seeraga Samba rice-based products, where prolonged thermal exposure may accelerate quality degradation.

$$\text{Microbial Load} = 2.68 - 0.63A - 0.34B + 0.22A2 - 0.18B2 - 0.08AB \qquad (5)$$

Where, A-Temperature and B-Time

3.1.3. F_0 Value and Process Lethality

The sterilization value (F_0) increased exponentially with an increase in retort temperature and holding time following the standard calculation procedure, $F_0 = \int 10^{((T-121.1)/10)}\,dt$ (Stumbo, 1973; Holdsworth & Simpson, 2015). Thus, at the lowest set point of 110°C for 5 min, the cumulative F_0 was 2.1 min, which is lower than the minimum required F_0 (3.0 min) for botulinum cook for low-acid food (Jimenez et al., 2024). By comparison, at 115.5°C for 10 min, the F_0 was 4.8 ± 0.3 min, whereas at the optimum condition of 121.1°C for 10 min, the F_0 was 10.3 ± 0.4 min, which is more than thrice the minimum required F_0 for botulinum cook to ensure commercial sterility. The obtained result was comparable to the earlier studies carried out on mutton kheema biryani, where the authors obtained F_0=6.0 (Lavanya et al., 2016).

The three-dimensional response surface plot revealed that the curvature for F_0 was positive and steep for temperature, suggesting that any increase in temperature above 119°C would impart significant lethality to the food system. The exponential relationship between temperature and lethality would allow the food technologist to choose high-temperature short-time (HTST) processing for achieving commercial sterility for the rice-based ethnic food product without compromising on quality.

3.1.4. Numerical Optimization and Validation

A numerical optimization method, which is based on desirability function, led to maximum composite desirability of d = 0.900. The corresponding optimal processing condition was 121.1°C and 10 minutes holding time, while the predicted responses were equal to Lovibond L= 52.1, moisture 58.1%, pH=6.3, microbial load 1.0logCFU/g, F0=10.3 minutes, and overall acceptability equal to 7.8/9. The response surface methodology was proved to be highly accurate and reliable prediction technique since the deviation between observed and predicted responses was less than 8% for all tested responses (Myers et al., 2009) and therefore the optimal condition was selected for further experimentation.

3.2. Commercial Sterility Confirmation

All retort processed Seeraga Samba vegetable biryani pouches (n=3 per batch) incubated at 37°C for 7days followed by 55°C for 7 days did not show any swelling, loss of vacuum, off-odours or any signs of spoilage. Further, the samples were found to be free from total viable count, total anaerobic and facultative anaerobic bacteria, yeast and moulds indicating commercial sterility of the product as per FSSAI (2011) guidelines and Chauhan and Jindal (2020). These results are in agreement with Thomas (2022), who reported commercial sterility of retort processed chicken biryani throughout the 120 days of storage, and Lavanya et al. (2016), who found no microbial growth in retort processed mutton kheema biryani.

3.3. Quality Changes During Ambient Storage

3.3.1. Physicochemical Changes

3.3.1.1. pH

The pH value was found to decrease with a statistically significant difference ($p<0.05$) from the initial value 6.50 ± 0.02 in the fresh sample to 5.90 ± 0.02 after 90 days, respectively. The decrease in pH was associated with the development of short chain organic acids like acetic, lactic and formic acids from Maillard reaction products, lipid hydrolysis, and starch degradation during ambient storage of Seeraga Samba biryani (Thomas, 2022). Lavanya et al. (2016) also reported a similar trend of decrease in pH value of retort processed mutton kheema biryani stored at room temperature.

It may be concluded that the pH of Seeraga Samba sample was found to be >5.5 throughout the storage period which is safe from the point of view of low-acid food (>4.6%) and also safe concerning the growth of Clostridium botulinum bacteria which require pH<4.6 to grow (Jimenez et al., 2024). A comparison between the present data and earlier studies reveal that rice and vegetables in biryani might have contributed to the buffer capacity against drastic change in pH as seen in case of meat products.

3.3.1.3. Colour (Lovibond L)

Lovibond L values decreased significantly ($p < 0.05$) from 53.4 ± 0.9 in the fresh sample to 50.1 ± 0.6 after 90 days, indicating progressive darkening of the product during storage (Table 4). This darkening was primarily attributed to non-enzymatic Maillard reactions between amino acids from spices and vegetables and reducing sugars produced from starch hydrolysis at ambient temperature (Jimenez et al., 2024). Similar gradual decreases in lightness during ambient storage were reported by Thomas (2022) for retort-processed chicken biryani stored for 120 days. Khunprama et al. (2022) also observed significant darkening in retort-processed fried rice immediately after processing at 116°C (F_0 = 5 min) due to Maillard browning; however, their study focused on processing-induced rather than storage-induced changes, limiting direct comparison with the present findings. The degree of darkening was more prominent in Seeraga Samba biryani than in Basmati-based biryanis, likely due to the short-grain variety's higher amylopectin content, which upon thermal hydrolysis yields greater concentrations of reducing sugars that intensify Maillard reactions during both processing and subsequent storage (Jeong et al., 2024).

Table 4. Sensory Evaluation of Retort-Processed Seeraga Samba Vegetable Biryani

Storage Period	Colour & Appearance	Aroma	Texture	Taste	Overall Acceptability
Day 0	8.2 ± 0.3	8.0 ± 0.4	7.9 ± 0.3	8.1 ± 0.3	8.1 ± 0.2
Day 30	7.8 ± 0.3	7.5 ± 0.3	7.4 ± 0.3	7.7 ± 0.3	7.6 ± 0.2
Day 60	7.3 ± 0.3	7.0 ± 0.3	6.9 ± 0.3	7.1 ± 0.3	7.0 ± 0.3
Day 90	6.9 ± 0.3	6.6 ± 0.3	6.4 ± 0.3	6.7 ± 0.3	6.7 ± 0.3

3.3.2. Nutritional Changes (Proximate Composition)

The physicochemical characteristics of the retort-processed product during 90 days of ambient storage are presented in Table 2. The carbohydrate content remained relatively stable throughout the storage period, ranging from 24.14 ± 0.20% to 25.47 ± 0.18%, indicating that retort processing and subsequent storage had minimal influence on carbohydrate composition. Similarly, the protein content showed an initial decrease from 2.90 ± 0.05% in the fresh sample to 2.48 ± 0.04% on Day 30, which may be attributed to thermal denaturation during sterilization. Thereafter, the protein content gradually increased to 2.80 ± 0.04% by Day 90, likely due to concentration effects associated with minor moisture fluctuations rather than actual changes in protein content (Catauro et al., 2012).

The fat content varied between 1.62 ± 0.03% and 2.37 ± 0.04% during storage, with no consistent increasing or decreasing trend, suggesting that lipid degradation was minimal under ambient storage conditions. Likewise, the moisture content remained relatively stable, ranging from 68.56 ± 0.28% to 70.76 ± 0.31%. A slight reduction in moisture was observed on Day 60, followed by recovery to 70.20 ± 0.26% on Day 90, demonstrating the effectiveness of the multilayer retort pouch in minimizing moisture migration and preserving product quality. Such minimal changes in moisture content have been widely reported in retort-pouched ready-to-eat foods stored under ambient conditions (Byun et al., 2010; Catauro et al., 2012).

The calorific value ranged from 121.54 ± 1.12 to 133.85 ± 1.25 kcal/100 g during storage, compared with 126.50 ± 1.15 kcal/100 g in the fresh sample. These slight variations are primarily associated with corresponding changes in moisture and fat content rather than substantial alterations in the nutritional composition of the product.

Ash content exhibited only marginal variation, decreasing from 1.10 ± 0.03% in the fresh sample to 0.94 ± 0.02% during the first 60 days, before slightly increasing to 1.05 ± 0.03% on Day 90. Since minerals are generally stable during thermal processing, these minor differences are most likely due to analytical variation and moisture redistribution rather than actual mineral losses.

Similarly, crude fibre content showed only slight fluctuations throughout storage. It increased to 0.86 ± 0.02% on Day 30, gradually decreased to 0.65 ± 0.02% on Day 60, and reached 0.53 ± 0.01% by Day 90, closely matching the fresh sample (0.54 ± 0.01%). These minor variations may be associated with structural softening of plant tissues and limited thermal degradation of fibre components during processing and storage (Mishra et al., 2026). Overall, the proximate composition remained relatively stable throughout the storage period, indicating that retort processing effectively preserved the nutritional quality of the ready-to-eat product under ambient storage conditions.

Table 5. TBARS Values of Retort-Processed Seeraga Samba Vegetable Biryani

Storage Period	TBARS (mg MDA/kg)
Day 0	0.42 ± 0.03
Day 30	0.67 ± 0.04
Day 60	0.91 ± 0.05
Day 90	1.18 ± 0.06

3.3.3. Lipid Oxidation (TBARS) During Storage

TBARS were found to increase significantly ($p<0.05$) with storage time (Table 5; Fig. 4), ranging from 0.42± .03 mg MDA/kg at Day 0 to 0.67± .04, 0.91± .05 and 1.18± .06 mg MDA/kg on Days 30, 60 and 90, respectively. Initially (Day 0), TBARS value can be attributed to lipid oxidation that occurred due to retort processing of the biryani. Polyunsaturated fatty acids in cooking oil, ghee and oleoresins of spices are highly susceptible to oxidation at elevated temperatures leading to higher levels of lipid oxidation products upon processing. The values of TBARS increased significantly during ambient storage at 28°C, indicative of the progress of lipid oxidation reactions due to the presence of residual oxygen in the hermetically sealed pouch during storage (Lavanya et al., 2016).

Lipid oxidation may have occurred due to presence of pro-oxidant spices such as cumin and coriander and iron from vegetable pigments in the spice mixture used for biryani. However, even on Day 90, the value of TBARS was 1.18 mg MDA/kg which is below the threshold value of 1.5-2.0 mg MDA/kg beyond which rancidity becomes noticeable to the consumer (Lavanya et al., 2016). Hence, it can be concluded that oxidation of lipids in spicy biryani was within permissible limits even after 90 days of storage at 28°C. The antioxidant activity of spice components such as cumin, coriander and turmeric in the biryani mixture might have contributed to the observed oxidative stability of lipids and reduced lipid oxidation during storage (Fig 4.).

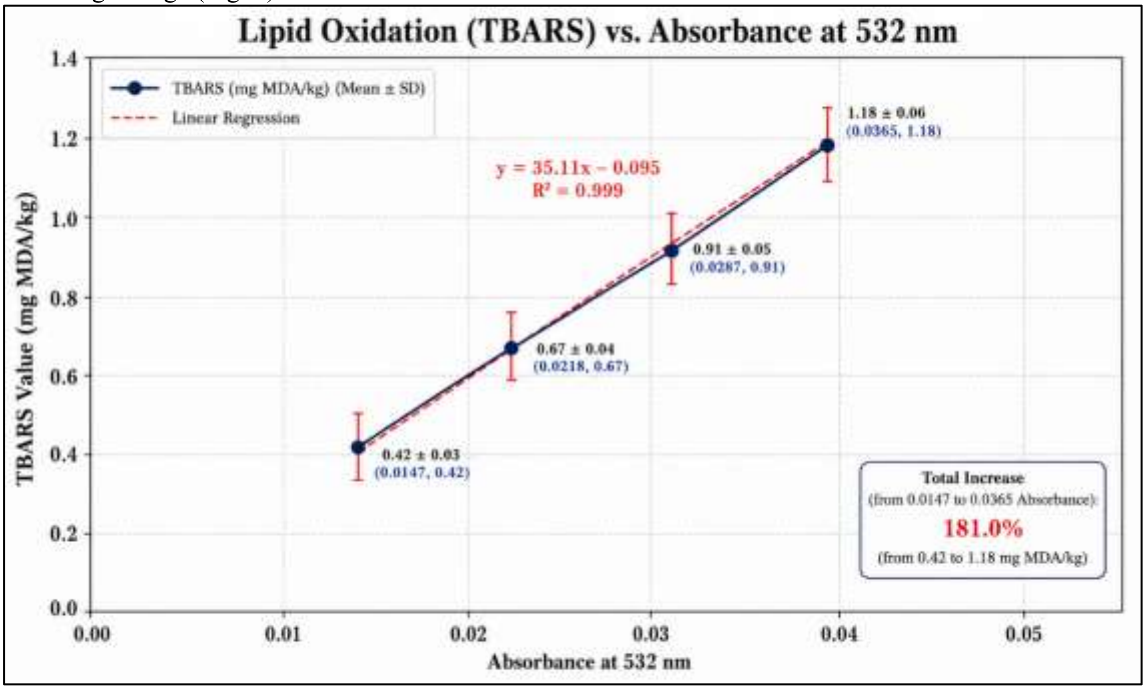

Fig. 4. Changes in lipid oxidation (TBARS, mg MDA/kg) during storage period

3.3.4. Microbiological Quality During Storage

The obtained results (Table 5) revealed that an aerobic plate count (APC), anaerobic count, and yeast and mould counts were below the detection limit (>1 log CFU/g) even after 90 days of storage at ambient temperature. No aerobic or anaerobic bacteria, yeasts, or moulds were detected on Days 0, 30, 60, and 90. Thus, it can be concluded that the 121.1°C, 10 min pressure steam heat (F_0 = 10.3 min) efficiently sterilized the biryani.

The results were supported by a similar study conducted by Thomas (2022), which showed no microbial growth after 120 days of retort chicken biryani storage. Another piece of research conducted by Jimenez et al. (2024) demonstrated that the proper sterilization of food products in an hermetically sealed pouch using the appropriate F0 allows keeping them for two years without compromising their safety and quality. Consequently, no microorganisms detected after 90 days of storage confirmed that the retort pouch made of four-layer PET/Al/PA/CPP was sterilized correctly during the process and protected against any post-processing contamination.

3.3.5. Sensory Evaluation During Storage

Sensory attribute scores for colour and appearance, aroma, taste, texture, and overall acceptability were determined using 9-point hedonic scale (Table 6). Freshly prepared product scored good acceptability (8.2 ± 0.3 for colour/appearance, 8.0 ± 0.4 for aroma, 8.1 ± 0.3 for taste, 7.9 ± 0.4 for texture, and 8.1 ± 0.2 for overall acceptability). As per Meilgaard et al. (1999), the freshly retorted samples showed high likeness (6 – like very much) as per the 9-point hedonic scale. The higher sensory scores could be attributed to the distinct aroma and absorption capacity of the Seeraga Samba rice (Bindusree et al., 2017) and optimized retort process parameters.

However, with prolonged storage, there was a significant reduction ($p < 0.05$) in all sensory scores. Reduction was more prominent for aroma from 8.0 ± 0.4 to 6.6 ± 0.4 (on Day 90). This could be explained by the loss of volatile compounds like 2-acetyl-1-pyrroline (2AP) that contributes to the aroma of Seeraga Samba rice (Bindusree et al., 2017). Similarly, there was a reduction in taste scores from 8.1 ± 0.3 to 6.7 ± 0.3 for the same duration. The lower taste scores could be attributed to the loss of spice blend aroma and altered salt to acid ratios. The texture scores also reduced from 7.9 ± 0.3 (for Day 0) to 6.4 ± 0.3 (for Day 90) due to retrogradation of starch during ambient storage (Jeong et al., 2024).

Interestingly, the overall acceptability scores were still in the moderately liked category (6.7 ± 0.3) on Day 90 as compared to the minimum acceptable score (6.0 – like slightly) for Seeraga Samba biryani. It can be concluded that the Seeraga Samba biryani maintained moderate liking with extended storage (up to 90 days). Similar trends were observed in earlier studies on retort processed chicken and mutton kheema biryani with prolonged storage (up to 90 days) (Lavanya et al., 2016; Thomas, 2022). However, Seeraga Samba rice showed better liking as compared to other rice varieties. This could be attributed to the unique physicochemical properties of Seeraga Samba rice that aid better retention of texture and aroma during thermal processing and storage.

Source	**Colour (L*) F-value**	**Colour *p*-value**	**pH Fvalue**	**pH *p*-value**	**Moisture F-value**	**Moisture *p*-value**	**Microbial Load F-value**	**Microbial Load *p*-value**
Model	9.53	0.0050*	9.90	0.0045*	19.75	0.0005*	6.48	0.0147*
A – Temperature	40.54	0.0004*	40.38	0.0004*	88.22	<0.0001*	29.34	0.0010*
B – Time	2.27	0.1757	4.99	0.0606	7.33	0.0303*	2.30	0.1732
AB	0.3445	0.5756	2.11	0.1893	0.0967	0.7649	0.0000	1.0000
A^2	0.6440	0.4486	0.1576	0.7032	2.05	0.1954	0.2081	0.6621
B^2	4.51	0.0713	1.23	0.3046	2.24	0.1782	0.7512	0.4148
Lack of Fit	1.17	0.4265	1.96	0.2618	0.4772	0.7152	0.3238	0.8094

Table 6. ANOVA Summary of Quadratic Models for the Responses of Retort-Processed Seeraga Samba Biryani

CONCLUSION

This study successfully demonstrated the feasibility of developing a commercially sterile, shelf-stable ready-to-eat (RTE) vegetable biryani using the indigenous aromatic Seeraga Samba rice through retort processing. The optimized processing conditions determined using response surface methodology (121.1°C for 10 min) achieved an F_0 value of 10.3 ± 0.4 min with high model desirability (0.900), ensuring adequate thermal lethality while maintaining product quality. During 90 days of ambient storage (28 ± 2°C), only minor changes were observed in physicochemical attributes, including moisture, pH, colour, proximate composition, and lipid oxidation, with TBARS values remaining below the critical rancidity limit. Complete microbial safety was maintained throughout storage, confirming commercial sterility, while sensory evaluation indicated that the product retained good consumer acceptability with an overall score above the acceptable threshold even after 90 days. The findings provide the first comprehensive scientific evidence on the retort processing behaviour, quality characteristics, and storage stability of Seeraga Samba rice-based RTE vegetable biryani, demonstrating its suitability for commercial production. The optimized process offers a valuable opportunity for value addition to this premium geographical indication rice cultivar, enhances its commercial utilization in the expanding RTE food sector, and has the potential to improve farmers' income by promoting high-value processed products. Overall, the study establishes a scientifically validated processing protocol that can serve as a foundation for the development of other Seeraga Samba-based convenience foods, while future research may focus on extended storage studies, packaging optimization, consumer acceptance, and advanced thermal and non-thermal processing technologies to further enhance product quality and shelf life.

Declarations

Abbreviations

ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; APC, aerobic plate count; BADH2, betaine aldehyde dehydrogenase 2; CCD, central composite design; CDT, come-down time; CFU, colony-forming unit; CUT, come-up time; DMRT, Duncan's multiple range test; F0, equivalent sterilisation value at 121.1°C; FSSAI, Food Safety and Standards Authority of India; GI, geographical indication; MDA, malondialdehyde; ND, not detected; RSM, response surface methodology; RTE, ready-to-eat; TBARS, thiobarbituric acid reactive substances; TPC, total plate count; 2AP, 2-acetyl-1-pyrroline.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. All relevant data supporting the findings of this study are included in the manuscript.

Declaration of Competing Interests
The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process
During the preparation of this work, the authors used AI-assisted tools for language editing and improving readability. The authors confirm that all scientific content, data analysis, interpretations, and conclusions are entirely their own. The authors take full responsibility for the content of this manuscript.

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Seenivasan S: Conceptualization, experimentation, data analysis, writing – original draft. Thirupathi V: Supervision and methodology review. Geetha P: Data validation, editing. Amuthaselvi G: Formal analysis. Ramalakshmi A: Review and final approval.

Ethical Statement
This study did not involve human participants or animals, and therefore ethical approval was not required.

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