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Optimization and Extension of Shelf-Life of Chapati Using Selected Natural Plant Extracts as Preservatives

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Abstract

Whole wheat flour is employed to prepare an unleavened flat-baked chapati product. The majority of people in Asian communities residing all over the world and the Indian subcontinent consume chapati as a traditional staple food. There is an increase in demand for ready-to-eat chapatis due to changes in lifestyle, so this study was initiated to increase the shelf-life of chapatis using selected plant samples through optimization. The optimized dough was seen to have a good leavening and soft texture with no change in their appearance, order, or taste. The spoilage of the prepared and stored chapattis was monitored. At the conclusion of the 20 trials, the range of shelf-life obtained was between 14 to 16 days. On studying the goodness of fit statistics in the model used for the optimization based on the low s value (0.3717), there is a better description of the response. 97.94% R^2 value was obtained with consideration of the response describing the fitness of the model. Similarly, its right and it determines adjacent R^2 value, and predicted R^2 value was not substantially less than the R^2 value making the model fit. On encoding the values 0.6625 for turmeric, 0.6625 for the sweet flag, and 0.7305 for tea powders can be used to obtain the maximized shelf-life of 16 days.

Keywords

Chapatti, Turmeric, Sweet Flag, Tea and Optimization

1. Introduction

Whole wheat flour is employed to prepare an unleavened flat-baked chapati product. The majority of people in Asian communities residing all over the world and the Indian subcontinent consume chapati as a traditional staple food.

Moreover, freshly prepared chapatis are prepared by customers as they stale swiftly during storage. There is an increase in demand for ready-to-eat chapatis due to changes in lifestyle [1]. Chapatis reduce constipation, diverticular disease, rates of chronic bowel disease, and diet-related cancers and provide abundant dietary fiber, and protein sources economically. Due to insufficient time and a high workload, difficulties are faced in preparing fresh chapatis at every meal as it is a highly needed daily food providing a sufficient amount of energy and calories for healthy living [2].

Bioactive molecules such as polyphenols (flavonols, anthocyanins, flavanols, benzoic acid, tannin, lignin, stilbenes, cinnamic acid, phenolic acids), terpenoids (carotenoids, terpenes, triterpenes, phytosterols, iridoids), organo-sulfurs, and alkaloids are seen in plant sources in abundance. The bioactive compounds extracted from various parts of the plant such as leaves, seeds, roots and so on exert antioxidant and antimicrobial effects which can be widely used as potential natural preservatives replacing synthetic preservatives [3]. It may be related to their chemical structure that can cause morphological changes in microorganisms, damage bacterial cell walls, and influence biofilm formation, yet their mechanism and action are still not completely understood. Polyphenol compounds present in plant compounds exerting antioxidant and antimicrobial activities also influence protein biosynthesis, change metabolic processes in bacteria cells and inhibit ATP and DNA synthesis, and can be used as an alternative to chemical preservatives [4]. The natural plant extracts employed in this study are derived from *Curcuma longa* (Turmeric), *Acorus calamus* (Sweet flag), and *Camellia sinensis* (Tea).

Turmeric is a popular herb of most traditional values with beneficial attributes like antibacterial, antiviral, antifungal, antioxidant, anti-arthritic, anti-tumor, antithrombotic, nematocidal, antihepatotoxic, anti-mutagenic, anti-choleretic, and even strong influence as antivenom activity, antispasmodic, cardiovascular, diuretic, carminative, astringent, cholagogue, digestive, appetizer, stimulant, and vulnerary property make essential in medicine, pharmaceutical, and food industry. It also stimulates the production of various digestive enzymes involved in the process improving the function of the small intestine, digestion, and metabolism by Nath, S *et al.* [5].

Acorus calamus grown as a marsh plant, leaves, and rhizomes are utilized as traditional medicine for years with interest for application as phytopharmaceuticals. Due to their flavoring properties and bioactivity, food products such as tea infusions, alcoholic beverages, and food supplements contain calamus preparations as additives. The phenylpropanoids (E)-1,2,4-trimethoxy-5-(1-propen-1-yl)-benzene (α-asarone, aA) and (Z)-1,2,4-trimethoxy-5-(1-propen-1-yl)-benzene (β-asarone, bA), which occur in the essential oil of leaves, roots, and rhizomes are attributed to the role of calamus as a food additive. Antimicrobial, antioxidative, and antispasmodic properties and several beneficial effects of calamus preparations and asarone isomers on human health are reported earlier by Hermes, L *et al.* [6].

Camellia sinensis is a shrub comprising major antioxidant and anticancer agents within its chemical composition. The oxidization of fatty acids and α-tocopherol are minimized due to tea (Camellia sinensis) and its phenolic constituents having antioxidant activity are reported and have the potential for use in several food products. Antioxidants have great importance to human beings and have a great interest in consumers and nutritionists to quantify them in various food sources [7]. Thus, the study is carried out to find out the interaction between the selected plant extracts and the dough composition involving the increase in half-baked chapatis shelf-life.

2. Materials and Methods

2.1. Raw Materials

The samples Turmeric (CURCUMA LONGA) were collected from local market in Erode, Sweet Flag (ACORUS CALAMUS) and Tea (CAMELLIA SINENSIS) were collected from the local field at Kotagiri, Nilgiris district.

2.2. Extraction of Natural Extracts from Powdered Samples

Extracts were prepared by adding 20 g of each selected plant samples to 80 mL of purified water at 90˚C. The obtained mixture was filtered with a 200-mesh sieve, and the filtrate contained in a beaker was cooled to 10˚C in an ice water bath, then centrifugated with a centrifuge for 15 min to remove impurities. The upper layer was collected and concentrated in a vacuum at 60˚C and then dried powdered extract was stored in airtight bottles and refrigerated at 4˚C until use.

2.3. Optimization of Usage of Plant Extract Using Response Surface Method (RSM)

From the observations made in cytotoxicity studies concerning the morphological changes and % cell viability (IC50), the range of Curcuma longa, Camellia sinensis and Acorus calamus (>50.01% ± 2.31% μg/ml, >172.2% ± 1.65% μg/ml and >52.67% ± 0.1% μg/ml) extract to be used was obtained. Based on the IC_{50} values, an optimization process for the extension of shelf-life using RSM-Central Composite Design (CCD) is carried out. The range table was constructed for the three variable factors with the K value 3 and α = 1.68179, shown in **Table 1**.

Table 1. Parameters levels and coded values used in the experimental design for the extracts.

Factors (mg/ml)	Range and Level				
	−1.682	−1	0	1	1.682
Turmeric (A)	0.001	0.011	0.026	0.041	0.051
Sweet flag (B)	0.001	0.011	0.026	0.041	0.051
Tea (C)	0.006	0.036	0.086	0.136	0.177

A central composite design with three factors and three levels including six replicates at the center point is used to fit a second-order polynomial equation. The fit quality of the polynomial model equation was expressed by the coefficient of determination R^2.

The CCD is chosen to develop the second-order polynomial model due to the reduced number of actual experiments without significant loss of information. Also, it is a good optimization tool in response to surface methodology because it permits: 1) estimation of the parameters of the quadratic model, 2) building of sequential design, 3) determination of lack of fit of the model and 4) use of blocks. This second-order polynomial model demonstrates the rapport between turmeric, sweet flag, and tea based on shelf-life. The number of experiments (n) required for the development of CCD is defined as n = 2k, where k is the number of experimental variables and Co is the number of experiments repeated at the center point (k = 3; Co = 6). As a result, a total of 20 sets of experiments have to be performed. All other experimental conditions are kept constant during the experiments, and the runs are randomized to exclude any bias.

Turmeric, sweet flag, and tea are the independent variables studied in the experimentation of shelf-life optimization. These three variables are tested at different levels by associated plus signs (+1.682) with high levels, zero (0) indicating the center value, and minus signs (−1.682) with low levels. **Table 1** shows the levels and coded values of independent variables used in the experimental design for shelf-life optimization.

$$X_j = \frac{X_i - X_0}{\Delta x} \quad i = 1,2,3,4,5 \tag{1}$$

The second-order polynomial regression model is given as Equation (2) to express Y as a function of the independent variables as follows, where β_0 is a constant, while β_i, β_{ii}, and β_{ij} are the linear, quadratic, and interactive coefficients, respectively. x_i and x_j are the levels of the independent variables:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=2}^{k} \beta_{ij} x_i x_j \tag{2}$$

The accuracy and ability of the above polynomial model could be evaluated by the coefficient of determination R^2 [8].

2.4. Preparation of Dough

From **Table 2**, composition of plant extracts to be used along with the wheat flour to prepare dough is determined. For the set of 20 experiments, 25 gms of wheat flour and the optimum amount of water along with oil, and salt was implied and kept constant to prepare the dough. Warm boiled and purified water was used to prepare the dough.

2.5. Preparation and Storage of Chapatis

The prepared dough was left to settle for about an hour in a container and out of the leavened dough the half-baked chapatis were prepared at optimal heat. The

Table 2. Central composite design matrix (uncoded values) for shelf-life optimization.

Run	Turmeric	Sweet Flag	Tea
1	0.011	0.011	0.036
2	0.041	0.011	0.036
3	0.011	0.041	0.036
4	0.041	0.041	0.036
5	0.011	0.011	0.136
6	0.041	0.011	0.136
7	0.011	0.041	0.136
8	0.041	0.041	0.136
9	0.001	0.026	0.086
10	0.177	0.026	0.086
11	0.026	0.001	0.086
12	0.026	0.051	0.086
13	0.026	0.051	0.006
14	0.026	0.026	0.177
15	0.026	0.026	0.086
16	0.026	0.026	0.086
17	0.026	0.026	0.086
18	0.026	0.026	0.086
19	0.026	0.026	0.086
20	0.026	0.026	0.086

half-baked chapatis prepared were stored in separate sealed packages and stored in a refrigerator at 4˚C.

2.6. Statistical Analysis and Evaluation of Stored Chapati

The statistical analysis was carried out using one-way ANOVA along with the Tukey test, Fisher test, Dunnett test, and Hsu MCB (multiple comparison method) tests to determine the significance of the interaction between the plant extracts and shelf-life using RSM software. The stored refrigerated chapatis were evaluated based on sensory attributes for the optimized points. The descriptive scheme was to evaluate the sensory attributes of chapattis with a non-expert panel of 20 members using 9 points hedonic scale (where 9-Like Extremely, 8-Like Very Much, 7-Like Moderately, 6-Like Slightly, 5-Neither Like nor Dislike, 4-Dislike Slightly, 3-Dislike Moderately, 2-Dislike Very Much, 1-Dislike Extremely). Each sample was evaluated for color, flavor, mouthfeel, taste, texture, and overall acceptability. The evaluation was done in triplicate and an average value was reported.

3. Results and Discussion

3.1. Optimization and Experimentation

From the experimentation trials given in Table 2, the dough was prepared and the half-baked chapattis were prepared. The dough was seen to have a good leavening and soft texture with no change in their appearances, order, or taste. The spoilage of the prepared and stored chapattis was monitored. In the conclusion of the 20 trials, the range of shelf-life obtained was between 12 to 16 days. At the end of the 16 days, the chapattis were found to absorb moisture, and cracks in the texture were observed in the next two days. No foul smell or difference in taste was absorbed on the 16th day of stored half-baked chapatti. The observations of shelf-life were made and noted down as a response to the RSM-CCD experimentation process. Based on the response (shelf-life) the design was analyzed concerning interactions between used variables such as turmeric, sweet flag, and tea respectively. The response surface regression was studied between shelf-life versus turmeric, sweet flag, and tea. To determine the contributing the most in experimentation to analyze the variability in the response, a Pareto chart (Figure 1) displays the absolute value of the standard effects in the decreasing order of their absolute values. Considering the Pareto chart comparing relative magnitude, the significance of main, square, and interaction effects in Figure 2, with a significance level of 0.05 the reference line (2.23) is drawn. Referring to the reference line the interaction between the square term of turmeric, sweet flag, and tea along with tea and turmeric are significant. The interaction of square term of turmeric has the highest effect and the interaction of sweet flag has the least effect. To determine the association between the shelf-life and the variables turmeric, sweet flag, and tea comparing their significance concerning coefficient, and P-value are shown in Table 3. The association between the main

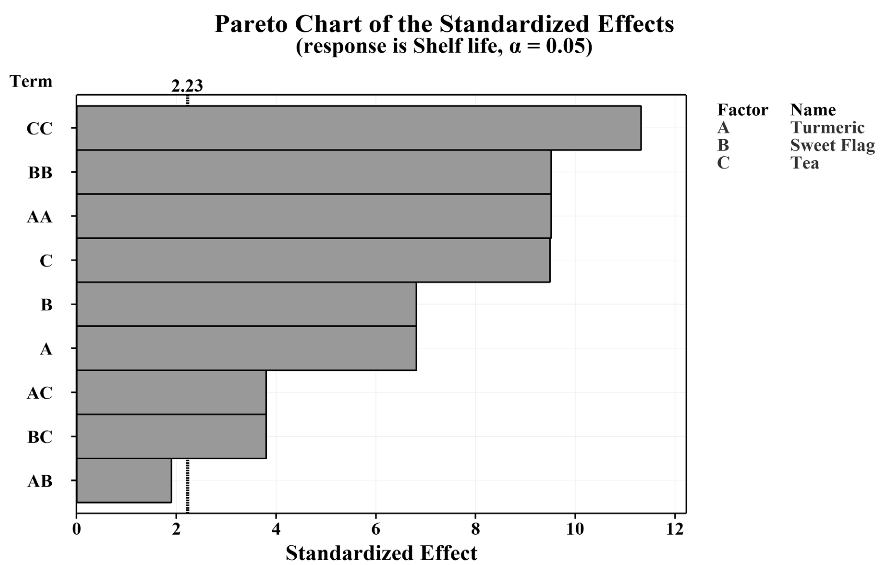


Figure 1. Pareto chart of the interaction between the variables to determine the contribution between the variables.

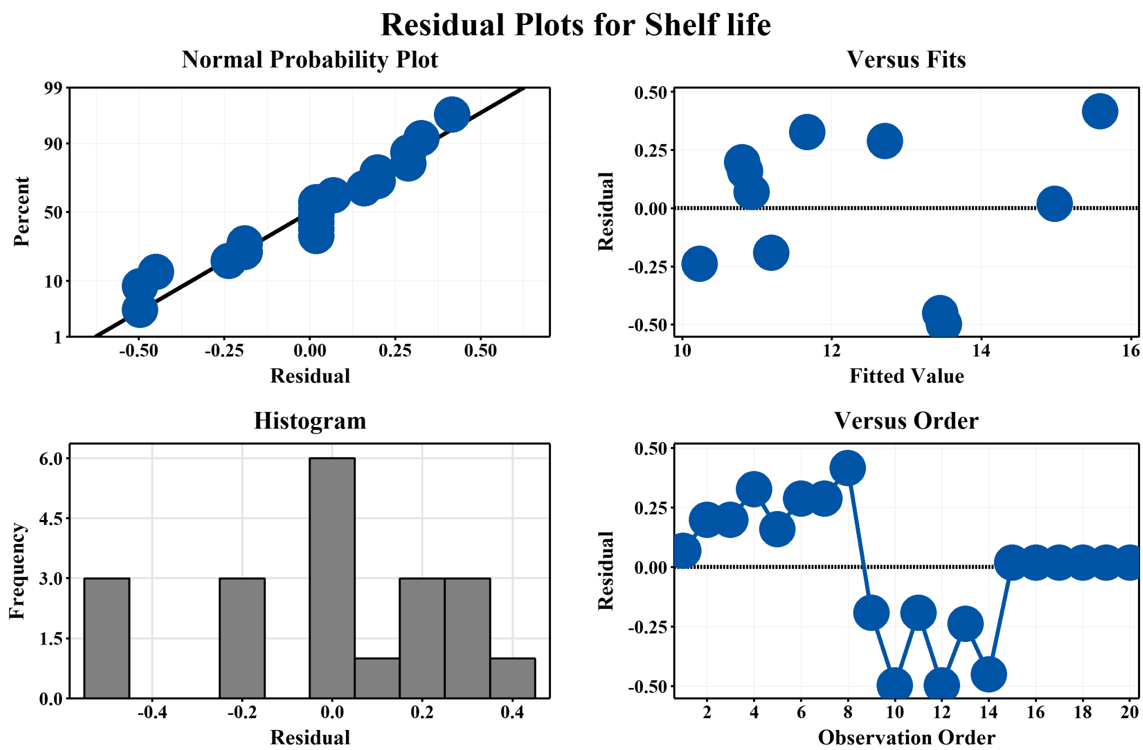


Figure 2. Residual plots to determine the adequateness of the model fitness.

Table 3. Coefficients of response surface based on the interaction between shelf-life versus turmeric, sweet flag, and tea.

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	14.980	0.152	98.79	0.000	
Turmeric	0.686	0.101	6.81	0.000	1.00
Sweet Flag	0.686	0.101	6.81	0.000	1.00
Tea	0.955	0.101	9.49	0.000	1.00
Turmeric*Turmeric	−0.9322	0.0979	−9.52	0.000	1.02
SweetFlag*Sweet Flag	−0.9322	0.0979	−9.52	0.000	1.02
Tea*Tea	−1.1090	0.0979	−11.32	0.000	1.02
Turmeric*Sweet Flag	0.250	0.131	1.90	0.086	1.00
Turmeric*Tea	0.500	0.131	3.80	0.003	1.00
Sweet Flag*Tea	0.500	0.131	3.80	0.003	1.00

and squared terms of interaction between turmeric, sweet flag, and tea are significant, even the interaction between tea and turmeric, and sweet flag and tea are associated between them. Considering the P-value, every term is significant and lies under the significance level of 0.05.

On studying the goodness of fit statistics in the model used for the optimization based on the low s value (0.3717) there is a better description of the response. 97.94% R^2 value was obtained with consideration of response describing the fitness of the model. Similarly, the adjacent R^2 value, and predicted R^2 value was not substantially less than the R^2 value making the model fit.

From Figure 2, residual plots are employed to determine the adequateness of the model. From the residual plots, the model was found to have good adequateness as they are randomly distributed around on both sides of zero with constant variances with an influential point far away from point zero. The experimental response was further analyzed, and the prediction process was carried out. The experimental values and predicted values of the shelf-life are given in Table 4. The predicted mean for every individual response was determined. From this, a likely single future value can be determined with a range examining the prediction interval.

The interaction effects are necessary to understand whether the interactions are parallel. The two-way interaction effects relate to the relationship between the response variable and predictors. The interaction effects between the

Table 4. Response values and Predicted values of shelf-life observed during experimentation.

Run	Turmeric	Sweet Flag	Tea	Observed Shelf-life	Predicted Shelf-life
1	0.011	0.011	0.036	11	10.9305
2	0.041	0.011	0.036	11	10.8017
3	0.011	0.041	0.036	11	10.8017
4	0.041	0.041	0.036	12	11.6730
5	0.011	0.011	0.136	11	10.8409
6	0.041	0.011	0.136	13	12.7122
7	0.011	0.041	0.136	13	12.7122
8	0.041	0.041	0.136	16	15.5835
9	0.001	0.026	0.086	11	11.1906
10	0.177	0.026	0.086	13	13.4968
11	0.026	0.001	0.086	11	11.1906
12	0.026	0.051	0.086	13	13.4968
13	0.026	0.051	0.006	10	10.2372
14	0.026	0.026	0.177	13	13.4502
15	0.026	0.026	0.086	15	14.9803
16	0.026	0.026	0.086	15	14.9803
17	0.026	0.026	0.086	15	14.9803
18	0.026	0.026	0.086	15	14.9803
19	0.026	0.026	0.086	15	14.9803
20	0.026	0.026	0.086	15	14.9803

shelf-life and turmeric, sweet flag, and tea have no parallel lines of interaction effects describing that the fewer parallel lines, the greater the strength of interactions between the response shelf-life and the predictor's turmeric, sweet flag, and tea. The interaction plot and main effects plot for shelf-life is shown in **Figure 3**.

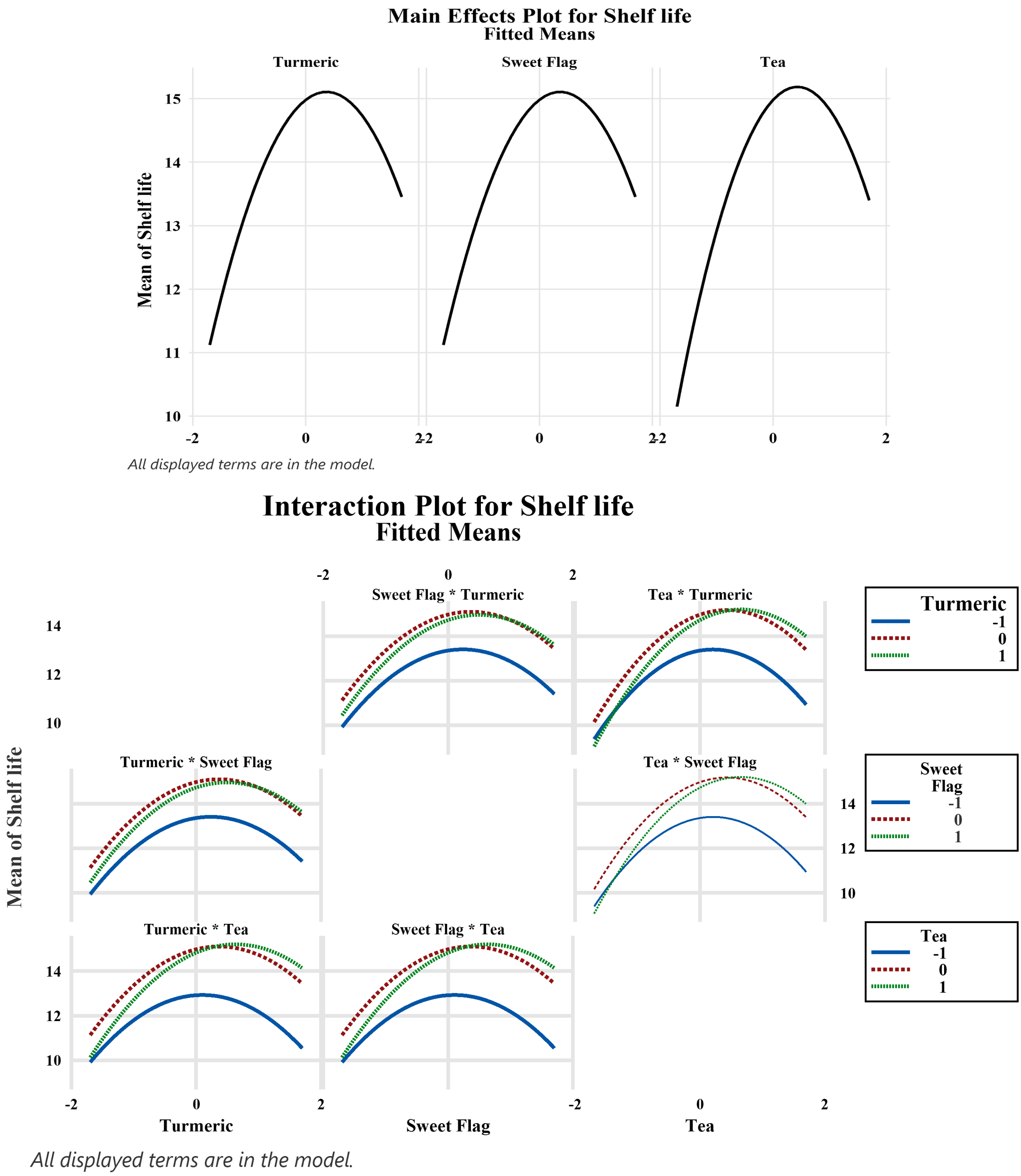


Figure 3. The two-way interaction plots (main effect plot and interaction plot) including the relationship between the response and predictors.

The contour plots and surface plots were studied to determine the quadratic terms and establish desirable response values relate to continuous variables with operating conditions. From **Figure 4**, the contour plot and surface plot of shelf-life versus sweet flag and turmeric with tea as held at value zero. The contour plot describes that the turmeric and sweet flag at the condition (0, 0) has a desirable response of 16 days shelf-life has the highest interaction effect, while the lowest interaction with quite a decent response of 12 days was obtained at the condition (−1.682, 0). Where two other conditions (0, 1.682) and (1.682, 0) had a good desirable response of 13 days shelf-life. Comparing the surface plots of the shelf life versus sweet flag and turmeric the highest interaction and response

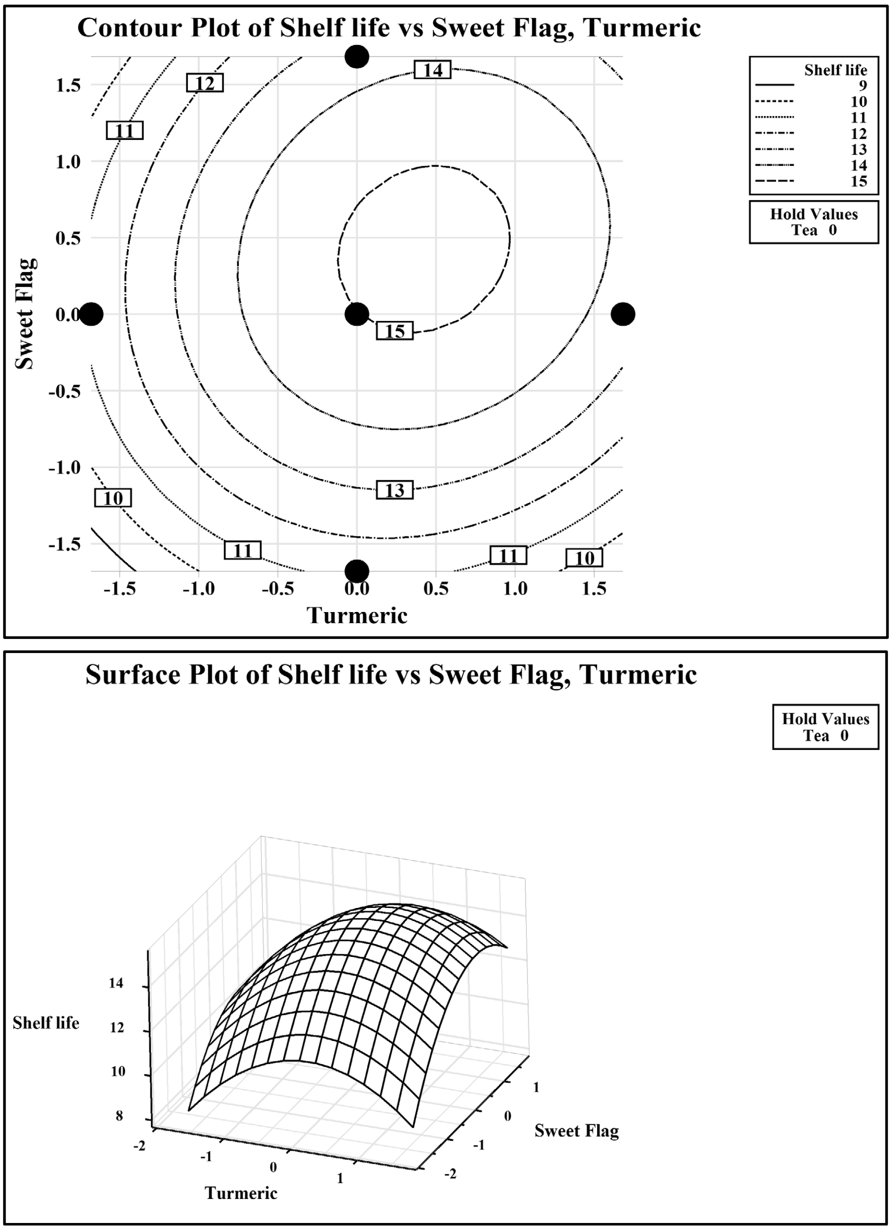


Figure 4. Contour and surface plots of shelf-life vs sweet flag and turmeric containing significant quadratic terms in the model.

was obtained at the middle range (0, 0) with a slight decrease with an increase in the range of turmeric and sweet flag and a decrease with decreasing range of turmeric and sweet flag.

From Figure 5, the contour plot and surface plot of shelf-life versus tea and sweet flag with turmeric as held at value zero. The contour plot describes that the tea and sweet flag at the condition (0, 0) has a desirable response of 14 days shelf-life has the highest interaction effect, while the lowest interaction with quite a decent response of 10 days was obtained at the condition (0, −1.682). Where two other conditions (1.682, 0) and (0, 1.682) had a good desirable response of 12 days shelf-life. Comparing the surface plots of the shelf-life versus tea and sweet flag the highest interaction and response was obtained at the middle range (0, 0) with a slight decrease with an increase in the range of tea and

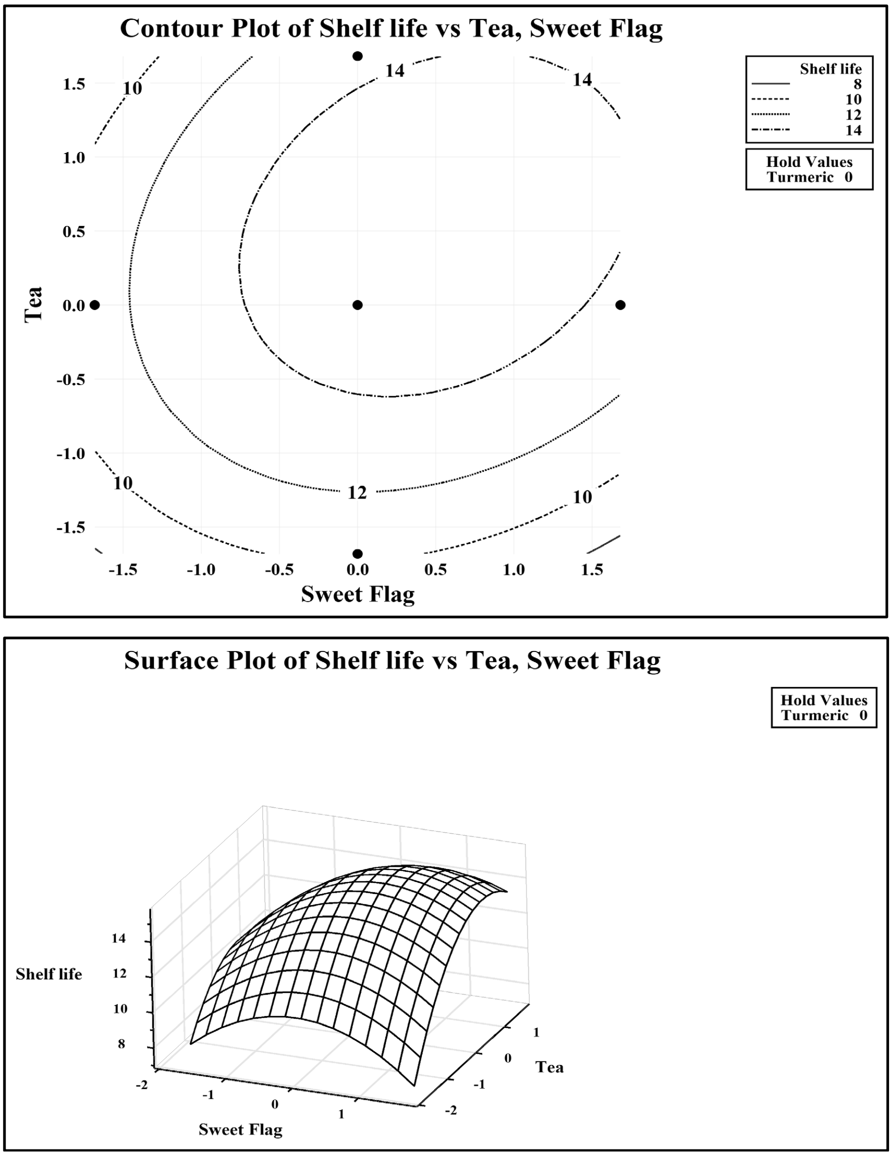


Figure 5. Contour and surface plots of shelf-life vs tea and sweet flag containing significant quadratic terms in the model.

sweet flag and a decrease with decreasing range of tea and sweet flag.

From **Figure 6**, the contour plot and surface plot of shelf-life versus turmeric and tea with the sweet flag as held at value zero. The contour plot describes that the turmeric and tea at the condition (0, 0) have a desirable response of 14 days shelf-life has the highest interaction effect, while the lowest interaction with quite a decent response of 10 days was obtained at the condition (0, −1.682). Where two other conditions (1.682, 0) and (0, 1.682) had a good desirable response of 12 days shelf-life. Comparing the surface plots of the shelf-life versus turmeric and tea the highest interaction and response was obtained at the middle range (0, 0) with a slight decrease with an increase in the range of turmeric and

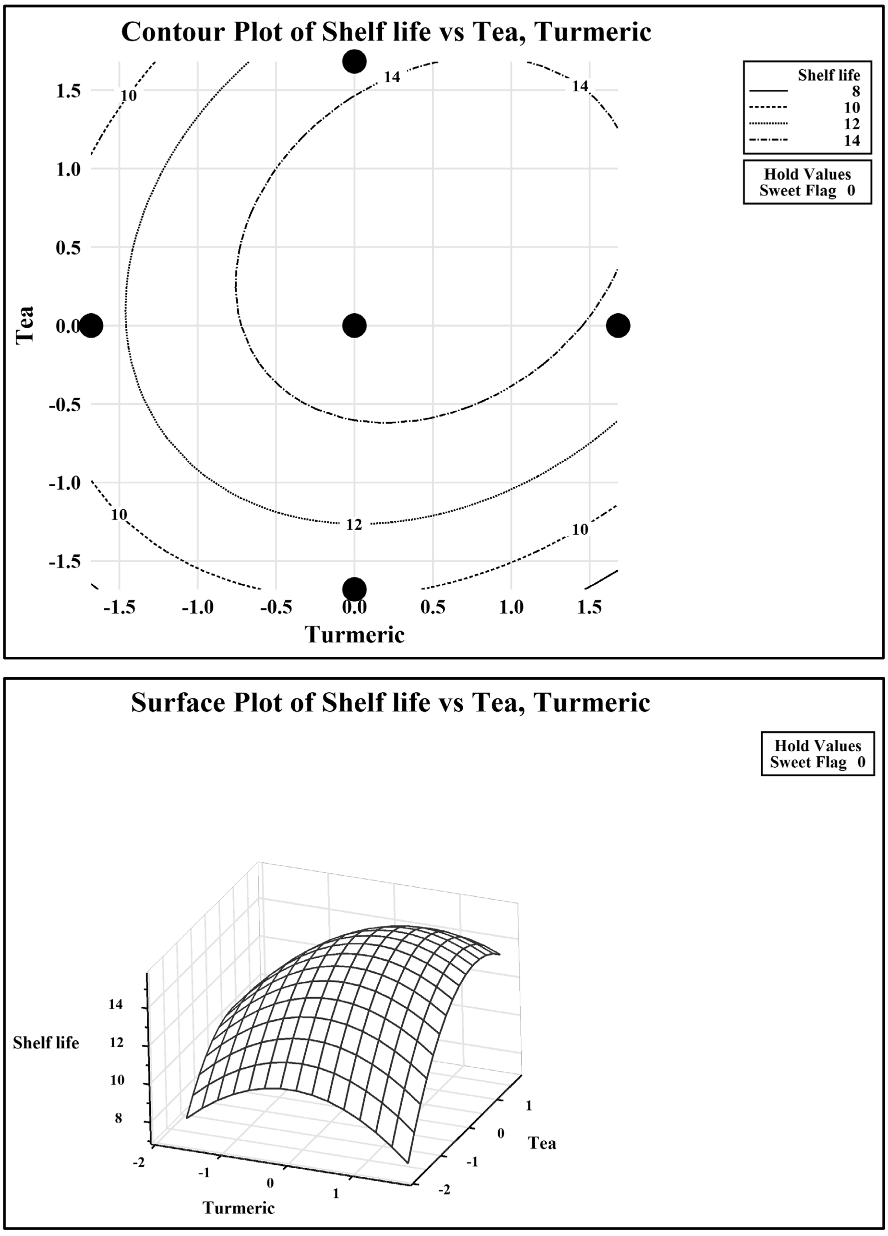


Figure 6. Contour and surface plots of shelf-life vs tea and turmeric containing significant quadratic terms in the model.

tea decrease with decreasing range of turmeric and tea.

3.2. Optimization, Statistical and Sensory Evaluation of Chapattis

Based on the responses, the model was optimized to determine the efficient range of turmeric, sweet flag, and tea to obtain the desired range of shelf-life as output shown in Figure 7. The D-optimal for this optimization process was maintained at 1. The higher and lower value for turmeric, sweet flag, and tea is 1.682 and −1.682. An optimization plot is obtained as a result showing the range values to be used to attain maximum shelf-life. The range values obtained were 0.6625 for turmeric, 0.6625 for the sweet flag, and 0.7305 for tea as coded values to obtain a maximum shelf-life of 16 days. On encoding the values 0.6885 mg/ml of turmeric, 0.6885 mg/ml of sweet flag, and 0.8165 mg/ml of tea powders can be used to obtain the maximized shelf-life of 16 days. During the response optimization process, the fit response of 15.770 was obtained from the 95% confident intervals (15.358, 16.162) and predicted intervals (14.845, 16.696).

Further one-way ANOVA along with Tukey's simultaneous test and Fisher's pairwise comparison test was implemented to study the individual significances between turmeric, sweet flag, and tea versus shelf-life using the coded values and the uncoded values are given in Table 2.

3.2.1. Turmeric versus Shelf-Life

On analyzing the interaction of turmeric versus shelf-life using one-way

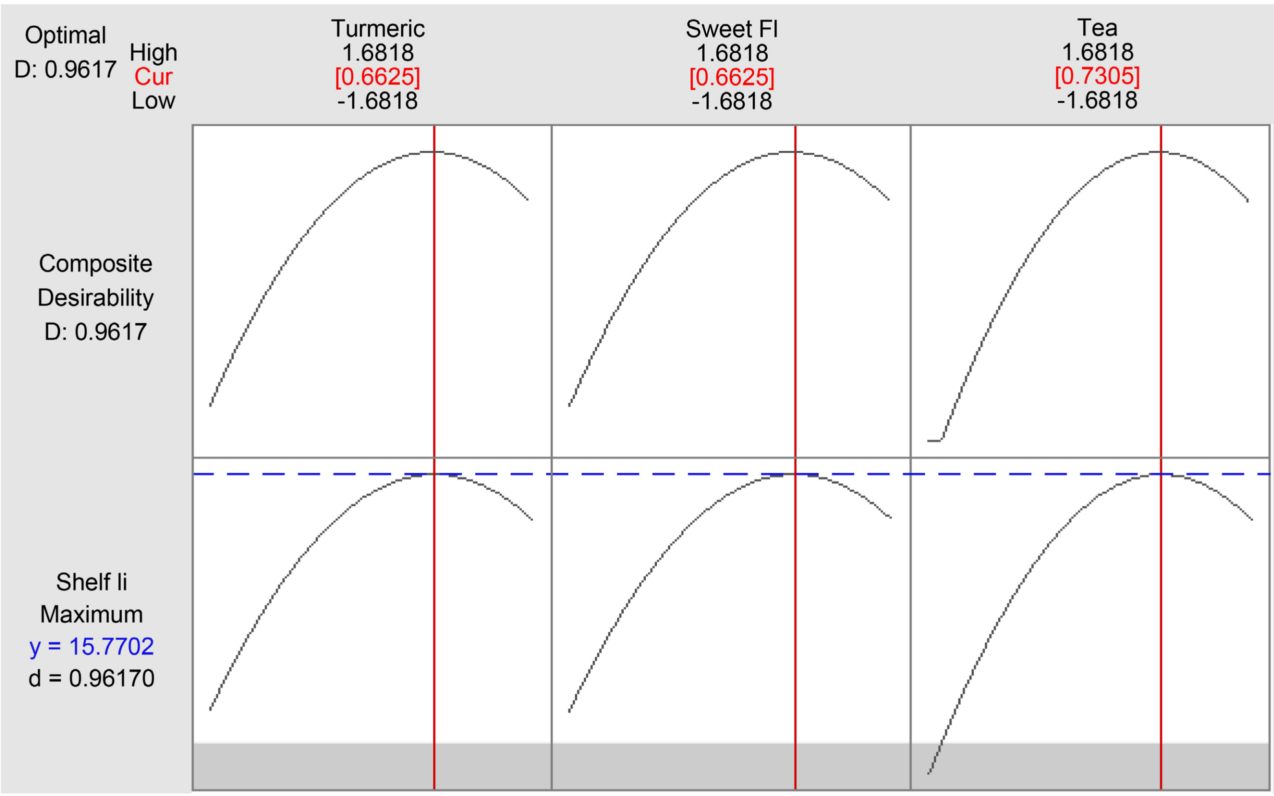


Figure 7. Optimization plot showing the optimal values for the predictors to be used for better shelf-life.

ANOVA, a significant P-value of 0.014 was obtained determining its high significance. 26.64% of variation or increase in the shelf-life is caused by the interaction of turmeric in the model. The S level was lower denoting that the turmeric interactions fit the model significantly.

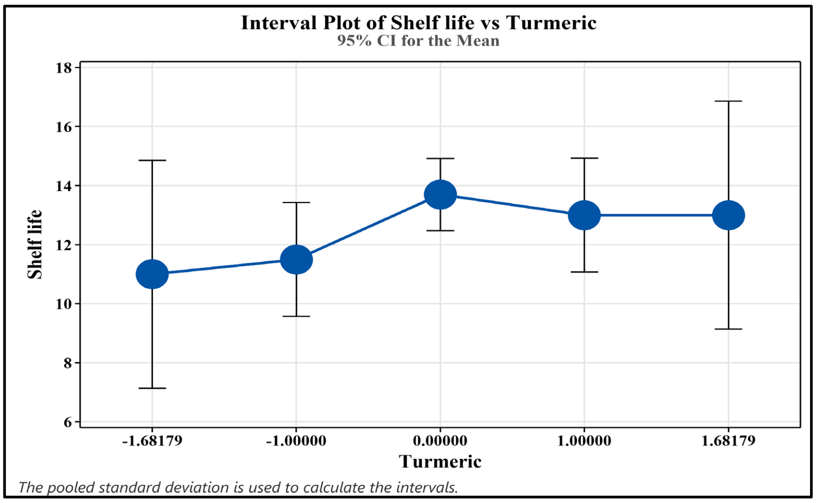
The interval plots for turmeric versus shelf-life obtained from the one-way ANOVA stated that the highest interaction was seen at zero level with a mean of 13.7 and the lowest interaction was obtained at −1.682 level with a mean of 11 shown in **Figure 8(a)**. Tukey comparisons test describes that the interaction between the turmeric and shelf-life had a 99.25% individual confidence level. From **Table 5**, which denotes the grouping information of all levels of turmeric comes under individual group respectively. The turmeric levels do not share different groups and there is no significant difference. **Figure 8(b)** shows that no levels of turmeric mean contain zero as its significant interval, justifying that the means of shelf-life obtained from turmeric are significantly different except at one level.

Using the Fisher method certain grouping was determined stating that all level of turmeric is grouped under A with the means of 13.700, 13.00, 13.00, 11.500, and 11 respectively described in **Table 6**. The turmeric level of zero had the highest interaction and −1.682 had the least interaction shown in **Figure 8(c)**, justifying that all interaction levels are significantly different. Fisher's method of grouping showed a simultaneous confidence level of 77.24% interaction between the levels of turmeric. Fisher individual tests showing the means of shelf-life obtained due to turmeric are significantly different.

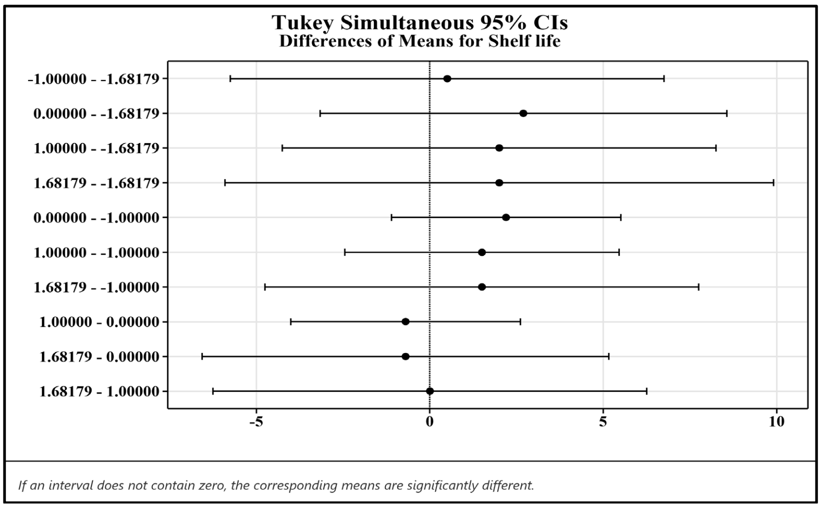
3.2.2. Sweet Flag versus Shelf-Life

On analyzing the interaction of sweet flag versus shelf-life using one-way ANOVA, a slight significant P-value of 0.104 was obtained determining its high significance. 26.66% of variation or increase in the shelf-life is caused by the interaction of the sweet flag in the model. The S level was lower denoting that the sweet flag interactions fit the model significantly.

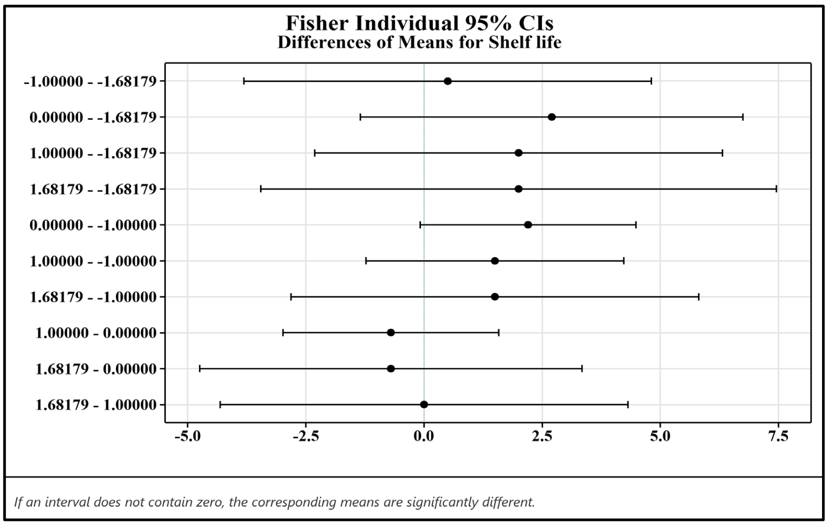
The interval plots for sweet flag versus shelf-life obtained from the one-way ANOVA stated that the highest interaction was seen at zero level with a mean of 13.5 and the lowest interaction was obtained at −1 level with a mean of 12.25 shown in **Figure 9(a)**. Tukey comparisons test describes that the interaction between the sweet flag and shelf-life had a 99.25% individual confidence level. **Table 7**, which denotes the grouping information of all levels of the sweet flag (−1.682, −1, 0, 1, and 1.682) comes under individual group A with the highest interaction level with a mean of 13.7 at zero level. Sweet flag with a −1 level with a mean of 11.00 had the lowest interaction level respectively. The sweet flag levels do not share different groups denoting those interactions of the sweet flag with shelf-life are not significantly different. **Figure 9(b)** shows that one level of interaction of sweet flag contains zero as its significant interval, justifying that the means of shelf-life obtained from sweet flag are significantly different except at one level of interaction.



(a)

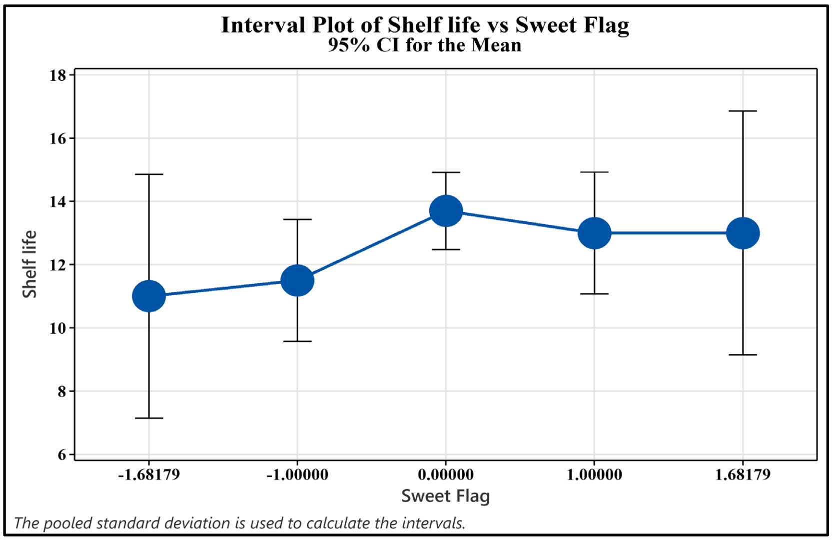


(b)

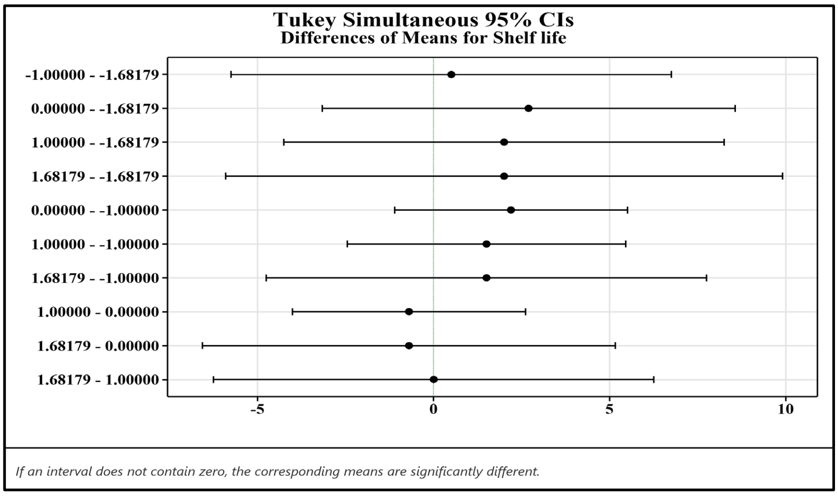


(c)

Figure 8. (a). Interval plot showing higher and least interaction of turmeric to obtain shelf-life. (b). Tukey comparisons test shows the means of shelf-life obtained due to turmeric are significantly different. (c). Fisher individual tests showing the means of shelf-life obtained due to turmeric are significantly different.



(a)



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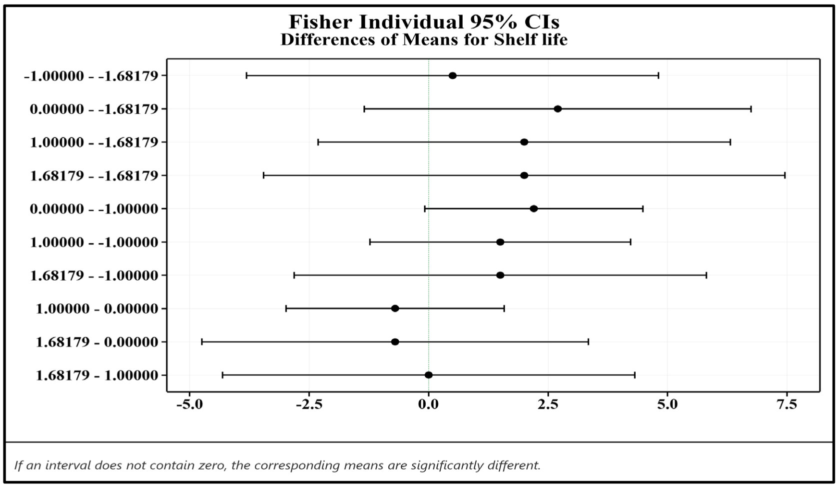


(c)

Figure 9. (a). Interval plot showing higher and least interaction of sweet flag to obtain shelf-life. (b). Tukey comparisons test showing the means of shelf-life obtained due to sweet flag are significantly different. (c). Fisher individual tests showing the means of shelf-life obtained due to sweet flag are significantly different.

Table 5. Grouping information using the tukey method and 95% confidence level of turmeric vs shelf-life.

Turmeric	N	Mean	Grouping
0.00000	10	13.700	A
1.68179	1	13.00	A
1.00000	4	13.00	A
−1.00000	4	11.500	A
−1.68179	1	11.00	A

Note: Means that do not share a letter are significantly different.

Table 6. Grouping information using the fisher LSD method and 95% confidence level of turmeric vs shelf-life.

Turmeric	N	Mean	Grouping
0.00000	10	13.700	A
1.68179	1	13.00	A
1.00000	4	13.00	A
−1.00000	4	11.500	A
−1.68179	1	11.00	A

Note: Means that do not share a letter are significantly different.

Table 7. Grouping information using the tukey method and 95% confidence level of sweet flag vs shelf-life.

Turmeric	N	Mean	Grouping
0.00000	10	13.700	A
1.68179	1	13.00	A
−1.68179	1	13.00	A
1.00000	4	11.500	A
−1.00000	4	11.00	A

Note: Means that do not share a letter are significantly different.

Using the Fisher method certain grouping was determined stating that which denotes the grouping information of all levels of the sweet flag (−1.682, −1, 0, 1, and 1.682) comes under individual group A with the highest interaction level with a mean of 13.7 at zero level. Sweet flag with a −1 level with a mean of 11.00 had the lowest interaction level respectively described in **Table 8**. The sweet flag level of zero had the highest interaction and −1 had the least interaction shown in **Figure 9(c)**. Fisher's method of grouping showed a simultaneous confidence level of 74.24% interaction between the levels of sweet flag. Fisher individual test showing the means of shelf-life obtained due to sweet flag are significantly different except at a single point of interaction level (1.682, −1.682) was determined as not significantly different.

Table 8. Grouping information using the fisher LSD method and 95% confidence level of sweet flag vs shelf-life.

Sweet Flag	N	Mean	Grouping
0.00000	10	13.700	A
1.68179	1	13.00	A
−1.68179	1	13.00	A
1.00000	4	11.500	A
−1.00000	4	11.00	A

Note: Means that do not share a letter are significantly different.

3.2.3. Tea versus Shelf-Life

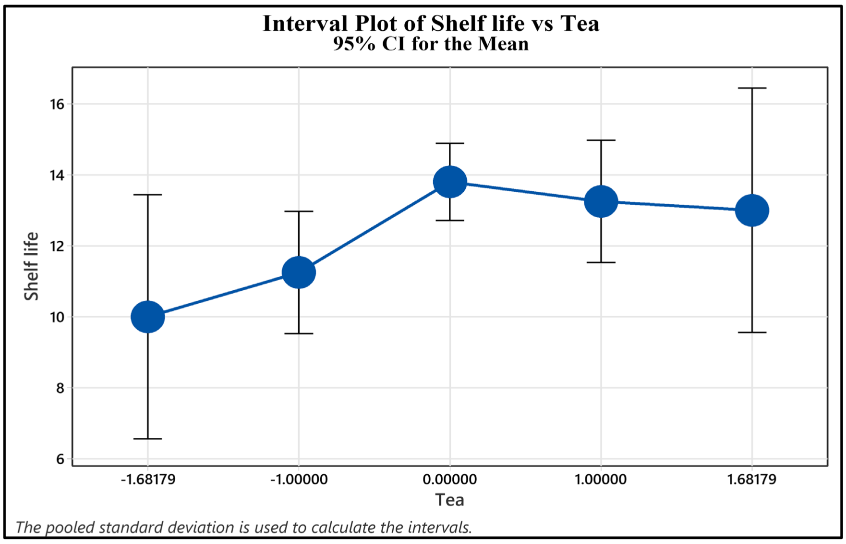
On analyzing the interaction of tea versus shelf-life using one-way ANOVA with a significant P-value of 0.071 was obtained determining its high significance. 41.60% of variation or increase in the shelf-life is caused by the interaction of tea in the model. The S level was lower denoting that the tea interactions fit the model significantly.

The interval plots for tea versus shelf-life obtained from the one-way ANOVA stated that the highest interaction was seen at zero level with a mean of 13.8 and the lowest interaction was obtained at −1 level with a mean of 10 shown in Figure 10(a). Tukey comparisons test describes that the interaction between the tea and shelf-life had a 99.25% individual confidence level. Table 9, which denotes the grouping information of all levels of tea comes under individual group A with the highest interaction level at zero. The tea levels do not share different groups denoting that those interactions of tea with shelf-life are significantly different at particular intervals. Figure 10(b) shows that three levels of interactions of tea contain zero at its significant interval, justifying that the means of shelf-life obtained from tea are significantly.

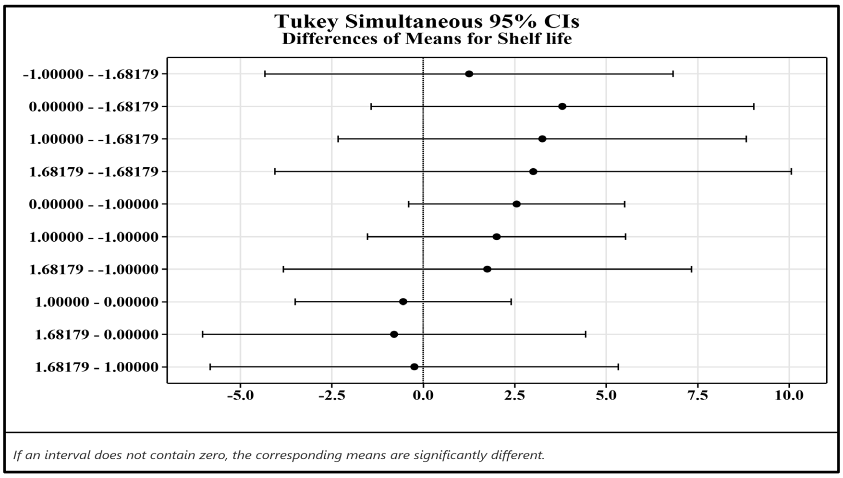
Using the Fisher method certain grouping was determined stating that zero level of tea is grouped under A with the mean of 13.8, while levels −1.682, and −1 are grouped under B with means of 11.25 and 10 respectively. 1.689 and 1 levels of tea are grouped under both A and B with the mean of 13.250 and 13.00, described in Table 10. The tea level of zero had the highest interaction and -1 had the least interaction shown in Figure 10(c). Fisher's method of grouping showed a simultaneous confidence level of 74.24% interaction between the levels of tea. Fisher individual test showing the means of shelf-life obtained due to tea are significantly different.

3.3. Sensory and Microbial Analysis of Optimized Chapattis

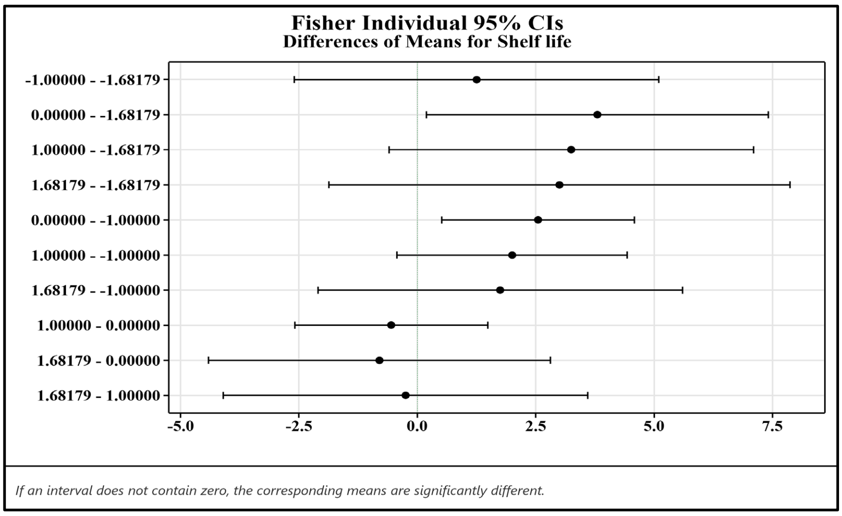
Using the values of 0.6625 for turmeric, 0.6625 for the sweet flag, and 0.7305 for tea powders obtained by the optimization process were used along the dough made of 250 g of wheat flour with an adequate amount of oil, water, and salt required. The dough was left for an hour to undergo the leavening process. The well-leavened dough had a soft texture with spongy nature, no changes were



(a)



(b)



(c)

Figure 10. (a). Interval plot showing higher and least interaction of tea to obtain shelf-life. (b). Tukey comparisons test shows the means of shelf-life obtained due to tea are significantly different. (c). Fisher individual tests showing the means of shelf-life obtained due to tea are significantly different.

Table 9. Grouping information using the tukey method and 95% confidence of tea vs shelf-life.

Tea	N	Mean	Grouping
0.00000	10	13.800	A
1.68179	4	13.250	A
1.00000	1	13.000	A
−1.68179	4	11.250	A
−1.00000	1	10.00	A

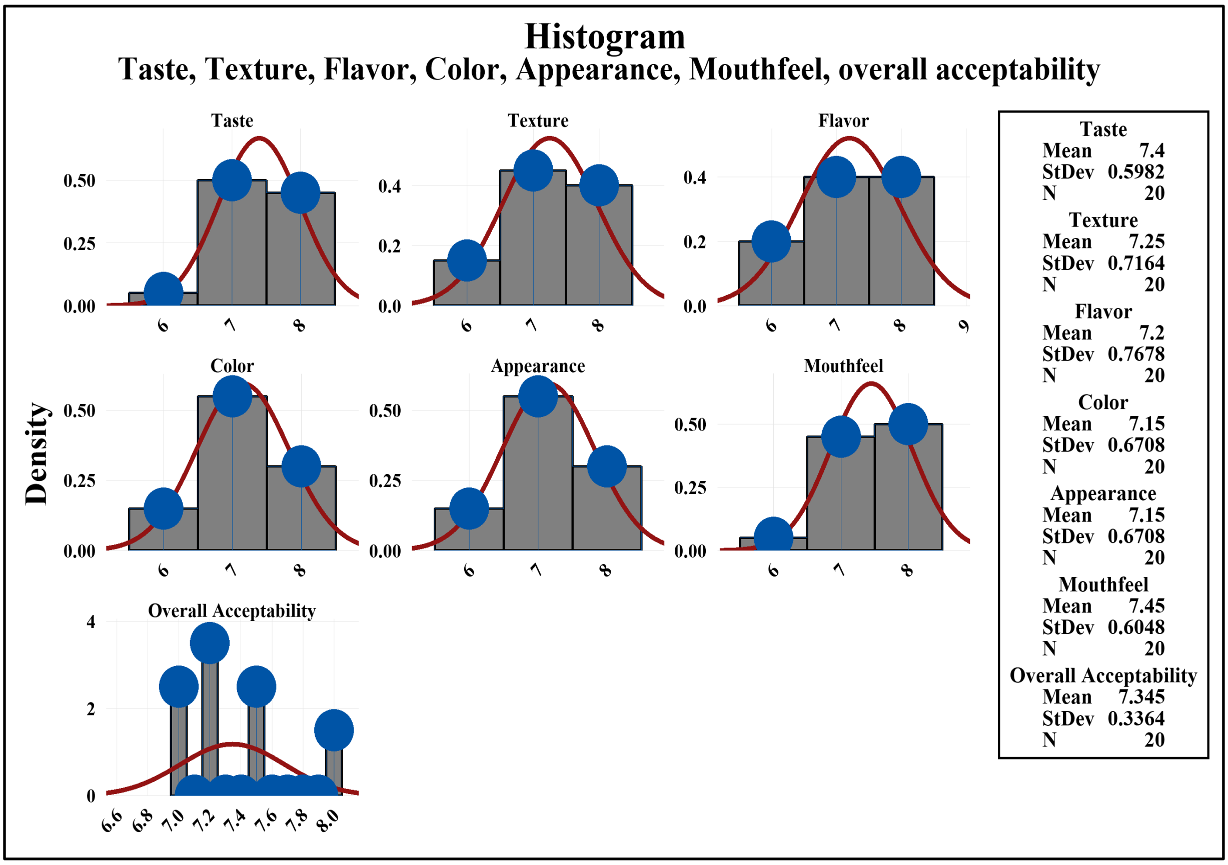
Note: Means that do not share a letter are significantly different.

Table 10. Grouping information using the fisher LSD method and 95% confidence of tea vs shelf-life.

Tea	N	Mean	Grouping	
0.00000	10	13.800	A	
1.68179	4	13.250	A	B
1.00000	1	13.000	A	B
−1.68179	4	11.250		B
−1.00000	1	10.00		B

Note: Means that do not share a letter are significantly different.

observed in the appearance of the dough with the addition of the extract powders. There was no change in its natural smell was observed along with taste and mouth feel. The microbial analysis was carried out regularly at two days intervals to visualize any microbial growth. There was no microbial growth observed until the 16th day. The chapattis texture was seen with cracks over its texture with no presence of microorganisms on the 18th day. The mold formation was visible on the 21st day resulting in the deterioration of chapattis. Thus, as a concluding aspect, 16 days can be fixed as the shelf-life period of the chapattis prepared out of the combinations of turmeric, sweet flag, and tea powders as preservatives. The sensory evaluation carried out fewer than 20 panelists based on taste, texture, flavor, color, and appearance was evaluated and given in **Table 11**. The overall acceptability of taste was around 7.4, the texture was 7.25, the flavor was 7.2, color was 7.15, and appearance was 7.15 respectively. The overall acceptability of the chapattis prepared using turmeric, sweet flag, and tea powders as preservatives were 7.35. To statistically justify the sensory attributes and overall acceptability a histogram along with a probability plot was constructed and shown in **Figure 11**. From the histogram, the mean obtained for taste, texture, flavor, appearance, and overall acceptability are 7.4, 7.25, 7.2, 7.15, 7.15, and 7.35 respectively. The histogram justifies that the sensory attributes and the overall acceptability have a good fit for the product with significance. The probability plots of the sensory attributes and the overall acceptability describe that all values are



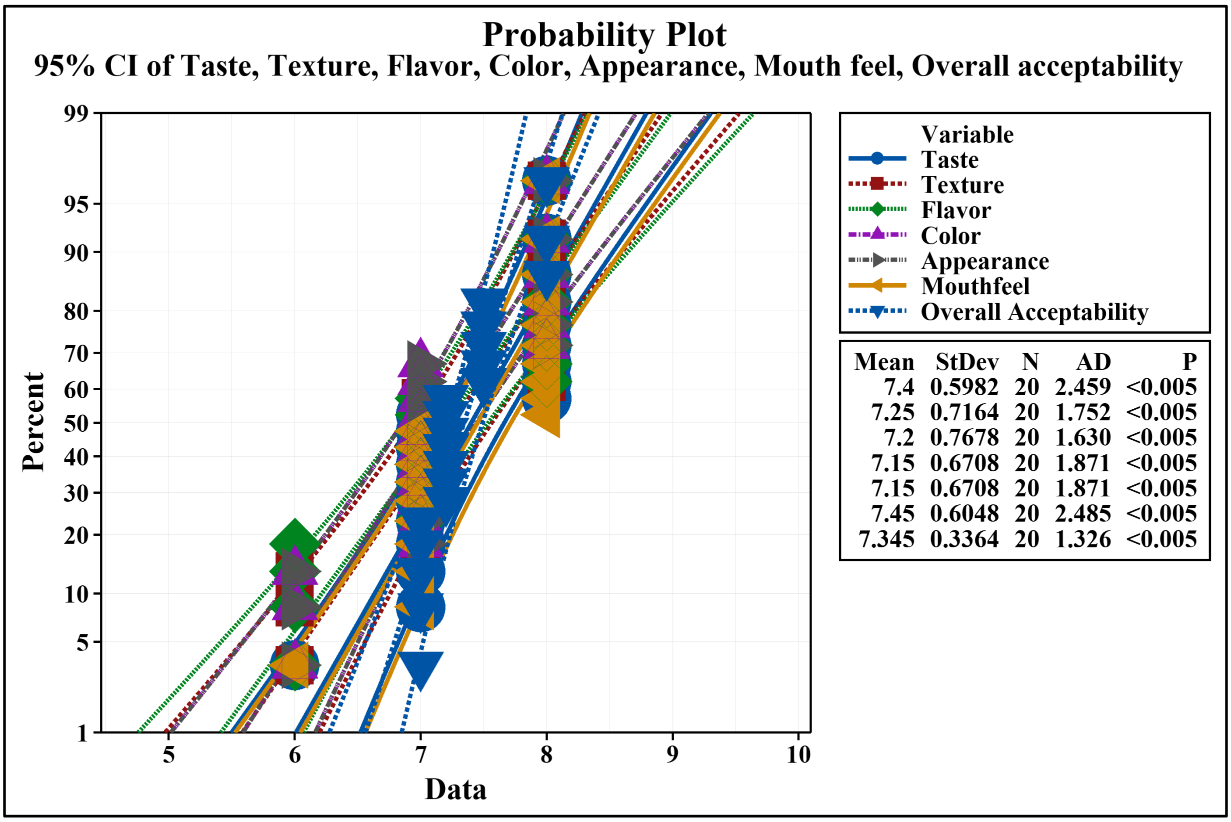


Figure 11. Histogram and probability plot of the sensory attributes which determine that all means are significantly different.

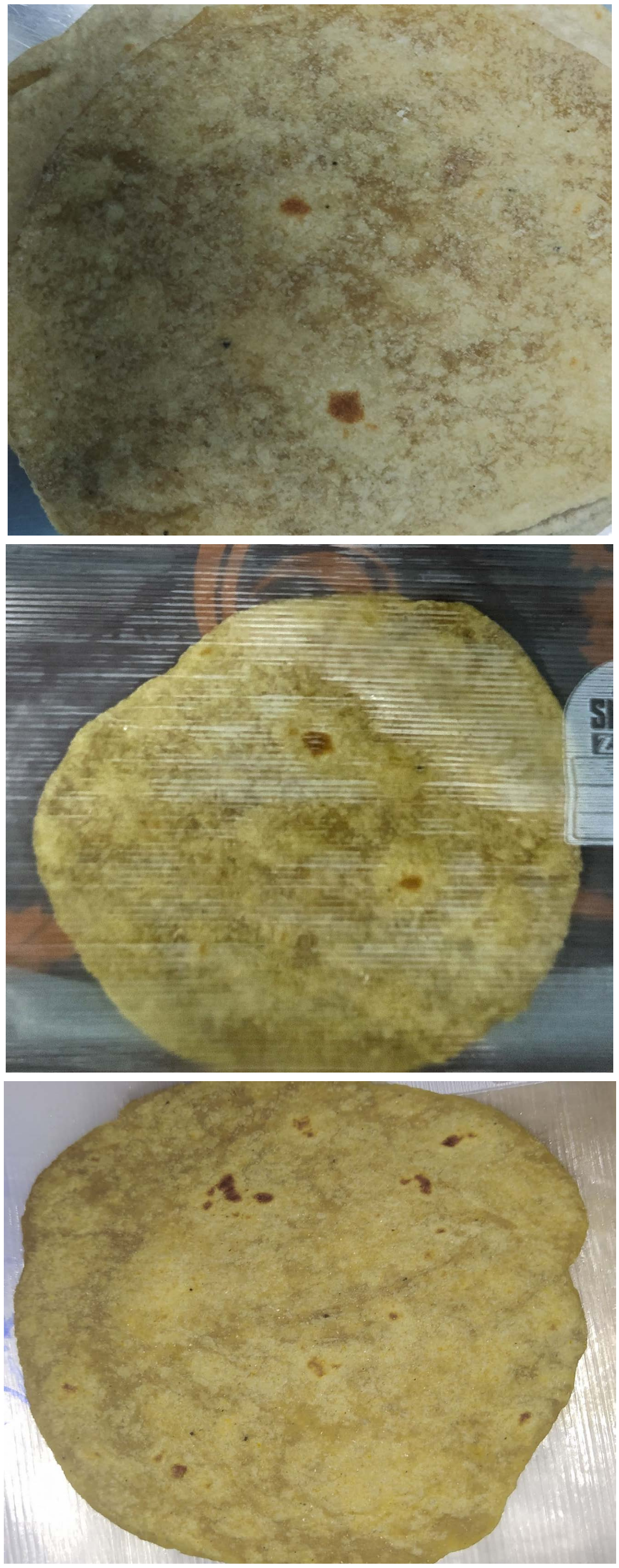


Figure 12. (a) First-day image of the prepared chapatti, (b) LPDE packaging of the chapatti for storage, and (c). 14th-day image of the prepared chapatti.

Table 11. Sensory attributes and the overall acceptability given by the panelists for the chapattis.

Panelists	Taste	Texture	Flavor	Color	Appearance	Mouthfeel	Overall Acceptability
1	8	8	7	8	7	7	7.5
2	7	8	8	7	8	8	8
3	7	7	8	7	7	7	7.2
4	8	8	8	7	7	8	8
5	7	8	8	7	8	8	8
6	8	7	7	8	7	8	7.5
7	7	8	8	7	8	7	7.5
8	8	7	7	8	7	8	7.5
9	7	8	8	7	8	7	7.5
10	8	7	6	7	7	8	7.2
11	7	6	7	8	8	7	7.2
12	8	7	6	7	7	8	7.2
13	6	6	7	8	8	7	7
14	7	7	8	7	7	6	7
15	8	8	7	6	6	7	7
16	7	6	6	7	7	8	7
17	8	7	7	8	6	7	7.2
18	7	7	6	6	7	8	7
19	8	8	7	7	6	7	7.2
20	7	7	8	6	7	8	7.2

significant with P-values lesser than 0.05. The overall acceptability of the chapatti product justified by the panelists was 75% which was significant in the histogram and the probability plots.

4. Conclusion

The dough was seen to have a good leavening and soft texture with no change in their appearance, order, or taste. The spoilage of the prepared and stored chapattis was monitored (**Figure 12**). At the conclusion of the 20 trials, the range of shelf-life obtained was between 12 to 16 days. On studying the goodness of fit statistics in the model used for the optimization based on the low s value (0.3717), there is a better description of the response. 97.94% R^2 value was obtained with consideration of response describing the fitness of the model. Similarly, the adjacent R^2 value, and predicted R^2 value was not substantially less than the R^2 value making the model fit. On encoding the values 0.6625 for turmeric, 0.6625 for the sweet flag, and 0.7305 for tea powders can be used to obtain the maximized shelf-life of 16 days. On the trial of the optimized values, no spoilage occurred up to the 16th day and the overall acceptability was good. Similarly, Minali Masih *et al*. [9] studied about shelf-life of chapatti and its flexibility improvement by adding several ingredients like hot water, choker, spinach, yeast, and oil in the wheat flour using the traditional method and preserving at 5˚C in a refrigerator in LDPE packaging material and then followed the results for the shelf life of chapatti in equal intervals, no spoilage was found in any sample and the overall acceptability was well rated. Results from the time measurements and microbiological tests showed that the product was acceptable in all conditions throughout the storage period.

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Conflicts of Interest

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

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