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*Research Article*

# A Ready-to-Eat Antioxidant Rich Appetizer Based on *Coleus aromaticus*: Its Development and Shelf Life Evaluation

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Antioxidant rich products are valued due to their health benefits and appetizers are required in several pathological and geographical stress situations such as prolonged exposure to altitude. The paper deals with the development of a shelf stable RTE (ready-to-eat) antioxidant rich herbal appetizer convenient to the consumer. Using ginger and Karpurvalli (*Coleus aromaticus*) as two independent variables, a central composite design with 13 experimental combinations was obtained. These combinations were processed by concentration and dehydration into the appetizer RTE munches using preprocessed ingredients and evaluated for antioxidant activity, vitamin C, and sensory characteristics. The product optimized using Design Expert Statistical Software had the proximate composition of 11.4% fat, 2.3% protein, and 75.0% carbohydrates, supplying about 82.36 Kcals per munch of 20 g. The munches packed in metalized polyester pouches had a shelf life of 10 months at 28 ± 5°C as well as 37°C storage. The RTE appetizer based on *Coleus aromaticus* was developed with excellent sensory properties and shelf stability.

## 1. Introduction

*Coleus aromaticus* (CA) commonly called as Country borage or Indian borage is a perennial herb grown throughout Indian subcontinent. Traditionally, the leaves of the plant are used for cold, cough, and fever as well as against skin irritations. The herb has been reported to have several therapeutic potentials. It has been reported that the leaves of the CA herb have antioxidant and radical scavenging activity [1, 2], as well as antibacterial and antifungal activities [3, 4]. The leaves are useful in urinary diseases and vaginal discharge [5]. The expressed juice is used in epilepsy and other convulsive disorders and plant extracts used in the treatment of gastrointestinal disorders [6] while the water extract of CA leaves was found to produce significant diuresis in rats [7]. However, the appetising potential of the herb was unexplored for development of products. Our studies have explored the appetising potential of the CA herb in the form of a shelf stable ready-to-drink beverage called Karpurvalli beverage through animal experiments [8] as well as in a study on human volunteers [9]. A hot water reconstitutable soup mix also has been reported based on the CA herb [10].

Appetite is governed by several factors such as cognizance and memories of the food and psychological and physiological status of the consumer as well as the type of the food and the way the food is being served. The various spices/herbs with pungent volatile compounds cause appetite stimulation through hormonal and metabolic factors. Ginger has been one of the essential ingredients in different appetite influencing foods such as RTE (ready-to-eat) appetizers [11, 12]. The RTE products are highly convenient to carry and to consume for sojourners to altitudes and cold areas. Such products are preferred by soldiers deployed at high altitudes, wherein they can avoid cooking, fuel use, and energy required for preparation of dehydrated mixes. It has been reported [13] that the consumers/sojourners at high altitude gave preference to the preferred sweet taste. The optimization of the product in the present study has been achieved by Statistical Design Software using RSM (Response Surface Methodology). The study aims to provide shelf stable sweet and spicy antioxidant rich appetizer with good acceptability.

## 2. Materials and Methods

*2.1. Raw Materials.* Karpurvalli (*Coleus aromaticus*) leaves were obtained from the laboratory's garden-plot specifically

Table 1: Experimental ranges with five levels of independent variables used in CCRD in terms of actual and coded values for Karpurvalli munch.

| Variables (range of levels) | A: ginger | B: Karpurvalli juice |
|---|---|---|
| Coded value | Actual value | Actual value |
| $-\alpha$ | 71.72 | 329.29 |
| −1 | 80 | 350 |
| 0 | 100 | 400 |
| +1 | 120 | 450 |
| $+\alpha$ | 128.28 | 470.71 |

cultivated. Jaggery, ajwain (*Trachyspermum ammi*) seeds, fresh matured ginger (*Zingiber officinalis* Roscoe), lemon (*Citrus limon*), raisins (*Vitis vinifera*), and ghee (Nandini brand) were procured from the local market. The pectin (HiMedia) and other chemicals and reagents used for analysis were of AR grade.

*2.2. Raw Material Processing.* The CA leaves were carefully plucked leaving aside the overmature leaves and washed, subjected to juice extraction using a screw type mechanical juice extractor, and filtered through muslin cloth. Jaggery, ajwain, and raisins were cleaned to remove impurities prior to further use. The raisins were fried in ghee at 160–180°C for 30–50 seconds and cooled thoroughly and were ground. Ginger (fresh) was washed in lukewarm (40–50°C) water, cleaned, peeled, and cut into pieces and then was ground into a paste using warring blender. Lemons (mature fruits) were washed in hot water and halved with knife and juice was extracted using squeezers and filtered. Ajwain seeds were cleaned and finely ground to pass through 60-mesh sieve.

*2.3. Experimental Design.* A five-level central composite rotatable design was constructed with two independent variables. The Design Expert version 8.0 Statistical Software package from Stat ease Inc., USA, was used to construct and to analyze the design. Karpurvalli juice and ginger paste were taken as independent variable with sensory score vitamin C and antioxidant activity as the responses. The experimental design with two variables had 13 design points including five centre point replications. The range of independent variables studied with coded and actual values are given in Table 1. The $\alpha$-values in the design outside the ranges were selected for rotatability of the design [14]. The center points were selected with ingredients at levels expected to yield satisfactory experimental results. The regression analysis of the responses was conducted by fitting suitable either linear or quadratic model.

*2.4. Appetizer Processing.* The Karpurvalli juice and ginger paste were weighed as per the design to form different formulation batches, while other ingredients such as lemon juice, jaggery, raisins, ajwain powder, and pectin were at fixed levels. Dissolved and filtered jaggery solution was taken in a LPG based open top concentrator (M/s. Solar arks, Kolhapur, India) and other ingredients were added one by one with continued stirring. The appetizer was prepared by concentration and dehydration technique with continuous stirring while heating at 160–200°C. The end point determination was a critical point identified when the concentrating semisolid mass begins leaving the pan surface and product exhibiting characteristic gloss. In hot condition, the product was poured into trays precoated with ghee. After cooling, 20 g samples were weighed and hand-moulded into oval shapes as individual munches and then packed in metalized polyester pouches and stored at ambient conditions 18–33°C and 37°C.

*2.5. Analytical Evaluation.* The proximate analysis which includes crude protein, fat, ash, and moisture content of Karpurvalli munch was carried out by standard AOAC procedures [15]. The acidity as citric acid and vitamin C were determined by titrimetry [16]. Changes in the thiobarbituric acid (TBA) value during storage were estimated by steam distillation method wherein the distillate was treated with TBA solution and OD was measured at 540 nm [17]. For estimation of sugars the sample was made protein free using lead acetate and potassium oxalate. The estimation of reducing and total sugars was done [15] using Fehling A and Fehling B solution and titration. The antioxidant activity based on 1,1-diphenyl-2-picrylhydrazyl free radical-scavenging activity was measured by the method reported by Braca et al. [18]. The sample (2 ± 0.01 g) preparation was done using methanolic solution (water : methanol, 1 : 2). The absorbance was measured at 517 nm using UV spectrophotometer (Model-UV-2550 from M/s. Shimadzu Corporation, Japan) and expressed as % scavenging activity. Total phenols estimation was carried out with Folin-Ciocalteu reagent (FCR) [19] wherein the sample 0.5–1 ± 0.01 g was weighed and ground with 10 times of 80% methanol in a pestle mortar. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was saved. The procedure was repeated again with 5 volumes of ethanol and the supernatant was pooled and evaporated at a low temperature. The residue was dissolved in 5 mL distilled water and 0.2–2 mL aliquots were pipetted in the test tubes. The final volume was made up to 3 mL, followed by 0.5 mL diluted FCR. After 3 min, 2 mL 7% sodium carbonate was added and the mixture was placed in a boiling water bath for 2 min. The tubes were cooled and absorbance was measured at 750 nm, against blank.

For estimation of dietary fibre [20], sample (1 g) was taken in Erlenmeyer flask, 25 mL of 0.1 M sodium phosphate buffer (pH 6.0) added to suspended uniformly. 100 mg of Termamyl was added and incubated in a boiling water bath for 15 min and cooled and 20 mL of distilled water was added and pH was adjusted to 1.5 with 4 N HCl (hydrochloric acid). 100 mg of pepsin was added and incubated at 40°C with agitation for 60 min and cooled and 20 mL of water was added and pH was adjusted to 6.8 with 4 N sodium hydroxide. 100 mg of pancreatin enzyme was added and incubated at 40°C with agitation for 60 min and cooled and pH was adjusted to 4.5

Table 2: Central composite rotatable design of experiments for Karpurvalli munch.

| Std. order | Run order | Factor 1 ginger (g) | Factor 2 Karpurvalli juice (g) | Response 1 antioxidant activity (%) | Response 2 vitamin C (mg/100 g) | Response 3 sensory score |
|---|---|---|---|---|---|---|
| 6 | 1 | 128.28 | 400.00 | 76.63 | 10.81 | 7.63 |
| 8 | 2 | 100.00 | 470.71 | 79.52 | 12.18 | 7.06 |
| 9 | 3 | 100.00 | 400.00 | 74.52 | 10.80 | 7.13 |
| 13 | 4 | 100.00 | 400.00 | 85.66 | 10.82 | 6.76 |
| 1 | 5 | 80.00 | 350.00 | 75.85 | 10.93 | 6.71 |
| 3 | 6 | 80.00 | 450.00 | 85.50 | 10.82 | 7.23 |
| 7 | 7 | 100.00 | 329.29 | 84.24 | 10.26 | 7.7 |
| 5 | 8 | 71.72 | 400.00 | 85.36 | 11.89 | 7.13 |
| 2 | 9 | 120.00 | 350.00 | 76.57 | 10.80 | 7.23 |
| 10 | 10 | 100.00 | 400.00 | 82.43 | 11.88 | 7.3 |
| 4 | 11 | 120.00 | 450.00 | 84.73 | 12.44 | 7.35 |
| 11 | 12 | 100.00 | 400.00 | 88.01 | 11.88 | 7.47 |
| 12 | 13 | 100.00 | 400.00 | 82.90 | 11.34 | 7.43 |

with 4 N HCl. The treated sample was then filtered through the dry Celite as the filter aid.

*Insoluble Fibre.* The residue was washed with 2×10 mL of 95% alcohol and acetone, dried at 105 ± 2°C to constant weight, and weight of the crucibles was noted ($D_1$) and incinerated at 550°C for 5 h. After cooling, weight of the crucibles was noted ($I_1$).

*Soluble Fibre.* The volume of the filtrate was made up to 100 mL, 400 mL of warm 95% alcohol was added, kept for 1 h to precipitate, then filtered through the dried and weighed crucible ($D_2$), and washed with 2 × 10 mL of alcohol and acetone and dried at 105 ± 2°C overnight. Incinerated at 550 ± 10°C for 5 h, cooled and weighed the crucibles ($I_2$).

*Blank (B).* Insoluble and soluble blanks were also run without sample following the same procedure:

$$\text{Insoluble dietary fibre (\%)} = \frac{D_1 - I_1 - B}{W} \times 100$$
$$\text{Soluble dietary fibre (\%)} = \frac{D_2 - I_2 - B}{W} \times 100, \quad (1)$$

where $W$ = weight of the sample.

*2.6. Sensory Evaluation.* All the combinations of the Karpurvalli munch were evaluated for their sensory attributes on 9-point hedonic scale by semitrained panel of 15 members during product development as well as storage study. The 9-point Hedonic scale grading was gradual from 9 = excellent to 1 = extremely poor. The statistical analysis for significance was carried out using trial version of SPSS 19 software.

## 3. Results and Discussion

Response surface designs have been proven to be quite useful in product and process optimisation. The responses used in the design for a development of products have sensory quality as one of the default response. Several researchers have studied sensory score along with other responses such as texture [21], acidity, total carotenoids, total sugars [10, 11], antioxidant activity, vitamin C [22], cutting force, and acidity [12] as one of the most important parameter of product development. Similarly in optimisation of the Karpurvalli munch developed in the present study, sensory score was one of the responses. As the product contains CA herb and ginger as crucial ingredients apart from other ingredients such as lemon juice and raisins, the antioxidant activity and vitamin C were taken as other responses in the study. The experimental design with actual levels of independent variables and the responses used in this study are given in Table 2. The observation recorded at different design points revealed that the % antioxidant activity ranged from 74.52 to 88.01, the vitamin C content from 10.26 to 12.44 mg/100 g, and the sensory score from 6.71 to 7.63. These responses were then subjected to model fitting by studying the ANOVA values and using the diagnostics tool available in the Design Expert Software. The ANOVA and the best fit polynomial models were obtained for all the three responses (Table 3), to assess how well the model represented the data. The quadratic model was fit for the antioxidant activity response with high model $F$-value of 50.97 while for the other two responses linear model was found suitable with nonsignificant lack of fit. The antioxidant activity was influenced by interaction among the ingredients used in product formulation; however, it was mainly increased with the level of Karpurvalli juice, while the ginger juice has opposite effect (Figure 1). The vitamin C was not affected much by the level of ginger, while the Karpurvalli juice had significant effect (Figure 2). The

Table 3: ANOVA and model statistics of the Karpurvalli munch.

| Sl number | Term | Response | | |
|---|---|---|---|---|
| | | Antioxidant activity | Vitamin C | Sensory score |
| 1 | Model | Quadratic | Linear | Linear |
| 2 | $F$-value | 50.97 | 8.48 | 19.44 |
| 3 | $P > F$ | <0.0001 | 0.0007 | 0.0004 |
| 4 | Mean | 81.57 | 11.41 | 7.29 |
| 5 | S.D.* | 0.88 | 0.43 | 0.15 |
| 6 | CV% | 1.08 | 3.79 | 2.03 |
| 7 | $R$ squared | 0.9733 | 0.6290 | 0.7955 |
| 8 | Adjusted $R$ squared | 0.9542 | 0.5547 | 0.7545 |
| 9 | Predicted $R$ squared | 0.8556 | 0.4206 | 0.6463 |
| 10 | Adequate precision | 21.673 | 7.951 | 12.429 |

*Standard deviation.

sensory score was affected by both ingredients (Figure 3), but the effect was more prominent with changes in levels of ginger. The regression equation obtained for the three responses are as follows:

$$\begin{aligned} \text{Antioxidant Acitivity} &= +83.31 - 2.82\text{A} + 3.68\text{B} \\ &\quad + 0.69\text{AB} - 1.32\text{A}^2 - 1.51\text{B}^2 \\ \text{Vitamin C} &= +11.41 - 0.23\text{A} + 0.58\text{B} \\ \text{Sensory score} &= +7.29 + 0.29\text{A} - 0.15\text{B}. \end{aligned} \tag{2}$$

The numerical optimisation approach of Design Expert was used to get the solution with criteria to maximize all the three responses. The optimized ingredient levels with the predicted responses are given in Figure 4. The product was prepared with the optimised ingredient levels and was evaluated for the responses to validate the prediction strength of the models used in the present study. The actual observed sensory score of the optimised product was 7.4 ± 0.12 while the antioxidant activity and vitamin C content were 81.14% and 11.77 mg/100 g. The predicted (Figure 4) and actual response values were in close concurrence with each other validating the robustness of the models. Thus, the final ingredient composition of the optimised Karpurvalli munch included Karpurvalli juice 42.6%, jaggery 26.5%, raisins 13.2%, ginger paste 11.3%, ghee 3.60%, and lemon juice 2.8%. In addition to these small quantities of ajwain powder and pectin were added to improve the flavour and texture of the product, respectively, during all the preparations.

The chemical composition of the optimised Karpurvalli munch (Table 4) revealed that the product is a good source of antioxidants and vitamin C with about 82.4 Kcal per munch of 20 g. It also has good amount of reducing sugars and dietary fibre as well. Similar appetiser munches with calorific value of 76–90 Kcal per munch of 20 g have been reported [11, 12]. The vitamin C content was of 37–43 mg/100 g in ginger based RTE appetisers; however, in the present context it is 11.77 mg/100 g of product. Antioxidant activity of foods has gained nutritional importance due to the potential protective effect on health. Kovačević et al. [23] reported that compared to fresh strawberry fruit, whose antioxidant activity was in the range of 90.74% to 92.41%, the jams also represent a noticeable source of antioxidant compounds, with the antioxidant activity of 83.76% to 85.65%. Another report [24] on low sugar bilberry jam states that the thermal processing resulted in decrease of ascorbic acid content, total phenolics, and ferric reducing antioxidant powers which further decrease during storage. However, in the present context the antioxidant activity is not dependent on one principal ingredient and is collectively contributed by CA

Table 4: Chemical analysis of optimised Karpurvalli munch.

| Parameter | Value |
|---|---|
| Moisture | 4.94% |
| Protein | 2.30% |
| Fat | 11.04% |
| Sugar | |
| (a) Total sugar | 59.25% |
| (b) Reducing sugar | 37.98% |
| Ash | |
| (a) Total ash | 2.90% |
| (b) Acid insoluble | 0.31% |
| Dietary fibre | |
| (a) Total dietary fibre | 4.13% |
| (b) Insoluble dietary fibre | 1.13% |
| Vitamin C | 11.77 mg/100 g |
| Antioxidant activity (DPPH free radical scavenging) | 81.14% |
| TBA value | 0.07 mg/Kg |
| Total phenols | 15.2 g/100 g |

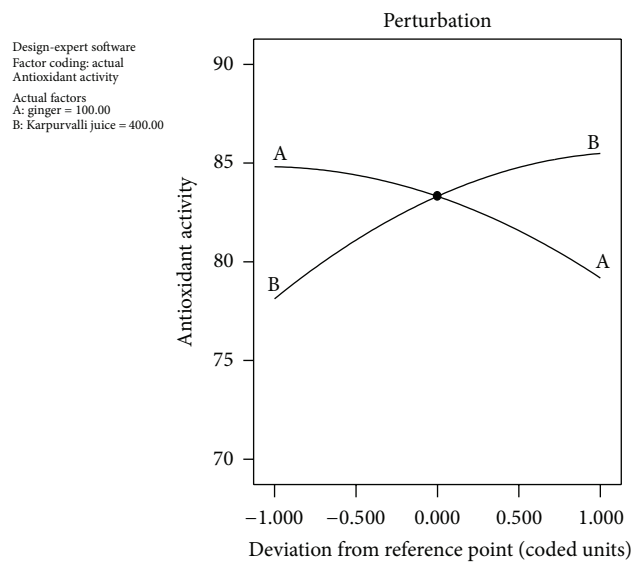


Figure 1: Effetct of different levels of variable on antioxidant acitivity of the product.

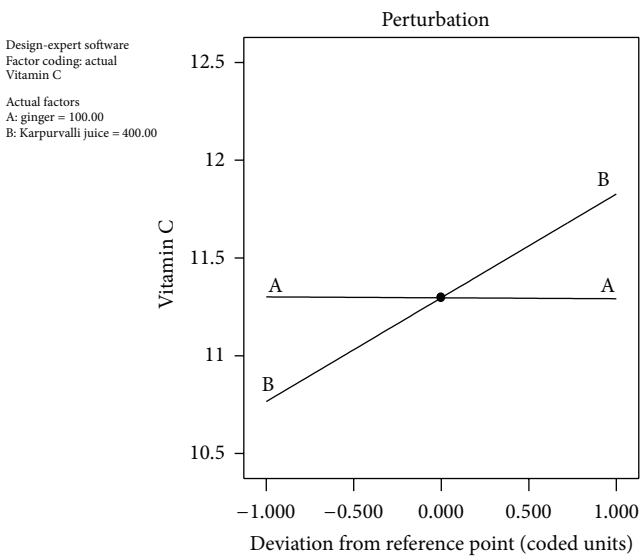


Figure 2: Effetct of different levels of variable on vitamin C of the product.

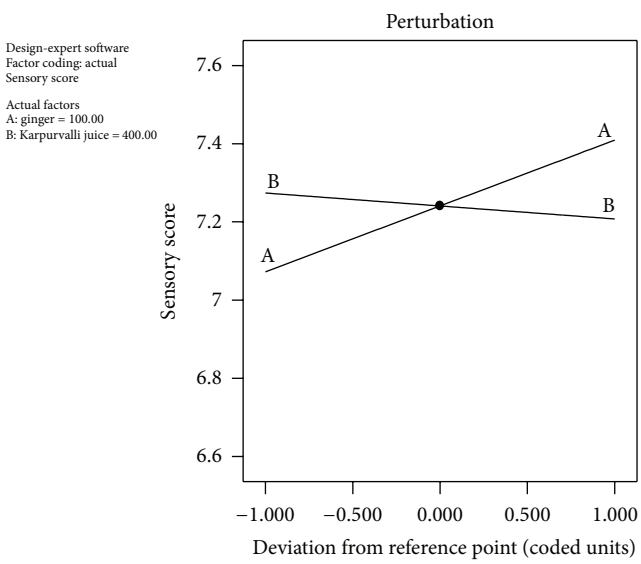


Figure 3: Effetct of different levels of variable on sensory score of the product.

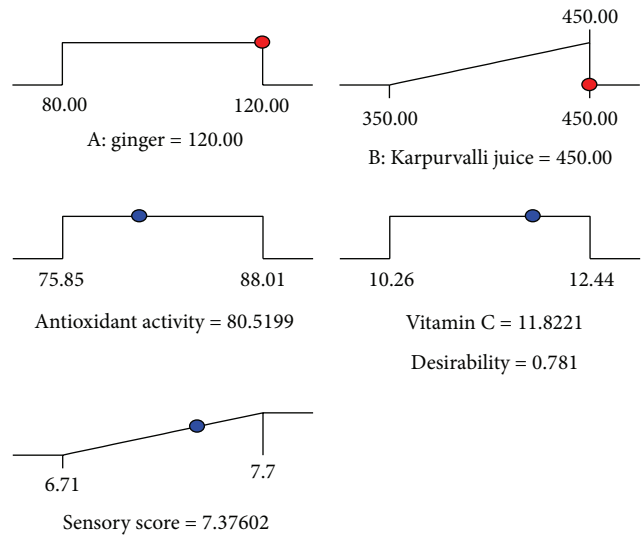


Figure 4: Optimised ingredient level and the predicted response values.

leaves, ginger, and lemon juice. The antioxidant activity in the optimised Karpurvalli munch was found to be 81 ± 0.5%.

The shelf stability of the product was studied in laminated pouches. Periodic evaluation of the sensory attributes and quality parameters such as TBA value, vitamin C, and antioxidant activity revealed (Table 5) that the Karpurvalli munch underwent slow and gradual deteriorative changes. The sensory scores gradually decreased during storage; however, the magnitude of reduction was more in samples stored at 37°C. The sensory score differed among samples stored at 5°C and RT after the 6 months of storage while the samples stored at 37°C the reduction was statistically significant ($P < 0.05$) after 4 months of storage. The TBA value which measures the lipid spoilage rendering rancidity and off-flavour to the product was low and did not show accelerated increase in it. Though after 10 months of storage at RT and 37°C it increased up to 0.4 and 0.6 mg malonaldehyde/kg, respectively; the product was acceptable for its sensory attributes. The retarded pace of oxidative deterioration may be attributed to inbuilt antioxidant activity in the product. The TBA values of the Karpurvalli munch differed significantly ($P < 0.05$) among the storage conditions of refrigerated storage and at room temperature as well as 37°C throughout the periodic evaluation. The vitamin C and antioxidant activity were reduced by more than 70% after 10-month storage at RT and 37°C; however, it was retained by more than 50% for samples stored at refrigerated conditions. There was significant difference in retention of vitamin C and antioxidant activity among the different storage temperatures. Thus the product was found stable for more than 10 months at ambient conditions.

A similar trend of storage change but in different product with different intensity was reported [25] wherein the antioxidant capacity in terms of % DPPH free radical scavenging ability and vitamin C was found to reduce during storage at different temperature conditions over a period of six months in case of certain orange juices. Burdurlu et al. [26] reported reductions in vitamin C content of citrus juice concentrates during storage. They reported that the ascorbic acid retention after storage at 28, 37, and 45°C was about 54.5–83.7%, 23.6–27%, and 15.1–20.0%, respectively. The gradual increase in TBA value during storage of similar products over the storage period of 8 months has been reported [11] wherein the TBA values were found to increase up to 0.7–0.9 mg malonaldehyde/kg with sensory scores going below 6.5 without any objectionable flavours. In case of an instant sweet mix based on soybean and semolina, increase in TBA values and a gradual decrease in sensory attributes had been reported [27] over the storage period of 6 months in PP pouches in ambient conditions. In another report [28] on

Table 5: Parameters studied for establishing shelf life of Karpurvalli munch ($n = 2$).

| Storage temp. | Storage period (months) | Sensory score*# | Vitamin C (mg/100 gm) | TBA value (mg/kg) | Antioxidant activity (%) |
|---|---|---|---|---|---|
| 5°C | 0 | $8.02 \pm 0.2^{a}$ | 11.77 | 0.070 | 82.14 |
| | 2 | $7.80 \pm 0.3^{a}$ | 11.55 | 0.082 | 79.45 |
| | 4 | $7.60 \pm 0.5^{a}$ | 10.92 | 0.097 | 71.23 |
| | 6 | $7.52 \pm 0.2^{a}$ | 9.38 | 0.123 | 67.89 |
| | 8 | $7.46 \pm 0.4^{a}$ | 8.14 | 0.148 | 62.15 |
| | 10 | $7.32 \pm 0.3^{a}$ | 6.79 | 0.179 | 51.82 |
| RT (28 ± 5°C) | 0 | $8.02 \pm 0.2^{a}$ | 11.77 | 0.070 | 82.14 |
| | 2 | $7.80 \pm 0.5^{a}$ | 11.28 | 0.191 | 77 |
| | 4 | $7.56 \pm 0.3^{a}$ | 10.17 | 0.236 | 60.17 |
| | 6 | $7.41 \pm 0.2^{b}$ | 8.13 | 0.261 | 51.30 |
| | 8 | $7.30 \pm 0.3^{b}$ | 6.51 | 0.307 | 40.79 |
| | 10 | $7.08 \pm 0.2^{b}$ | 4.78 | 0.412 | 29.83 |
| 37°C | 0 | $8.02 \pm 0.2^{a}$ | 11.77 | 0.070 | 82.14 |
| | 2 | $7.70 \pm 0.3^{a}$ | 11.27 | 0.269 | 75.05 |
| | 4 | $7.32 \pm 0.2^{b}$ | 8.76 | 0.356 | 51.48 |
| | 6 | $7.18 \pm 0.3^{bc}$ | 5.41 | 0.401 | 43.58 |
| | 8 | $7.01 \pm 0.4^{bc}$ | 3.19 | 0.473 | 31.72 |
| | 10 | $6.60 \pm 0.4^{bc}$ | 2.30 | 0.609 | 19.98 |

RT: room temperature; *score on nine-point Hedonic scale ($n = 15$ panelists).
#Different alphabet superscripts in the same column indicate statically significant difference.

two RTE appetizer munches, the acceptability of the products was very good initially with a sensory score of 3.8 and 3.4 on 5-point hedonic scale for pepper munch and lemon munch which reduced to 2.7 and 2.9 after 9 months of storage, while the TBA value increased from 0.05 and 0.02 to 0.28 and 0.3 mg malonaldehyde/kg, respectively for the two appetizers. In another study, a shelf life of a similar ready to eat appetiser based on *Trachyspermum ammi* has been reported [12] wherein the acidity, TBA value, and sensory cores were monitored. The initial values of sensory score was reduced from 8.6 to 7.2 and 6.5, acidity was increased from 0.48% to 0.83 and 0.89%, while the TBA value increased from 0.03 to 0.2–0.28 mg malonaldehyde/kg at RT and 37°C, respectively, over the storage period.

These studies indicated that it is a trend in similar products during storage and the results obtained in the present context are comparable to the other reports on such products.

## 4. Conclusion

It can be concluded that a shelf stable antioxidant rich appetising product was prepared using the herb *Coleus aromatics*. The herb has been successfully explored for its use in an RTE product never reported earlier. The product has a shelf life of 10 months at ambient conditions when stored in sealed metalized polyester pouches.

## Conflict of Interests

The authors report no conflict of interests.

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