Assessment of Shelf-life of Curry Leave Extract Added Paneer Made from Cow Milk

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Abstract- Historically curry leave (*Murraya koenigii L.*) has been used for centuries worldwide in food not only to increase its flavor and taste but also for its antioxidant properties. The antioxidant effect which is facilitates the shelf life of the food product including dairy product. The present study was conducted to assess the different concentrations of curry leave extract on the shelf life of paneer for a storage period of 28 days at refrigeration temperature (7±1˚C). In this study, paneer was developed with five different concentration of curry leaves extract viz. 0.0% (control), 0.2%, 0.4%, 0.6% and 0.8%. The prepared samples of paneer were analyzed for chemical, physical and sensory evaluation at day 1, day 7, day 14, day 21 and day 28 storage at 7°±1˚C . At the day one, the chemical attributes, such as ash (1.66±0.02), dry matter (49.60±1.85), acidity (0.20±0.02) and antioxidant activity at 593 absorbance (0.292) showed higher value in paneer treated with 0.8% curry leaves extract added paneer. On the other hand, pH (5.88±0.01) and free fatty acid (0.21±0.02) were higher in paneer made without curry leaves extract. At the day 7 of storage period, paneer treated with 0.8% curry leaves extract showed higher mean value for dry matter (51.28±1.52), ash (1.73±0.01), pH (5.85±0.01) and antioxidant activity at 593nm (0.295). While paneer treated without curry leaves extract showed higher mean value for titratable acidity (0.27±0.01) and free fatty acid (0.23±0.00). At the day 28 of storage period, paneer treated with 0.8% curry leaves extract received higher mean value for ash (1.96±0.01), dry matter (54.69±0.30), pH (6.10±0.02). Similarly, paneer made without curry leaves extract received higher mean value for titratable acidity (0.44±0.00) and free fatty acid (0.45±0.21). Both mineral content and antioxidant activity were increasing during storage period and increased with increasing of concentration of curry leaves. Sensory attributes were changed among the all types of paneer and paneer made from 0.6% curry leaves extract showed the highest preference of sensory attributes at day14 and day 21 of storage

Index Terms- *Antioxidant, Curry leaves, Panner, Physicochemical properties, Shelf –life*

I. INTRODUCTION

Paneer the indigenous variety of soft cheese is obtained by heat and acid coagulation of milk at high temperatures [1]. Paneer is a traditional non- fermentative milk product result from coagulation of heated milk by using citric acid. Paneer can be prepared from cow milk, buffalo milk and mixture of them [2]. Paneer has become a popular milk product within consumers because of its high nutritive value. Proteins, fats, minerals especially calcium and phosphorus and fat soluble vitamins like nutrients are highly contain in paneer [3]. However, paneer is a suitable food for nursing mothers, infants, growing children, and adults due to its high

energy and it has a rich source of animal protein available at a comparatively lower cost and forms important source of animal protein for vegetarians [4]. Also it can be recommended for diabetic and coronary heart disease patients due to its high protein and low sugar content. Presence of essential amino acids of proteins origin from animal is another valuable reason for consuming paneer to vegetarians [3]. Major barrier of paneer production in commercial level is shorter shelf life. Shelf life of paneer is expressed as six days under refrigeration at 10°C, but its freshness is lost within three days [5].

In recent year's herbs, spices and their extracts have been used as an antioxidant and antimicrobial activities, which enhance shelf life of food and dairy products [6]. Curry leaves acts as a preservative in many food components because of its antioxidant and other functional properties [6]. In addition to that it has been used in food to increase its flavour [8-10]. Curry leaves has been identified as a herb in traditional medicine for treatment of various kind of diseases like heart diseases, kidney disorders and diabetes. Also it increase digestive secretion and reduce the risk of cancer [11]. Adding of the curry leaves to paneer may help to reduce the deterioration and increase the shelf life due to its antioxidant effect. Generally curry leaves are discarded from the dish while eating as a consequence, the nutrition potential of curry leaves remains underutilized. It is better to promote curry leaves in an edible form where larger quantities can be incorporated in paneer preparation [12]. The information on making of paneer using curry leaves extract is lacking. Therefore, this study was designed to make paneer by incorporation of different concentrations of curry leaves extract and to assess the physicochemical properties during the storage period.

II. MATERIALS AND METHODS

A. Preparation of Curry Leaves Extract

Curry leaves were sterilized by distilled water for 5 min and 1g of *Murraya koenigii (L.)* fresh leaves were grinded with 10 ml of distilled water (1:10, w/v) and the mixture was squeezed and filtered through muslin cloth and No. 1 Whatman Filter Paper, respectively.

B. Preparation of Paneer

Paneer was prepared from after standardizing the cow milk to 4.5% fat and 8.5% SNF using method described by Gokhale *et al*. [1] with modifications. The standardized milk fat was heated to 95 °C for 5 min and cooled to 70 °C. Then Curry leaves extract 0%, 0.2%, 0.4%, 0.6 % and 0.8% was added after pasteurized milk. Then Citric acid (1.0% solution) was added with continuous agitation until the coagulation was complete. The coagulum was allowed to settle for 5 min for complete coagulation. Whey was drained through a muslin cloth and pressed for 20 min. Then the freshly prepared paneer sample was chilled in cold water at 4^0C for 2 hours. The paneer removed from chilled water were allowed to drain for 5 min. Finally the paneer was stored for 28 days at 7-8^{o}C in the vacuumed packed in Cryovac bags and panner was taken 1, 7, 14, 21 and 28 days after manufacture.

C. Chemical analysis

The panner samples stored at 7-8ºC were analyzed for the chemical characteristics at a regular interval of 7 days during the storage. Moisture content in paneer was determined in triplicate for dry matter by oven drying at 105 °C to get constant weight according to method described by AOAC [13] and percentage of dry matter was calculated. Ash content was determined by using muffle furnace at 550 °C for 4 h as described by AOAC [13]. Titratable acidity of paneer samples was determined by following the method prescribed for cheese by the AOAC [14]. Free fatty acids content of paneer was estimated using the method described for cheese by Thomas [15]. The pH of paneer was determined by using a pH meter as the method described by Arora and Gupta [16].

D. Determination of mineral content

The type and concentration of specific minerals in yoghurt samples were determined by the use of a flame atomic absorption spectroscopy. According to official method 968.08 of AOAC [17]. Two gram of different types of yoghurt samples were ashes in a muffle furnace at 550 °C for 6 hours, followed by acid digestion of the ash. After ashing, the residue was quantitatively transferred to a digestion tube by using 2 washes each with 5 ml of 25% HCl. The digestion tubes were then placed onto a preheated (125 °C), digestion block and samples were digested for 30 min. The digests were removed from the digestion block, cooled to room temperature, diluted to a volume of 100 ml with deionized water, and finally analyzed for various mineral elements by flame atomic emission spectrometer.

E. Determination of antioxidant activity

The FRAP assay was done according to the procedure described by Benzie and Strain [18]. Briefly the FRAP reagent was prepared by mixing 1 ml of (10mmol/L) TPTZ solution in 40 mmol/L HCl, 1 ml of FeCl3 (20mmol/L) and 10 ml of acetate buffer, (0.3 mol/L). Twenty microliters of 0.1 g /ml sample was mixed with 1 ml of FRAP reagent and the absorbance at 593 nm was measured spectrophotometrically after incubation at room temperature for 4 minutes.

F. Sensory evaluation

Each block of paneer was cut into rectangular pieces of approximately 1 cm × 2 cm. The prepared samples of paneer were subjected to sensory evaluation by a panel of 30 judges when fresh and at regular interval of 7 days during storage using 9-point hedonic scale according the methods Buch *et al.* [5]. The sensory characteristics, such as colour, flavour, taste, texture and overall acceptability of the paneer samples were judged by the panelists at day 1, 7, 14, 21 and 28 of storage period.

G. Statistical analysis

Samples were randomly collected; parametric data were analyzed by using Multivariate Analysis of Variance (MANOVA) and used to determine the significance level of the treatments, while the Duncan's Multiple Range Test (DMRT) was used for mean separation. The sensory analysis was carried out using Friedmans test for non-parametric data analysis.

III. RESULTS AND DISCUSSION

A. Nutritional Attributes and pH of Paneer Made from Curry Leaves Extract at Day One.

The result is indicating in Table 1 that pH and titratable acidity did not show any significant changes among concentration at day 1. It might be due to that considerable amount of changes not occurred in biochemical and microbial reactions. Dry matter content of paneer increased with an increase in the level of curry leaf extract. High value of dry matter content (49.60±1.85%) was noticed in paneer treated with 0.8% curry leaf extract, where as the lowest (47.81±0.23%) was recorded in paneer without curry leaf extract. When adding various concentrations of curry leaves extract to paneer contributes to increase dry matter content of samples treated with different concentration of curry leaves extract. This result was supported with finding of Buch *et al*. [5]. Ash content of paneer was increased with increasing curry leaves extract. It could be due to that addition of higher concentration of curry leaves extract increases the ash contents in paneer. This result was agreed with finding of Buch *et al*. [5].

The pH (6.10±0.02) of 0% curry leaves extract added paneer showed high value than other treatments. The changes in the pH value differ according to the different concentration extracts added in the panner. The pH value of paneer samples were gradually decreased with increasing the curry extracts concentration due to acidic compound of curry leaves. Saini and Reddy [19] reported pH value of curry leaves as 6.3-6.4. Acid compounds like ascorbic acid, folic acid may reason for the acidity of curry leaves extract. When curry leaves extract was increasing, decrease the pH value of paneer due to increasing acidity. The lowest acidity (0.17±0.02%) was observed in paneer prepared without curry leaf extract and mean value of acidity increased with increase in the level of curry leaf extract due to acidic compound [20].

Free fatty acid (0.18±0.02%) of paneer with 0.8% curry leaves extract showed the lowest value than other treatments. Wahifiyah *et al*. [21] reported that caffeine like alkaloid neutralized the free fatty acid formed. So alkaloid like Murrayanine, Murrayastine, Murrayafoline contained in curry leaves extract are natural bases [22]. They have an ability to neutralize the free fatty acid formed as oil hydrolysis. Therefore, free fatty acid formation was reduced when increasing the concentration of curry leaves extract. The result was evidenced with the study conducted by Buch *et al*. [5].

Table I: Nutritional status and pH of paneer made from curry leaves extract at day one**.**

Attributes	**Treatments**				
	T_1	**T_2**	**T_3**	**T_4**	**T_5**
Dry matter (%)	47.81 ± 0.2^{b}	48.32 ± 0.27^{ab}	48.63 ± 0.42^{ab}	49.13 ± 0.26^{b}	49.60 ± 1.85^{a}
Ash (%	1.53 ± 0.01^{d}	1.57 ± 0.06^{cd}	1.60 ± 0.02^{bc}	1.64 ± 0.01^{ab}	1.66 ± 0.02^{a}
pH	5.89 ± 0.01^{a}	5.89 ± 0.02^{a}	5.88 ± 0.01^{a}	5.86 ± 0.01^{b}	5.86 ± 0.02^{b}
Acidity (%)	0.17 ± 0.02^{b}	0.18 ± 0.07^{b}	0.20 ± 0.01^{ab}	0.21 ± 0.02^{a}	0.21 ± 0.02^{a}
FFA (%)	0.21 ± 0.02^{a}	0.21 ± 0.01^{a}	0.20 ± 0.01^{ab}	0.19 ± 0.01^{ab}	0.18 ± 0.02^{b}

T_1-Paneer without curry leaves, T_2 – Paneer with 0.2% curry leaves extract, T_3- Paneer with 0.4% curry leaves extract, and T_4- Paneer with 0.6% curry leaves extract, T_5 –Paneer with 0.8% curry leaves extract. Values are means ± standard deviations of replicate determination means with same letters are not significantly different at ($p > 0.05$).

B. Dry Matter Content and Ash Content in Paneer during Storage Period

The dry matter content in the paneer among the storage period is showed in Table 2. Dry matter content of paneer was ($p<0.05$) increased with the storage period. In present study, dry matter may increase due to loss of moisture content and whey drainage during storage period. At the end of storage period paneer treated with 0.8% curry leaves extract showed the highest value of dry matter content (54.69±0.30%) and paneer without curry leaves extract showed the lowest value of dry matter content (52.03±0.41%). When increasing concentration of curry leaves extract increases dry matter of panner. It due to increasing the concentration of curry leaves extract added in the paneer.

Ash content is an indicator of total amount of minerals present in the paneer. As shown in Table 2, at the day 7 of storage, 0.8% curry leaves extract added paneer exhibited ($P < 0.05$) higher mean value (1.79±0.03%) than paneer made from without curry leaves extract (1.56±0.02%). At the day 28 of storage, 0.8% curry leaves extract added paneer also showed higher mean value (1.96±0.01%), and paneer made without curry leaves extract received lower mean value (1.73±0.01%). It could be due to that addition of higher concentration of curry leaves extract increases the ash contents in paneer. This value increased during storage period. The results agree with the finding of Buch *et al*. [5].

C. pH Content and Titratable Acidity in Paneer during Storage Period.

The pH of paneer was ($p<0.05$) changed with different concentration of curry leaves extract added paneer while pH of the paneer was decreased with the storage period (Table 2). The changes in pH may due to the breakdown of lactose into the lactic acid during fermentation process by microorganism. During the storage period, pH significantly ($P<0.05$) varied among the treatments. However, at the day 28, paneer without curry leaves extract added paneer showed the lowest value of pH (5.53± 0.01) than other treatments. It might depend on incorporation of curry leaves extract in which has an antimicrobial property it leads to the slow rate of development of acidity during storage period. On the other hand, the titratable acidity of paneer differed ($p<0.05$) with different concentration of curry leaves extract added paneer. The titratable acidity of paneer was decreased with the storage period. At the day 28, paneer treated without curry leaves extract showed the highest value of titratable acidity (0.44±0.00%) than other treatments. Previously, Kumar and Bector [23], Venkateswarlu *et al.* [24] and Yellamanda *et al.* [25] suggested that normal range of titratable acidity is 0.47–0.59%. In this study, the contents of titratable acidity was little lesser than reported values. The titratable acidity was progressively increased with the storage period, it due to metabolism of lactose by lactic acid bacteria and produce acids. This result was agreed with finding of Pal *et al.* [20] and Shanaziya *et al.* [26]. Acidity level of paneer was also decreased with increasing of concentration of curry leaves extract added in the paneer.

D. Free Fatty Acid in Paneer during Storage Period

The data obtained for changes in FFA content of paneer during storage at 7 °C are presented in Table 2. Free fatty was increased during storage due to lipids undergo hydrolysis (lipolysis) through the microbial enzymes thereby increasing the free fatty acids (FFA) content of the product. At the end of the storage period highest value of free fatty acid showed in paneer without curry leaves extract (0.45±0.21). Results of the study were

Table II: Nutritional status and pH of paneer made from curry leaves extract during storage at 7°C.

Treatments	Day 7	Day 14	Day 21	Day 28
		Dry matter (%)		
T_1	48.15±0.05^{f}	48.32±0.59^{ef}	49.22±0.55^{def}	52.03±0.41^{b}
T_2	48.88±0.71f^{def}	49.46±1.14^{de}	51.52±0.41^{b}	53.81±0.64^{a}
T_3	49.56±0.44^{de}	49.91±0.31^{cd}	51.9±0.29^{b}	54.09±0.26^{a}
T_4	50.80±0.30^{bc}	51.01±1.32^{bc}	51.29±0.29^{b}	54.31±0.19^{a}
T_5	51.28±1.52^{b}	52.03±0.93^{b}	53.77±0.63^{a}	54.69±0.30^{a}
		Ash (%)		
T_1	1.56±0.02^{l}	1.66±0.02^{j}	1.74±0.01^{fgh}	1.79±0.03^{e}
T_2	1.61±0.02^{k}	1.69±0.02^{i}	1.76±0.02^{f}	1.82±0.02^{d}
T_3	1.62±0.02^{k}	1.71±0.00^{hi}	1.81±0.02^{de}	1.91±0.01^{b}
T_4	1.65±0.01^{j}	1.74±0.02^{fg}	1.85±0.01^{c}	1.94±0.01^{a}
T_5	1.73±0.01^{gh}	1.75±0.02^{fg}	1.87±0.02^{c}	1.96±0.01^{a}
		pH		
T_1	5.83±0.01^{abc}	5.76±0.03^{f}	5.59±0.01^{i}	5.53±0.01^{j}
T_2	5.84±0.01^{a}	5.79±0.01^{de}	5.65±0.01^{h}	5.60±0.01^{i}
T_3	5.84±0.02^{a}	5.80±0.01^{cd}	5.72±0.01^{g}	5.67±0.01^{h}
T_4	5.84±0.01^{a}	5.81±0.01^{bcd}	5.75±0.03^{f}	5.71±0.01^{g}
T_5	5.85±0.01^{a}	5.83±0.01^{ab}	5.79±0.02^{de}	5.77±0.01^{ef}
		Titratble acidity (%)		
T_1	0.27±0.01^{ef}	0.32±0.03^{cd}	0.36±0.02^{bc}	0.44±0.00^{a}
T_2	0.25±0.87^{f}	0.25±0.01^{f}	0.33±0.14^{b}	0.34±0.11^{bcd}
T_3	0.25±0.02^{f}	0.25±0.09^{f}	0.32±0.00^{cd}	0.32±0.14^{b}
T_4	0.24±0.87^{f}	0.24±0.09^{f}	0.30±0.11^{bcd}	0.30±0.01^{de}
T_5	0.23±0.01^{ef}	0.24±0.03^{cd}	0.25±0.02^{bc}	0.25±0.00^{f}
		Free fatty acid (%)		
T_1	0.23 ± 0.00^{jk}	0.26 ± 0.02^{fg}	0.33 ± 0.01^{cd}	0.45 ± 0.21^{a}
T_2	0.23 ± 0.01^{hij}	0.24 ± 0.00^{ghij}	0.29 ± 0.01^{ef}	0.37 ± 0.01^{b}
T_3	0.22 ± 0.02^{jk}	0.24 ± 0.02^{ghij}	0.25 ± 0.01^{ghi}	0.30 ± 0.02^{c}
T_4	0.20 ± 0.02^{lk}	0.23 ± 0.01^{ijk}	0.26 ± 0.01^{gh}	0.31 ± 0.03^{de}
T_5	0.19 ± 0.00^{lk}	0.23 ± 0.01^{hijk}	0.24 ± 0.01^{ghij}	0.30 ± 0.01^{e}

T_1-Paneer without curry leaves, T_2 – Paneer with 0.2% curry leaves extract, T_3- Paneer with 0.4% curry leaves extract, and T_4- Paneer with 0.6% curry leaves extract, T_5 –Paneer with 0.8% curry leaves extract. Values are means ± standard deviations of replicate determination means with same letters are not significantly different at (p> 0.05).

agreed with Kumar and Bector (1991) who reported that the free fatty acids content of paneer increased from 0.28 to 0.5 on 22 days of storage at 5 °C. Furthermore, FFA content among treatments is varied due to different concentration of curry leaves extract. Free fatty acid formation was reduced when increasing the concentration of curry leaves extract. Buch *et al*. [23], Bandyopadhyay [22] and Wahifiyah *et al.* [21] reported that alkaloid like murrayanine, murrayastine, murrayafoline in curry leave reduces the free fatty acid formation.

E. Mineral content of curry leaves extract added paneer

As mentioned in Table 3, mineral content of panner with different concentration of curry leaves extract at day 7 of storage at 7 °C. Minerals such as Calcium, Phosphorus, and Magnesium were higher that Zinc and Manganese but Cobalt was not present on the carry leave extract added panner. Gahlawat *et al*. [27] reported that the main minerals contained curry leaves are calcium, phosphorus, iron and Magnesium, which was reflect this finding.

Table III: Mineral content of curry leaves extract added paneer

Mineral content (mg/g)	T_1	T_2	T_3	T_4	T_5
Ca	2717	6811.5	7556.7	7770.4	9309.6
P	3700	3948.7	4184.1	4262.7	4391.4
Mg	93.7	278.9	306	310	342.5
Cu	3.9	3.9	4.4	4.8	69.7
Zn	0.8	25.2	25.9	-	34.3
Co	-	-	-	-	-
Mn	1.9	2.6	2.9	2	5.8

T_1-Paneer without curry leaves, T_2 – Paneer with 0.2% curry leaves extract, T_3- Paneer with 0.4% curry leaves extract, and T_4- Paneer with 0.6% curry leaves extract, T_5 –Paneer with 0.8% curry leaves extract

F. Antioxidant activity in paneer during storage period

Antioxidant activity of paneer was performed at day 1 and day 7 of storage at 7 °C (Table 4). Antioxidant activity was increasing during storage period. It might be chemical and biochemical reactions of antioxidant compound and released from curry leaves extract during storage. It may be a reason to increase antioxidant activity during storage period. Also antioxidant activity was increased with increasing curry leaves extract. It may be occurred due to increasing amount of antioxidant compound like flavonoid and phenolic with increasing concentration.

Table IV: Antioxidant activity in paneer during storage period

Treatment	Antioxidant activity absorbance at 593 nm	
	Day 1	Day 7
T_1	0.103	0.106
T_2	0.178	0.182
T_3	0.245	0.248
T_4	0.273	0.277
T_5	0.295	0.298

T_1-Paneer without curry leaves, T_2 – Paneer with 0.2% curry leaves extract, T_3- Paneer with 0.4% curry leaves extract, and T_4- Paneer with 0.6% curry leaves extract, T_5 –Paneer with 0.8% curry leaves extract

G. Sensorial Attributes of Curry Leave Extract Added Paneer

The sensory attributes of curry leaves extract added paneer samples were evaluated on the basis of colour, appearance, taste, flavor, texture and overall acceptability. Figure 1depicts the data for sensory evaluation of curry leave extract added paneer packed under Cryovac bags and stored at 7 °C. At the day 1 and day 7 most of the panelists preferred to 0.0% curry leaves added paneer due to its white color appearances. The highest value of odor, taste, and overall acceptability were observed in 0.6% curry leaves extract added paneer at day 14 and 21. According to Singh *et al.* [28], reported that essential oil as pinene, sabinene, caryophyllene, cadinol and cadinene contained in curry leaves supply good aroma and flavor to paneer product. The sensory scores for colour, appearance, taste, flavor, texture and overall acceptability varying significantly declined after 21 days of storage in all types of paneer. The sensory attributes were significantly affected when increasing of concentration beyond 0.6% curry leaves extract, it due to it gives bitter taste for paneer and poor colour appearance. The paneer prepared using 0.6% curry leaves extract showed the highest preference of sensory attributes at day14 and day 21 of storage.

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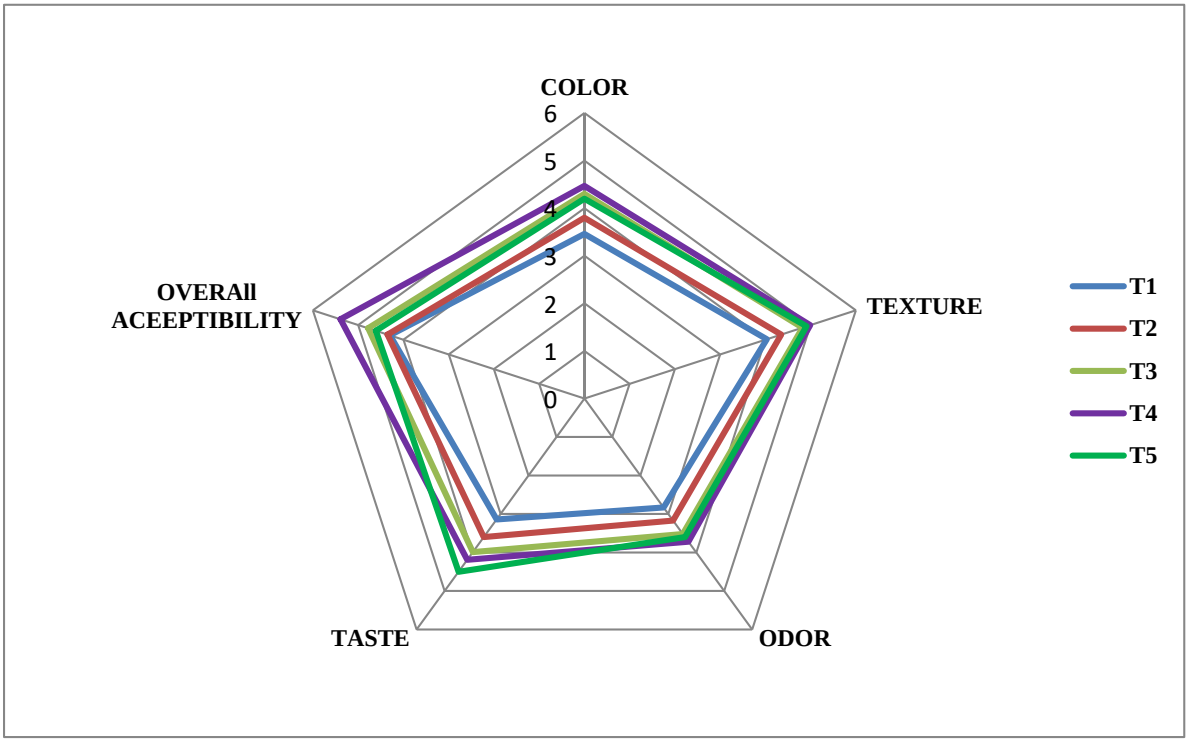

T_1-Paneer without curry leaves, T_2 – Paneer with 0.2% curry leaves extract, T_3- Paneer with 0.4% curry leaves extract, and T_4- Paneer with 0.6% curry leaves extract, T_5 –Paneer with 0.8% curry leaves extract

Figure I: Overall quality of product during storage period

VI. CONCLUSION

Based on the results of this study incorporation of curry leave extract can be used successfully for extending the shelflife of paneer. Curry leave extract can be used make a panner with acceptable physicochemical properties and sensory attributes. However, panner made with 0.6% curry leaves extract showed the highest preference of sensory attributes at day14 and day 21 of storage.

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