

## **EFFECT OF TULSI LEAF EXTRACT ON PHYSICO-CHEMICAL AND MICROBIAL QUALITY OF RAW MILK**

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**Abstract:** A study was conducted to assess the effect of tulsi leaf extract on physico-chemical and microbial quality of raw milk. Raw milk collected hygienically from an organized dairy farm was assessed by addition of aqueous extracts of tulsi leaves at different concentrations viz., 0.25, 0.5, 0.75 and 1 per cent and studied for physico-chemical and microbial parameters at regular intervals at 37 °C. It was found that addition of aqueous extract of tulsi leaves extracts at 0.5 per cent (v/v) extended the shelf life of raw milk by 5 hours by clot on boiling test. There was a controlled increase in pH and titratable acidity in the milk samples added with 0.5%, 0.75% and 1% level of tulsi leaves extract. The Statistical analysis of data revealed that standard plate count of the aqueous extract of tulsi leaves treated raw milk showed highly significant difference ( $P \leq 0.01$ ) was noticed within treatments during the storage as well as between treatments from 3 to 12 hours of storage.

**Keywords:** Tulsi leaves, Clot on boiling test, titratable acidity, standard plate count

### **Introduction**

Milk and other dairy related products comprise a major chunk of food products for their nutritive values. However rich source of proteins and vitamins in them turn out to be very good growth medium for several pathogenic microorganisms. Since milk is perishable, special measures and considerations are necessary to ensure that it reaches the market in an acceptable condition. Currently there is a growing interest to use natural antimicrobial compounds like extracts of herbs and spices for the preservation of food. The mode of action of natural preservatives is inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in milk. Phenols and polyphenols are water soluble compounds which can be easily mixed with milk. The use of plant extracts as a source of phenols is preferred as a natural method (Gad and Salam, 2010). The present study was carried out to study the effect of tulsi leaf extract on physico-chemical and microbial quality of raw milk.

## Materials and Methods

Fresh tulsi leaves (*Ocimum sanctum*) procured from the local market in Chennai was shade dried and powdered as per the method of Preethi *et al.* (2010). Tulsi leaf powder (10 g) was immersed in 100 ml of sterile distilled water, mixed and allowed to soak for 24 hours, then filtered through whatman No.4 filter paper to obtain a clear extract. The extract was diluted with sterile distilled water to a concentration of 100 mg/ml and then stored in air tight containers at refrigerated temperature.

Raw cow milk was collected hygienically from the organized dairy farm in sterilized sample bottles (100 ml) with aqueous tulsi extract at 0.25, 0.5, 0.75 and 1 per cent (v/v) concentrations. One sample was maintained as a control without extract, and all the samples were stored at a temperature of 37 °C. The samples were analysed for physico-chemical parameters like Clot on boiling test (COB), pH, titratable acidity and microbial quality (Standard plate count) done as per the (BIS, SP: 18 (Part XI)-1981).

## Results and Discussion

COB test reveals that control milk sample were acceptable up to 6 hours with negative COB test. The maximum shelf life of raw milk was observed up to 11 hours at 0.5, 0.75 and one per cent addition of aqueous extract of tulsi leaves. The higher concentration of 0.75 and one per cent did not improve the shelf life of raw milk these findings were similar to that of Ray (2008) who found that higher concentration of banana pseudostem extract did not further increase the shelf life of milk at  $30 \pm 2^\circ\text{C}$ .

From the Table 1 it was evident that the control milk sample remained acceptable up to 6 hours of storage. The samples treated with 0.5, 0.75 and one per cent of aqueous extracts of tulsi leaves showed a reduction in pH to  $6.10 \pm 0.004$ ,  $6.10 \pm 0.024$  and  $6.10 \pm 0.004$  respectively at 12<sup>th</sup> hour of storage period.

The statistical analysis of data showed a highly significant difference ( $P \leq 0.01$ ) between treatments from 2 to 12 hours of storage. The highly significant difference within treatment may be due to presence of the bacteria which were capable of increasing the acidity during the log phase (Urbiene and Leskauskaite, 2006). The highly significant difference between treatments may be due to the presence of phenolic compounds which were able to overcome the inhibitory effect of compounds in the food matrix as mentioned by Ersoz *et al.* (2011).

From the Table 2 it was observed that untreated raw milk was acceptable up to 6 hours with titratable acidity of  $0.19 \pm 0.004$ , whereas, in the treated samples, there was a gradual increase in the titratable acidity up to 12<sup>th</sup> hour of storage. These results are in agreement with

the findings of Abbas and Osman, 1998 who reported that the titra table acidity in milk increased gradually during storage period

From the Table 3 the analysis of data showed that standard plate count of untreated raw milk increased during the storage period up to 12 hours, whereas the 0.25, 0.5, 0.75 and one per cent samples showed a restricted increase in the standard plate count during the storage, which might be due to antimicrobial activity of phenolic compounds causing destruction of bacterial cell wall and cytoplasmic membrane resulting in the leakage of cytoplasm as reported by Shan *et al.*, 2007. Highly significant difference ( $P \leq 0.01$ ) was noticed within treatments during the storage as well as between treatments from 3 to 12 hours of storage.

### Conclusion

In this present study of effect of tulsi leaf extract on physico-chemical and microbial changes of raw milk it was concluded that addition of 0.5 percent tulsi leaf extract (v/v) to raw milk remained acceptable upto 11 hours of storage at 37°C. Addition of higher concentrations up to 0.75 and 1 per cent level of the aqueous extracts of tulsi leaves did not increase the shelf life of raw milk beyond 11 hours.

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**Table 1**  
**pH of the raw milk with aqueous extract of tulsi leaves at different concentrations (v/v) at 37°C (Mean±SE)<sup>@</sup>**

Storage time (Hrs)	Control raw milk	T1 0.25% aqueous extract of tulsi	T2 0.5% aqueous extract of tulsi	T3 0.75% aqueous extract of tulsi	T4 1% aqueous extract of tulsi	F value
0	6.65±0.005 <sup>i A</sup>	6.65±0.004 <sup>A</sup>	6.65±0.003 <sup>hA</sup>	6.65±0.002 <sup>gA</sup>	6.65±0.004 <sup>A</sup>	0.000 <sup>NS</sup>
1	6.60±0.007 <sup>i A</sup>	6.62±0.044 <sup>h A</sup>	6.63±0.052 <sup>ghA</sup>	6.64±0.004 <sup>igA</sup>	6.63±0.005 <sup>hA</sup>	0.119 <sup>NS</sup>
2	6.51±0.004 <sup>h A</sup>	6.56±0.022 <sup>h B</sup>	6.62±0.044 <sup>ghB</sup>	6.64±0.031 <sup>igB</sup>	6.61±0.004 <sup>hB</sup>	5.747 <sup>**</sup>
3	6.46±0.005 <sup>h A</sup>	6.55±0.004 <sup>ghB</sup>	6.60±0.004 <sup>fghB</sup>	6.60±0.006 <sup>efgB</sup>	6.60±0.004 <sup>ghB</sup>	8.734 <sup>**</sup>
4	6.40±0.004 <sup>gh A</sup>	6.52±0.005 <sup>gB</sup>	6.58±0.014 <sup>fgC</sup>	6.58±0.004 <sup>efC</sup>	6.58±0.003 <sup>ghB</sup>	59.440 <sup>**</sup>
5	6.35±0.003 <sup>fg A</sup>	6.48±0.004 <sup>fgB</sup>	6.55±0.004 <sup>fB</sup>	6.55±0.004 <sup>eB</sup>	6.55±0.004 <sup>gB</sup>	11.990 <sup>**</sup>
6	6.25±0.014 <sup>f A</sup>	6.44±0.016 <sup>efB</sup>	6.48±0.007 <sup>ec</sup>	6.48±0.004 <sup>dc</sup>	6.48±0.004 <sup>fc</sup>	79.966 <sup>**</sup>
7	6.10±0.004 <sup>e A</sup>	6.41±0.004 <sup>efB</sup>	6.45±0.005 <sup>eB</sup>	6.46±0.011 <sup>dc</sup>	6.45±0.014 <sup>efB</sup>	48.869 <sup>**</sup>
8	5.98±0.044 <sup>d A</sup>	6.36±0.011 <sup>deB</sup>	6.42±0.004 <sup>deB</sup> c	6.44±0.004 <sup>dc</sup>	6.41±0.004 <sup>deBC</sup>	64.396 <sup>**</sup>
9	5.92±0.004 <sup>d A</sup>	6.30±0.004 <sup>dB</sup>	6.36±0.004 <sup>dB</sup>	6.36±0.004 <sup>cb</sup>	6.36±0.004 <sup>deB</sup>	62.430 <sup>**</sup>
10	5.82±0.061 <sup>c A</sup>	6.22±0.004 <sup>cb</sup>	6.28±0.007 <sup>cc</sup>	6.28±0.004 <sup>bc</sup>	6.28±0.035 <sup>cc</sup>	171.178 <sup>**</sup>
11	5.65±0.005 <sup>b A</sup>	6.10±0.002 <sup>bb</sup>	6.20±0.016 <sup>bc</sup>	6.22±0.003 <sup>bc</sup>	6.21±0.004 <sup>bc</sup>	51.043 <sup>**</sup>
12	5.52±0.048 <sup>a A</sup>	5.98±0.014 <sup>ab</sup>	6.10±0.004 <sup>ac</sup>	6.10±0.024 <sup>ac</sup>	6.10±0.004 <sup>abC</sup>	40.352 <sup>**</sup>
<b>F value</b>	104.378 <sup>**</sup>	62.355 <sup>**</sup>	56.545 <sup>**</sup>	68.170 <sup>**</sup>	74.358 <sup>**</sup>	

Different superscripts in a row (capital letters) and column (lowercase letters) differ significantly

NS – Non significant (P>0.05)

\*\* Highly significant (P≤0.01)

**Table 2**  
**Acidity<sup>#</sup> of the raw milk with addition of aqueous extract of tulsi leaves at different concentrations (v/v) at 37°C (Mean±SE)<sup>@</sup>**

Storage time (Hrs)	Control raw milk	T1 0.25% aqueous extract of tulsi	T2 0.5% aqueous. extract of tulsi	T3 0.75% aqueous extract of tulsi	T4 1% aqueous. extract of tulsi	F value
0	0.14±0.002 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.14±0.003 <sup>aA</sup>	0.14±0.003 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.577 <sup>NS</sup>
1	0.15±0.004 <sup>abA</sup>	0.15±0.002 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	1.875 <sup>NS</sup>
2	0.16±0.005 <sup>bB</sup>	0.16±0.004 <sup>ab</sup>	0.15±0.005 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.14±0.003 <sup>abA</sup>	5.642 <sup>**</sup>
3	0.16±0.002 <sup>cb</sup>	0.16±0.006 <sup>B</sup>	0.16±0.002 <sup>ba</sup>	0.15±0.005 <sup>abA</sup>	0.15±0.004 <sup>abA</sup>	17.417 <sup>**</sup>
4	0.17±0.004 <sup>cc</sup>	0.17±0.002 <sup>bb</sup>	0.16±0.006 <sup>ba</sup>	0.15±0.002 <sup>ba</sup>	0.15±0.002 <sup>abA</sup>	13.173 <sup>**</sup>
5	0.18±0.002 <sup>cb</sup>	0.17±0.003 <sup>ba</sup>	0.17±0.002 <sup>ba</sup>	0.16±0.004 <sup>bcA</sup>	0.16±0.006 <sup>bcA</sup>	6.176 <sup>**</sup>
6	0.19±0.003 <sup>cc</sup>	0.17±0.002 <sup>bcdA</sup>	0.17±0.003 <sup>ba</sup>	0.16±0.006 <sup>bcA</sup>	0.17±0.002 <sup>deA</sup>	5.878 <sup>**</sup>
7	0.20±0.002 <sup>dc</sup>	0.18±0.005 <sup>bcdB</sup>	0.17±0.002 <sup>ba</sup>	0.16±0.002 <sup>bcA</sup>	0.17±0.002 <sup>cdA</sup>	12.694 <sup>**</sup>
8	0.21±0.006 <sup>eb</sup>	0.18±0.002 <sup>cdeA</sup>	0.18±0.003 <sup>ba</sup>	0.17±0.005 <sup>ca</sup>	0.18±0.033 <sup>efA</sup>	21.774 <sup>**</sup>
9	0.23±0.006 <sup>id</sup>	0.19±0.002 <sup>efC</sup>	0.18±0.002 <sup>ba</sup>	0.17±0.002 <sup>dAB</sup>	0.18±0.002 <sup>efgBC</sup>	17.900 <sup>**</sup>
10	0.25±0.002 <sup>gc</sup>	0.19±0.003 <sup>efAB</sup>	0.19±0.004 <sup>cAB</sup>	0.18±0.002 <sup>deA</sup>	0.19±0.002 <sup>ghB</sup>	16.861 <sup>**</sup>
11	0.27±0.004 <sup>B</sup>	0.20±0.002 <sup>fA</sup>	0.19±0.002 <sup>ca</sup>	0.19±0.006 <sup>efA</sup>	0.19±0.005 <sup>fghA</sup>	32.500 <sup>**</sup>
12	0.32±0.005 <sup>hc</sup>	0.24±0.003 <sup>gB</sup>	0.20±0.002 <sup>dA</sup>	0.20±0.003 <sup>fA</sup>	0.20±0.004 <sup>hA</sup>	156.594 <sup>**</sup>
<b>F value</b>	135.215 <sup>**</sup>	34.375 <sup>**</sup>	25.647 <sup>**</sup>	23.593 <sup>**</sup>	30.240 <sup>**</sup>	

Different superscripts in a row (capital letters) and column (lowercase letters) differ significantly

NS – Non significant (P>0.05)

\*\* Highly significant (P≤0.01)

# Titratable acidity expressed as percentage of lactic acid

**Table 3**  
**Standard Plate Count ( $\log_{10}$ cfu/ml) of the raw milk with aqueous extract of tulsi leaves**  
**at different concentrations (v/v) at 37°C (Mean $\pm$ SE)<sup>@</sup>**

Storage time (Hrs)	Treatments					
	Control raw milk	T1 0.25% aqueous extract of tulsi	T2 0.5% aqueous .extract of tulsi	T3 0.75% aqueous. extract of tulsi	T4 1% aqueous. extract of tulsi	F value
0	5.31 $\pm$ 0.017 <sup>aA</sup>	5.31 $\pm$ 0.017 <sup>aA</sup>	5.31 $\pm$ 0.017 <sup>aA</sup>	5.31 $\pm$ 0.017 <sup>aA</sup>	5.31 $\pm$ 0.017 <sup>aA</sup>	0.000 <sup>NS</sup>
3	5.92 $\pm$ 0.004 <sup>bD</sup>	5.51 $\pm$ 0.014 <sup>bA</sup>	5.55 $\pm$ 0.009 <sup>bB</sup>	5.64 $\pm$ 0.011 <sup>bC</sup>	5.63 $\pm$ 0.011 <sup>bC</sup>	265.181 <sup>**</sup>
6	6.15 $\pm$ 0.029 <sup>cC</sup>	5.87 $\pm$ 0.060 <sup>cB</sup>	5.64 $\pm$ 0.043 <sup>bA</sup>	5.90 $\pm$ 0.043 <sup>cB</sup>	5.62 $\pm$ 0.083 <sup>bA</sup>	15.493 <sup>**</sup>
9	6.81 $\pm$ 0.007 <sup>dD</sup>	6.27 $\pm$ 0.027 <sup>dC</sup>	6.13 $\pm$ 0.025 <sup>cB</sup>	6.10 $\pm$ 0.032 <sup>dB</sup>	6.00 $\pm$ 0.027 <sup>cA</sup>	163.747 <sup>**</sup>
12	7.06 $\pm$ 0.023 <sup>eB</sup>	6.56 $\pm$ 0.038 <sup>eA</sup>	6.42 $\pm$ 0.060 <sup>dA</sup>	6.38 $\pm$ 0.094 <sup>cD</sup>	6.36 $\pm$ 0.086 <sup>dA</sup>	18.755 <sup>**</sup>
<b>F value</b>	1384.752 <sup>**</sup>	218.305 <sup>**</sup>	174.570 <sup>**</sup>	71.118 <sup>**</sup>	53.287 <sup>**</sup>	

Different superscripts in a row (capital caps) and column (small caps) differ significantly

NS – Non significant (P>0.05)

\* - Significant (P $\leq$ 0.05)

\*\* Highly significant (P $\leq$ 0.01)