

## **EFFECT OF ETHANOLIC EXTRACT OF TULSI LEAF IN EXTENDING THE KEEPING QUALITY OF COW MILK**

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**Abstract:** A study was conducted to assess the effect of ethanolic extract of tulsi leaf on physico-chemical quality of cow milk. Cow milk collected hygienically from an organized dairy farm was assessed by addition of ethanolic extracts of tulsi leaves at different concentrations viz., 0.25, 0.5, 0.75 and 1 per cent and studied for physico-chemical parameters at regular intervals at 37 °C. It was found that addition of ethanolic extract of tulsi leaves extracts at 0.25 per cent (v/v) extended the shelf life of raw milk by 9 hours by clot on boiling test. Higher concentrations (0.5, 0.75 and 1 per cent), did not appreciably extend the shelf life and showed COB positive at 8<sup>th</sup> and 7<sup>th</sup> hour itself. There was a controlled increase in titratable acidity in the milk samples added with 0.25%, level of tulsi leaves extract. Statistical analysis showed a highly significant difference ( $P \leq 0.01$ ) in titratable acidity within treatments from 1 to 10 hours of storage period and no significant difference ( $P > 0.05$ ) between treatments from 0 to 5 hours of storage.

**Keywords:** Tulsi leaves, Ethanolic extract, Clot on boiling test, titratable acidity.

### **Introduction**

In India milk production is 187.7 million tonnes and per capita availability of milk is 394 gms/day (BAHS ,2019). Milk and dairy products are good sources of high-quality protein. Protein is important during weight loss and subsequent weight maintenance due to the high satiating effect which helps to prevent over-consumption of energy and thereby reduces body fat stores, Bendsten *et.al* (2013). However milk is rich source of proteins and vitamins in them it 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. Tulsi (*Ocimum sanctum*) is a aromatic plant which has medicinal properties (Singh et al.2012). It contains several phyto-constituents such as eugenol, cubenol, borneol, vallinin (Kadian and Parle, 2012) due to which it posses antibacterial, antiviral, antifungal, antioxidant properties (Cohen,2014). 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 ethanolic extract of tulsi leaf on physico-chemical quality of cow milk.

### **Materials and Methods**

Fresh tulsi leaves (*Ocimum sanctum*) were procured from the local market in Chennai. The leaves were washed with sterile water and shade dried. The dried leaves were powdered and stored in airtight bottles for further studies at room temperature as per the method of Preethi *et al.* (2010).

Ethanolic extraction was done as per the method of Chakraborty and Shah (2011) with slight modifications. Ten grams of dry herbal leaf powder was subjected to soxhlet extraction with 300 ml of ethanol as solvent. Extraction was carried out for 3 hours, 10 cycles and a temperature of 65° C was maintained. The extract obtained in the round bottom flask was transferred into a clean and pre-weighed tube. The solvent was removed from the extract by using rotary evaporator. The extract was removed as slurry and the yield was calculated, then diluted to 100 mg /ml by 95 per cent ethanol and stored in a container at refrigerated temperature.

Raw cow milk was collected hygienically from the organized dairy farm in sterilized sample bottles (100 ml) with ethanolic tulsi leaf extracts 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 for further studies. The samples were analysed for physico-chemical parameters ie. Clot on boiling test (COB) and titratable acidity were done as per BIS, SP: 18 (Part XI)-1981 at regular intervals. The data were tabulated and subjected to statistical analysis by means of analysis of variance (ANOVA) as per the procedure given by Snedecor and Cochran (1994).

### **Results and Discussion**

The results of Clot on boiling test of the control and ethanolic extract of tulsi leaves treated samples at 37 °C are given in Table 1. It was observed that all untreated milk samples (control) and samples treated with one per cent of the ethanolic extract of tulsi leaves were acceptable up to 6 hours at 37°C. Samples treated with 0.5 and 0.75 per cent remained acceptable up to 7 hours of storage, whereas, cow milk sample treated with 0.25 per cent remained acceptable up to 9<sup>th</sup> hour of storage which was similar to the observations of Jandal (1996) who reported that milk added with onion extract and ethanol, stored at  $25 \pm 2$

°C extended the shelf life. Higher concentrations (0.5, 0.75 and one per cent), did not appreciably extend the shelf life and showed COB positive at 8<sup>th</sup> and 7<sup>th</sup> hour itself due to the precipitation of casein. This might be due to presence of ethanol in the tulsi leaves extract leading to destabilization of casein as indicated by Fox and Sweeney, 2003.

The results related to the titratable acidity (per cent lactic acid) of the control and ethanolic extract of tulsi leaves treated raw milk samples at 37°C are shown in Table 2. In the control and 1 per cent treated milk samples, the titratable acidity (per cent lactic acid) increased from initial value of  $0.14 \pm 0.002$  to  $0.20 \pm 0.003$  and  $0.20 \pm 0.002$  respectively at the 7<sup>th</sup> hour of storage. In the treated samples at 0.5, 0.75 per cent levels, the titratable acidity increased to  $0.20 \pm 0.003$  and  $0.20 \pm 0.002$  at the 8<sup>th</sup> hour of storage period and the 0.25 per cent treated sample showed a titratable acidity of  $0.20 \pm 0.002$  at 10<sup>th</sup> hour of storage.

Statistical analysis showed a highly significant difference ( $P \leq 0.01$ ) in titratable acidity within treatments from 1 to 10 hours of storage period and no significant difference ( $P > 0.05$ ) between treatments from 0 to 5 hours of storage. However, a highly significant difference ( $P \leq 0.01$ ) was observed between treatments from 6 to 10 hours of storage.

From the Table 2, it was clear that in the untreated and one per cent ethanolic extract of tulsi leaves treated raw milk samples, the titratable acidity increased from initial value of  $0.14 \pm 0.002$  to  $0.20 \pm 0.003$  at 7<sup>th</sup> hour of storage. The addition of 0.5, 0.75 and 1 per cent levels of the ethanolic extracts of tulsi leaves did not markedly improve the shelf life as presence of ethanol at higher concentration in the extracts caused precipitation of protein which was in accordance with the findings of Ray (2008).

### **Conclusion**

In this present study effect of ethanolic extract of tulsi leaf on physico-chemical changes of raw cow milk was carried out. It was concluded that addition of 0.25 percent tulsi leaf extract (v/v) to raw milk remained acceptable upto 9 hours of storage period at 37°C. Addition of higher concentrations up to 0.5, 0.75 and 1 per cent level of the ethanolic extracts of tulsi leaves did not increase the shelf life of raw milk.

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**Table -1**  
**Clot on boiling test for cow milk with ethanolic extract of tulsi leaves at different concentrations (v/v) at 37 °C<sup>@</sup>**

Storage time (Hrs)	Control cow milk	T1 0.25% ethanolic extract of tulsi leaves	T2 0.5% ethanolic extract of tulsi leaves	T3 0.75% ethanolic extract of tulsi leaves	T4 1% ethanolic extract of tulsi leaves
0	-ve	-ve	-ve	-ve	-ve
1	-ve	-ve	-ve	-ve	-ve
2	-ve	-ve	-ve	-ve	-ve
3	-ve	-ve	-ve	-ve	-ve
4	-ve	-ve	-ve	-ve	-ve
5	-ve	-ve	-ve	-ve	-ve
6	-ve	-ve	-ve	-ve	-ve
7	+ve	-ve	-ve	-ve	+ve
8	+ve	-ve	+ve	+ve	+ve
9	+ve	-ve	+ve	+ve	+ve
10	+ve	+ve	+ve	+ve	+ve

@Average of six trials

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

Storage time (Hrs)	Control cow milk	T1 0.25% ethanolic extract of tulsi leaves	T2 0.5% ethanolic extract of tulsi leaves	T3 0.75% ethanolic extract of tulsi leaves	T4 1% ethanolic extract of tulsi leaves	F value
0	0.14±0.002 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.14±0.002 <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±0.003 <sup>abA</sup>	0.15±0.004 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	0.14±0.002 <sup>aA</sup>	1.579 <sup>NS</sup>
2	0.15±0.002 <sup>abcA</sup>	0.15±0.004 <sup>aAb</sup>	0.15±0.003 <sup>abA</sup>	0.14±0.003 <sup>aA</sup>	0.15±0.002 <sup>abA</sup>	1.635 <sup>NS</sup>
3	0.16±0.004 <sup>bcdA</sup>	0.15±0.002 <sup>abcA</sup>	0.15±0.002 <sup>bcA</sup>	0.15±0.002 <sup>abA</sup>	0.16±0.004 <sup>bcA</sup>	0.911 <sup>NS</sup>
4	0.16±0.002 <sup>cdA</sup>	0.16±0.005 <sup>bcdA</sup>	0.16±0.002 <sup>cdA</sup>	0.16±0.005 <sup>bcA</sup>	0.17±0.003 <sup>cdA</sup>	1.583 <sup>NS</sup>
5	0.17±0.002 <sup>dAB</sup>	0.16±0.002 <sup>cdA</sup>	0.17±0.002 <sup>deA</sup> B	0.17±0.002 <sup>cdA</sup> B	0.18±0.002 <sup>deB</sup>	2.174 <sup>NS</sup>
6	0.19±0.005 <sup>eB</sup>	0.17±0.004 <sup>deA</sup>	0.18±0.007 <sup>eB</sup>	0.18±0.004 <sup>deB</sup>	0.19±0.005 <sup>eB</sup>	5.640 <sup>**</sup>
7	0.20±0.003 <sup>eB</sup>	0.18±0.002 <sup>efA</sup>	0.19±0.002 <sup>eA</sup>	0.19±0.004 <sup>efA</sup>	0.20±0.002 <sup>fB</sup>	6.815 <sup>**</sup>
8	0.22±0.002 <sup>fD</sup>	0.18±0.002 <sup>fA</sup>	0.20±0.003 <sup>fBC</sup>	0.20±0.002 <sup>fAB</sup>	0.22±0.002 <sup>gCD</sup>	7.288 <sup>**</sup>
9	0.25±0.004 <sup>gC</sup>	0.19±0.003 <sup>fgA</sup>	0.22±0.002 <sup>gB</sup>	0.22±0.002 <sup>gB</sup>	0.24±0.003 <sup>hC</sup>	29.969 <sup>**</sup>
10	0.27±0.005 <sup>hC</sup>	0.20±0.002 <sup>gA</sup>	0.25±0.006 <sup>hBC</sup>	0.24±0.004 <sup>hB</sup>	0.27±0.002 <sup>iC</sup>	37.476 <sup>**</sup>
<b>F value</b>	<b>72.282<sup>**</sup></b>	<b>21.514<sup>**</sup></b>	<b>75.676<sup>**</sup></b>	<b>64.571<sup>**</sup></b>	<b>93.615<sup>**</sup></b>	

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

NS – Non significant (P>0.05)

\*\* Highly significant (P≤0.01)

# Titrateable acidity expressed as percentage of lactic acid