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# Physico-chemical and microbiological changes in cow milk added with ethanolic extracts of tulsi leaf

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**Abstract**
A study was conducted to assess the physico-chemical and microbiological changes in cow milk added with ethanolic extracts of tulsi leaf. 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 its physico-chemical ie. Titratable acidity, pH and microbiological parameters at regular intervals. It was concluded that addition of 0.25 percent ethanolic extracts of tulsi leaf (v/v) to raw milk remained acceptable upto 9 hours of storage period at 37°C. Addition of higher concentration of up to 0.5, 0.75 and 1 per cent level of the ethanolic extracts of tulsi leaf in the milk did not increase the shelf life of raw milk and milk remains acceptable upto 6 hours. 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. There was a restricted increase in standard plate count of the samples added with 0.25,0.5, 0.75 and 1 percent of ethanolic extracts. Statistical analysis of data showed a highly significant difference ($P \leq 0.01$) within treatments and also between treatments at 3, 6, 9 hours of storage.

**Keywords:** Cow milk, tulsi leaves, titratable acidity, pH, 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. Natural antibacterial substances like extracts of herbs like betel, tulsi, can be used in preservation of milk. Tulsi (*Ocimum sanctum*) is a aromatic plant which has medicinal properties (Singh *et al*. 2012) [14]. It contains several phyto-constituents such as eugenol, cubenol, borneol, vallinin (Kadian and Parle, 2012) [8] due to which it posses antibacterial, antiviral, antifungal, antioxidant properties (Cohen, 2014) [4]. The mode of action of these substances 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) [6]. Herbal extracts when added to milk in storage can suppress the growth and enzymatic activities of bacteria, thus increasing the quality and shelf life of milk The present study was carried out to study the effect of ethanolic extract of tulsi leaf on physico-chemical and microbiological 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) [10].
Ethanolic extraction was done as per the method of Chakraborthy and Shah (2011) [3] 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 titratable acidity was done as per BIS, SP: 18 (Part XI)-1981 [2] at regular intervals. pH of the samples was estimated using digital pH meter. Standard plate count was done at every three hours interval up to 12 hours as per the procedure described in BIS,SP:18 (Part XI) – 1981 [2]. 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) [13].

## Results and Discussion

Table -1 shows the results of titratable acidity (per cent lactic acid) of the control and ethanolic extract of tulsi leaves treated raw milk samples at 37°C. In the control and 1 per cent treated milk samples, the titratable acidity (per cent lactic acid) increased from initial value of 0.14 ± 0.002 to 0.20 ± 0.003 and 0.20 ± 0.002 respectively at the 7th hour of storage. In the treated samples at 0.5, 0.75 per cent levels, the titratable acidity increased to 0.20 ± 0.003 and 0.20 ± 0.002 at the 8th hour of storage period and the 0.25 per cent treated sample showed a titratable acidity of 0.20 ± 0.002 at 10th 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. 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) [11].

**Table 1:** Titratable Acidity of the cow milk with addition of ethanolic extract of tulsi leaves at different concentrations (v/v) at 37°C (Mean±SE)@

| 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 | $0.14\pm0.002^{aA}$ | $0.14\pm0.002^{aA}$ | $0.14\pm0.002^{aA}$ | $0.14\pm0.003^{aA}$ | $0.14\pm0.002^{aA}$ | $0.577^{NS}$ |
| 1 | $0.15\pm0.003^{abA}$ | $0.15\pm0.004^{aA}$ | $0.14\pm0.002^{aA}$ | $0.14\pm0.002^{aA}$ | $0.14\pm0.002^{aA}$ | $1.579^{NS}$ |
| 2 | $0.15\pm0.002^{abcA}$ | $0.15\pm0.004^{aAb}$ | $0.15\pm0.003^{abA}$ | $0.14\pm0.003^{aA}$ | $0.15\pm0.002^{abA}$ | $1.635^{NS}$ |
| 3 | $0.16\pm0.004^{bcdA}$ | $0.15\pm0.002^{abcA}$ | $0.15\pm0.002^{bcA}$ | $0.15\pm0.002^{abA}$ | $0.16\pm0.004^{bcA}$ | $0.911^{NS}$ |
| 4 | $0.16\pm0.002^{cdA}$ | $0.16\pm0.005^{bcdA}$ | $0.16\pm0.002^{cdA}$ | $0.16\pm0.005^{bcA}$ | $0.17\pm0.003^{cdA}$ | $1.583^{NS}$ |
| 5 | $0.17\pm0.002^{dAB}$ | $0.16\pm0.002^{cdA}$ | $0.17\pm0.002^{deAB}$ | $0.17\pm0.002^{cdAB}$ | $0.18\pm0.002^{deB}$ | $2.174^{NS}$ |
| 6 | $0.19\pm0.005^{eB}$ | $0.17\pm0.004^{deA}$ | $0.18\pm0.007^{eB}$ | $0.18\pm0.004^{deB}$ | $0.19\pm0.005^{eB}$ | $5.640^{**}$ |
| 7 | $0.20\pm0.003^{eB}$ | $0.18\pm0.002^{efA}$ | $0.19\pm0.002^{eA}$ | $0.19\pm0.004^{efA}$ | $0.20\pm0.002^{fB}$ | $6.815^{**}$ |
| 8 | $0.22\pm0.002^{fD}$ | $0.18\pm0.002^{fA}$ | $0.20\pm0.003^{fBC}$ | $0.20\pm0.002^{fAB}$ | $0.22\pm0.002^{gCD}$ | $7.288^{**}$ |
| 9 | $0.25\pm0.004^{gC}$ | $0.19\pm0.003^{fgA}$ | $0.22\pm0.002^{gB}$ | $0.22\pm0.002^{gB}$ | $0.24\pm0.003^{hC}$ | $29.969^{**}$ |
| 10 | $0.27\pm0.005^{hC}$ | $0.20\pm0.002^{gA}$ | $0.25\pm0.006^{hBC}$ | $0.24\pm0.004^{hB}$ | $0.27\pm0.002^{iC}$ | $37.476^{**}$ |
| F value | $72.282^{**}$ | $21.514^{**}$ | $75.676^{**}$ | $64.571^{**}$ | $93.615^{**}$ | |

Different superscripts in a row (capital letters) and column (lowercase letters) differ significantly
NS – Non significant ($P > 0.05$)
** Highly significant ($P \leq 0.01$)
# Titratable acidity expressed as percentage of lactic acid

The results of pH of the untreated raw milk (control) and samples treated with ethanolic extract of tulsi leaves at 37 °C were shown in Table-2. There was a decrease in the pH value (6.65 ± 0.004 to 6.10 ± 0.004) in the untreated (control) samples at 7th hour of storage. The samples treated with 0.5, 0.75 and one per cent of ethanolic extracts of tulsi leaves showed a decrease in pH at 8, 8 and 7 hours of storage. The decrease in pH of milk at higher concentration of extract earlier when compared to 0.25 percent might be attributed to the presence of ethanol in tulsi extracts which caused the precipitation of proteins. Rokhsana *et al*. (2007) [12] also found that there was a drop in pH of milk during storage. The samples treated with tulsi leaf extract at 0.25 per cent showed a gradual decrease in pH (6.10 ±0.003) in 10 hours of storage period when compared with that of the control. The increase in storage period in the treated sample may be due to antimicrobial activity of *Piper betel* leaves which inhibit all the spoilage microorganisms in milk as per Kriangkrai and Penkhae (2009) [9]. The shelf life extension of milk with ethanolic tulsi extracts at 0.5, 0.75 and one per cent levels was less when compared to 0.25 per cent which might be due to coagulation of protein in milk in the presence of acid and alcohol as per Fox and Sweeney (2003) [5]. Statistical analysis of the data showed a highly significant difference ($P \leq 0.01$) within treatments from 0 to 10 hours of storage.

**Table 2:** pH of the raw milk with ethanolic extract of tulsi leaves at different concentrations (v/v) at 37 °C (Mean±SE)@

| 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 | $6.65\pm0.004^{A}$ | $6.65\pm0.004^{gA}$ | $6.65\pm0.004^{A}$ | $6.65\pm0.004^{A}$ | $6.65\pm0.004^{A}$ | $0.000^{NS}$ |
| 1 | $6.58\pm0.005^{hA}$ | $6.62\pm0.014^{gA}$ | $6.61\pm0.020^{A}$ | $6.60\pm0.004^{hA}$ | $6.60\pm0.010^{hA}$ | $0.159^{NS}$ |
| 2 | $6.55\pm0.004^{ghA}$ | $6.61\pm0.005^{gA}$ | $6.55\pm0.014^{hA}$ | $6.55\pm0.022^{ghA}$ | $6.55\pm0.041^{ghA}$ | $0.783^{NS}$ |
| 3 | $6.50\pm0.004^{fgA}$ | $6.56\pm0.004^{fgA}$ | $6.48\pm0.004^{ghA}$ | $6.48\pm0.004^{fghA}$ | $6.48\pm0.004^{fghA}$ | $1.070^{NS}$ |
| 4 | $6.48\pm0.003^{fA}$ | $6.50\pm0.007^{efA}$ | $6.42\pm0.011^{fgA}$ | $6.42\pm0.003^{efgA}$ | $6.42\pm0.005^{efgA}$ | $1.475^{NS}$ |
| 5 | $6.36\pm0.013^{eA}$ | $6.45\pm0.004^{deA}$ | $6.36\pm0.004^{efA}$ | $6.36\pm0.004^{efA}$ | $6.36\pm0.004^{efA}$ | $1.957^{NS}$ |
| 6 | $6.24\pm0.004^{dA}$ | $6.41\pm0.004^{cdeC}$ | $6.28\pm0.005^{deB}$ | $6.28\pm0.048^{deB}$ | $6.28\pm0.002^{deB}$ | $32.343^{**}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | 6.10±0.004$^{cA}$ | 6.38±0.004$^{cdC}$ | 6.20±0.004$^{cdB}$ | 6.20±0.004$^{cdB}$ | 6.10±0.004$^{cdA}$ | 23.650$^{**}$ |
| 8 | 5.98±0.004$^{bA}$ | 6.32±0.006$^{cC}$ | 6.10±0.004$^{cB}$ | 6.10±0.015$^{bcAB}$ | 6.00±0.004$^{bcAB}$ | 21.114$^{**}$ |
| 9 | 5.86±0.007$^{aA}$ | 6.22±0.002$^{bB}$ | 5.98±0.002$^{bA}$ | 5.98±0.004$^{abA}$ | 5.90±0.044$^{abA}$ | 5.510$^{**}$ |
| 10 | 5.61±0.020$^{aA}$ | 6.10±0.004$^{bC}$ | 5.85±0.021$^{aB}$ | 5.82±0.004$^{abB}$ | 5.64±0.006$^{abA}$ | 5.674$^{**}$ |
| F value | 168.444$^{**}$ | 23.438$^{**}$ | 44.961$^{**}$ | 29.780$^{**}$ | 64.170$^{**}$ | |

@Average of six trials
Different superscripts in a row (capital letters) and column (lowercase letters) differ significantly
NS – Non significant ($P$>0.05)
** Highly significant ($P\leq$ 0.01)

Table -3 showed that standard plate count of untreated raw milk (5.31 ± 0.017 $\log_{10}$ cfu/ml) increased rapidly during the storage period up to 9 hours (6.46 ± 0.008 $\log_{10}$cfu/ml), whereas the 0.25, 0.5, 0.75 and 1 per cent tulsi leaves extract treated samples showed restricted increase in standard plate count during 9 hours of storage period. Statistical analysis of data showed a highly significant difference (P ≤0.01) within treatments and also between treatments at 3, 6, 9 hours of storage. These results were in accordance with Abdalla *et al.* (2007) [1] who found that the total bacterial count of milk treated with mango seed kernel extract increased slowly when compared to control.

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

| 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 | 5.31±0.017$^{aA}$ | 5.31±0.017$^{aA}$ | 5.31±0.017$^{aA}$ | 5.31±0.017$^{aA}$ | 5.31±0.017$^{aA}$ | 0.000$^{NS}$ |
| 3 | 5.81±0.018$^{bD}$ | 5.39 ±0.015$^{bC}$ | 5.36±0.012$^{aBC}$ | 5.33±0.017$^{aB}$ | 5.35±0.029$^{aA}$ | 135.160$^{**}$ |
| 6 | 5.98±0.015$^{cD}$ | 5.68 ±0.028$^{cC}$ | 5.57±0.045$^{bBC}$ | 5.51±0.055$^{bB}$ | 5.41±0.100$^{bA}$ | 20.568$^{**}$ |
| 9 | 6.46±0.008$^{dD}$ | 5.89 ±0.017$^{dC}$ | 5.72±0.029$^{cB}$ | 5.61±0.033$^{cA}$ | 5.59±0.024$^{cA}$ | 273.521$^{**}$ |
| F value | 992.791$^{**}$ | 186.501$^{**}$ | 46.430$^{**}$ | 18.529$^{**}$ | 7.989$^{**}$ | |

@ Average of six trials
Different superscripts in a row (capital letters) and column (lowercase letters) differ significantly
NS – Non significant ($P$>0.05)
** Highly significant ($P\leq$ 0.01)

## Conclusion

In this present study effect of ethanolic extract of tulsi leaf on physico-chemical and microbiological changes of raw cow milk were 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, thus it increased the shelf life of milk. 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 and milk remains acceptable upto 6 hours.

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