RESIDUAL EFFECT OF COMMON ANTIBIOTICS USED FOR MASTITIS TREATMENT AT THE FARM LEVEL ON THE GROWTH OF BACILLUS SUBTILIS AND STREPTOCOCCUS THERMOPHILUS

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ABSTRACT
The common antibiotics used for the treatment of mastitis are Benzylpenicillin, Gentamycin, Tetracycline and Oxytetracycline. The antibiotic residues are being secreted to milk if the animal milk is drawn within 72 hr. These antibiotic residues in milk inhibit the growth of lactic acid bacteria used for fermented milk products as they inhibit on the cell wall synthesis and protein synthesis. The most sensitive organism for these antibiotics is Bacillus subtilis, Bacillus stearothermophilus and Streptococcus thermophilus. In this experiment the antibiotics are being tested against the indicator bacteria Bacillus subtilis by using the disc assay method and inhibitory zone is determined at the lowest concentration level. The benzylpenicillin produced inhibitory zone of 15 sq mm at the concentration of 0.24 µg/ml, Gentamycin produced 20 and 14.5 sq mm at the concentrations of 0.4 and 0.04 µg/ml, oxytetracycline produced 30 and 28 sq mm at the concentrations of 50 and 5µg/ml. S.thermophilus when used as indicator organism was showed that the S.thermophilus was not inhibited at concentrations of 0.024 and 0.0024IU/ml of benzylpenicillin, gentamycin inhibited the organism at the concentrations of 0.4 and 0.04 µg/ml oxytetracycline inhibited the organism at the concentrations of 50 and 5 µg/ml respectively.

Key words: Antibiotics, Inhibitory zone, Disc assay, Bacillus subtilis, Streptococcus thermophilus.

INTRODUCTION
The usefulness of antibiotics and drugs against mastitis and other diseases in animals have rendered them almost indispensable in veterinary medicine. The administration of these antibacterial substances results in the secretion of their residues into milk (Radostitis, 1994). The consumption of such contaminated milk has physiological implication in human beings and technological implications in preparation of fermented milk product (Fallon et al. 1996). Once the antibiotic residues find their way into milk, it is very difficult to get rid of them, heat treatment is not usually effective. It has become very common to use the antibiotics when the animals are vulnerable to diseases like mastitis. The most commonly used antibiotics for the diseases treatment are Benzylpenicillin, Streptomycin, Gentamycin, Tetracycline and Oxytetracycline (Kosikowski et al., 1952). If the farmer does not withhold the milk for 72 hrs after the last treatment then these residual antibiotics may find their way into the milk and even heat treatments like pasteurization, sterilization or other means of process may not fully reduce their presence in the final product. If this antibiotic contaminated milk is used for preparation of milk based fermented products, the starter organisms could be inhibited in the presence of these residual antibiotics affecting the characteristics of the final products. To detect the presence of these antibacterial substances various tests are being used by using most sensitive indicator organisms. The most commonly used are Bacillus subtilis and Bacillus stearothermophilus the later one is most sensitive and acid producing organism were being employed in the microbial inhibitory test (IDF, 1991). But the use of lactic acid bacteria is less commonly known. Streptococcus thermophilus is known for its sensitivity against...
The product was stored in LDPE pouches for further studies at refrigeration temperature (4±1°C) for one week.

**Analytical procedures:** The pH of product was determined as per the method of O’Keeffe et al. (1976) by using a digital pH meter (Systronics Digital pH Meter 802, Serial No. 603).

The moisture, protein, fat and ash content of the product were determined by standard methods using hot air oven (Yorco sales Pvt. Ltd. India, Model-YS1-431, S. No. 02B2843), Soxhlet extraction apparatus, Muffle furnace and Kjeldhal assembly respectively (AOAC, 1995). Method of Witte et al. (1970) was used for measuring 2-Thiobarbituric acid (TBA) values.

Psychrotrophic and total plate count of the samples were determined as per the methods described by APHA (1984). Preparation of samples and serial dilutions were done near the flame in a vertical laminar flow apparatus (Thermo Electron Corporation. D-63505 Langenselbold, Robert Boschstr.1, Germany) which was pre-sterilized by ultraviolet irradiation observing all possible aseptic precautions.

**Sensory evaluation:** The sensory evaluation of the product was carried for appearance, flavour, juiciness, texture and the overall acceptability by a panel of trained members composed of scientists and research scholars of the division based on a 8-point hedonic scale, wherein 8 denoted “extremely desirable” and 1 denoted “extremely undesirable”.

**Statistical analysis:** The experiment was replicated six times and the data generated up to seven days of storage were expressed as mean ± SE. The statistical differences between the means were assessed by ANOVA-one way classification (Snedecor and Cochran, 1980). A difference at P<0.05 was considered statistically significant.

### RESULTS AND DISCUSSION

The mean values of various physicochemical parameters of Kashmiri saffron phirni are presented in the Table-2. The pH recorded a non-significant (P>0.05) trend with increase in the level of semolina and the mean values of pH at 5, 7.5 and 10 % levels were comparable to each other. Jain (2003) also reported a similar effect of different levels of binder (SMP) on pH of skimmed milk nuggets.

A non significant (P>0.05) influence of the level of semolina was also observed on the various proximate parameters of the product. The mean values of the proximate parameters were comparable at all the three levels. Although, a non-significant (P>0.05) decreasing trend was observed in all the proximate parameters with the increase in level of semolina.

The mean values of various sensory parameters of Kashmiri saffron phirni containing 5, 7.5 and 10 % semolina are presented in the Table-3. Treatments (levels of semolina) had a significant (P<0.05) influence on flavour, juiciness, texture and overall acceptability of Kashmiri saffron phirni. Appearance scores, although having a non-significant trend was highest for the product containing 7.5% semolina. Flavour and texture scores showed a significant (P<0.05) increasing trend with increase in the level of semolina. However, the flavour and texture scores of the product prepared with 7.5 % semolina were comparable with the scores of other two products containing 5 and 10 % semolina. Jain (2003) also reported a similar effect of different levels of binder on texture and flavour scores of whole milk and skimmed milk nuggets. Juiciness scores of the Kashmiri saffron phirni showed a significant (P<0.05) decrease with increase in the level of semolina. Juiciness of the product was perceived by the juices released during first few chews. However, the juiciness scores of the product prepared with 7.5% semolina were comparable with the scores of other two products containing 5 and 10% semolina. Similar results were also reported by Jain (2003). Overall acceptability score was significantly (P<0.05) highest for the product containing 7.5% semolina in comparison to the other two which were comparable to each other. Thus, based on the various

| TABLE 1: Ingredients used for preparation of Kashmiri saffron phirni. |
|-------------------|------------------|
| Ingredients       | Amount           |
| Milk              | 1 Kg             |
| Semolina          | 5, 7.5 and 10% w/w |
| Sugar             | 10% w/w          |
| Saffron           | 0.01% w/w        |
| Dry fruits (almonds and resins in 2:1 ratio) | 4% w/w |

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physicochemical and sensory parameters, 7.5% level of semolina with 10% level of sugar was optimized as best in the product development and was selected for further studies at refrigeration temperature (4±1°C) for one week.

Mean values of various physicochemical parameters of aerobically packaged Kashmiri saffron phirni during refrigerated storage at 4±1°C are presented in Table-4. The mean pH values of the product increased significantly (P<0.05) during the refrigerated storage. The value observed on day 0 (7.01) was significantly lower than the values recorded on day 4 (7.23) and day 7 (7.47) which also varied significantly (P<0.05) with each other. This increase in product pH during storage might be due to the liberation of metabolites resulting from increased bacterial activity. This was in agreement with the findings of Jain (2003) who also reported an increase in pH of milk nuggets during refrigerated storage.

The TBA values of the product also rose significantly (P<0.05) with increase of storage period but overall the values were within the limits of acceptability. This increase in TBA values during the storage period might be due to the oxidation of fatty acids. This was in agreement with the findings of Pal et al. (1993) who also reported a similar increase in TBA values of processed paneer at refrigeration temperature. Kumar et al. (2011) also reported a significantly increasing trend in the TBA values of shrikhand under refrigerated storage.

The mean values of various microbiological characters of the product containing 7.5% semolina are also presented in the Table-4. Studies indicated that the samples had lower aerobic counts as cooking is reported to have pronounced effect in reducing bacterial load if done for longer times at higher temperature (Bryan et al, 1980).

The mean values of the total plate count increased significantly (P<0.05) throughout the storage period but overall the values were within the limits of acceptability. Bhat et al. (2010) reported a similar increase in the total plate count of dietetic phirni prepared from reconstituted skimmed milk during refrigerated storage. Kumar et al. (2011) also reported a significantly increasing trend in the total plate count of shrikhand during refrigerated storage. Pal et al. (1993) and Jain (2003) also observed a similar increase of total plate counts while
TABLE 4: Effect of refrigerated storage on chemical and microbiological quality of packaged (in LDPE) Kashmiri saffron phirni with 7.5% semolina. (Mean ±SE)*

<table>
<thead>
<tr>
<th>Quality attributes</th>
<th>Storage Period (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>7.01 ±0.01A</td>
</tr>
<tr>
<td>TBA (mg malonaldehyde /Kg)</td>
<td>0.32±0.01A</td>
</tr>
<tr>
<td>Total plate count (Log10 Cfu/g)</td>
<td>1.57±0.06A</td>
</tr>
<tr>
<td>Psychrotrophic count(Log10 Cfu/g)</td>
<td>1.15±0.32A</td>
</tr>
</tbody>
</table>

*Mean ±SE with different superscripts in a row for different parameters differs significantly (P<0.05).
n1 (pH) = 3, n2 (TBA, TPC & PC) = 6 for each treatment.

studying the microbiological quality of paneer and milk nuggets at refrigeration temperature.

The mean values of the psychrotrophic count increased significantly (P>0.05) throughout the storage period and overall the values were within the limits of acceptability. Bhat et al. (2010) and Kumar et al. (2011) reported a similar increase in the psychrotrophic counts of dietetic phirni and shrikhand respectively during refrigerated storage. Jain (2003) also observed a similar increase of psychrotrophic count while studying the effect of refrigerated storage on the quality of milk nuggets.

CONCLUSION

Kashmiri saffron phirni prepared from semolina at 7.5% and sugar at 10% was highly acceptable to the panelists and had good physicochemical and sensory properties. Product gave the aerobic counts in acceptable range during 7 days of refrigerated storage. Thus it may be concluded that semolina at 7.5% level and sugar at 10% level in milk can be successfully utilized for preparation of Kashmiri saffron phirni which is well acceptable upto 7 days of refrigerated storage at 4±1°C.

REFERENCES


