

## LETHAL EFFECT OF CARBONATION ON PSEUDOMONAS FLUORESCENS AND P.FRAGI IN RAW MILK\*

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Carbon dioxide is commonly employed in food manufacturing to improve the shelf-life of refrigerated foods by inhibiting the growth of many spoilage microorganisms such as psychrotrophic bacteria (Daniels, *et al.*, 1985). In cheese making, CO<sub>2</sub>-preserved, degasified and pasteurized milk was inoculated with a smaller amount of starter than the control, the Clotting Time was reduced, cheese yield was higher, and curd hardness and whey losses were increased (Madiedo *et al.*, 1998). Hence, a study was conducted to inhibit the multiplication of *Pseudomonas* species (dominant flora in refrigerated milk) in raw milk by using CO<sub>2</sub>.

The total viable count and psychrotrophic counts of the raw milk samples (113 samples collected from farm, milk vendors and dairy plants) were carried out as per the Standard methods for Examination of Dairy Products (APHA, 1978). Standard Plate Count Agar was used for plating and *Pseudomonas* agar base with CFC supplement (Himedia Laboratories, Mumbai) was used for the selective cultivation of *Pseudomonas* species. Carbon dioxide is commonly employed in food

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The isolates were identified by morphological, cultural and biochemical tests following the methods described by Buchanan and Gibbons (1974). Millipore (0.45 nm) syringe filter was used for the sterilization of milk. Milk was sterilized after fixing the membrane between the filter pads. The screw was tightly fixed. The assembly was packed in aluminium foil and sterilized at 121°C for 15 minutes. After sterilization the assembly was utilized for filter sterilization of milk. Sterilization was done in a sterile environment using the laminar flow system. The sterilized raw milk was collected in sterile glassware.

*Pseudomonas* species (18 isolates of *P. fluorescens* and 18 isolates of *P. fragi*) were inoculated into the filter sterilized raw milk samples up to the level of 3 to 4 log<sub>10</sub> cfu per ml and the milk samples were sparged with CO<sub>2</sub> (beverage grade) in a sealed cylinder up to pH of 6.4, and 6.2 and 6.0. The CO<sub>2</sub> was initially bubbled in to the tubes until the pH range between 6.3 to 6.5 for the pH 6.4 treatment, 6.1 and 6.3 for the 6.2 pH treatment and 5.9 to 6.1 for the 6.0 pH treatment similar to the procedure adopted by Madiedo *et al.* (1996). The pH of the milk was measured using pH meter. During milk collection and preparation, efforts were made to avoid bacterial contamination. After carbonation milk was stored at 4°C. Milk samples were analyzed on day 4 of storage for psychrotrophic count by plating and incubating at 7°C. The data collected were subjected to statistical analysis as per Snedecor and Cochran (1989).

Addition of CO<sub>2</sub> to reduce the pH of raw milk to 6.4, 6.2, and 6.0 reduced the count of *P. fluorescens* to 4.08 ± 0.06, 3.95 ± 0.02 and 3.57 ± 0.07 log<sub>10</sub> cfu/ml, respectively, after storage at 4°C for 4 days, from the count of 5.22 ± 0.07 (log<sub>10</sub> cfu/ml) in the untreated samples stored at 4°C for 4 days (Table 1). Similarly, the reduction in the population of *P. fragi* was 4.16 ± 0.06, 3.99 ± 0.04 and 3.67 ± 0.08 log<sub>10</sub> cfu/ml, respectively, after storage at 4°C for 4 days, from the count of 5.31 ± 0.08 (log<sub>10</sub> cfu/ml) in the untreated milk stored at 4°C for 4 days.

Reduction of the pH of the filter sterilized raw milk to 6.4, 6.2, 6.0 by carbonation reduced the count of *P. fluorescens* to 26.77, 44.29, 73.31 per cent, respectively and the population of *P. fragi* was reduced to 27.97 and 51.32, 76.07 per cent, respectively, from the untreated sample after storage at 4°C for 4 days. The reduction in the count was significant (P<0.01) between the pH 6.2 and 6.0. Acidification to pH 6.0 was more inhibitory than pH 6.2. These results are in close agreement with the findings of Uceda (1994) and Madiedo *et al.* (1996). Ma *et al.* (2003) reported similar results after 7 days of storage at 4°C. Addition of CO<sub>2</sub> (30 mM) to milk was found to increase the generation time of psychrotrophs to 16.7 h at 7°C from 6.3 h (Roberts and Torrey 1988). It may be concluded that for the milk meant for special purposes such as cheese making, carbonation may be done to improve the shelf life of raw milk stored at 4°C without affecting the components of milk by heat treatment. Since, carbonation is safe, cheap and the reaction is reversible by vacuum treatment.

**Table 1**  
Lethal effect of carbonation on *P. fluorescens* and *P. fragi* in raw milk

| S.No | Species                            | Untreated      | Untreated after 4 days      | Count after 4 days          |               |                             |               |                             |               |
|------|------------------------------------|----------------|-----------------------------|-----------------------------|---------------|-----------------------------|---------------|-----------------------------|---------------|
|      |                                    |                |                             | pH 6.4                      | Reduction (%) | pH 6.2                      | Reduction (%) | pH 6.0                      | Reduction (%) |
| 1    | <i>P. fluorescens</i><br><i>ns</i> | 3.79<br>± 0.04 | 5.22 <sup>a</sup><br>± 0.07 | 4.08 <sub>b</sub><br>± 0.06 | 26.77         | 3.95 <sub>b</sub><br>± 0.02 | 44.29         | 3.57 <sub>c</sub><br>± 0.07 | 73.31         |
| 2    | <i>P. fragi</i>                    | 3.70<br>± 0.05 | 5.31 <sup>a</sup><br>± 0.08 | 4.16 <sub>b</sub><br>± 0.06 | 27.97         | 3.99 <sub>b</sub><br>± 0.04 | 51.32         | 3.67 <sub>c</sub><br>± 0.08 | 76.07         |

Means bearing different superscripts in a row differ significantly (P<0.01)

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