

## ROLE OF ANTIMYCOTIC AGENTS IN CONTROLLING THE GROWTH OF MYCOTOXIGENIC *Penicillium citrinum* IN CHEESE

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### ABSTRACT

Swiss cheese samples collected aseptically from private dairies were analysed for the presence of *Penicillium citrinum* and for the efficacy of antimycotic agents against *Penicillium citrinum*. The spore suspension of *Penicillium citrinum* and antimycotic agents like pimaricin and potassium sorbate at specified concentrations were added to cheese and stored at 25<sup>o</sup> C for 21 days. The keeping quality of cheese was assessed at 0, 7, 14 and 21 days. On statistical analysis, pimaricin at 10 ppm concentration was found to be highly significant ( $P < 0.01$ ) over 5 ppm of pimaricin, 500 and 1000 ppm of potassium sorbate with regard to anti-mycotic effect.

**Key words:** Cheese - *Penicillium citrinum* – control – antimycotic agents

Milk and milk products contain all kind of nutrients that favour the growth of microorganisms. Milk products like cheese containing high moisture content serve as a favorable medium especially for the growth of yeast and moulds. Spoilage of dairy products by moulds is of frequent occurrence. The tropical environmental conditions prevailing in India favour the growth of moulds and aggravate the situation. Since, cheese is being held at refrigerated storage for few months during ripening, there is every possibility of toxin production in cheese, if the storage temperature is above 12<sup>o</sup> C and cause health hazards to the consumers.

Citrinin, one of the mycotoxins produced mainly by *Penicillium citrinum* is nephrotoxic and has been implicated in disease outbreaks in both animals and human (Betina, 1984). As the shelf life of cheese depends upon the microbiological quality, an investigation on the prevalence of moulds in cheese will be of immense use to the cheese industry. Screening and identification of *Penicillium citrinum* from cheese that produces citrinin will be of critical importance from the public health point of view.

Natamycin (Pimaricin) and Sorbic acid and its water soluble potassium salt are of major

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commercial significance as preservatives for food products including cheese. Keeping the above points in view, a study was conducted to control the growth of mycotoxigenic *Penicillium citrinum* in cheese by using antimycotic agents thereby to extend its keeping quality.

Swiss cheese samples aseptically collected from private dairies were analysed for the incidence of *Penicillium citrinum*. Antimycotic agents viz. Pimaricin (Natamycin) of Gist brocades, Holland and Sorbic acid (Potassium salt) of Glaxo chemicals were used in the study.

Potato dextrose agar (BIS, 1966) and Malt extract agar (Lodder, 1970) were used for enumeration of moulds in this study. The role of certain preservatives like pimaricin and potassium sorbate was studied as per the method of Hassan Gourama and Bullerman (1988).

*Penicillium citrinum* isolates isolated from cheese were grown on potato dextrose agar slants for 14 days at room temperature. The spores were harvested by adding 10 ml of distilled water to the slants and gently brushing the conidiophores with a sterile inoculating loop. This procedure was repeated twice and the harvested spore suspensions were pooled.

The spore suspension were then filtered through four layers of sterile cheese cloth and diluted to a concentration of approximately  $10^3$  conidia per ml and was inoculated into the cheese surface and divided into five parts. One part was kept as control. The antimycotic agents like pimaricin and

potassium sorbate at specified concentrations were added to the rest and stored at  $25^{\circ}\text{C}$  for 21 days. The keeping quality was assessed by estimating *Penicillium citrinum* count at regular intervals of 0, 7, 14 and 21 days. The data were analysed as per procedure of Snedecor and Cochran (1994).

The counts of *Penicillium citrinum* at 0, 7, 14 and 21 days in control cheese and cheese in which antimycotic agents added at specified concentrations were presented in Table 1.

The *Penicillium citrinum* counts in control cheese at 0, 7, 14 and 21 days were  $1.8 \times 10^3$ ,  $2.2 \times 10^3$ ,  $2.8 \times 10^3$  and  $3.5 \times 10^3$  cfu per gram respectively with the mean value of  $2.49 \times 10^3$  cfu per gram. At a concentration of 500 ppm potassium sorbate in cheese, the count on 0, 7, 14 and 21 days were  $1.8 \times 10^3$ ,  $9 \times 10^2$ ,  $6.1 \times 10^2$  and  $2.2 \times 10^2$  cfu per gram respectively with the mean value of  $6.83 \times 10^2$  cfu per gram. The counts were  $1.8 \times 10^3$ ,  $8 \times 10^2$ ,  $9 \times 10^1$  and  $2.8 \times 10^1$  cfu per gram at 0, 7, 14 and 21 days respectively with the mean value of  $2.45 \times 10^2$  cfu per gram at 1000 ppm of potassium sorbate in cheese.

At 5 and 10 ppm concentrations of pimaricin in cheese, the counts at 0, 7, 14 and 21 days were  $1.8 \times 10^3$ ,  $7.6 \times 10^2$ ,  $5.1 \times 10^1$ ,  $2.2 \times 10^1$  and  $1.8 \times 10^3$ ,  $9 \times 10^1$ ,  $1.4 \times 10^1$  and  $1 \times 10^1$  cfu per gram respectively with the mean values of  $1.98 \times 10^2$  and  $6.9 \times 10^1$  cfu per gram respectively. On analysis of variance, it revealed that there was a significant difference between different trials of anti-mycotic agents on *Penicillium citrinum* isolates.

The *Penicillium citrinum* count was the highest in control cheese ( $2.49 \times 10^3$  cfu per gram), whereas the lowest was found in cheese with pimaricin at 10 ppm concentration ( $6.9 \times 10^1$  cfu per gram). Pimaricin at 10 ppm concentration was found to be the most effective in controlling *Penicillium citrinum* in cheese when compared to 5 ppm of pimaricin and 500, 1000 ppm of potassium sorbate. These observations were similar to the results

of Bullerman (1977), Ray and Bullerman (1982) and Hassan Gourama and Bullerman (1988).

**Table 1** : Effect of antimycotic agents on *Penicillium citrinum* in cheese

Particulars	<i>Penicillium citrinum</i> Count (cfu per gram)					F / SE / CD
	0 Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>st</sup> Day	Mean	
Control	$1.8 \times 10^3 \pm 0.61$	$2.2 \times 10^3 \pm 0.6$	$2.8 \times 10^3 \pm 0.59$	$3.5 \times 10^3 \pm 0.59$	$2.49 \times 10^3 \pm 0.66$	F : 32.21** S.E: 0.104 C.D: 0.449
Potassium sorbate 500 ppm	$1.8 \times 10^3 \pm 0.61$	$9 \times 10^2 \pm 0.58$	$6.1 \times 10^2 \pm 0.59$	$2.2 \times 10^2 \pm 0.60$	$6.83 \times 10^2 \pm 1.2$	
Potassium sorbate 1000 pm	$1.8 \times 10^3 \pm 0.61$	$8 \times 10^2 \pm 0.58$	$9 \times 10^1 \pm 0.58$	$2.8 \times 10^1 \pm 0.59$	$2.45 \times 10^2 \pm 3.42$	
Pimaricin 5 ppm	$1.8 \times 10^3 \pm 0.61$	$7.6 \times 10^2 \pm 0.58$	$5.1 \times 10^1 \pm 0.59$	$2.2 \times 10^1 \pm 0.60$	$1.98 \times 10^2 \pm 4.13$	
Pimaricin 10 ppm	$1.8 \times 10^3 \pm 0.61$	$9 \times 10^1 \pm 0.58$	$1.4 \times 10^1 \pm 0.62$	$1 \times 10^1 \pm 0.64$	$6.9 \times 10^1 \pm 5.41$	

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