D-28: Contaminating molds in curd

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Curd is a fermented milk product obtained from coagulation of cow or buffalo milk by a group of lactic acid bacteria. The high acidity of curd provides a suitable environment for growth of fungi, and the keeping quality of curd is related to contaminants and molds. The objective of this study was to detect the keeping quality of curd by enumeration of yeasts and molds. Identification of molds and determination of toxisgenicity of certain species of molds were also carried out.

Sixty samples of curd in consumer packages obtained from different producers were analysed for yeasts and other molds over a period of 8 months. 10 g of the sample were added to 90 ml of sterile 0.1% peptone water and serial dilutions of this were made. The dilutions were plated on yeast extract, dextrose, chloremphenicol medium and incubated at room temperature for 7 days. Enumeration of yeasts and molds were done at the end of the incubation period. Different mold species were subcultures and identified by agar block method. Species of *Aspergillus flavus* and *A. parastiticus* in curd were examined by inoculating the serial dilutions of curd in *Aspergillus flavus/ A. parasiticus* agar (Oxoid CM 731) and incubating at 30°C for 42-43 h. 12 isolates showing typical colony characteristics on *Aspergillus flavus/ A. parasiticus* agar were differentiated into the 2 species using colony colour and morphological characters in slide cultures. The above 12 were grown in Potato dextrose agar with Zn dust and $NaNO_3$ medium for 7 days and examined for aflatoxin production.

Aflatoxins were extracted with 70% acetone, and the extract was purified by precipitation with lead acetate and extraction with chloroform. The residue of the extract after evaporating chloroform was dissolved in a known volume of benzene acetonitrile (98 : 2 v/v) and thin layer chromatography was performed on silica gel plates together with aflatoxin standards. R_f values of samples were compared with SLS standards.

All samples of curd examined contained yeast and mold counts which ranged from (1-5) 10^5/CFU. The fungal isolates were distributed in 3 genera: *Aspergillus, Penicillium*, and *Mucor*. *Aspergillus flavus* and *A. parasiticus* was present in 31.25% of the samples with colony forming units ranging from 10-12/g. 12 of the above isolates were subjected to detailed examination. Eight isolats which showed yellow colonies at first and turned bright to dark yellow green later with biserate conidiophores, were identified as *Aspergillus flavus*. The 4 isolates that produced dark green colonies with comparatively short uniserate conidiosphores were identified as *A.parasiticus*. Of the 8 isolates of *Aspergillus flavus*, one isolate produced aflatoxin B_1, B_2 and 5 produced G_1 and G_2. Two isolates of *A. parasiticus* were able to produce aflatoxin B_1, B_2 and 2 other isolates produced G_1 and G_2 forms of aflatoxin.

The curd tested could be graded as very poor quality as the contaminating yeasts and molds of the samples were excessive. Further, the *Aspergillus* species were found to be mycotoxin producers in an artificial medium.