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ANALYSIS OF FUNGAL CONTAMINATION OF SOME MILK ANALOGUE (TEA WHITENER) IN KARACHI CITY, PAKISTAN

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ABSTRACT

Tea is an instant energy providing national drink of Pakistan and consumed by the people of all social classes. In our culture dairy milk or milk analogue (tea whitener) is added in black tea to enhance its taste, color and aroma. The present study is designed to investigate the physical characteristics and hygienic level of the different brands of tea whitener either in liquid (TWL) or powder (TWP) form that are commonly available in Karachi city. Six species (*Aspergillus candidus*, *A. flavus*, *A. niger*, *A. nidulans*, *Neurospora cressa* and *Penicillium notatum*) were recovered in summer season, while a total four species (*Aspergillus niger*, *Mucor mucedo*, *Neurospora cressa* and *Penicillium notatum*) were isolated in winter season. In this regard hygiene of samples was checked by applying culture plate technique for fungal growth and isolation.

Key-words: Fungal contamination, milk, tea, Karachi.

INTRODUCTION

Tea whiteners are milk based commercial products available in different packaging. The acceptance of such milk analogue by the consumer is not only because of its easy handling but also adequate ingredients especially for cholesterol conscious persons and cardiac patients since animal fat or milk fat (saturated fat) is substituted by vegetable oil (unsaturated fats). One of the main advantages is that the consumer does not have to boil the tea whitener. The product differs considerably from dairy milk and there is growing concern about its nutritional quality. It is not recommended for infant feeding (Hui, 1992).

In developing countries fungus and mycotoxins commonly exist in edibles due to the defect in processing. The assessment of contaminants in edibles, minimizes the food security issues and to follow national and international mycotoxin regulatory standards (Makun *et al*., 2010 and Petrus *et al*., 2010).

Fungal spores prevail in all segments of the environment and its persistence in tea whitener either in liquid form or powder state could never be neglected, although liquid tea whiteners are pasteurized or subjected to UHT. But pasteurization cannot guarantee the absence of microorganisms, when they are present in large numbers in raw milk or due to post-pasteurization contamination (Salmeron *et al*., 2002).

Rao *et al*., (2009) reported that spores of a number of fungal species remain in the atmosphere of Karachi city throughout the year. Afzal *et al*., (2004) pointed out that the high abundance of air-spora of Karachi is mainly attributable to high humidity and temperature of the atmospheric environment. It is well-known that the growth of the moulds is greatly dependent on climatic, geographical and seasonal effects (Pesic-Mikulec *et al*., 2005 and Marija *et al*., 2009). Keeping these considerations in view, the present study focused on the fungal contaminants of various tea whiteners commonly used in the city of Karachi.

Materials and Methods

Samples of homogenized and UHT exposed milk analogue (tea whitener) packed in aluminum lined paperboard cartons or tetra pack and powdered samples in aluminum lined polyethene packets sachet packs of various brands were purchased from local markets in summer and winter season. All samples were analyzed as early as possible. Analysis of fungi in the samples was carried out by culture media Petri plate method. Potato dextrose agar and Sabroud's dextrose agar, two types of media were prepared for the purpose of the recovery of fungi from the samples. For the sake of establishing the high precision 1ml of liquid samples whereas approximately 1% solution of powdered samples (1.0g in 100 ml sterile distilled water) were inoculated in each media plates. Treatments were replicated thrice. Incubation period was 5-8 days at room temperature (30-40^{o}C) in summer season and 8 to 15 days at room temperature (15-25^{o}C) in winter season. In each culture fungi were

analyzed on the basis of their morphological and microscopic characteristics following these manuals (Ellis 1971, 1976; Domsch *et al*., 1980). The data were subjected to one-way analysis of variance (ANOVA) followed by Fisher's least significant test (Zar, 2009).

Table 1. Average CFU unit of fungal species in milk analogues samples in summer.

Name of species	TWL1	TWL2	TWP1	TWP2
Aspergillus candidus	--	--	--	1±0.4
Aspergillus flavus	0.666±0.3	1.333±0.6	--	--
Aspergillus niger	--	--	--	0.666±0.3
Aspergillus nidulans	--	--	2±0.8	--
Neurospora cressa	--	3±1.7	--	--
Penicillium notatum	7±4.2	--	--	--

TWL= Tea whitener liquid, TWP= Tea whitener powder

Table 2. Average CFU unit of fungal species in milk analogues samples in winter.

Name of species	TWL1	TWL2	TWP1	TWP2
Apergillus niger	--	--	1±0.5	0.666±0.3
Mucor mucedo	1±0.4	--	--	1±0.4
Neurospora cressa	--	1±0.6	--	--
Penicillium notatum	--	--	1±0.4	1.333±0.6

TWL= Tea whitener liquid, TWP= Tea whitener powder

Result and discussion

The average CFU of fungal species isolated from different brands of liquid/powder tea whiteners in summer and winter season have been shown in tables 1 and 2, respectively. In all samples one or two mould species were found except sample TWP2 of winter season. From the results three fungal species (*Aspergillus niger*, *Neurospora cressa* and *Penicillium notatum*) were recovered both in summer and winter season. *Aspergillus flavus* and *Penicillium notatum* persist during the heat treatment and spoil the product by post pasteurization. Although cooking and freezing stop their growth but do not eliminate mycotoxins already produced (Wardlaw, 2004).

In Karachi, summer season is long about 6 months whereas winter season is short as ~ 2 to 3 months. From Table 1, a total of 6 species (*Aspergillus candidus*, *A. flavus*, *A. niger*, *A. nidulans*, *Neurospora cressa* and *Penicillium notatum*) were found in summer season, while a total four species (*Aspergillus niger*, *Mucor mucedo*, *Neurospora cressa* and *Penicillium notatum*) were isolated in winter season as presented by Table 2. Results of ANOVA showed no significant difference for summer (F = 2.23, ns) and winter (F= 0.29, ns) with respect the prevalence of *Aspergillus* species taken together.

In summer most frequently recovered mould was *Aspergillus* genus, whereas species *Aspergillus niger* isolated in both season. *Aspergillus* had greatest diversion as compared to other genera. It is the major toxigenic fungi (D'Mello and Macdonald, 1997) that produces aflatoxins which are mycotoxins produced as carcinogenic, teratogenic and mutagenic secondary metabolites (Frisvad *et al*., 2005). *Aspergillus flavus* isolated minimum in TWL1 and significantly in TWL2, that is the most prevalent species among the *Aspergillus* spp. in the indoor and outdoor of a warm climate area (Hedayati *et al*., 2010). It grows rapidly at 30-55°C, slowly at 12-15°C and ceases to grow at 5-8°C (Agrios, 2005). Hence in this study absence of *Aspergillus flavus* in winter season as shown in Table 2 favor this statement as well. Commonly *Aspergillus flavus* creates skin infections, whereas granulomatous sinusitis, dermatitis, wound septic and osteomyelitis diseases as a result of chronic exposure have been recorded (Hedayati *et al*., 2007).

In winter season two fungal species *Mucor mucedo* and *Neurospora cressa* were found in TWL1 and TWL2 respectively. Three fungal species (*Aspergillus niger, Mucor mucedo* and *Penicillium notatum*) were isolated from TWP2 in winter season (Table 2) and two species (*Aspergillus candidus* and *Aspergillus niger*) were found in the summer sample (Table 1). *Penicillium* and *Mucor* are almost exclusively used for the commercial production of

extracellular acidic proteases (Thakur *et al*., 1990; Chrzanowska *et al.*, 1995; Wong *et al*., 2008) necessary in the cheese industry at milk clotting step.

Results of this investigation disclosed that fungal spores were detected without affecting the quality in all samples and in both summer as well as winter seasons.

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