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ESTIMATION OF MICROBIAL POPULATION IN SOME CONFECTIONARY PRODUCTS

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ABSTRACT

Microbial analysis is essential part of food safety. Confidence in the safety and integrity of the food supply is an important requirement for consumers. For the evaluation we used following confectionary items such as Dairy milk, Jam, Jelly, biscuit (Parle-G, Priya gold, and Britannia). For microbiological test all sample were used in triplate form. Specific media were used for identification of different microbes (Yeast, Mould, Bacteria). For the identification and microbial analysis TPC, PDA, Macconkey media were used and for confirmation of gram positive bacteria gram staining have done. Number of gram positive seen in jelly and Mould was seen in biscuit. Aim of our studies were compare specified confectionary product and find out the amount of microbes found in all confectionary products and also compare all on the basis of microbes presence in all confectionary products.

Keywords: Mould, Yeast, *E.coli*, Confectionary, Microbial analysis, TPC.

[I] INTRODUCTION

In the Global food market, confectionary industry play essential role. It represent wide array of confectionary such as Candies, Jam, Jelly, Toffe, Fudge etc. The top five companies supplying confectionary are Cadbury, Nestle, Kraft, Mars and Parle-G, Priya gold, Britannia etc. Jams/jellies tend to be high in sugar and low in nutrients. The typical jam, jelly or marmalade offers little more than concentrated energy with high sugar content - one tablespoon contains about 40 calories. In 2001, estimated retail sales of chocolate, other candy, and gum in the United States had reached $24 billion, and more 1,400 new items of candy were introduced. Classic wheat flour biscuits having Zn and Cu contents and enriched in whole wheat grain, soya and skimmed milk [1]. In confectionary various critical parameter were used which such as control of raw material, environmental factor, processing techniques. In confectionery Major hazard has been found in the form of Salmonella. Testing for this organism at specific control points provides the best means of quality control [2]. For prevention of contamination Constant surveillance and good manufacturing practice are the best methods [3]. For the reduction of microbiological loads and extending shelf life of harvested chestnut combination of freeze drying and chocolate-coating give great result [4]. Differences in microbial activities between

different heap fermentations can result in dried fermented cocoa beans and chocolates with different flavour characteristics. Flavour of chocolate may control by fermentation control [5] [6]. Major routes for the production of flavors are enzymatic and microbial process, that complied with the definition of "natural" in the Federal Register which focused on microorganism, enzymes, substrates, and significant odorants and tastants which is described by [7]**.** Optimization of food processing help to conservation of bioactive compound which is present in fruits, that is approximately 73% total phenolics and 65% antioxidant [8]. There is a new method of fruit content determination, which is based on hemicelluloses quantification, used for yielding the amount of fruit content [9] and the antioxidant activity of fruit studied by High performance liquid chromatography/diode array detection [10] and DPPH method which is helpful for free radical scavenging activity of the ellagic acid derivatives for the evaluation of phenolic compound [8] [11]. Caffeic, ellagic acids, kaempferol, quercetin, myricetin and morin were selected phenolic aglycon in nine types of berries [10] and the highest content of ascorbic acid was found in Jam [8] [12].

The objective of our study was microbial analysis of different confectionary product on different media and assures quality of confectionary product.

[II] MATERIALS AND METHODS

2.1. Sample collection

For this study confectionary products were selected from different brands like as Dairy milk, Jam, Jelly, Biscuit and all samples were collected from local market of Gwalior and expiry date also saw on the all samples. For the microbial analysis of all samples were taken in triplicates.

2.2. Media preparation

The appropriate amount of distilled water was added to the flask by using a measuring cylinder (clean and dry).The ingredients in appropriate amounts (except Agar), were taken with clean and dry spatula and put into the conical flask. Dissolved all ingredients and then pH was adjusted, using 0.1 N NaOH or 0.1 N HCl solutions, as per media requirements, Agar then added. Agar was dissolved by keeping the flask in a water bath at 55^0C or using a magnetic stirrer on a pre-warmed surface at 60^0C or by heating over a flame with regular mixing. Plug the flask with non-adsorbant cotton and autoclave it. In this study, different specific media like PDA (for Mould and Yeast, for 48-96 hrs at 25^0C and Plate count agar (for choliform) for 72 hrs at 37^0C, EMB agar media for 24 hrs at 35^0C , Macconkey agar for 48 hrs at 30^0C were selected for microbial analysis.

2.3. Sample preparation

10g sample weighed in a clean, dry and sterile conical flask and under the aseptic conditions 90 ml of pre-warmed (45-50^0C) peptone water was added and mix thoroughly, dilution (1:10) was preferred. Sterile pipette was used for the inoculation of 0.1 ml, 0.5 ml, &1.0 ml of sample in sterile petriplates. In these petriplates specific media's were used, which was around 20ml. Gently the petriplates were rocked in horizontal, vertical and circular directions to mix the solution with media and allow the medium to set, incubate the all petriplates in the inverted position at particular specific temperature and also an uninoculated control plate should also be incubated along with the sample plate.

[III] RESULTS

Result of total plate count

For this test all gave positive result, this means these having highly sugar content and small opaque white colour colonies (minute) were considered as bacterial colonies. In case of doubt, confirmation could be done by Gram staining, short rods /cocci where as big oval shape was

yeast. Control did not show any growth till 72 hrs it means there is no contamination so we calculated it as following and it gave **1.1x 10^4/mg/ml TPC**.

TPC of the sample is calculated as follows:

TPC/mg/ml= ΣC/ (n1+0 .1n2) d.f.

Where,

$\Sigma_{C=}$ sum of the colonies counted of all petriplates

n_1= the no of plates with 1st dilution

n_2=the no. of plates counted 2nd dilution

D.F. =Dilution Factor corresponding to the 1st dilution.

3.2. Results of PDA agar plate & coliforms

This test shown negative result it means there was no growth of yeast, mould and bacteria because PDA is specific for yeast and mould growth and Macconkey is specic for the growth of coliform bacteria, result shows that there was not any type of mishandling or packaging problem.We can conclude that these all confectionary item having proper packaging.

3.3. Comparative results of different biscuits on same media

In this test Priya gold and Brittania gave negative result but Patle-G biscuit (I, II) gave positive result towards test. After testing we found that the colony was appeared, which were small round shape as considered bacteria and other were fungal colonies as shown in **figure (1).**

Figure-1: Shows the contamination of biscuits on EMB agar media.

3.4. Comparative results of Parle-G biscuit on different media (EMB agar/LB agar/Macconkey agar

In this test we used three different types of media and Parle-G biscuit used as a sample and in which Parle-G gave positive results on EMB agar media and negative results has been shown on Macconkey agar media. Small round shape colony was observed which was considered as bacterial colony and other fungal colonies were also appeared as shown in **figure (2).**

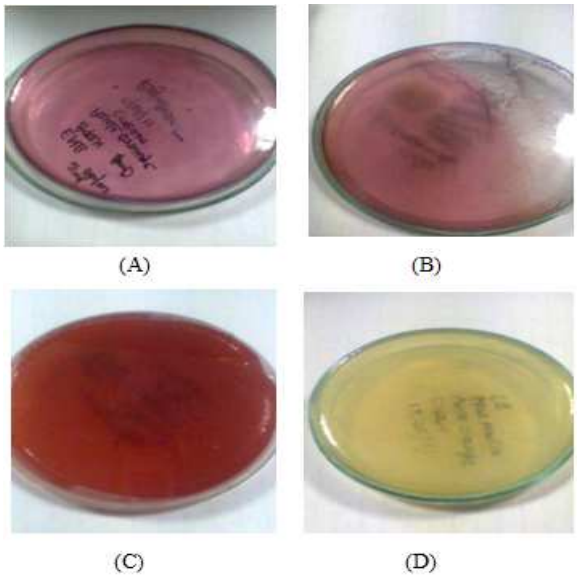

Figure-2: Shows the microbial contamination of biscuits on different media (where A & B=EMB agar plates; C= LB agar plate; D=Mackonkey agar plate).

[IV] DISCUSSION

Confectionary product mainly free from microbial hazard but some time due to improper packaging and mishandling it could be hazardous. Mainly confectionary product having high sugar so it is attracts some bacteria towards it. In our experiment we analyses four confectionary samples Jam, Jelly, Dairy milk, biscuit and also compared three brands of biscuits (Parle-G, Priya gold & Britannia). After analysis we found presence of microbes on the some confectionary products as compare to other confectionary

products Parle-G biscuits show more contamination.

[V] CONCLUSION

We conclude after all test or experiment that in the plate count agar growth occur but in PDA media there was no growth found it means the growth was not yeast or mould because PDA is selective media for yeast and mould and bacteria was confirmed after staining. For identification or differentiation of bacteria we used Macconkey agar which is a selective media for gram negative and it suppress the growth of gram positive bacteria but the Macconkey gave no growth so we conclude that the bacteria which was grow that was gram positive bacteria (*Bacillus subtilis*) and the reason of bacterial growth was not mishandling or improper packaging, the bacteria was grown because these confectionary having high sugar content. But the Parle-G biscuit shown growth of yeast it means its packaging was not properly handled. So we conclude that although Parle-G having higher sugar content but it is not as safer as Britannia and Priya gold regarding packaging.

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