

Bioload of the Marketed Available Pickels Brand in Karachi

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ABSTRACT

Fermented foods are now widely using by the people as they are very easy to make and longer shelf life. Many fermented products are very common and available in the market nowadays; one of them is pickle which is well known in the whole world especially in Eastern countries.. Although as being a fermented product it also contains Lactobacilli but unfortunately when there is a high load of other contaminated harmful organisms they fail to compete with them and the other harmful organisms become dominant which not only spoil the pickle but also cause harm to the people. Therefore, it was the need to examine the pickle and search the ways which leads to accompanying the pure or hygienic pickle. Our study based on the determination of microbial load present in this type. We found out growth of bacteria and Fungi like *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* and bacterial growth like *Escherichia coli*, *Bacillus* and *Lactobacillus acidophilus* were isolated from the pickles sample used in this study. We found out that the pickle of Rizwan Company had 1100 colonies in 100 ml of sample which can be extremely dangerous for consumption and the pickle from another marketed available brand Company showed the lowest amount i.e. 28 colonies in 100 ml of sample. Other brands that were tested also showed higher amount of organisms in between 150 to 460 colonies in 100 ml of sample. Elimination of the microbial load of pickle also performed on the pickle of National Company that gave a marvelous result the amount of organisms is fallen down from 150 colonies to 11 colonies in 100 ml of sample. This study provides preliminary work and open new doors in assessing and improving the quality of pickles available in the market.

Keywords: Fermentation, Probiotics, Pickle, Elimination, Consumption

INTRODUCTION*

Microorganisms are an assorted gathering on earth present as the biggest populace that involves their space with the assistance of focused instrument or relationship. The cooperative microscopic organisms are available all over the place which have impact on the growth (Braendle *et al.*, 2003; Koropatnick *et al.*, 2004), sustenance (Baumann, 2005; Backhed *et al.*, 2005), advancement and phylogenesis (Hurst and Werren, 2001; Bandi *et al.*, 2001; Hurst and Jiggins, 2000; Stouthamer *et al.*, 1999), give a shield against the pathogenic operators (Piel, 2002; Scarborough *et al.*, 2005; Oliver *et*

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al., 2003), and dispensation (Macdonald and Monteleone, 2005). There is an illustration to assess their populace on the planet that "our mouth contains 20 billion microscopic organisms" (Bill Landers *et al.*). The vast majority of them are valuable which are generally utilized as a part of dairy items, drugs and fermented items. In any case, the primary concern is to take out as more microorganisms as conceivable from our everyday life which is exceptionally hard to happen. Fermented products are drinks, wieners, yogurt, cheddar, bread and pickle. Presently the inquiry emerges what are fermented items? How are they delivered? They are delivered by the procedure of aging. Fermentation is the term utilized

by microbiologists to portray any procedure for the generation of an item by method for the mass society of microorganisms. The item can either be: 1. The cell itself: alluded to as biomass creation. 2. A microorganism's own metabolite: alluded to as an item from a characteristic or hereditarily enhanced strain. A microorganism outside item: alluded to as an item from recombinant DNA innovation or hereditarily designed strain, i.e. recombinant strain (Brian Pumphrey, Christian Julien and Benelux May 1996). Dry salted lime pickle is a well known natively constructed aged item. In India, the pickle is extremely fiery and hot due to the expansion of bean stew. Indian gooseberry and mango pickles are readily available. Khalpi is a customary cucumber pickle utilized as a part of the Himalayan district of India (Tamang JP New Delhi 1998). Fermented pickles also have beneficial bacteria that can control harmful intestinal microbes. The crude material is put away in brackish water tank where they can be protected for twelve months. Pickling of plants is a moderately old strategy for sustenance protection. It is assessed that the primary pickles were delivered more than 4,000 years back utilizing cucumbers. Pickling of plants is a moderately old strategy for sustenance protection. It is assessed that the primary pickles were delivered more than 4,000 years back utilizing cucumbers. In this way, the dangers of defilement are killed amid the concentrating so as to prepare of pickle on the abnormal or exceptional danger elements; this can prompt the hygienic pickle (Akbulut B, Ozer MH, Uylaser V, *et al.*, 2007). For the examination of microbial burden it is the need to apply MPN strategy the most plausible numbers to gauge about the vicinity of microbial verdure in the pickle and is it suitable for utilization or not. The "most likely number" (MPN) technique is a valuable, if underutilized, instrument for the microbiologist.

The first and best stride to dispense with the

hurtful microscopic organisms from pickle is the expansion of apple juice i.e. unadulterated vinegar which makes the pickle acidic which is the unfavorable environment for microorganisms (Carol S. Johnston and Cindy A. Gaas, 2006). Another approach to dispose of the parasite from pickle is the expansion of asafoetida which is dynamic for antifungal action. The old conventional phytomedicine asafoetida, an oleo-gum-sap got from the bases of various *Ferula assa-foetida*, is utilized as a part of various nations for different purposes (Milad Iranshahy and Mehrdad Iranshahi, March 2011). Garlic can likewise be added to beat the microbial development; it contains an antibacterial specialist supposed allicin. One gram of allicin is equivalent to 15 units of penicillin. Allicin in its unadulterated structure was found to display i) antibacterial action against an extensive variety of Gram-negative and Gram-positive microorganisms, including multidrug-safe enterotoxigenic strains of *Escherichia coli* (Serge ankari and David Mirelman February 1999)..

MATERIALS AND METHODS

Marketed available sealed Pickle's samples were collected from different companies. Dilution of pickle samples were made according to the protocol then streaked on MRS. Next day plates were observed for bacterial and fungal colonies.

Identification: The Fungal and bacterial colonies were then identified using Scotch Tape Method (for fungus) And Gram Staining (for bacteria and yeast. identified with the help of microscope

MPN Technique: Take a sample and make solution of 5ml in saline. Then prepare the 10 fold dilution in series. 1ml samples of each dilution are inoculated into triplicate broth culture tubes and incubated at 35-40°C for 24 hours. All tubes are examined for turbidity and the pattern of growth in the tubes score

Table I: Microbial Load Using MPN technique

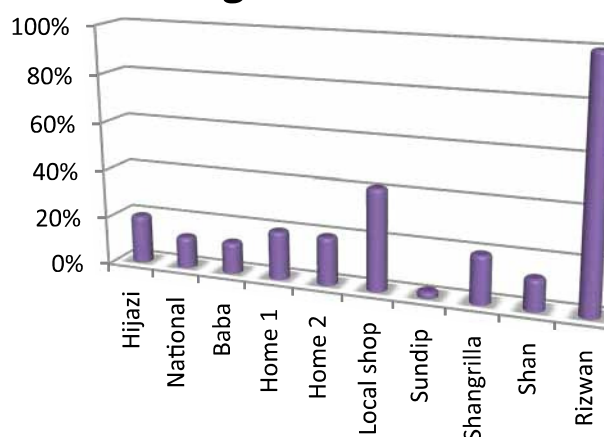
Name of Samples	Triplicate tubes 10ml	Triplicate tubes 1 ml	Triplicate tubes 0.1ml	Probable No. of organisms
Hijazi pickle	3+	2+	2+	220colonies /100 ml
National	3+	2+	1+	150 colonies / 100 ml
Baba	3+	2+	1+	150 colonies / 100 ml
Home 1	3+	2+	2+	220 colonies /100 ml
Home 2	3+	2+	2+	220 colonies /100 ml
Local shop	3+	3+	1+	460 colonies /100 ml
Sundip	3+	2+	1+	28 colonies /100 ml
Shangrila	3+	2+	2+	220 colonies /100 ml
Shan	3+	2+	1+	150 colonies /100 ml
Rizwan	3+	3+	2+	1100 colonies /100 ml

Table II: Microbial Load After Elimination Of Microorganisms

Name Of Pickle Samples	Triplicate tubes 10ml	Triplicate tubes 1 ml	Triplicate tubes 0.1ml	Probable No. Of organisms
Test Pickle	1+	1+	1+	11 colonies /100 ml

Processing Of Pickle: The oil, spices and vegetables should be pure and free of contamination. Add 3gm garlic and 3ml of apple cider in 75gm of pickle also add 3gm of asafoetida in 75gm of pickle. Apply the MPN technique after the 3 days of incubation for the test pickle.

RESULTS



In the given isolated six organisms have been isolated that are *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus*, *Escherischia coli*, *Bacillus* spp and *Lactobacillus acidophilus*. And the results of MPN method are Pickle Hijazi with 220colonies /100 ml, Pickle National with 150 colonies / 100 ml, Pickle Baba with 150 colonies / 100 ml , Pickle Home 1 with 220colonies /100 ml , Pickle Home 2 with 220colonies /100 ml, Pickle Local shop with 460 colonies /100 ml, Pickle Sundip with 28 colonies /100 ml , Pickle Shangrila with 220 colonies /100 ml , Pickle Shan with 150 colonies /100 ml, Pickle Rizwan with 1100 colonies /100 ml. After the application of eliminating procedures the load of the pickle was reduced to 11 colonies/100 ml.

DISCUSSION

Pickles are the most contaminated fermented product that is shown by the recent studies.

The colonies of *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp., *Escherichia coli*, *Bacillus* spp. and *Lactobacillus acidophilus* have been observed on MRS and Nutrients Agar plates. As we studied previously that various species of *Aspergillus*, *Rhizopus* and *Alternaria* are dominant in the pickle of mixed vegetables like carrots, cabbages, cucumbers and peppers (Walbeck, 1973). *Lactobacillus* spp are the probiotics which are usually present in fermented products so it must be isolated from pickles but rest of the organisms are included in contamination and they may be harmful for our health. As the study said that the normal host can be affect by *Aspergillus* infections which can lead to the superficial to systemic infections (G. P. Bodey and S. Vartivarian, May 1998).

Further studies on pickles were conducted to examine the microbial load in pickles by MPN method. The samples were taken from National, Shan, Shangrila, Sundip, Rizwan, Hijazi and Baba companies, homemade pickles were also taken to check their microbial load and suggest that it is favorable for consumption or not. According to the results, the most favorable pickle for consumption is Sundip Company because it has 28 colonies/100 ml of Sundip pickle sample. On the other hand most dangerous pickle for consumption is from Rizwan Company because we got 1100 colonies/100 ml from Rizwan pickle sample. Rest of the samples gave such results, 220 colonies/100 ml of Hijazi pickle sample, 150 colonies/100 ml of National pickle sample, 150 colonies/100 ml from Baba pickle sample, 150 colonies/100 ml from Shan pickle sample, 220 colonies/100 ml from Shangrila pickle sample, 2 homemade pickle samples from two different places both gave 220 colonies /100 ml and one pickle sample taken from local shop gave 460 colonies/100 ml.

So, the results showing that pickle is the most contaminated product which can be harmful

for consumption due to known reasons. This can be eliminated by following good hygienic practices and applying those methods which affect the growth of undesired organisms. Therefore, to make the pickle suitable for consumption, some methods were applied to reduce contamination in the pickle of National Company. Apple cider was added in pickle, except this chopped garlic and asafoetida was also added in pickle. After applying these methods there were a great difference in the results, MPN was again conducted of that treated pickle the results was incredible. There was only 11 colonies in 100 ml of sample was left. This load can be further reduced by increasing the amount of any of these three substances. Many studies also evidences about there antimicrobial activity such as;

Garlic can also be added to overcome the microbial growth; it contains an antibacterial agent so called allicin. One gram of allicin is equal to 15 units of penicillin. It is the most readily available dried substance which has the higher affinity of the antimicrobial activity (Serge ankari and David Mirelman, February 1999).

Asafoetida is a dried latex gum which can be obtained by the roots of a plant *Ferula assafoetida* which was readily used in the past centuries as traditional medicines and for other purposes. It is a type of spice and mainly used in almost all the foods which are difficult to digest and also for the many diseases such as asthma, gastrointestinal disorders, and intestinal parasites and many others. It also has antifungal, anti-diabetic, anti-inflammatory, anti-mutagenic and antiviral activities (Milad Iranshahy and Mehrdad Iranshahi, March 2011).

Vinegar is well known for the antibacterial activity and have been using from the past centuries for healing wounds and septic cuts or burns “The use of vinegar to fight infections

and other acute conditions dates back to Hippocrates (460-377 BC), who recommended a vinegar preparation for cleaning ulcerations and for the treatment of sores. Oxymer, a popular ancient medicine composed of honey and vinegar, was prescribed for persistent coughs by Hippocrates and his contemporaries, and by physicians up to modern day" (Carol S. Johnston, and Cindy A. Gaas, 2006).

CONCLUSION

The study showed that the pickle is the most contaminated fermented product due to the unhygienic handling and some factors which lead to the microbial growth. Many organisms were isolated from pickle including *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* spp., *Escherichia coli*, *Lactobacillus acidophilus* and *Bacillus* spp. By applying MPN method we got the highest number of colonies which is really a threat for the individuals who use pickle at daily bases. It must be free from harmful microorganisms and favorable for the consumption for people. In this study, the techniques were used for the elimination of microbial load that gave the efficient results. It means that these methods can be very useful to reduce bioload in pickles that would be very beneficial for pickle industries and suitable for consumption.

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