Determination and Elimination of Microbial Load from Pickle's Brand in Karachi

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Abstract: The study is the elementary step to determine the causes of spoilage of fermented products and factors that could be remove to acquire the hygienic fermented products. Pickle is one of the mostly used fermented products especially as a side dish in Eastern countries, but it is developing a high rate of infectious diseases either due to its failing probiotic activity or due to increase harmful microbial flora in the pickle which are dominating the probiotics. The study is based on the determination of microbial load present in the pickle using MPN technique. The growth of fungi such as *Aspergillus niger, Aspergillus flavus,* Rhizopus, and bacteria *Escherichia coli, Lactobacillus acidophilus and* Bacillus were isolated from the pickles samples used in this study. By using MPN technique, It was observed 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 brand Sundip 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 dropped 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 one of the largest populations on earth and living in the different associations for their survival. There is different type of associations formed by the microorganisms. The mutual microorganisms are present in the association which have impact on the growth [1,2], nutrition [3], development and phylogenesis [4,5], give a shield against the pathogenic operators [6], and dispensation [7].

There is an illustration to assess their population on the planet that "our mouth contains 20 billion microorganisms" [8]. 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. Now, the question arises what are fermented products? How are they delivered? They are delivered by the procedure of fermentation.

Fermentation is the term utilized by microbiologists defined as "the chemical breakdown of a substance by bacteria, yeasts, or other microorganisms, typically involving bubbles and the giving off of heat" [9]. Dry salted lime pickle is a well known domestically manufactured fermented item. In India, the pickle is extremely sizzling and hot due the expansion of bean stew. Indian gooseberry and mango pickles are also well known. Khalpi is a customary cucumber pickle utilized as a part of the Himalayan district of India [10].

Fermented pickles also have beneficial bacteria that can control harmful intestinal microbes. The raw material is put away in briny water tank where they can remain preserved for twelve months. Pickling of plants is a moderately old strategy for nutritional resources. It is assessed that the primary pickles were delivered more than 4,000 years back utilizing cucumbers. In this way, the risk of contamination could be prevented with the purpose to prepare the pickle without irregular or exceptional hazardous elements; this can provide the hygienic pickle [11]. For the examination of microbial load, it is the need to apply MPN technique the Most Probable Numbers to determine about the zone of microbial abundance in the pickle and is it suitable for utilization or not? The "Most Probable Number" (MPN) technique is a valuable, if underutilized, tool for the microbiologist.

The first and best step to dispense with the harmful microorganisms from pickle is the increase quantity of apple juice i.e. pure vinegar which makes the pickle acidic which is the unfavorable environment for microorganisms [12]. Another approach to elimination of the parasite from pickle is the increase amount of asafoetida which is highly active for antifungal activity. 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 [13]. Garlic can likewise be added to

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prevent or kill the microbial load; it contains an antibacterial compound named as allicin. One gram of allicin is equivalent to 15 units of penicillin. Allicin in its pure structure was found to display i) antibacterial activity against a wide variety of Gram-negative and Gram-positive microorganisms, including multidrug-resistant enterotoxicogenic strains of Escherichia coli [14].

MATERIAL AND METHOD

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 Agar. Next day plates were observed for bacterial and fungal colonies.

Identification

The fungal and bacterial colonies were than identified using Scotch Tape Method (for fungus) and Gram Staining (for bacteria and yeast), then we observed them under microscope.

MPN Technique

Took a sample and made solution of 5ml in saline. Then prepared the 10 fold dilution in series, 1 ml solution of each dilution are inoculated into triplicate broth culture tubes and incubated at 35-40°C for 24 hours. All tubes were examined for turbidity and the pattern of growth in the tubes score.

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 study, 6 microorganisms are isolated that are *Aspergillus niger, Aspergillus flavus,* Rhizopus*, Escherischia coli,* Bacillus and *Lactobacillus acidophilus*. The results of MPN technique are Pickle Hijazi with 220 colonies/100 ml, Pickle National with 150 colonies/100 ml, Pickle Baba with 150 colonies/100 ml, Pickle Home 1 with 220 colonies/100 ml, Pickle Home 2 with 220 colonies/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.

Observations

Table 1: Microbial Load Using MPN Technique

Name of Samples	Triplicate tubes 10ml	Triplicate tubes 1 ml	Triplicate tubes 0.1ml	Probable No. of organisms
Hijazi pickel	3+	2+	2+	220 colonies/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 2: 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

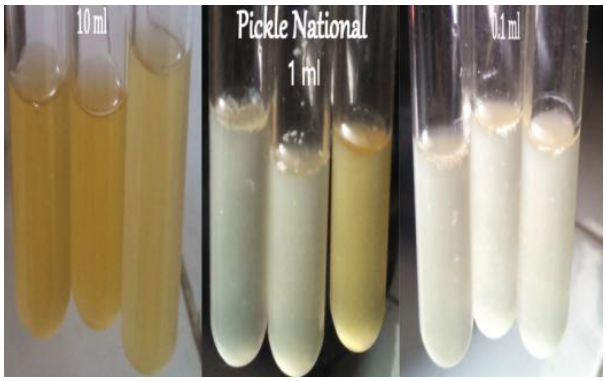

Figure 1: MPN Technique before elimination of Microbial Load.

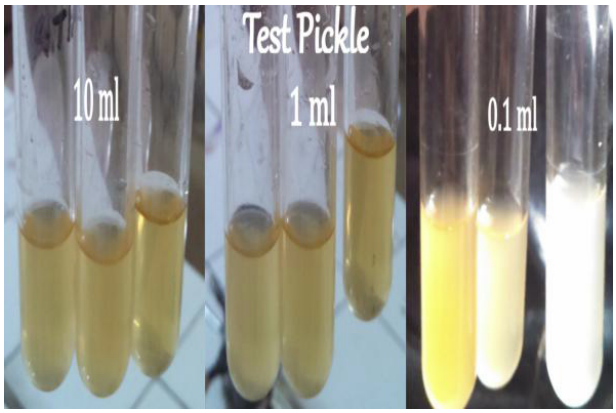

Figure 2: MPN Technique after elimination of Microbial Load.

DISCUSSION

Pickles are the most contaminated fermented product that is shown by the recent studies. The colonies of *Aspergillus niger, Aspergillus flavus,* Rhizopus spp*, Escherischia coli,* Bacillus 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 [15]. Lactobacillus species 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 [16].

Further studies on pickles were conducted to examine the microbial load in pickles by MPN technique. 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 evidence about their 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 [14].

Asafoetida is a dried latex gum which can be obtained by the roots of a plant *Ferula assa-foetida* 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 [13].

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. Oxymel, 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” [14].

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 *Aspergillas niger*, *Aspergillas flavus*, Rhizopus, *Escherischia coli*, *Lactobacillus acidophillus* and Bacillus. By applying MPN technique, we obtained 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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APPENDIX

Composition of MRS Agar

MRS Agar Typically Contains (w/v)

- 1.0 % peptone
- 0.8 % egg extract
- 0.4 % yeast extract
- 2.0 % glucose
- 0.5 % sodium acetate trihydrate
- % polysorbate 80 (also known as Tween 80)
- % dipotassium hydrogen phosphate
- % triammonium citrate
- 0.02 % magnesium sulfate heptahydrate
- 0.005 % manganese sulfate tetrahydrate
- 1.0 % agar
- pH adjusted to 6.2 at 25°C

Composition of Nutrient Broth

Distilled water	1	L 500 ml
beef extract	1 g	0.5 g
Peptone	2 g	1 g
Sodium chloride (NaCl)	5 g	2.5 g
Yeast extract	5 g	2.5 g

REFERENCE

[1] Braendle C, Miura T, Bickel R, Shingleton AW, Kambhampati S, Stern DL. Developmental origin and evolution of bacteriocytes in the aphid–Buchnera symbiosis. PLoS Biol 2003; 1(1): e21. https://doi.org/10.1371/journal.pbio.0000021

[2] Koropatnick TA, Engle JT, Apicella MA, Stabb EV, Goldman WE, McFall-Ngai MJ. Microbial factor-mediated development in a host-bacterial mutualism. Science 2004; 306(5699): 1186-8. https://doi.org/10.1126/science.1102218

[3] Baumann P. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu Rev Microbiol 2005; 59: 155-89. https://doi.org/10.1146/annurev.micro.59.030804.121041

[4] Bandi C, Dunn AM, Hurst GD, Rigaud T. Inherited microorganisms, sex-specific virulence and reproductive parasitism. Trends in Parasitology 2001; 17(2): 88-94. https://doi.org/10.1016/S1471-4922(00)01812-2

[5] Hurst GD, Jiggins FM. Male-killing bacteria in insects: mechanisms, incidence, and implications. Emerging Infectious Diseases 2000; 6(4): 329. https://doi.org/10.3201/eid0604.000402

[6] Piel J. A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of Paederus beetles. Proceedings of the National Academy of Sciences 2002; 99(22): 14002-7. https://doi.org/10.1073/pnas.222481399

[7] MacDonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. Science 2005; 307(5717): 1920-5. https://doi.org/10.1126/science.1106442

[8] Landers B. oral bacteria how many how fast. the-landers-file 29(7).

[9] Pumphrey B, Julien C, BV NB. An Introduction to Fermentation. Fermentation Basics, New Brunswick Scientific (UK and The Netherlands) 1996; 1-24.

[10] Tamang JP. Lactic acid bacteria in traditional food fermentation. In Fermentation biotechnology-industrial perspectives proceedings of the symposium, all India Biotech association and Department of Biotechnology, Government of India p 1998; pp. 102-108.

[11] Akbudak B, Ozer MH, Uylaser V, Karaman B. The effect of low oxygen and high carbon dioxide on storage and pickle production of pickling cucumbers cv.'Octobus'. Journal of Food Engineering 2007; 78(3): 1034-46. https://doi.org/10.1016/j.jfoodeng.2005.12.045

[12] Johnston CS, Gaas CA. Vinegar: medicinal uses and antiglycemic effect. Medscape General Medicine 2006; 8(2): 61.

[13] Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)—A review. Journal of Ethnopharmacology 2011; 134(1): 1-0. https://doi.org/10.1016/j.jep.2010.11.067

[14] Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes and Infection 1999; 1(2): 125-9. https://doi.org/10.1016/S1286-4579(99)80003-3

[15] Bodey GP, Vartivarian S. Aspergillosis. European Journal of Clinical Microbiology and Infectious Diseases 1989; 8(5): 413-37. https://doi.org/10.1007/BF01964057

[16] Walbeck YW. Fungal toxins in food. Canadian Food Technol 1973; 6: 96-10. https://doi.org/10.1016/S0315-5463(73)73986-9

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