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Mycoflora involved during pickle formation in mango (*Mangifera indica* L.)

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In the present study, three kinds of pickle (oily, non-oily and salted) were made from green mango to observe changes in fungal population, pH and total acidity. The fungi were isolated from the unwashed and washed mangoes and the three types of mango pickle at different stages of fermentation by serial dilution-agar plate method on the malt extract agar medium. *Aspergillus, Penicillium, Curvularia* (Deuteromycetes), *Mucor, Rhizopus* (Zygomycetes), *Saccharomyces* sp. and *Hanseniaspora* sp. (yeasts) (Blastomycetes) were identified. Fungal population of unwashed mangoes was found higher as compared to washed mango fruits. There was 60% (qualitative) and 40.3% (quantitative) reduction of fungal population after washing. Maximum fungal population was recorded in non-oily pickle followed by oily pickle and minimum in salted pickle. The total acidity expressed as % lactic acid produced during formation of three kinds of pickle varied between 0.14% and 2.7% and pH varied between 2 and 4. The oily and salted pickles were the best method to preserve the mango slices.

Key words : *Aspergillus,* mango, molds, non-oily, oily, pickle, spoilage, yeasts

INTRODUCTION

Mango (*Mangifera indica* L.), a member of the family *Anacardiaceae,* is the most popular and choicest fruit of India, where the area of mango cultivation is about 60-70% of the total fruit-growing area (0.81 million ha). It has an excellent flavour, fragrance, taste, and high nutritional value that have made it one of the choicest fruits (Hussain *et al.,* 2002; Reddy and Reddy, 2005; Bally, 2006; Tharanathan *et al.,* 2006; Ojokoh, 2007).

Amongst preserved foods, pickles are common appetizer in South Asian countries, particularly in India and Pakistan (Khan *et al.,* 2005). The surplus amounts are preserved in the form of dried slices, pickle, canned pulp and also as juice and powder (Reddy and Reddy, 2005). Mango pickle (*Am Ka Achar*) with or without oil is the most popular green mango product in India. In most preserved foods, microbial growth causes undesirable changes in the odour, colour, taste, texture and appearance of the food. In most cases, ingredients of homemade pickles act as carriers of microbial contamination (Rhyall and Pentzer, 1974; Byran, 1974). The general technique of producing brine-cured pickles have been in use since many years, but it often leads to serious economic losses because of microbial spoilage of pickle due to some bacteria and molds from such conditions as blackening (*Bacillus nigrificans*), bloaters (*Enterobacter* spp. lactobacilli and pediococci), softening (*Bacillus, Fusarium, Penicillium, Phoma, Cladosporium, Alternaria, Mucor, Aspergillus* and other spp.), colour off and so on. Several molds especially aspergilli and penicillia produce mycotoxins such as aflatoxin, ochratoxin, cyclopiazonic acid, sterigmatocystin, fumitremorgens, territrems, echinulin cyclohalasins, tryptoquivalines, penitrime A, secalonic acid D, patulin and citrinin that exhibit a wide range of toxicities (Doyle *et al.,* 2001; Jay *et al.,* 2005). During the production of fruit and vegetable products, the processes of harvesting, washing, cutting, slicing, packaging and shipping can create additional conditions where contamination can occur (Lee, 2004). Barriers to the growth of the molds may be necessary to reduce the risk of microbial spoilage of mango slices

manufactured under commercial conditions. Keeping in view the dangerous consequences of the mycotoxins, there is a need for controlling microbial load in pickles for protecting the consumers from food poisoning.

The objective of the present study has been to study the surface mycoflora of mangoes and the enumeration of molds and yeasts from oily, non-oily and salted pickles of mango and the changes in pH and total acidity during the fermentation process.

MATERIALS AND METHODS

Preparation of pickle

Three kinds of pickle (oily, non-oily and salted) were made in the laboratory from freshly collected green mangoes from an orchard located at the town Behat, Saharanpur, Uttar Pradesh. The constituents of the three types of pickle per kilogram fo mango slices used were : 40 g sodium chloride (nonionized), 50 g fenugreck seeds, 50 g ginger, 20 g turmeric, 25 g red chillies, 30 g black pepper, 30 g fennel and 300 ml mustard oil (oily pickle); 200 g salt, 75 g chillies and 10 g asafoetida (non-oily pickle) and 200 g salt (salted pickle) (Anonymous, 2004). The fermentation of mango slices was allowed to continue at room temperature for 28 days (Fig. 1). Sampling was made for isolation and enumeration of molds and for determination of pH and lactic acid at an interval of 14 days.

Isolation and enumeration of fungi

Serial dilution-agar plate method (also called viable plate count method) was used for the quantitative and qualitative determination of fungi (molds and yeasts) (Aneja, 2003) from unwashed and washed mangoes and the three kinds of pickle at different stages of fermentation. In this method, 1 g mango slices or 1 ml brine/liquid samples were added into sterile 9 ml water blanks and further dilutions were made up to 10^{-4}. Aliquots of 0.1 ml from various dilutions were added into the sterile Petri plates. To these inoculated plates, molten malt extract agar (MEA) supplemented with streptopenicillin was added. The plates were incubated at 25°C for 3-7 days in an inverted position. All the experiments were performed in triplicates. The isolated and purified molds and yeasts were maintained on MEA slants for identification and further use.

The fungal isolates were identified and colonies appearing on different plates of various dilutions were recorded. Quantitative estimation of yeasts and molds were made at different stages of fermentation. Colony forming unit/s (CFU/s) were calculated by applying the formula:

$$\text{CFU(s).g* or ml} = \frac{\text{Number of colonies (mean)} \times \text{dilution factor}}{\text{Volume plated (0.1 ml)}}$$

*Colony forming units/s per gram (not per ml) were calculated from unwashed and washed pomosphere of mango and from the three types of mango pickle at the stage (21st day of fermentation) when brine was not available for isolation of fungi.

Fungal identification

Representatives from each colony were selected from the serial dilution agar plates at each sampling time differing in the morphology i.e., shape and colour. Isolates were purified by repeated streaking/ subculturing techniques and were identified following the Manuals of Gilman (1967), Ellis (1971), Domsch *et al.* (1980) and Frazier and Westhoff (2003).

Determination of pH

The pH of each kind of mango pickle was determined by pH paper strips because brine was too less to be measured by pH meter.

Determination of total acidity

Total acidity is expressed as % lactic acid. Lactic acid was determined following the method Cappuccino and Sherman, (1996). Ten ml undiluted juice sample was added to an Erlenmeyer flask, followed by addition of 10 ml of distilled water. The contents were boiled for 1 minute to drive off the dissolved carbon dioxide. Five drops of phenolphthalein (1%) was added to the cooling contents. The titration with 0.1 N NaOH was carried out until a light pink colour persisted. The per cent lactic acid was calculated by using the formula:

$$\% \text{ lactic acid} = \frac{\text{Vol. of alkali used} \times \text{normality of alkali} \times 9}{\text{Vol. of sample taken}}$$

RESULTS AND DISCUSSION

A total of 7 molds (*Aspergillus luchuensis, A niger,*

A. flavus, Penicillium oxalicum, Curvularia clavata, Rhizopus stolonifer and *Mucor* sp.) and 2 yeasts (*Saccharomyces* sp. and *Hanseniaspora* sp.) were identified from the unwashed and washed whole mangoes as well as three types of pickle at different stages of fermentation.

Microbiological analysis of the unwashed whole mangoes revealed the association of four molds namely, *A luchuensis, C. clavata, R. stolonifer* and *Mucor* sp. and a yeast *Saccharomyces* sp., the fungi which are commonly found in the aerospora. Washed mangoes showed the presence of only two taxa, *A. luchuensis* and *Saccharomyces* sp., thus indicating a 60% qualitative reduction of the microbial load. The findings of quantitative estimation assayed by serial dilution-agar plate method showed that there was a reduction of fungal population from 137 $\times$ 10^4 cfu/g to 5.96 $\times$ 10^3 cfu/g on washing of mangoes. Thus, washing resulted both in reduction of qualitative and quantitative population of fungi, thus substantiating the findings of Ngarmsak *et al.* (2006), who also reported reduction in microbial population of mangoes when washed in water with or without chlorine.

Qualitatively, non-oily pickle during the course of fermentation revealed the presence of *A. luchuensis, A. niger, A. flavus, P. oxalicum, Hanseniaspora* sp. and *Saccharomyces* sp. The molds and yeasts varied qualitatively during the course of fermentation in mango pickle (*Am Ka Achar*). *A. luchuensis, A. niger* and *Saccharomyces* sp. were common to all three types of pickle. *Hanseniaspora* sp. was common to non-oily and salted pickles, while *A. flavus* and *P. oxalicum* were exclusively associated with non-oily pickle. *Hanseniaspora* sp. was not isolated from oily pickle (Table 1) thus, revealing that the maximum numbers of fungi are associated with non-oily pickle. *Hanseniaspora* sp. has been

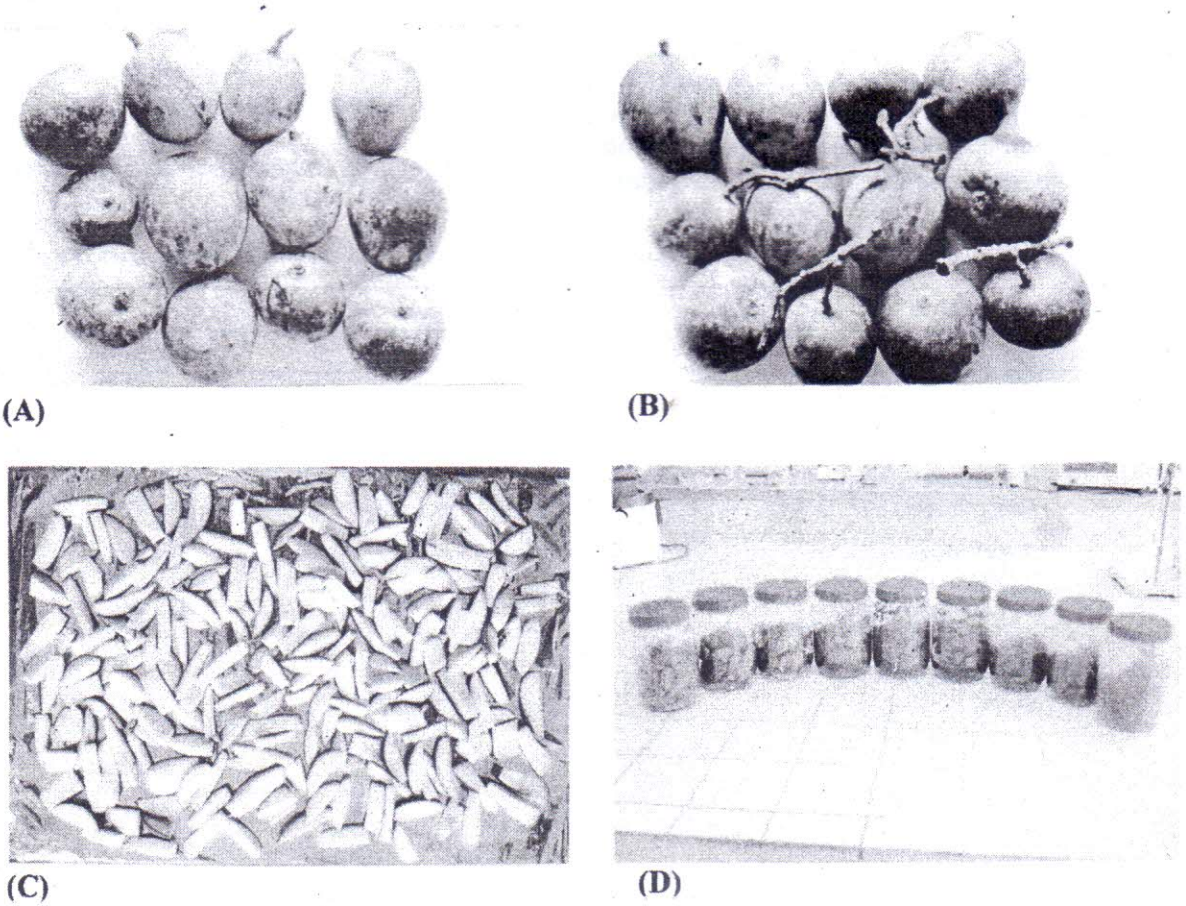

Fig. 1: Unwashed mango fruits (A), washed mango fruits (B), mangoes pieces for pickle preparation cut with sterile knife (C), three kinds of pickle allowed to ferment in glass jars. (Left to right) First three jars of salted pickle followed by three jars of oily pickle and finally three jars of non-oily pickle (D)

found to be associated with a variety of foods, especially figs, tomatoes, strawberries, citrus fruits, and cacao bean fermentation (Jay *et al.,* 2005). Presence of *A. niger* in all the three kinds of pickle may be both beneficial and harmful to humans as this mold is known as a producer of citric and gluconic acids, which are used as a food additives and carrier for calcium and sodium (Prescott *et al.,* 2005). On the other hand, *A. niger* may be harmful as some strains are the producer of a heat stable toxin (ochratoxin A), which are hepatotoxic and nephrotoxic in nature (Jay *et al.,* 2005). Some strains of *A. flavus* produce mycotoxins such as aflatoxin B_1, B_2 and cyclopiazonic acid, which are acutely and chronically toxic in animals and humans, producing acute liver damage, liver cirrhosis, tumour induction, and teratogenesis (Doyle *et al.,* 2001). Other mold, *P. oxalicum* is a known producer of secalonic acid D, which has significant animal toxicity (Doyle *et al.,* 2001), thus non-oily pickle is not safe for human consumption.

Oily pickle showed the presence of *A. luchuensis, A. niger* and *Saccharomyces* sp. in the beginning. A quantitative reduction in molds was observed in the oily pickle after 3rd day of fermentation. It may be attributed to the creation of anaerobic conditions and secondly due to the presence of allyl isothiocyanate (AIT) which has been reported to be inhibitory in action against molds, yeasts and bacteria in the range of 16 to 110 ng/ml in vapour phase (Issiki *et al.,* 1992).

The mycoflora recorded from the salted pickle was similar to the non-oily pickle, excepting two molds *A. flavus* and *P. oxalicum* (Table 1) which were not observed in the salted pickle. There was a quantitative reduction in the molds in salted pickle after 3rd day of fermentation because of the resulting osmotic removal of water from the food which reduced the water activity to a level according to the quantity of salt added and thus rendered conditions less favorable to microbial life (Lee, 2004).

Molds and yeasts varied qualitatively during the course of succession in three types of pickle (Table 1). Fungal population decreased from initial to the later stages of fermentation. A similar trend was observed in the fungal load during the course of fermentation. Interestingly the oil resulted in the reduction of both qualitative and quantitative population of molds and yeasts, suggesting that the oily pickle is safe for human consumption.

The total acidity expressed as % lactic acid

Table 1: Qualitative and quantitative changes in molds and yeasts at different stages of fermentation in three kinds of pickle.

Day of fermentation	Molds/Yeasts	CFU/ml		
		Oily pickle	Non-oily	Salted pickle
1	*Aspergillus luchuensis*	9.67×10^2	4.67×10^2	9.66×10^2
	A. niger	4.00×10^2	3.00×10^2	1.00×10^2
	A. flavus	—	2.67×10^2	—
	Saccharomyces sp.	5.67×10^2	6.00×10^2	1.66×10^2
	Hanseniaspora sp.	—	8.66×10^2	1.66×10^2
3	*A. luchuensis*	6.33×10^2	3.00×10^2	7.60×10^2
	A. niger	1.00×10^2	—	3.00×10^2
	Saccharomyces sp.	—	13.7×10^2	1.00×10^2
	Hanseniaspora sp.	—	18.3×10^2	—
7	*A. luchuensis*	—	2.33×10^2	—
	Saccharomyces sp.		2.66×10^2	—
	Hanseniaspora sp.		4.00×10^2	—
14	*P. oxalicum*	—	3.33×10^2	—
	Saccharomyces sp.		2.67×10^2	—
	Hanseniaspora sp.		1.67×10^2	—
21	*A. luchuensis*	$1.33 \times 10^{2*}$	—	$3.33 \times 10^{2*}$
	Saccharomyces sp.	—	1.67×10^2	—
28	No growth	—	—	—

— ; No growth; *Brine was too less so qualitative and quantitative changes in molds and yeasts were done per gram of mango slices at the 21st day of fermentation.

produced during three kinds of pickle formation varid between 0.14 and 2.7% and pH varied between 2 and 4. The spoilage of the pickles by bacteria and molds is encouraged if the pH reaches 4 as reported by Jay *et al.* (2005). Fruits which are low in pH are usually spoiled by molds and yeasts due to their acid tolerance nature (Rhyal and Pentizer, 1974).

Table 2. Comparison of total fungal population at different stages of fermentation in three kinds of pickle

Types of mango pickle	Total fungal CFU/ml ($X10^2$)					
	Fermentation time (days)					
	1	3	7	14	21	28
Oily	19.34	7.33	–	–	1.33*	–
Non-oily	25.0	35.0	8.99	7.67	1.66*	–
Salted	13.98	11.60	–	–	3.33*	–

'—' No growth; *Brine was too less so quantitative changes in molds and yeasts were done per gram of mango slices at the 21st day of fermentation.

Conclusively, a comparison of the data of the fungal population in the three kinds of pickle revealed that non-oily pickle has the maximum population followed by the oily pickle and molds and yeasts in salted pickle has the lowest population (Table 2). It may be suggested that oily and salted pickles seem to be best for human consumption since, no molds and yeasts were recorded after 3rd day of fermentation and the populations too were less ever at the first sampling i.e. 3rd day of fermentation. The reoccurrence of fungi in very few numbers at the 21st day of fermentation may be due to the aerial contamination. Presence of mycotoxin producing molds such as *A. flavus* and *P. oxalicum* in the non-oily pickle at different stages of fermentation during study suggests that this type of pickle preparation is to be avoided.

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