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Some Studies on Spoilage Fungi of Pickles

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ABSTRACT

Samples of spoiled mixed pickles in oil collected from several locations showed high water activity and pH, which is promising for fungal contamination. Various filamentous lipolytic fungi viz; *Aspergillus, Penicillium, Alternaria, Rhizopus* and *Mucor* were isolated. These isolations were able to produced lipolytic enzymes, which are responsible for the spoilage and rancidity of oil pickles. The lipolytic activity exhibited by spoilage fungal strains of pickles varied in the order of *Penicillium* > *Rhizopus* > *Aspergillus* > *Mucor.*

Key Words: Spoilage fungi; Pickles; Lipolytic enzymes; *Penicillium; Rhizopus; Aspergillus; Mucor*

INTRODUCTION

Amongst preserved foods, pickles are common appetizer in "South Asian" countries, particularly India and Pakistan. Generally, pickles are of two types; oil pickles and vinegar pickles. However, oil pickles are more common in India and Pakistan. The spoilage of home made pickles in mainly because of poor processing and ineffective preservation (Somithri, 1967). The quality of processed food is influenced by the quality of ingredients. Although, certain ingredients may contribute a small part of the total food value, they may add substantial microorganisms (Benerwart & George, 1981). Therefore, spoilage of food commodities with special reference to spoilage has gained a considerable intention for the maintenance of the health of the society (Tomikins, 1951). In most preserved foods, microbial growth causes undesirable changes in the odor, colour, taste, texture, or appearance of the food. In most cases, ingredients of pickles act as carriers of microbial contaminants (Rhyall & Pentzer, 1974). The utilization of poor quality vegetables, fruits and spices also influences the spoilage intensity in the preserved food, particularly in the homemade pickles. Spices are often the source of high microbial numbers (Byran, 1974). Pepper is the main ingredient of potentially consumed and usually highly contaminated with bacteria and fungi. Generally, oil pickles containing fruits are spoiled by *Penicillium italicum, P. digitatum* and *P. expansum.* Berries are predominantly carriers of *Botrytis cinerea* and *Mucor mucedo.* When carrots, cabbage, cucumbers and peppers are in pickles, various species of *Aspergillus, Rhizopus* and *Alternaria* become dominant (Walbeck, 1973). Some oils as mustard oil has some preservative effective (Anderson *et al*., 1973). In general, ordinary or common types of food spoilage are caused by changes of a biological nature. Food may, however, be spoiled by the introduction of undesirable or toxic chemicals. The biological changes attributed to the growth of bacteria, yeast, molds change caused by enzymes and in some cases changes resulting from auto–oxidations, but in most cases, food spoilage microorganisms are responsible. Bacon (1958) reported that minimal water activity for the production of a mycotoxin by a wide variety of mold genera and species is 0.81. This study was carried out to (i) isolate and identify spoilage fungi from oil pickles, (ii) evaluate their lipolytic activities, and (iii) study the inhibitory effect of some chemical preservatives on the growth of these fungi.

MATERIALS AND METHODS

Seven spoiled pickles samples were collected from various localities in wide neck sterilized glass bottles. Isolation and identification of spoiled fungi was carried out by methods recommended by Somithri, (1967). One loopful from spoiled sample of mixed pickle in oil was taken and spread by streaking out to potato dextrose agar medium. Five plates from each sample were prepared. The plates were incubated at 30ºC for 72 h. The plates were observed under stereomicroscope and the characteristics of colonies were recorded. Fungal colonies from each plate were selected at random and purified on malt agar slants prior to incubation. The isolated and purified fungal cultures were maintained on malt extract agar slants for further studies.

One ml of monoxal 0.1 as suspending agent was used to suspend fungal spores in slant. The suspending agent was prepared by dissolving 0.2 mL monoxal 0.1 in 400 mL distilled water. Effectiveness of different concentrations of chemical preservatives (Sodium benzoate 0.5%, Potassium sorbate 0.5%, Glycerol 2%, Sorbitol 5%, Propylene 5%) was evaluated by using potato dextrose agar in test tube. The experiment was repeated thrice. Nile blue agar medium was used to determine the lipolytic activity of isolated fungi.

RESULTS AND DISCUSSION

Table I shows different samples of spoiled mixed oil pickles collected from various localities, their ingredients, pH and water activity (aw). This table also shows variations in ingredients of oil pickle in each locality. Despite these, a slight variation in pH also indicates the rancidity or acidity

Table I. Proximate composition, water activity and pH of spoiled oil pickle collected from various sources

Sample No.	*Collection Source*	*Proximate Ingredients*	*Aw (Water activity)*	*pH*
1	Village	Mango, Lemon, Lassora, Spices, Oil, Salt.	0.938	5.0
2	Village	Mango, Lasoora, Carrot, Spices, Oil, Salt.	0.835	5.0
3	Village	Mango, Green Chillies, Horse, Radish, Spices, Oil, Salt.	0.90	6.1
4	Data Darbar Market	Mango, Lemon, Carrot, Lassora, Spices, Oil, Salt.	0.93	6.0
5	Sanda Road	Mango, Lassora, Spices, Oil, Salt	0.86	5.6
6	Krishan Nagar	Mango, Lassora, Garlic, Lemon, Oil, Spices, Salt.	0.91	6.0
7	Mozang Market	Green Chillies, Mango, Lemon, Spices, Oil Salts.	0.81	6.0

aw at 25 °C

in the spoiled pickles attributed to the growth of fungi. It is well established that fruits have low pH which inhibits bacterial growth, however, usually spoiled by yeasts and molds due to their acid tolerant nature (Smith, 1955; Rhyall & Pentizer, 1974).

In addition, vegetables and fruits have low buffering capacity, since the use of small amount of acid also lowers the pH significantly which provide promising growth condition for spoilage fungi. Similarly, all the samples have aw 0.81 to 0.938 while aw should be less than 0.81 in order to preserve the oil pickles for a longer period (Bone, 1973).

Results presented in Table II show lipolytic activity of spoilage fungi isolated from mixed pickle oil. It has been observed that lipolytic characteristics of fungal isolates depend upon the fat in the substrate (Shaw & Change, 1988). In addition taxonomically close strains may also produce lipases of different types. Therefore, lipase–producing isolates of *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* have shown considerable variation in the zones formation in nile blue solution.

It is evident that there are variations in the lipolytic activity in each isolate of *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., and *Mucor* sp. which are attributed to the instability of the auto synthetics activities of the cellular material during the growth process as a result of genetic variability in the mold and to certain physiological constrains such as a result of genetic variability in the mold and to certain physiological constraints such as the allostrec

Table II. Lipolytic activity of different fungi at different pH levels

	Lipolytic activity					*pH*				
Isolates	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Species										
Penicillium sp.	+	+	+	+	+	6.0	6.0	6.1	7.0	7.0
Rhizopus sp.	+	+	+	+	+	6.8	6.7	7.0	7.1	7.0
Mucor sp.	+	–	–	–	–	6.0	6.0	6.0	6.0	6.8
Aspergillus sp.	–	–	+	+	+	6.0	6.0	6.8	6.3	6.6
Alternaria sp.	+	+	+	+	+	6.0	6.0	6.0	6.0	6.0

+ Isolates showed lipolytic activity; – Isolates showed no lipolytic activity

Table III. Effect of various chemical preservatives on fungal growth

	Concentration of preservatives				
Organisms	**Sodium Benzoate (0.5%)**	**Potassium Sorbate (0.5%)**	**Glycerol (2.0%)**	**Sorbitol (5.0%)**	**Propylene glycol (5.0%)**
Alternaria sp.	–	–	–	–	+
Mucor sp.	+	+	–	–	–
Penicillium sp.	+	–	–	–	+
Rhizopus sp.	–	–	–	–	–
Aspergillus sp.	–	–	–	+	–

mechanisms. This study also confirmed that increased size of zones indicating lipase production was higher in *Penicillium* sp. and *Rhizopus* sp. The pH of fermented mash ranged between 6–7 during lipase production. Conclusively lipolytic activity varies in the order of *Penicillium* > *Rhizopus* > *Aspergillus* > *Mucor*.

Table III shows a large variation in the effectiveness of these chemical preservatives on the growth of spoilage fungi. The 2.0% glycerol v/v was one of the most effective preservative and absolutely inhibited the growth of fungi. However, certain fungi such as *Penicillium & Mucor* exhibited the resistance to 0.5% sodium benzoate. The addition of 5% sorbitol almost inhibited the growth of all the fungi except *Aspergillus* comparatively. *Alternaria* and *Penicillium* were able to grow in the presence of 5% propylene glycol concentration. In general the effectiveness of the chemical is increased with the increase in concentration. It has suggested that very high levels are not desired due to potential adverse effect on food quality and toxicity to human.

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