

# FUNGAL DETERIORATION OF SOME EDIBLE FRUITS AND THEIR BIOCHEMICAL CHANGES OF AMYLASE PRODUCTION.

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**Abstract**— Production of microbial enzymes at the industrial scale and their commercialization has gained a lot of focus and importance. Some of the industrially important enzymes from microbial origin include Lipase, Amylase, Proteases, Xylanases, Pectinases. The current work involves the use of fungi isolated from the decaying fruit for extraction of amylase. The organism were isolated from decayed fruit were cultured in modified CZ agar (soluble starch used instead of sucrose) plate as the isolation of fungi. The organisms were screened for the production of amylase using modified CZ media. Microscopic identification, isolation and morphology study revealed that the fungi isolated were *Fusarium sp.*, *Aspergillus niger*, Unidentified fungal fruit rotter, *Aspergillus candidus*, *A. flavus*. Qualitative estimation and quantitative estimation of amylase were done. The results they obtained can conclude that the use of *Fusarium sp.*, *Aspergillus niger* and Unidentified fungal fruit rotter are highly beneficial for the production of amylase.

**Index Terms**— Cucumber, Pomegranate, Lemon, *Fusarium*, *Aspergillus*, Amylase

## I. INTRODUCTION

In human nutrition, fruits play an important role for balanced diet. They provide not only energy and also packed with vital protective nutrients. Comparatively fruits are the cheapest source of nutritive food which helps in building resistant against diseases and the diseased fruits loss their nutritive and other beneficial properties.

Fruit rot is a general term has been used in describing about the occurrence of softening and deterioration of a fruit. Commonly, fruit rot can be grouped into two categories, known as field rot, which occur before harvest; and storage rot, which occur after harvest. There are some fungal species classified exclusively as field rot or storage rot pathogens, while others may cause rot in both field and storage. Since fruit rot is by multiple fungal species and other microorganism as well as interacting factors, hence it is sometimes called as a 'disease complex' (McManus, 2001). Fruit rot is categorized under post-harvest disease type, which the main causes are fungal infection, physical injury and physiological disorders (Sangeetha et al., 2009). Post harvest decays of fruit account for significant levels of losses upto 40% of the total crop.

From the moment a fruit is harvested it begins to decay. Senescence also induces the decay simulation of fruit. Microorganisms in the environment rush to take up residence feeding on moisture and nutrients. At the same time, chemical reactions breakdown cell from the inside out. In nature speedy decomposition is a positive thing, allowing plants to get their seeds into the soil. But in the kitchen we prefer to stall this process as long as possible. Major causes of loses are attributed to fungal decays, chilling injury and rapid maturation that enhance senescence process. There are some causes which inhibits the decaying of fruit:-

## II. RIPENING OF FRUIT PROMOTES FRUIT ROT

Fresh fruits undergo a natural stage of development known as ripening. This occurs when fruit has ceased growing and is said to be mature. Ripeness is followed by ageing (often called senescence) and easy breakdown of fruit. Soften fruit having thin cell wall rotted faster and fruits having thick cell wall may remain in an edible state for several days.

## III. MICROORGANISMS ARE EVERYWHERE

The fungi and bacteria that cause the spoilage in plant foods need water and nutrients to grow and reproduce. Diseases caused by fungi and bacteria commonly result in losses of fresh produce. There are also post-harvest losses in fresh produce. Fungal and bacterial diseases are spread for the most part by microscopic spores which are widely distributed in the air and soil and dead and decaying plant material. Fruits can become infected:

Through injuries caused by careless handling, by insect or other animal damage or through growth cracks.

Through natural pores in the above and below ground parts of plants, which allow the movement of air and water vapors into and out of the plant.

Post-harvest diseases can also be spread by:

Field boxes contaminated by soil or decaying produce or both.

Contaminated water used to wash produce before packing.

Contaminating healthy produce in packages.

There are several factors that may influence the occurrence of fruit diseases such as commodity type, cultivar susceptibility to the disease, storage situation (temperature, relative moisture, and air composition), yield maturity and ripeness phase, handlings used for disease control, yield

control approaches and storage hygiene (the tools used, the pickers' hands and containers to keep the yields). Fruit rot diseases are often categorized according to the infection is started. They are also named 'quiescent' or 'latent' infections, where the pathogen induces infection of the host at certain point in a period (typically at pre-harvest), but then pass in a period of inactivity until the physiological status of the host tissue changes in such a way, where the infection can continue. The quiescent infections may triggered by intense physiological changes of the fruit during ripening process (Coates & Johnson, 1997).

Extent and rapidity of deterioration of fruit depend on several physical and chemical factors. The physical factors such as pH, temperature, moisture and the chemical factors like minerals influence the fungal growth are evidently important in deterioration of fruit rot. There are three conditional point viz., minimum, maximum and optimum which serve to indicate growth requirement of a fungus for a particular factor. The optimum requirement supporting best growth, the minimum and maximum represent two extreme points beyond which growth become negligible. Growth which is an overall cumulative expression of a variety of metabolic activities, includes a large no of enzymatic reactions each of which may have its own requirement of physical as well as chemical condition.

#### IV. EFFECT OF pH ON DETERIORATION OF FRUIT

The influence of pH on fungal growth and metabolism is complex. Fungi like other organisms are susceptible to the reactions of surrounding medium upon which their growth and survival depends. From the available literatures, (Cochrane, 1958; Tandon, 1961) it is evident that most fungi grow over a fairly wide range of pH of the media, the limits varying with the species while few possess a narrower range. Studies on the effect of pH on growth of fungi are usually concerned with the initial pH of the medium. Optimum pH of the substrate not only favours growth and higher enzyme activity but also facilitates substrate utilization. Different enzymes have different pH optima for their activities, the favourable range generally varies between pH 4 to 8.

The mechanism of action of pH on growth is different such as influencing enzyme action, altering metal solubility, modifying surface reactions, preventing or facilitating entry of vitamin, organic acid or minerals into the hypha. A great deal of work is still needed to elucidate the mechanism of the effect of pH on growth of fungi.

#### V. EFFECT OF TEMPERATURE ON DETERIORATION OF FRUIT

Temperature has either a direct or indirect effect on almost all cellular activities. It plays an extremely important role in affecting various metabolic activities of fungi. A number of investigators like Cochrane (1958), Deverall (1965) have investigated the influence of temperature on fungal growth. They have shown that for each fungus there is a maximum, an optimum and a minimum temperature for metabolic activities and that exist in the neighborhood 30°C – 45°C, 20°C – 30°C and 0.5°C respectively. Thus a temperature growth curve has the typical skewed pattern which tends to become more pronounced as the optimum is higher but becomes more and more symmetrical as the optimum is lower.

The experimental work is designed with the fungal members causing rot of cucumber (*Cucumis sativus*),

pomegranate (*Punica granatum*) and sweet lemon (*Citrus limetta*) were carried out along the following lines :-

Isolation, characterization, identification and determination of the colonization frequency of fruit rotting fungi.

Qualitative detection for amylase production

Quantitative estimation for amylase production.

Relative efficacy of the isolates for the production of amylase in natural habitat.

### VI. MATERIALS & METHODS

#### A. isolation and identification of fruit rotting fungi

#### B. sample collection:

Rotten fruits are collected from market and brought to the laboratory.

#### 1) ISOLATION OF FUNG

1ml. of 1% anti-bacterial antibiotic (Gentamycin) was taken and put into sterilized Petri plate followed by pouring sterilized modified Czapek-Dox Agar(CDA) media (soluble starch used instead of sucrose) in each Petri plates. Then fruits were surface sterilized by 90% ethanol (for 1 minute), followed by 3% NaOCl (8 minutes) and finally 90% ethanol (30 seconds) and then washed with sterilized distilled water (2–3 times). Small blocks (1cm) of rotted portion were cut and placed into modified CDA media by sterilized scalpel and forceps. Petri plates were sealed by parafilm and put into incubator. Previous all processes were done aseptically. After 3–7 days when infected block shows further growth, subculturing was done in culture tube containing modified CDA media and put into incubator at 30°C. After 4–7 days of incubation when growth shown identification was done from subcultured specimen.

#### 2) IDENTIFICATION:

The isolated fungi from rotting fruit were identified based on Burnett and Hunter (1998), Gilman (1967), Whatnabe (2010) and internet information.

#### COLONIZATION FREQUENCY OF FRUIT ROTTING FUNGI:

The colonization frequency (CF %) Of a single fruit rotting fungi was calculated by using following formula (Hata and Futai, 1995)

CF % = (The no of segment colonized by fruit rotting fungal species)/(total no.of rotten fruit block) × 100

#### VII. QUALITATIVE DETECTION FOR AMYLASE PRODUCTION

Subcultured specimens were transferred at the centre of the Petri plates containing modified CDA media. After 4 days of incubation at 32°C. Petri plates with fungal growth were flooded with 1% iodine solution and left for 2 minutes. Then excess solution was decant from the Petri plates and observed and diameter of the utilized sugar was measured.

#### VIII. QUANTITATIVE DETECTION FOR AMYLASE PRODUCTION

EFFECT OF pH: 200 ml. of Czapek- Dox Broth media was taken in Erlenmeyer's flasks. The pH of 3 sets were adjusted to 5.2, 5.6, 6 with 2 (M) KOH. The flasks were then plugged , sterilized and inoculated and spore suspension of dominant fungus at 32°C ±1°C for 12 days. Mycelial

mates were then taken out from the flask and soluble sugar was estimated by DNS method.

### IX. EFFECT OF TEMPERATURE

Identical sets of flasks containing media at optimum pH 5.6 and inoculated as before were incubated at 28°C, 32°C and 36°C with a temperature fluctuation of  $\pm 1^\circ\text{C}$  for 12 days. Soluble sugar was estimated as stated earlier

Subcultured specimens were transferred at the sterilized Erlenmeyer's flask containing 200 ml. modified Czapek-Dox Broth media and kept in incubator for 12 days at optimum temperature 32°C and optimum pH 5.6. the culture filtrate was then obtained by filtering the 12 days old cultured broth through glass wool placed on a Buchner funnel under condition of vacuum filtration. 1 ml. of 1% soluble starch solution, 1 ml of sodium acetate buffer (pH 5.1) and 2 ml of culture filtrate were taken in test tubes and mixed to prepare reaction mixture. 3 such replicates for each set were maintained. A blank set containing 1 ml. of 1% soluble starch, 1 ml. of buffer and 2 ml. of controlled set also maintained as control. Then the mixtures were maintained in incubation at 37°C for 30 minutes. The test tubes containing mixtures after incubation the reaction was stopped by keeping the test tubes in boiling water bath for 10 minutes. 1ml of reaction mixture was taken in a test tube and mixed with 1 ml. of DNS reagent. The controlled set was also treated in a similar way. After treatment with DNS reagent the test tubes were kept in boiling water bath for 10 minutes till the colour develops. 1 ml. of Rochelle salt (40%) was mixed when the mixture was still warm. Then the tubes were cooled under tap water. The optical density were measured at 540 nm. wavelength against the control set. The released amount of soluble sugar was calculated by using a standard curve prepared by maltose solution.

### X. RELATIVE EFFICACY OF THE ISOLATES FOR THE PRODUCTION OF AMYLASE IN NATURAL HABITAT

Rotten fruits were cleaned and rotted portion was cut out by sterilized scalpel. Then rotted portion was crushed by mortar and pestle. The homogenate was centrifuged at 10000 rpm for 20 minutes. Supernatant was taken from centrifuged material and used as the culture filtrate and the subsequent steps were similar to the DNS method.

### XI. RESULTS & DISCUSSIONS

The fungal organism causing fruit rot of cucumber (*Cucumis sativus*), pomegranate (*Punica granatum*) and sweet lemon (*Citrus limetta*) were subjected to plate culture using modified Czapek-Dox Agar media to determine the characteristics features for identification purpose.

The cultural characteristics features and microscopic features noted from isolated fruit rotting fungi are as follows:

### XII. FRUIT ROTTING FUNGUS OF CUCUMBER (*CUCUMIS SATIVUS*)

#### A. *Fusarium sp.*

Plate study: Upper surface: 3 days old modified CDA culture shows moderately rapid growth, colony is whitish, cottony, slimy.

B. *Reverse: Central portion creamy, extreme margin creamy white.*

#### C. *Microscopic study*

Hyphae branched and conidiophores found laterally, bearing two types of conidia— macroconidia and microconidia. Macroconidia are sickle shaped blunt apex and may be 1 septate (length approximately 16.42µm, breadth approximately 2.94µm), 2 septate (length 17.45µm–18.69µm, breadth 3.22µm–3.8µm). Microconidia are small, oval and many in number. Length of microconidia is 6.30µm–7.91µm and breadth 1.00µm–1.93µm and smooth walled. Lacking of chlamydospore.

### XIII. FRUIT ROTTING FUNGUS OF POMEGRANATE (*PUNICA GRANATUM*):

#### A. *Aspergillus niger*

Plate study: Upper surface: 5 days old modified CDA culture shows moderately rapid growth. Colony is wooly, black, peripheral part of the colony is brownish

Reverse: Whitish. Microscopic study: Septate hyphae, long conidiophores (length approximately 256µm, breadth approximately 11.41µm), vesicle globose (diameter approximately 88.09µm), conidia globose with spiny wall, diameter of conidia 3.71µm–4.41µm.

### XIV. FRUIT ROTTING FUNGI OF SWEET LEMON (*CITRUS LIMETTA*):

Unidentified fungal fruit rotter :

Plate study: Upper surface: 7 days old modified CDA culture shows rapid growth, colony white, mat like, blackish dot on mat surface.

Reverse: Creamy white.

Microscopic study: Hyphae septate, length of each septal part is 24.22µm–31.79µm and breadth of hyphae approximately 3.40µm. Chlamydospore may be terminal (length approximately 9.67µm and breadth approximately 2.82µm) or intercalary (length approximately 9.44µm and breadth approximately 6.20µm). Conidia are rounded, 2.99µm–3.28µm in diameter.

#### A. *Aspergillus niger*

Plate study: Upper surface: 5 days old modified CDA culture shows moderately rapid growth. Colony is wooly, black, peripheral part of the colony is brownish

Reverse: Whitish.

Microscopic study: Septate hyphae, long conidiophores (length approximately 256µm, breadth approximately 11.41µm), vesicle globose (diameter approximately 88.09µm), conidia globose with spiny wall, diameter of conidia 3.71µm–4.41µm.

#### B. *Aspergillus candidus* :

Plate study: Upper surface: 7 days old modified CDA culture shows white floccose colony.

Reverse: whitish yellow.

Microscopic study: Mycelia branched, septate, conidiophores (length approximately 55.31µm and breadth approximately 6.41µm) bearing globose vesicle (approximately 21.13µm in diameter), conidia oval (length 5.76µm–7.63µm and breadth 2.88µm–3.76µm).

### C. *Aspergillus flavus* :

Plate study: Upper surface: 7 days old modified CDA culture shows greenish velvety colony with yellowish peripheral part.

Reverse: Creamy yellow.

Microscopic study: Septate hyphae with rather long conidiophores, vesicle round (approximately 45.63µm in diameter), conidia remain in chain, globose (3.40µm–4.13µm in diameter), conidial wall spiny.

The rotten fruit blocks of cucumber, pomegranate and sweet lemon were subjected to plate culture using modified CDA media showed the fungal growth in different frequencies (CF%). It is evident from Table-1 that, the colonization frequency of the isolated fungi varied the range from 88.89% - 22.22%. The highest colonization frequency was noted in both *Fusarium* sp. of cucumber and unidentified fungal fruit rotter of sweet lemon (88.89%). It was followed by *Aspergillus niger* of pomegranate and *Aspergillus niger* of sweet lemon (77.78%), *Aspergillus candidus* of sweet lemon (66.67%) and the lowest colonization frequency was noted in *Aspergillus flavus* of sweet lemon (22.22%).

Amongst the three substrate it has been found (Table-2) that, sweet lemon fruit shows higher efficiency in utilizing the modified CZ agar media and it was 25.505 cm<sup>2</sup> which was followed by pomegranate (19.625 cm<sup>2</sup>) and cucumber (18.086 cm<sup>2</sup>) in Petri plate study.

The organisms isolated from rotten fruit blocks of cucumber, pomegranate and sweet lemon were subjected to subcultured to subculture in 250 ml. of Erlenmeyer's flask containing 200 ml. of CZ broth media. They were allowed to grown separately. It has been found in Table-3 that, after 12 days incubation the organism unidentified fungal fruit rotter of sweet lemon released higher amount of amylase as represented by the amount of sugar (6.8 mg/µl/h) followed by *Aspergillus niger* of pomegranate (6.4 mg/µl/h) and *Fusarium* sp. of cucumber (6 mg/µl/h) respectively. So in optimum pH 5.6 and optimum temperature 32°C (shown by higher amount of mycelia growth) unidentified fungal fruit rotter shows highest efficiency than *Aspergillus niger* and *Fusarium* sp. respectively.

The rotten fruits of cucumber, pomegranate and sweet lemon were taken for estimation of sugar. The rotten fruits were cut separately into blocks (1 cm.) and sugars were estimated directly following earlier method. It has been found in Table-4 that, the amount of released sugar was highest in pomegranate (4 mg/µl/h) followed by sweet lemon (3.6 mg/µl/h) and cucumber (2.5 mg/µl/h) respectively. So it is evident that the rotting associated organism in pomegranate probably shows highest efficiency than those of other two substrates i.e. sweet lemon and cucumber. So the extant of production of amylase as reflected by the amount of released sugar shown highest in pomegranate than sweet lemon and cucumber in natural habitat.

From the result it has been found that in natural and laboratory condition the amount of released sugar is more in laboratory condition than natural habitat. In laboratory condition (in optimum pH 5.6 and optimum temperature 32°C) the organism shows higher growth. So quite naturally the organism shows higher efficiency in utilizing the substrate, as a result the amount of released sugar was higher in sweet lemon (6.8 mg/µl/h) followed by pomegranate (6.4 mg/µl/h) and cucumber (6 mg/µl/h) respectively. Similar result was found in Petri plate study by iodine test (i.e. utilizing of sugar highest in sweet lemon followed by pomegranate and cucumber respectively). But in natural

habitat the efficiency of the microorganism was lower. So naturally the amount of sugar released was lower. In laboratory condition, the microorganism do not face any competition than that of natural habitat condition. So the amount of released sugar was higher. This work was supported by Chaudhary et al. (1980) where they reported that organisms like *Pestalotia anonicola*, *Stachybotrys* sp. And *Trichoderma viridae* were decrease the total sugar and increase the reducing sugar. The amount of sugar content was also increased in mango when degraded by *Aspergillus niger* (Tandon, 1970; Pandey et al., 1974; Fush et al., 1980; Reddy and Laxminarayana, 1984). Similarly in loquat and capegoose-berry *Cladosporium oxysporum* and *Drechslera rostrata* utilized the total sugar within 10 days and the reducing sugar was also increased (Chaudhary et al., 1980). Singh and Sinha (1982) found that *Aspergillus flavus* and *A. parasiticus* cause depletion of total reducing and non-reducing sugars of guava fruit. Bilgrami et al. (1983) reported that there was a sharp decline in the level of total reducing and non-reducing sugars of dry fruits during *Aspergillus flavus* infestation. But our present investigation was also supported by Sawant and Gawai (2011). They found that, *Rhizopus stolonifer*, *Aspergillus flavus*, *Penicillium digitatum*, *Curvularia lunata* and *Fusarium moniliforme* were responsible for decrease in total sugar content of papaya fruit. Sawant and Gawai (2011) also reported that, *Aspergillus niger*, *Fusarium roseum*, *Rhizopus stolonifer* and *Gleospodium musarum* were decreases the total sugar and increases the reducing sugar content of banana fruit. This work corroborated our present investigation. Gadgile (2011) reported that *Aspergillus niger* caused the changes in sugars in mango pulp.

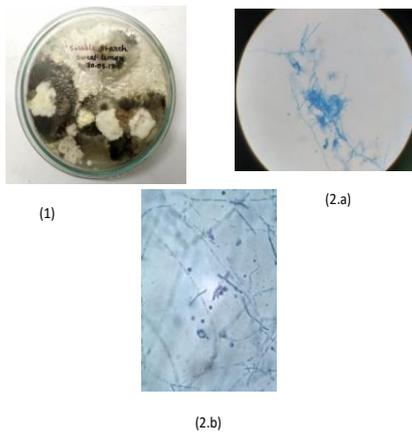


*Aspergillus niger*  
1. Plate study  
2. Microscopic study



### Unidentified fungal fruit rotter

1. Plate study
2. Microscopic study (a & b)



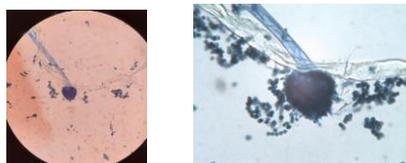
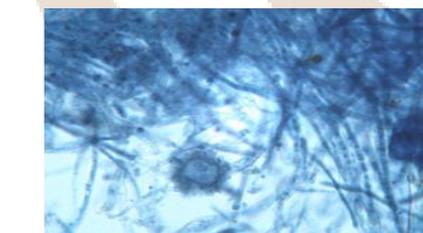
**Aspergillus niger**  
1. Plate study  
2. Microscopic study



**Aspergillus candidus**  
1. Plate study  
2. Microscopic study (a & b)



**Aspergillus flavus**  
1. Plate study  
2. Microscopic study (a & b)



NAME OF THE ISOLATED FUNGAL FRUIT ROTTER	TYPES OF PLANT MATERIAL USED	NO. OF ROTTEN FRUIT BLOCKS USED	NO OF ROTTEN FRUIT BLOCKS SHOWING FUNGAL GROWTH	COLONIZATI ON FREQUENCY (CF %)
<i>Fusarium m sp.</i>	Rotten fruit blocks	9	8	88.89%
<i>Aspergillus niger</i>			7	77.78%
Unidentified fungal fruit rotter			8	88.89%
<i>Aspergillus niger</i>			7	77.78%
<i>Aspergillus candidus</i>			6	66.67%
<i>Aspergillus flavus</i>			2	22.22%

Table-1 Colonization frequency of the fungal organisms in different rotten fruits

ORGANISM	SUBSTRATE	INCUBATION TIME	DIAMETER OF PETRI PLATE	AREA OF PETRI PLATE (X)	DIAMETER OF UTILIZED ZONE	AREA OF UTILIZED ZONE (Y)	UNUTILIZED AREA (X-Y)
<i>Fusarium sp.</i>	Cucumber	4 days	9 cm.	63.585 cm <sup>2</sup>	4.8 cm.	18.086 cm <sup>2</sup>	45.49 cm <sup>2</sup>
<i>Aspergillus niger</i>	Pomegranate		9 cm	63.585 cm <sup>2</sup>	5 cm.	19.625 cm <sup>2</sup>	43.96 cm <sup>2</sup>
Unidentified fungal fruit rotter	Sweet lemon		9 cm	63.585 cm <sup>2</sup>	5.7 cm.	25.505 cm <sup>2</sup>	38.08 cm <sup>2</sup>

Table-2

Production of amylase (reflected by utilized zone) by the fungal isolates in different fruit substrates

ORGANISM	SUBSTRATE	DAYS OF INCUBATION	pH	TEMPERATURE	AMOUNT OF RELEASED SUGAR
<i>Fusarium sp.</i>	Cucumber	12	5.6	32°C	6 mg/μl/h
<i>Aspergillus niger</i>	Pomegranate				6.4 mg/μl/h
Unidentified fungal fruit rotter	Sweet lemon				6.8 mg/μl/h

Table-3

Production of amylase (reflected by the amount of released sugar) by the fungal isolates in different fruit substrates

SUBSTRATE	AMOUNT OF RELEASED SUGAR IN NATURAL HABITAT
Cucumber	2.5 mg/μl/h
Pomegranate	4 mg/μl/h
Sweet lemon	3.6 mg/μl/h

Table-4

Production of amylase (reflected by amount of released sugar) in natural habitat by the fungal fruit rotter in different fruit substrates

## XV. ACKNOWLEDGEMENT

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