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Research Article

# Extraction of Carotenoids from Fungi Isolated from Different Food Sources

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## Abstract

Interest in carotenoids has increased considerably, due in part of the growing evidence have shown increased benefits in agriculture, aquaculture and poultry industry. Utility of Carotenoid as colouring agents for cooled sausage, soft drinks, baked goods and as additive is well known. Microbial synthesis offers a promising method for production of Carotenoids. These Explains increasing interest in production of microbial carotenoids as alternative synthetic food colorants. Contaminated food material was cut into small segments, sterilized, plated on Potato dextrose agar (PDA) and then incubated at 27 0C for 5 days. It resulted in pure culture and maintained by sub-culturing. Sub cultured biomass was grown and oven dried. Dried Biomass of *A. Niger* and *Penicillium* species were churned with the help of glass beads with combination of different solvents. Different colours were obtained by using various fungal species and with the combination of different solvents, we got different ranges of colours.

**Keywords:** Contaminated Food Material; Fungi; Extraction; Solvents; Carotenoids

## Introduction

Carotenoids are major pigments of carrot. They are the most important constituents in food as natural colorants. Colours of food surface is very important to show its freshness and safety. Carotenoids are fat soluble plant pigments that provide much of the colour in nature. They are yellow to orange red pigments. Natural food dye in replacement of synthetic ones is used because of undesirable market. They can also be produced by bacteria, yeast and fungi. There are about 600 known carotenoids that can be grouped as carotenes or carotenoids and xanthophylls. They can also be used as antioxidants among them lutein is one of the valuable antioxidants. Beta carotene is non- toxic additive which can be used in ice cream, orange juice and candies.

According to some yearly reports, it is shown that 20% of fruits and vegetables produced are lost to spoilage [1]. which could serve as the potential sources for extraction of carotenoids due to their high content of antioxidants. *A. Niger* and *Avenaceuim* were more widespread among all the spoil fruits which were followed by *Saccharomyces* species, whereas *P.Digitatum* and *A.Flavus* were only found in tomato [2]. Dominance of *A. Niger* by being a main cause of post-harvest spoilage in sweet orange and acid lime [3]. There was also another report showing the dominance of *A.Niger*, [4] it was highest occurring species in pineapple, watermelon, oranges, paw paw and tomatoes with a frequency of 38%. . Contamination of Fungi of any agricultural products including tomatoes states inf field [5] as well as biological and physical damages during transportation phases along with large amount of water moles products more susceptible to be spoiled by fungi. Another study on tomato to indicated 5 species of fungi; *Aspergillus, Penicillium, Fusarium, Eladosporium* and *Rhizopus* in which *Aspergillus, Penicillium* and *Fusarium* were dominant [6]. Moisture content was one of the major factors supporting the fungal growth in dates [7]. Type of fungi which is common in spoilage of Date fruits were *Aspergillus species* and *Alternaria* [8].

Extraction method include procedure with acetone and the selective removal of Chlorophylls and esterified fatty acids from

the organic phase using a strongly basic resin. Extraction of Carotenoids from samples includes polar solvents; acetone, methylic alcohol, dichloro methane and mixture of solvents. Extraction is mainly based on solubility of chemicals of interest. There is no standard method of extraction of carotenoids from food samples. Methanol and diethyl ether, methanol and chloroform, methanol and hexane, methanol and acetone-hexane were used for research [9]. For carrot carotenoid analysis, more complex mixture hexane-acetone-methano-toluene (10:7:6:7, v/v) was used [10].

According to a study, yeast isolates were grown in 250ml flasks containing growing medium. Culture was inoculated with 10% inoculum and incubation was carried out at 300C for 8days in static culture for primary screening. After primary screening, shaking culture at 100rpm was done as a part of secondary screening for 7days. For primary screening, criteria were colour of colonies and absorbance at 570nm and total carotenoids as secondary criteria. Later, the isolates were classified and colour of yeast pallets grown on extract malt agar slants was matched with Munshell colour charts. Another study was carried out to extract β-carotene from filamentous fungus *Mucor azygoporus*. All the chemicals used for this study was analytical grade. The fungus *Mucor azygoporus* was produced and mass cultivation was carried out in 14L lab fermenter. There were many solvents used for extraction from *Mucor azygoporus* among which hexane: ethyl acetate (1:1, v/v) was found to be ultimate of all. Purification was done by column chromatography. At the end of all processes, the recovery of β-carotene was observed to be 92% and quality was comparable to commercial preparation [11]. According to study carried out in China who targeted fungal cell wall and organic solvents by one factor at a time. In this research work, four methods were used for breaking cell wall and compared. The methods were ultrasonic treatment, liquid nitrogen grinding, quartz sand grinding and acid heating method. For each method, 0.5gm of *C. militaris* fruit body powder was used. For ultrasonic treatment, ultrasonic powder was set at 500W for 10mins. For acid heating method, the dried biomass was saturated in 1M HCl at 300C for 20 minutes. After centrifugation at 5000rpm for 40 minutes, supernatant was removed and residue was washed with distilled water.

Microbial sources are used just because of its potential content, easy down streaming process, easier extraction, high yield through strain improvement and no seasonal variation. Overall for the extraction of carotenoids, breakdown of cell wall is the most important step. It can be done by either mechanical methods or chemical methods.

## Materials and Methods

### Isolation of fungi

Food samples from six different categories, Dairy Products, Fruit, Vegetable, Pulses, Bakery Item, Grain were selected and they were cheese, apple, carrot, moong, brown bread, chapatti. Selected food samples were collected using hand gloves into sterile plastic containers for 15 days.

**Figure 1:** All selected contaminated Food Samples in a container after degradation of 15 days.

All the glass wares used for experiment were properly washed, dried and sterilized in the autoclave. The entire working surface was cleaned by methanol to reduce contaminants. Potato Dextrose Agar (PDA) was prepared and poured into Petri-dishes. The Inoculums sample was prepared in distilled water and was homogenised using stirring rod in sugar tube. A loop full amount of Tween 80 was added as a surfactant to reduce bacterial contaminants in inoculums.

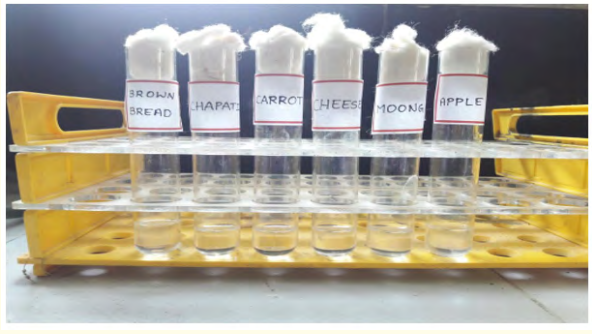



**Figure 2:** Inoculum of the respective for food samples for isolation.

### Microscopic observation

Observation was done through Lacto-Phenol Blue Method. Species were taken on slants through wire loop under aseptic conditions. Slants then were stained with Lacto-phenol Blue. Stained slants were then observed under microscope and photo graphed through microscopic lens.

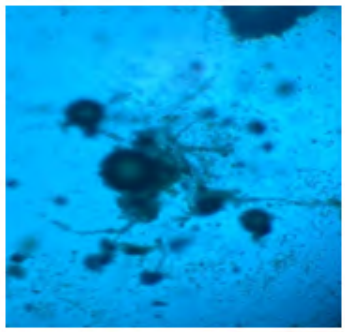


**Figure 3:** *A. Niger* Species through Lacto-phenol Blue staining method under microscope.

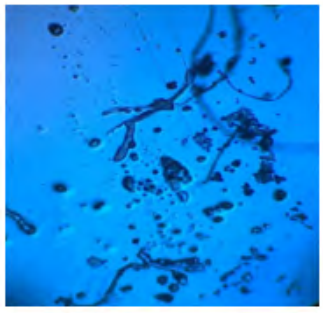


**Figure 4:** *Pencilium* Species through Lacto-phenol Blue staining method under microscope.

### Method for extraction of carotenoid

Extraction was done through Churning Method. Species were isolated and kept for growth in PDA broth prepared domestically for 10 days in a rotary shaker at 100 rpm. Biomass obtained was oven dried and was in a range of 5 - 10 grams for *A.niger* and *Pencilium* species. Obtained biomass was then churned for 15 - 20 minutes with glass beads along with different combination of solvents such as Hexane + Ethyl acetate (4:2), Acetone + Petroleum ether (4:1), Chloroform + Ethanol (1:1). The churned biomass was filtered using Whatman filter papers (125 mm ø Cat No. 1001 125).

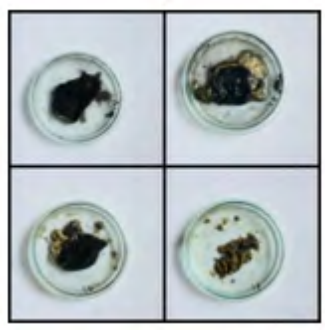


**Figure 5:** Biomass Grown of *Pencillium* and *A. Niger* species weighed ranging 5-10 grams.

**Figure 6:** Churning of Obatined Biomass for 15-20 mins with combination of different solvents.

## Results and Discussion

Six food samples were collected in a plastic air tight container and was preserved for 15 Days with temperature range of 27 - 330C. On 16th day, PDA was prepared and was used for growth of fungi. Streak Plate Method was used for initial isolation of organisms. Isolated colonies were observed after 5 days of incubation (Figure 7).

Picture 5(a) shows the growth of fungi, taken from contaminated bread sample. Species were identified on the plate by its colour, appearance, morphology. Plate of bread sample had 4 species such as *A. niger* which was black in colour and appeared to be most, as it covered most part on the plate. Other three were in small amount.

Yeast which was pure white in colour, Mucor was also white in colour and appearing similar to that of cotton. *A. flavus* also appeared in the form of pink colour colonies. Growth of Fungi from apple showed only two species, *A. niger* and Mucor. Species found from carrot sample were *A. niger, Pencilium*, appeared to be green colour having sight fibrous white layer over it, covring small area on the plate. Plate prepared from chapatti sample also had two species and they were *Mucor*, which was in major proportion and *A. niger*. Plate of cheese sample had a three fungi species on it. They were *A. niger*, which was dominant and *Penicillium* and *Mucor* were in small amount, appearing to be same as in previous plates. For Biomass cultivation PDA(Broth) was selected which was prepared using liquid obtaining after potatoes and 2% sugar. Biomass cultivation was initiated by keeping the flasks in rotary shaker for 15 days at 100rpm. The Biomass was obtained (Figure 8).

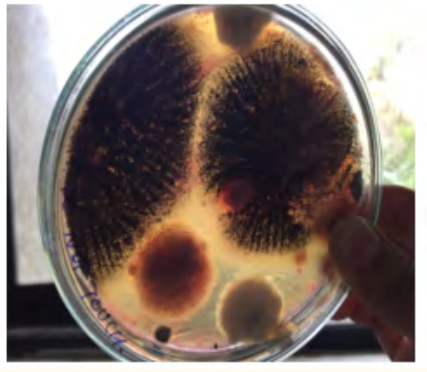


**Figure 7(a):** Fungi from Bread.

**Figure 7(b):** Fungi from Apple.

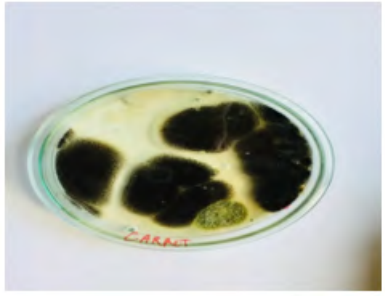


**Figure 7(c):** Fungi from Carrot.

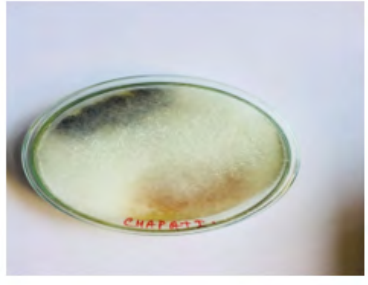


**Figure 7(d):** Fungi from Chapati.

**Figure 7(e):** Fungi from Cheese.

**Figure 8:** Cultivated Biomass being dominated by species of *A. Niger*.

Biomass, obtained had a great dominance of *A. niger* species, as five out of four flasks had a *A. niger* species in it, though *Penicillium* biomass was obtained in one flask. The range of A. niger Biomass in all four flasks were 6 - 10 grams. Biomass obtained from flask containing *Penicillium* was 8 - 9 grams.

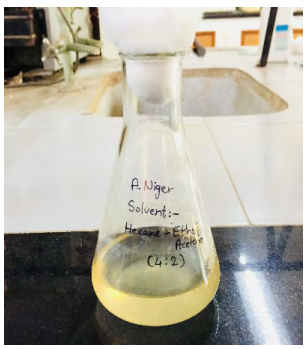
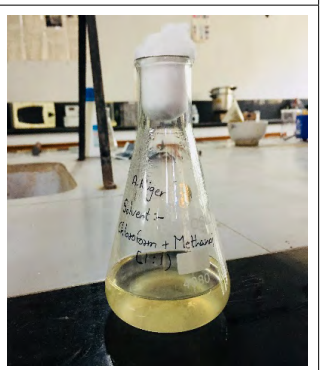
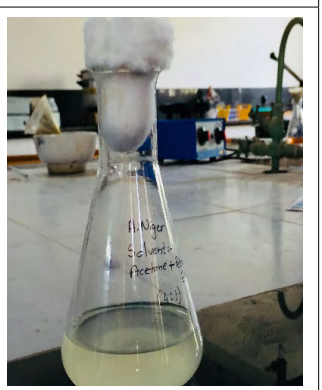
| No. | Solvent | Results |
|---|---|---|
| 1. | Hexane + Ethyl Acetate (4:2) |  |
| 2. | Chloroform + Methanol (2:1)) |  |
| 3. | Acetone+ Petroleum Ether (4:1) |  |

**Table 1:** Results of A. Niger species experimented with different solvents.

Extraction was done through churning method. Glass beads were used for providing Abrasive action for the disruption of Fungi. The Biomass was churned for 10-15 min and was filtered using Whatman filter paper with a fixed volume of 100ml.

**Figure 9:** Orange colour obtained through extraction of *Penicillium* species with solvent Hexane + Ethyl Acetate (4:2).

Orange colour was obtained through churning of Biomass. Churning of *A. niger* species was done with different solvents. There was difference in colour intensity was observed for three experimented solvents, among which highest was from Hexane + Ethyl Acetate (4:2), second among the three was of chloroform + methanol (2:1), lowest intensity was observed among acetone + petroleum ether (4:1). On the basis of this it can also be included that selection of appropriate solvent would play a vital role for extracting carotenoids.

## Conclusion

Selected food samples of Bread, Carrot, Moong, Cheese, Chapati, and Orange yielded species such as *A. niger, A. flavus, Yeast, Penicillium, Mucor. Among them A. niger, Penicillium* and *Mucor* were the species obtained in the sufficient amount for cultivating biomass. Obtained Biomass was churned using glass beads. It was of *A. niger* and *Penicillium* which resulted in the pigments of Yellow and Orange colour. Number of different solvents were tried but highest colour intensity was observed in solvent Hexane + Ethyl Acetate (4:2).

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