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 methods for production of Carotenoids. These Explains increasing interest in production of microbial carotenoids as alternative synthetic food colorants. Spoiled food material was cut into small segments, sterilized, plated on Potato dextrose agar(SDA) and then incubated at 27 degree celcius 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 *Pencilium* species were churned with the help of glass beads with combination of different solvents.

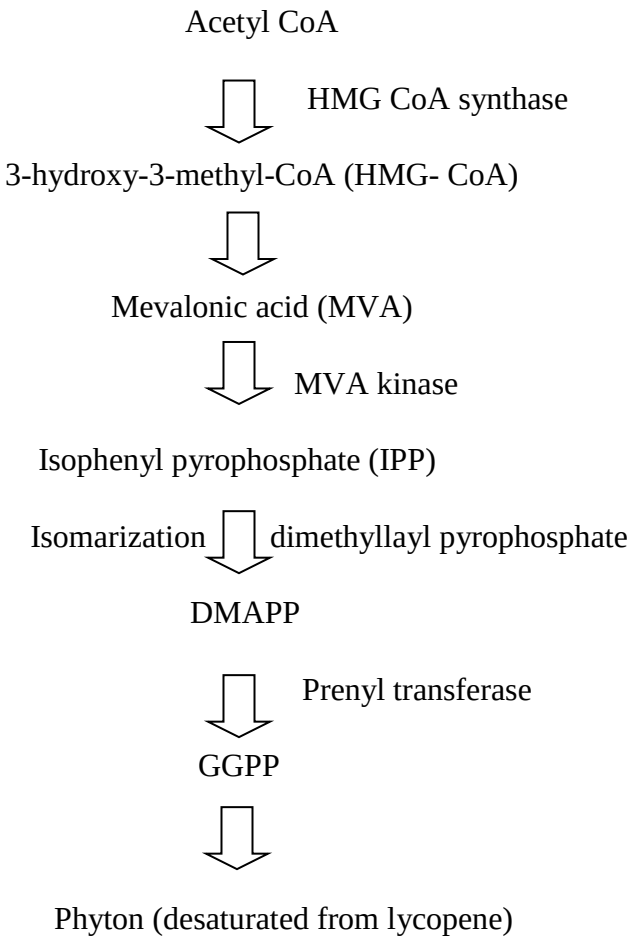
Key Words: Contaminated Food Source, Fungi, Extraction, Solvents, Carotenoids

INTRODUCTION

Cartoenoids are major pigments of carrot. They are the most important constituents in food as natural colorants. Carotenoids are fat soluble plant pigments that provide much of the colour in nature. There are about 600 known carotenoids that can be grouped as carotenes or carotenoids and xanthophylls. Beta carotene is non- toxic additive which can be used in ice cream, orange juice and candies. In green plants, it is found in chloroplasts which is responsible for biosynthesis system but are more coloured in roots, fruits and flowers. It gives colours ranging from pale yellow to bright orange to deep red. Natural colorants are used in baby foods, breakfast cereals, pastas, sauces, processed cheese, fruit drinks, vitamin enriched milk products and energy drinks. Colours of food surface is very important to show its freshness and safety. Natural food dye in replacement of synthetic ones is used because of undesirable market. 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. They can also be used as antioxidants among them lutein is one of the valuable antioxidants. The main focus of this study is to analyse various fungal infected food and our concern is to extract carotenoids from fungi. They can also be produced by yeasts and bacteria. There is a current need for natural colorants which are cost effective. There are certain other sources of extraction of carotenoids. One of the method such as shrimp processing developed by Anderson.Spinelli and Mahnken developed a 3-stage counter current extraction process for recovery of astaxanthin containing oil from red crab waste.

There is a current need for natural colorants which are cost effective. There are certain other sources of extraction of carotenoids. Procedures for extraction of carotenoids involves various types of solvents, combinations of solvents and other procedures.

One general pathway of carotenoid synthesis was described by Simpson but later it was modified by Goodwin which is as follows:


Acetyl CoA
HMG CoA synthase
3-hydroxy-3-methyl-CoA (HMG- CoA)
Mevalonic acid (MVA)
MVA kinase
Isophenyl pyrophosphate (IPP)
Isomarization
dimethyllayl pyrophosphate
DMAPP
Prenyl transferase
GGPP
Phyton (desaturated from lycopene)

LITERATURE REVIEW

Carotenoids are yellow to orange red pigments and ubiquitous in nature. Fruits and vegetables contain different amount and types of carotenoids and other components. Procedures for of extraction of carotenoids involves various types of solvents, combination of solvent and procedures.

Fruits and Vegetables contain different amount and types of carotenoids,(Pauli).Yearly reports have shown that 20% of fruits and vegetables produced are lost to spoilage, (Barth.M.Harleinson) which could serve as the potential sources for extraction of carotenoids due to their high content of antioxidants. Another study have reported occurrence of 36%, 25%, 22% and 17% for *R.Stolonifer, A.Flavus, A.Fumigatus and A.Niger* respectively in sweet oranges,(Tabina). *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, (Gadzile). Dominance of *A.Niger* by being a main cause of post harvest spoilage in sweet orange and acid lime, (Bali), there was also another report showing the dominance of *A.Niger,* (Samuil Mailafia) , it was highest occurring species in pineapple, watermelon, oranges, paw paw and tomatoes with a frequency of 38% . Another species such as *Fusariumavenaceum* with frequency of 31% followed by *Rhizopus Stolonifer* which was having least frequency of 4%. Other species such as *Saccharomyces* 10% *Fusarium solani* 8% and *A.Flavus* 5% frequency were noted. Contamination of Fungi of any agricultural products including tomatoes states inf field (Aran) 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 indicated 5 species of fungi; *Penicilium, Fusarium, Eladosporium* and *Rhizopus* in which *Aspergilus, Penicilium* and *Fusarium* were dominant, (Diphne, Neka Ezikenyi). Species of *S.Aureus* and *Bacillus* and two fungi species; *A.Flavus* and *R.Stolonifer* , in tomatoes, whereas present work was dominated by over other fungi species, (Asan,Oriji, Bashir). Mixed vegetables the members of *ascomycetes* and *deutromycetes* comprised species of *alternariadlternata, Cladosporiumherbarum* also *Fusarium* species also had remarkable presence. After *deutromycetes, zygomycetes* were found which consist of the mucorals of genre *Rhizopus, Mucor*. In the month of May and June, *aspergilus* species were dominant whereas *Penicilium* species also grew over wide range. *A.Flavus* were predominant species on all food commodities, (Gazala, Tabassum).

Moisture content was one of the major factor supporting the fungal growth in dates (Hill and Waller). Type of fungi which is common in spoilage of Date fruits were *Aspergilus species* and *Alternaria* , (Djerbi). Results of experiments on dates in Nigeria marked growth of Fungi and which were dominant are in following in order, *Rhizopus* sp(100%) , *Mucor* species(100%) followed by *Torula* species (16.67%) and *Alternaria* species (13.33%) respectively, (Ibrahim S,Rahma MA).

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 solvetns; 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 (Hart and Scot). For carrot carotenoid analysis, more complex mixture hexane-acetone-methano-toluene (10:7:6:7,v/v) was used (Chen).

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 30 degrees Celsius 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 was color of colonies and absorbance at 570nm and total carotenoids as secondary criteria. Later, the isolates were classified and color of yeast pallets grown on extract malt agar slants was matched with Munshell color 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, (Valduga). 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 30degrees Celsius for 20mins. After centrifugation at 5000rpm for 40mins, supernatant was removed and residue was washed with distilled water. After breakdown of cell wall, acetone-petroleum ether was added. Carotenoid extraction was performed by centrifugation.

Overall, this review is about different microorganisms from which carotenoids can be extracted and different methods used by various researchers to extract carotenoids from fungi. The main focus was breakage of cell wall of fungi and mechanical methods were mostly used for breakdown of cell wall.

MATERIALS AND METHOD

ISOLATION OF FUNGI

Food samples from six different categories , Dairy Products, Fruit, Vegetable, Pulses, Bakery Item, Grain were selected and they where 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 15days.

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.

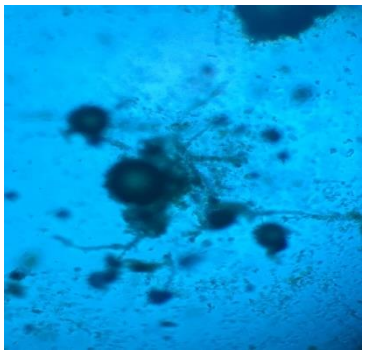


Figure 3

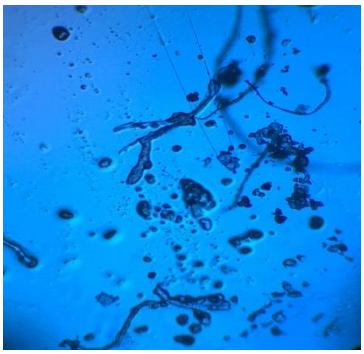


Figure 4

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 CAROTENOIDS

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 gms 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).

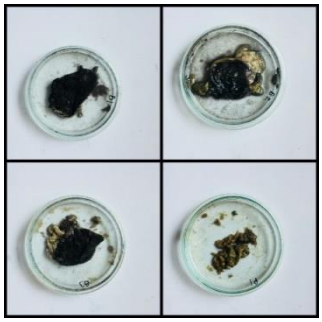


Figure 5

Figure 6

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.

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RESULTS AND DISCUSSIONS

Six food samples were collected in a plastic air tight container and was preserved for 15 Days with temperature range of 27-33 degree Celsius. 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.

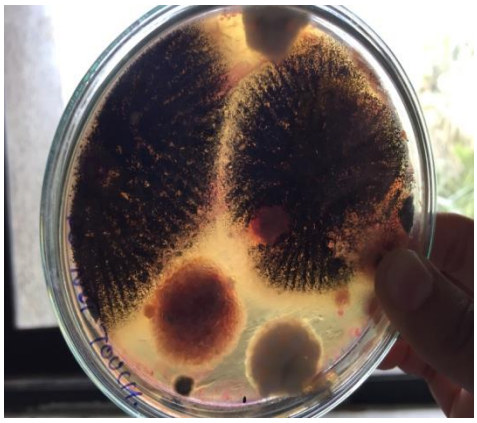


Figure 7(a) Fungi from Bread

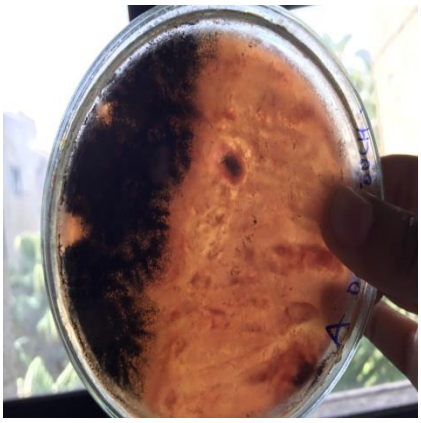


Figure 7(b) Fungi from Apple

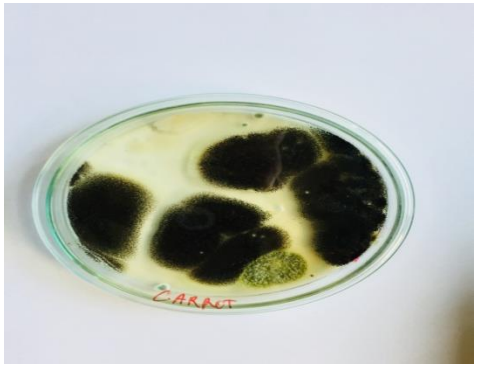


Figure 7(c) Fungi from Carrot

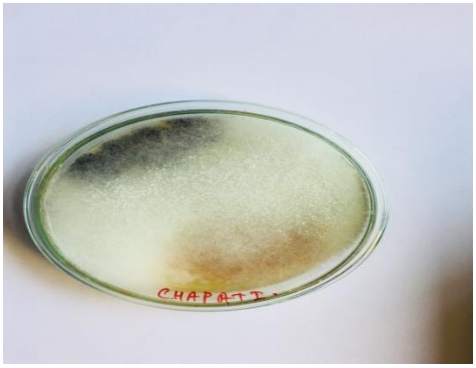


Figure 7(d) Fungi from Chapati

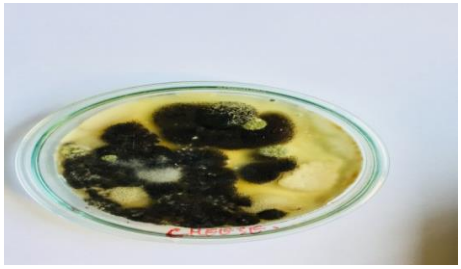


Figure 7(d) Fungi from Cheese

Picture 5(a) shows the growth of fungi, taken from contaminated bread sample. Species were identified on the plate by its color, appearance, morphology. Plate of bread sample had 4 species such as A.Niger which was black in color 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 color, Mucor was also white in color and appearing similar to that of cotton. A.Flavus also appeared in the form of pink color 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 color 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 proportionand A.Niger. Plate of cheese sample had a three fungi species on it. They were A.Niger, which was dominant and Pencilium and Mucor were in small amount, appearing to be same as in previous olates. For Biomass cultivation PDA(Broth) was selected which was prepared using liquid obtaining after potatoes and 2% sugar. Biomass cultivation was iniated by keeping the flasks in rotary shaker for 15 days at 100rpm. The Biomass was obtained.

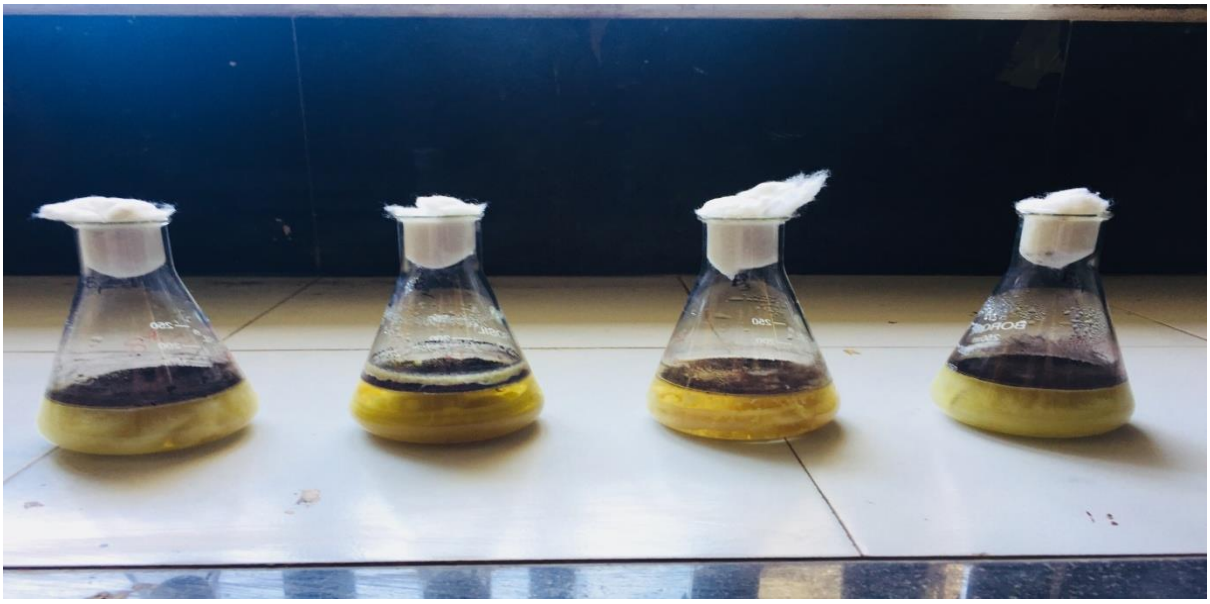


Figure 8 :- Cultivated Biomass being dominated by species of A.Niger

Biomass, obtained had a great dominance of A.Niger species, as five out of four flasks had a A.Niger species in it, though Pencilium biomas was obtained in one flask. The range of A.Niger

Biomass in all four flask were 6-10 grams. Biomass obtained from flask containing penicilium was 8-9 grams.

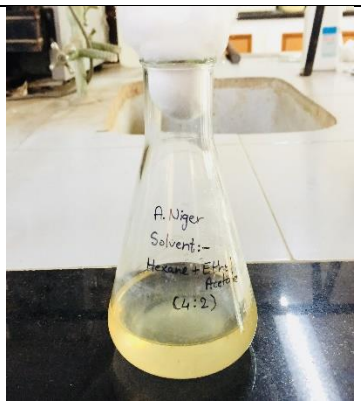
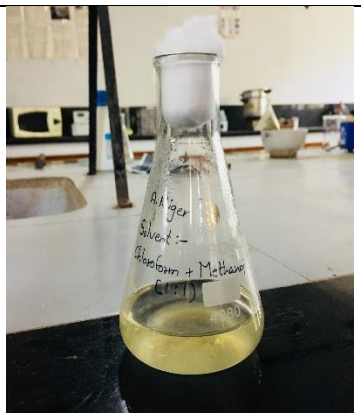
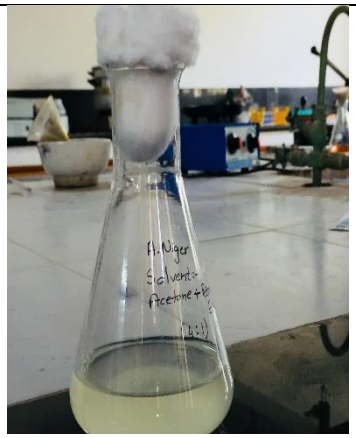
No.	Solvent	Results
1.	Hexane + Ethyl Acetate (4:2)	
2.	Chloroform + Methanol (2:1))	
3.	Acetone+ Petroleum Ether (4:1)	

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 Whatmann filter paper with a fixed volume of 100ml.

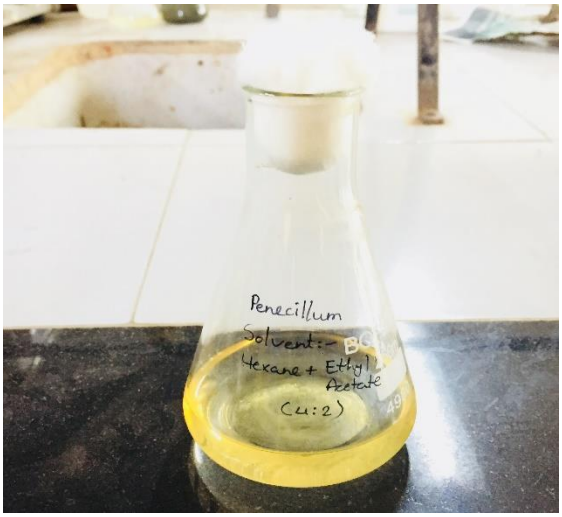


Figure 9 :- Orange colour obtained through extraction of *Penecilium* species with solvent Hexane + Ethyl Acetate (4:2)

Orange color was obtained through churning of Biomass. Churning of A.Niger species was done with different solvents. There was difference in color 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.

CONCLUSIONS

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

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BMC CANVAS

- **BUSINESS MODEL CANVAS**

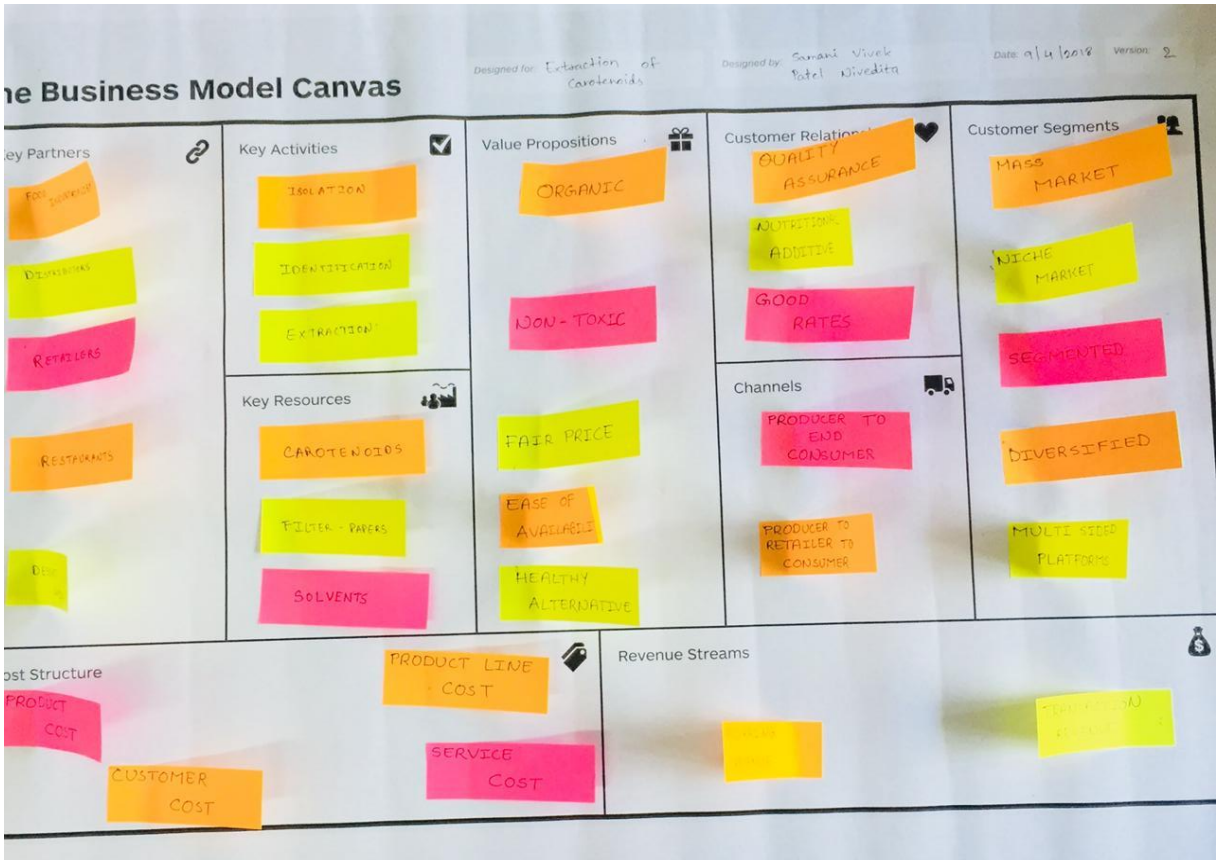


Figure 10:- Business model canvas

BUSINESS MODEL CANVAS (BMC) REPORT

Buissness Model Canvas-What is it?

The **Business Model Canvas** is a one page overview that lays out both what you do (or want to do), and how you go about doing it; enabling structured conversations around management and strategy by laying out the crucial activities and challenges involved with your initiative and how they relate to each other. This visual format, first introduced by Osterwalder and Pigneur, is useful for both existing and new organisations and businesses. Existing programmes can develop new initiatives and identify opportunities while becoming more efficient by illustrating potential trade-offs and aligning activities. New programmes can use it to plan and work out how to make their offering real.

The individual elements prompt thoughts within the separate activities or resources, while the capability to have the complete overview encourages fresh perspectives and ideas about how those pieces fit together. This structure also helps to keep group discussions more focused and bring everyone onto the same page.

Buisness Model Canvas-How to use it?

To make a Business Model Canvas, the easiest way to start is by filling out what you do. This helps keep the focus on your main goal as you fill out the other building blocks of the canvas. From there you can build on that goal and see how it can be achieved by adding details about the other activities and resources you have.

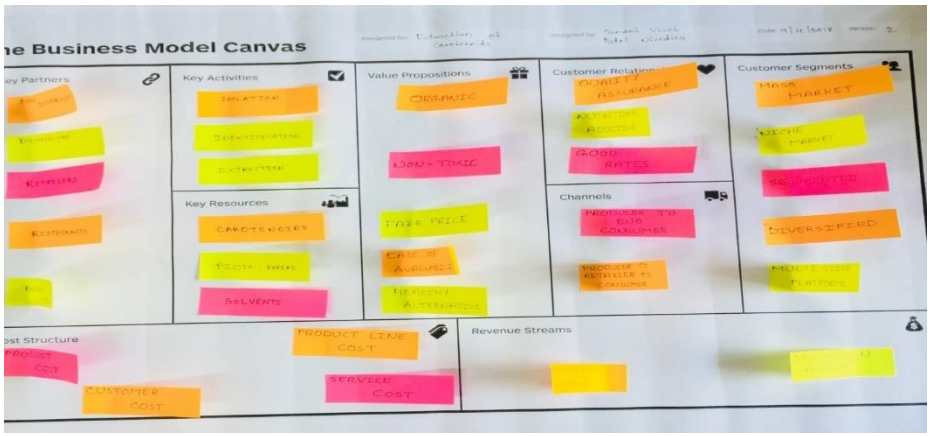
Start from a blank canvas and add notes with keywords to each building block of the canvas. If you use ‘sticky notes’ for this, you can move ideas around as you fill out each building block in the canvas. You may want to colour-code elements related to a specific client segment.

However, be careful not to fall in love with your first idea and instead sketch out alternative business models for the same product, service, or technology.

Building Blocks (Main Features)

- Key Partners
- Key Activities
- Key Resources
- Value Propositions
- Customer Relationships
- Channels
- Customer Segments
- Cost Structure
- Revenue Stream

Buisness Model Canvas



1) **Key Partners**

   Mainly the key Partners for the project were Food Industrialist as the product which was to be developed in the particular context was completely related to this particular industry. The other partners in brief mainly include Distributors and Retailers to increase for increasing the reach of the products. The main users of this product were Restaurants and

Desserts Chain as these is mainly use by those people in bulk whereas it can also be used Domestically.

2) **Key Activites**

Activities which were involved in the primary phase of the project was the selection of food samples and then determining the fungi yield of the particular sample. The next step were identification and isolation of the species obtained from samples. Second Phase included the growth of biomass of the isolated species. Work was done on selecting the appropriate extraction method along with the solvents. The method was selected and pigments were obtained.

3) Key Sources

Key Sources for these project mainly were waste food samples. There after the sources which can be included are Fungi, the PDA broth and agar for isolating and growing of biomass. Then comes the solvents and apparatus used for the extraction method of the obtained biomass for obtaining the pigments.

4) **Value Propositions**

The Value Propositions can be the simple way to understand your customers needs and design products and services they want. The main needs which can be satisfied is that it is organic and non-toxic and also it would be available at fair price in comparison to other organic products available in the market. Work would be done in order to ease the availability of the product as these would be an healthy alternative with respect to products which are non-healthy and are in current use.

5) **Customers Relationships**

Customer Relationship can be blossomed by quality assurance. Also reach of the product must be at the ease so it would be easy for the product to win the trust. It has also got the feature of nutrional additive which are things always looked by the customers when they are looking for any food product also the product should be offering good rates as well.

6) **Channels**

The channels through which product should be made available to customer where decided to be of mainly of two types and they were. Producer to direct end customer whereas the other one was involving the third party and was producer to retailer and then to the end customer.

7) **Customer Segments**

Customer segments defines the groups of people or organizations you aim to reach or serve. Every company needs profitable customers in order to survive.A mass market focuses on a large group of customers without really distinguishing between different types of customers, and aims to satisfy a set of broadly similar needs and problems.A niche market is quite opposite from a mass market, focusing on a very specific group of customers.A segmented market is one in which you have multiple different groups of customers with different sets of needs and problems. In this case you would provide the same product or service with slightly different value propositions to meet the varying customer needs.A diversified market is similar to a segmented except that it utilizes entirely different sets of value propositions to cater to unrelated customer segments rather than just slightly altering the product. Multi sided platform markets serve interdependent customer segments. For example, a credit card company interacts with both the card holder, and the merchants who accept those cards.

8) **Cost Structure**

This defines the cost of running a business according to a particular model. Businesses can either be cost driven i.e. focused on minimizing investment into the business or value driven i.e. focused on providing maximum value to the customer. Following are some traits of common cost structures. Fixed Costs costs that remain the same over a period of time. Variable Costs as the name suggests, these costs vary according to a variance in

production. Economies of Scale costs decrease as production increases. Economies of Scope costs are decreased by investing in businesses related to the core product.

9) Revenue Streams

A revenue stream is the methodology a company follows to get its customer segments to buy its product or service. When setting up revenue streams, it is important to recognize that an effective price for the product and/or service will be arrived at through the process of elimination. Different iterations of prices should be listed and evaluated. It is important, in the end to take a break ad reflect on possible avenues open to you as a business.