ISOLATION AND IDENTIFICATION OF FUNGI RESPONSIBLE F

OR SPOILAGE OF CARROTS

BY

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CERTIFICATION

This is certify that this project is the original work carried out and reported by **ADERIBIGBE RASHIDAT OYINDAMOLA** with matric number **HND/23/SLT/FT/0221** to the Department of Science Laboratory technology, Microbiology unit, Institute Of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved In partial fulfillment of the requirements of the Award of Higher National Diploma (HND) In Science Laboratory Technology

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DEDICATION

I dedicate this project to Almighty Allah who is my creator and my first source of inspiration. He's kept and protected me until this moment and me capable of doing this project. This project report is also dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the large task can be accomplished if it's done one step at a time and to my immediate younger brother, who has contributed greatly to my journey, believed in me and been with me eve step of the way. You inspire me YUSUF and I'm grateful for the gift of you.

Finally to my parents who has never failed to give me financial, moral, emotional and spiritual support. I do not take any of it for granted.

I love and celebrate you all

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Abstract

Over the past decades vegetable consumption specifically carrot has been on the rise however, its wastage due to microbial spoila ge has been estimated at around 20% annually. In this study, spoi lage fungi associated with carrots were identified by employing standard microbiological procedures. Various tests were used to characterize carrots with soft rot symptoms. This study was aim ed at assessing fungi associated with spoilage of carrots. Five (5)fungal species were detected via morphology and biochemical screening. The results showed that Aspergillus niger was recorde d the highest (40%)while the least prevalence of the fungi was M ucor sp. (9%). Results from this study affirmed that spoilage fun gi are present in carrots, therefore care must be taken in handlin g ,washing and processing carrots before consumption so as to prevent spoilage that might lead to infections and food-borne ou t breaks due to fungi.

Keywords: Carrots, Food spoilage, Food-borne outbreaks, Fungi

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Carrots(*Daucuscarota*)isabiennialherbaceousspecies,itispartofthe ApiaceaeFamily.Carrots are classified into two mainly; Western car rots and Eastern carrots and this is based on carrot pigmentation. The origin of western carrots is not yet known while the eastern car rots is said to originate from Afghanistan. Most carrots root is purp le and some are yellow. The leaves are slightly dissected and roots branched. Currently the more widely cultivated carrots in the world are the orange carrots and are more popular. (Que, F., Hou, Xl, Wan g, Gl*et al.*, 2019)

Carrots are grown in sandy loam or silt loam soil most at times to enhance water holding capacity and drainage. Planting carrots in r aised beds can further help in proper water drainage. Carrots need soil that has adequate air and water drainage because wet and co mpacted soils can cause a deformed growth. The temperature of the soil three inches below the surface should be 50°F or lower. Carrots can withstand PH ranging from5.5 to 8.0 because there are hard crops, however, light sandy soil with a neutral PH and under full sun exposure, this is opposite to very clay-like or wet, chalky soil. Tillage of soil is done toloosen the compacted ground before seeding. To have the best root development and growth, carrots should have approximately 18-24 inches of ell-tilled soil that has adequate drainage. Abnormal shaped or forked carrots that are unmarketable are grown due to the presence of pebbles and stones in the soil. Pythium root die back, nematodes, and exposure to frost are other factors that could causes tub bed off or ked roots (Anupama *et al.*, 2020)

Carrots are crop that are able to adequately extract nutrient from the soil due to their deep-rooting nature. It is necessary for soil test to be carried out before planting and throughout development to me asure soil nutrient such as Nitrogen, Potassium, Phosphorus, Magnesium, Manganese, Boron and Sulphur. However, nutrient can be added before seeding and during crop maturation with the use of side dressing or broadcasting. Precaution should be taken as excess nitrogen in the soil causes root cracking during harvest. Due concern for food safety and high nitrogen, addition of fresh manure is not advisable. (Pensack-Rinehart and Buning 2015).

Carrots are the most important crop in the Apiaceae family .Carrots was first used for medicinal purpose and later used as food. Orange carrots the most popular was cultivated in 15th and 16th centuries in central Europe. The reason for popularity of orange carrots was because it was observedtocontainhighProVitamineA.ThemajorAntitoxidantfoundincarrotsareCarotenoids and Anthocyanin. Yellow carrots are highly rich in Alpha and Beta carotene and rich in ProVitamine A(daSilvaDias2014).Lutein present in carrots is responsible for its yellow color and plays an important role in macular degeneration pre vention. Carotene level gradually increases with growth and is more concentrated at the corticle than the core. Carrots have high nutritional value.It is a good source of dietary fiber and of trace minerals molybdenum (Nicolle*etal.*,2004).

Carrots is a root vegetable that contain carotenoid, flavonoids, polyacetylenes, vitamins an minerals, all of these possess numerous nutrition and health benefits. They were an old adage that carrots are good for the eyes. Carotenoid, polyphenol and vitamins present in carrots act as antitoxidant, anticarcinogenics, and immune enhancers. Antidiabetic cholesterol and cardiovascular disease, lowering, antihypertension, hepatoprotective, renoprotective and wound healing benefits of carrots also have been reported (da Silva Dias 2014).

Processed vegetables, the spoilage of horticultural products justifies the use of preservative techniques. This processing not only adds value to the products, but as well makes the products more conv enient to be consumed by consumers. Consumers requested for high quality, a fresh, nutritiveandconvenientlypreparedvegetablehasincreasedsomuchintherecentyears.This has led to the development of lightly processed vegetables. Preparation of lightly processed carrots is done by peeling the epidermal layer of the carrot roots; this is one of the most popular products that are available in the United States .One of the disadvantages of this processing method is that it makes carrots susceptible to different physiological changes that cut short their shelf-life. The peeling of the epidermal layer of the carrots increase these potential for carotene oxidation during storage, this also may further increase the respiration of carrots tissue resulting in increased degradation of protein ,carbohydrates, lipids and the development of off-flavors (PeiyinandBarth 1998). A new protective layer called white blush is developed when the epidermal layer is peeled off and this result in dehydration and lignification on the carrots surface (Bolin andHuxsoll,1991).

Though carrots are important sources of nourishment to human beings (Kaur*et al.*, 2017),specifically vitamins, and could serve as an important ingredient in enhancing health and proper diets. However, they are notable sources of chemical and microbial contaminants.(Uzeh*et al.*, 2009). Velusamy*et al.* (2010) stated that vegetables have been linked with illnessesarising from food borne because notable pathogens grow on them. Unfortunately, carrots and other vegetables are consumed for their enormous nutritional benefits without thoughts of possible contamination with disease causing microorganisms. These organisms are not able contaminants of vegetables and raw fruits through faecal, untreated irrigation and surface water, and sewage channels (Kaur*et al.*,2017). The level of food borne outbreaks caused by spoilt fruits and vegetables has been on a rising side in recent years, thus, a quest to isolate and identify these pathogens, in particular fungi that causes spoilage should be recommended as a control measure.

1.2 Statement of Problem

Increase in awareness of the health benefits of carrots has resulted in an increase in consumption. Many vegetables are consumed ra w to retain the natural taste and heat labile nutrients. It is claimed t hat Microbes are found all over the globe with some few exception s, including sterilized surfaces.They include normal flora that is no n pathogenic,which contribute to the larger percentage and pathog enic species which are few (Gadafi et al., 2020). The safety of raw vegetables especially carrots is a great concern. This research and experiment are therefore centered on isolation and identification of fu ngi responsible for spoilage of fresh carrot, to also know possible food borne fungi pathogen on carrots (Anupama *et al.*, 2020).

1.3 Aim

The aim of this research is to isolate and identify possible pathoge nic fungi

On carrots sold in Ipata Market, Ilorin, Kwara State.

1.4 Objectives

The main objective of this study is to isolate and identify fungi respo nsible for the spoilage of carrots.

Specifically, this research will do the following:

i. Isolate and identify possible pathogenic fungi on carrots sold i n Ipata Market, Ilorin.

ii. Determine antifugal susceptibility patterns of the pathogens fro m carrots sold in Ipata Market, Ilorin.

1.5 Research Questions

i. What method was used to isolate and identify possible pathoge nic fungi on carrots sold in Ipata Market, Ilorin?

ii. What are the antifugal susceptibility patterns of the pathogens from carrots sold in Ipata Market, Ilorin?

1.6 Significance of the Study

Carrots are root vegetables that are highly consumed in every famil y. It is essential to health because of its high nutritional value. It pro vides nutrients such as vitamins and mineral sand also is of medica l important. Carrots are liable to contamination from various sources s uch as soil, man, water, air, and insects(Yong,2014).Therefore, Isolat ion and identification of pathogenic bacteria from fresh carrots is necessary, to enlighten consumer of various ways of hygienic pract ices that leads to reduction of microbial load and a determination of the antifungal susceptibility patterns of the isolates in case of f ood borne outbreak inthe country (Anupama*et al.*, 2020).

CHAPTERTWO

LITERATUREREVIEW

2.1 Carrot

2.1.1 Origin and Domestication

The Carrot (*Daucuscarota)* is a root vegetable, usually orange in col or, though purple, black, red, white, and yellow cultivars exist. By the existence of orange carrots, purple root color was apparently more common in eastern regions, yellow more common in the west. Eas tern carrots tend to have less deeply divided leaflets with heavy lea f pubescence in some cultivars. For any carrot production, early flowering is unsatisfactory, eastern carrots have a greater tendency toward early flowering than western carrots, likely due to the somewhat warmer climates over the eastern production range. Beyond the yellow, purple, and orange root colors, eastern carrots have long included red-rooted types while western carrots included white-rooted types. Carrot use has also varied across production areas, with a more predominant use as animal forage in the east but largely human use as a root vegetable in the west (Philipp *et al.,* 2020).

Carrot is the most widely grown member of the Apiaceae or Umbelliferae. They area domesticated form of the wild carrot, *Daucus carota*a native to Europe and Southwestern Asia. This diverse and complex plant family includes several other vegetables, suchas parsnip, fennel, celery, root parsley, celeriac, arracacha, and many herbsand spices (Rubatzky*et al.,* 1999). The plant probably originated in Persia and was originally cultivated for its leaves and seeds (Wikipedia 2021).

Underlying varietal distinctions based upon storage root color and shape is adaptation to cool versus warm growing temperatures. Carrot is categorized as a cool-season vegetable and the majority of effort on carrot breeding has been towards improving production in temperate regions where cool temperatures(<~10C) can stimulate early flowering or “bolting” .More recently there have been successful efforts in broadening the adaptation of carrot to warmer subtropical climates where excessive heat can retard plant growth, inhibit root color development, and stimulate the development of strong flavor in unadapted germplasm (Anupama*et al.*, 2020). The ‘Brasília’ cultivar, for example, grows successfully in agricultural regions near the Equator. The development of temperate (late-flowering) and subtropical (early-flowering) types has resulted from a greater emphasis on ability to withstand early bolting in cooler climates for temperate types, incontrasttoagreateremphasisontheabilitytoproduceamarketablecrop in warm climates for subtropical types (Philipp *et al.,* 202

0). Subtropical carrots tend to grow faster than temperate types suggesting a complex interaction between root growth, flowering induction, and temperature that is not well understood. It should be noted that, unlike many crops, there is little evidence for a photo period effect on carrot root production and flowering so that the same cultivar theoretically could be grown anywhere in the world, if temperature requirements are met.

In fact, many carrot cultivars are widely adapted and can be grown over such extreme production temperatures as represented by north of the Arctic Circle to highland subtropical climates.(Philipp *et al.,* 2020)

Like other plants of this family, carrot seeds are aromatic and consequently have long been used as a spice or herbal medicine. Infact, carrot seed was found in early human habitation sites as long as 3

000 to 5000 years ago in Switzerland and Germany(Laufer,1919).This seed is thought to be from wild carrot used for flavor or medicine. It also forms a major ingredient in the food processing industry, a significant constituent of cosmetic products and its image has long been used to symbolize healthy eating. The leaves are also consumed in salads and the seeds made into an herbal tea (John *et al.,* 2011).

In terms of both areas of production and market value, carrot is part of the top-ten most economically significant crops vegetable in the world (Rubatzky*et al.*, 1999; Simon, 2000; Fontes and Vilela, 2003;Vilela,2004). In 2005,world production approached 24Mton1.1million hectares. The total global market value of the more widely traded carrot seed crop has been estimated to be in the range of $100 million(Simon,2000),but such estimates have little reliable data to confirm them and true value is likely much more. The development of cultivars adapted for cultivation in both summer and winter seasons on all continents has allowed a year-round availability of carrot pro ducts with relatively stable pricesto consumers. Some production areas harvest crops year-round. Carrot improvement today includes several academic, private and government research programs around the world that work in concert with local, regional, and global industries. Both grower and consumer needs are addressed by public and private carrot breedersthat incorporate modern technologies into the classical breeding process (Philipp *et al.,* 2020).

The genetic improvement of carrot has been an ongoing effort throughout its cultivation and domestication. Before the 20th century, carrot production was small scale in family or community gardens. A portion of the crop was likely protected in the field over winter with mulch ,or the best roots saved in cellars were replanted the subsequent spring to produce a seed crop. There is no written record of what traits were evaluated or any other detail of the selection process in this period, but all domesticated carrot differs from its wild progenitors in forming larger, smoother storage roots, so it is clear t hat these traits also were improved through regular selection. Selection for low incidence of premature flowering was also necessarily among the most important traits selected during domestication, as it is now, since with the initiation of flowering, eating quality diminishes dramatically (Philipp *et al.,* 2020). One can say that color and flavor were primary selection criteria since they were the traits used to distinguish among carrots recorded by historians, cooks, and eventually seed catalogues. Carrot root color also changed dramatically during domestication. While wild carrot roots are white or very pale yellow, purple and yellow

Carrots were the colors of the first domesticated carrots. These were the only colors recorded until the16thto 17th century when orange carrots were first described and soon came to be preferred in both the eastern and western production areas (Rubatzky et al. 1999, Simon, 2000). Banga compiled an extensive list of comments about carrots over history and while purple carrots were usually (butnot always) regarded as better flavored than yellow, the darkstainstheyl eftonhands, cookware,andincookingwaterraisednegativecomment sbysomeauthors.Wedonotknowwhy early carrot breeders shifted th eir preference to orange types, but this preference has had a signifi cant effect in providing a rich source of vitaminA, fromalpha-andbe ta-carotene,to carrot consumer sever since. Soon after orange carr ots became popular, the first named carrot cultivars came to be de scribed in terms of shape, size, color, and flavor, and the first com mercially sold carrot seed included reference to this growing list of distinguishing traits.

2.1.2 Disease Resistance

Disease and pests limit carrot production to some extent in all car rot production regions. Leaf blights caused by *Alternariadauci*, *Cer cosporacarotae*, and *Xanthomonascampestris*pv. *carotae*, powdery mildew (*Erisipheheraclei*), carrot fly (*Psilarosae*), cavity spot (*Pythium* species and perhaps other pathogens), and several nematodes (e.g.

*Meloidogyne*spp., *Heteroderacarotae*, *Pratylenchus*spp.) are among the most widespread carrot diseases and pests, occurring worldwide. Several other pathogens and pests can cause very serious damage in more limited regions (Rubatzky*et al.,* 1999). Carrot breeders have relied upon natural infection in production areas where there is regular disease occurrence to make progress in selecting for genetic resistance for most diseases. Often highly susceptible cultivars or inbreeds are interspersed among entries to be tested in the field and in some cases natural inoculation is supplemented with inoculum from artificially infested plants. This approach has been used in selecting for resistance to *Alternaria* leaf blight (Boiteux*et al.,*1993;Simon andStrandberg,1998),and aster yellows (Gabelman*et al.,*1994). For soil borne disease and pests, heavily infested disease evaluation plots have been established for *Meloidogyne incognita, M. javanica*(Vieira*etal.,*2003),Methodsfor evaluating resistance to *Alternaria* leaf blight (Simon and Strandberg, 1998; Pawelec*et* *al*., 2006), cavity spot and *Rhizoctoniasolani*resistance (Breton *et al.,* 2003), *M. hapla*(Wang and Goldman, 1996), and *M. javanica*(Simon*e tal*.,2000)in controlled environments such as a greenhouse or gro wth chambers have also been developed.

2.1.3 Consumer Quality

Selection for uniform orange color has been exercised by carrot br eeders for the last century.The nutritional quality conferred by the p rovitamin. A carotenoid that account for the orange color of carrots has received the attention of carrot breeders since the 1960s begin ning with extensive efforts of W.H. Gabelman and his students(Um iel *et al* 1972;Buishand*et al* 1979).As a result, selection has raised pro vitamin carotene content in typical U.S. carrot varieties by70% between 1970 and 1992 (Simon, 1992). Yellow, purple, red, and wh ite carrots have received a renewed level of interest in recent years as growers look for new niche markets and consumers become m ore aware of the nutritional benefits of pigments. To support select ion with objective measurements of color, an evaluation tools have been developed (Surles *et al.*, 2004).

Orange carrot color is primarily due to alpha-and beta-carotene, yellow and red carrot color are caused by carotenoids lutein and lycopene, respectively, and purple carrot color is caused by anthocyanins (Surles *et al.,* 2004). When no pigments accumulate, carrots are white. The commercial interestin carrots of unusuall colors has stimulated research to determine the genetics underlying carrot color. Genes for carotenoid accumulation described by Gabelman's group account for yellow and red color classes (Buishand *et al.*, 1979). Their efforts described seven major genes accounting for difference among orange,white,yellow,and red root color. More recently the *Y* and *Y2* genes were mapped, a SCAR marker developed for *Y2* (Bradeen and Simon, 1998), and 20 QTL mapped for carotenoid content (Santos and Simon, 2002). A single major gene, *P1*,confers purple storage root color but this gene only accounts for part of the variation obse rved for purple color, as a wide range of pigmentation patterns occur, and at least one other gene, *P2*, influences pigmentation in aerial plant parts (Simon, 1996). To develop breeding stock with potential commercial application, carrot breeders utilize traditional regional carrots and long- ignored heirloom cultivars with unusual colors in crosses with adapted, good-flavored orange carrots to combine unusual color with acceptable flavor for modern consumers (Erdman *et al.*, 2020).

Nitrates are important for their anti-nutritional value, especially for carrots used to make baby food. The inheritance of nitrate content in carrot is complex with incomplete dominance so that low-nitrate parents are necessary to obtain low–nitrate hybrids. In fact, while heteros is has significant positive effects upon many carrot production attributes, it is not observed for carotenoid or nutrient content, as mid parent values are observed in the majority of hybrids (Philipp *et al.*, 2020).

Carrot flavor is a very important variable influencing consumer decisions. Flavor differences were noted between purple and yellow carrots hundreds of years ago and among modern orange carrot root types today, sweet and juicy flavor can be found in a wide range of types such as ‘Nantes’, ‘Kuroda’ and ‘Imperator’. With a broad genetic range in carrot flavor and the development of high value carrot products, including lightly processed “baby” or“ cut and peel” carrots, improved raw carrot flavor has become a major breeding goal of carrot breeders in North America (Simon, 2000). Sweet flavor and succulent juicy texture are two of the major targets for improving raw carrot flavor. In addition to these two attributes, lack of harsh or turpentine flavor, caused by volatile terpenoids is the primary flavor component evaluated in selecting for improved flavor since high levels in harsh carrots can mask sweet flavor. Laboratory –facilitated selection is sometimes used for sweetness, using refractive index, colorimetric, or HPLC methods to quantify sugars; and for harsh flavor, using gas chromatography to quantify volatile terpenoids (Simon *et al.*, 1982).

The genetics of raw carrot sweet and harsh flavor has been described and the patterns of inheritance are complex. Sweet flavor, not surprisingly, is associated with higher sugar content which is polygenic, although as in glemajorgene, *Rs*, determines whether reducing sugars glucose and fructose, or sucrose, are the primary storage carbohydrates (Stommel and Simon, 1989). While texture is an important component of raw carrot flavor, little attention has been paid to the genetics of this trait. Since variation in texture interacts with perception of sweetness and harshness, breeder selection of carrot flavor generally relies upon tasting roots in the field and/or during the period they are being stored for verbalization. Relatively little change occurs in carrot flavor or carotene content during early post-harvest storage so it isa convenient timeto evaluate quality attributes. Unfortunately, the brittleness that accompanies crisp texture tends to have a negative impact on the“durability” of carrots in mechanical harvesti ngand washing (Philipp *et al.,* 2020).

2.2 Nutritional Value of Carrot

2.2.1 Bioavalaibility of β-Carotene

Deficiency in Vitamin A remains a major nutritionalproblem in mos t economically disadvantaged areas of the world (Olson 1994a, Som mer*et al.,*1996), this makes the population to rely on dietary sources o f provitamin.A carotenoid to meet the need of vitamin A. It has bee n considered that the most appropriate solution to this problem is t he strategies developed by Public health which enhanced the incre ased intake of carotenoid rich vegetables and fruits (Solomonand Bulux 1993). Various factors affect the bioavailability of carotenoid s, such as characteristics ofthefood source, interaction withother di etaryfactors andvarioussubject characteristics (Bowen *et al.,* 1993, Erdman *et al.*, 1993, Olson1994b, Parker 1996), Size ofthe particle, thelocation of the carotenoid in the plant source(i.e. the pigment protein complexes of cell chloroplasts vs. the crystalline form in chloroplasts). Factors that affect proper micelle formation are included in characteristic that can affect carotenoid uptake and absorption (Erdman *et al.*, 1993, Rock *et al* 1992,Zhou*etal.,*1996). However, suggestions have been made that heat treatment may improve the bioavailability of carotenoids from vegetables(Poor *etal.,*1993). During feeding of processed vs. raw vegetables the percentage changes in plasma of cis-β-carotene and α-carotene concentration remains the same. Daily consumption of processed carrots within 4 weeks will result production of plasma β-carotene response compare to the consumption of the same amount of the raw vegetables. Study has shown that thermal processing of this vegetable had substantially increased the proportion of cis-β-carotene isomers. Result from stud ies have also made a suggestion that is omersofcis-β-carotene have less of pro vitamine A activity than that of all-trans- β-carotene, and lower bioavailability may also be explained by some absorption and discrimination of isomers(Erdman *etal.*,1993,Gaziano*etal.,* 1995,de Pee *etal.*, 1995). Consumption of food riches in carotenoid that have been treated with mild heat has sometimes but not always have been observed to enhanced the serumβ-carotene or retinol concentration in population whose marginal vitamin A status is poor than (Bulux *et al.,* 1994, de Pee *et al.,* 1995, Solomon *et al.,* 1993, Solomon 1996). The following are factors that can seriously affect carotene absorption: high rates of parasitic infections, very low- fat diets consumption, and impaired absorption capability as a result of malnutrition (Bowen *et al.,* 1993, Erdman *et al.* 1993, Olson 1994b, Parker 1996).

2.2.2 Calcium Transport Activity in Carrot

Intake of low dietary calcium can impact health negatively and enhanced the risk of diseases known as osteoporosis. Fruits and vegetables offer a diverse mixture of nutrients that promote good health, and it is generally believed that they will be more beneficial to human health than dietary supplements. One way to increase the nutrient content of some vegetables is to increase their bioavailable calcium levels. Carrots are among the most popular vegetables in the United Statesand contain high levels of beta carotene (the precursor to Vitamin A) and other vitamins and minerals; however, like many vegetables, they are a poor source of dietary calcium. By engineering carrots and other vegetables to contain increased calcium levels, one may boost calcium uptakeand reduce the incidence of calcium deficiencies(Roger *et al.*, 2007).

Generally, calcium (Ca) levels in plants can be engineered through