DEPARTMENTOFSCIENCELABORATORYTECHNOLOGY

ISOLATION AND IDENTIFICATION OF FUNGI RESPONSIBLE FOR SPOILAGE OF CARROT.

BY

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CERTIFICATION

This is to certify that this project work presented by; BAMISAYE REBECCA OLUWATOYIN withMatriculationNumber(HND/23/SLT/FT/0244) hasbeenread,approved and submitted to the department of Science Laboratory Technology (Microbiology unit), institute of applied science, Kwara State, Ilorin.

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DEDICATION

This project is dedicated to Almighty God, the most merciful, the most gracious, who as protected me through the completion of this academic program. May HIS name be praised forever.

Also to my family for their support throughout this project and to the department of microbiology.

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Contents

ABSTRACT

ISOLATION AND IDENTIFICATION OF FUNGI RESPONSIBLE FORSPOILAGE OF CARROTS

Abstract

Over the past decades vegetable consumption specifically carrot has been on the rise however, its wastage due to microbial spoilage has been estimated at around 20% annually. In this study, spoilage fungiassociated with carrots were identified by employing standard microbiological procedures.Various tests were used to characterize carrots with soft rot symptoms. This study was aimed 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 recorded the highest (40%)while the least prevalence of the fungi was Mucor sp. (9%). Results from this study affirmed that spoilage fungi are present in carrots, therefore care must be taken in handling ,washing and processing carrots before consumption so as to prevent spoilage that might lead to infections and food-borne out breaks due to fungi.

Keywords:Carrots,Foodspoilage,Food-borneoutbreaks,Fungi

CHAPTER ONE

INTRODUCTION

1.1 Backgroundtothestudy

Carrots(*Daucuscarota*

)isabiennialherbaceousspecies,itispartoftheApiaceaeFamily.Carrots are classified into two mainly; Western carrots and Eastern carrots and this is based on carrot pigmentation. The origin of western carrots is not yet known while the eastern carrots is said to originate from Afghanistan. Most carrots root is purple and some are yellow. The leaves are slightly dissected and roots branched. Currently the more widely cultivated carrots in the world arethe orange carrotsand are more popular. (Que, F., Hou, Xl, Wang,G*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 raised beds can further help in proper water drainage. Carrots need soil that hasadequate air and water drainage because wet and compacted 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 underfull 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 pebblesandstonesinthesoil.Pythiumrootdieback,nematodes,andexposureto

frost are other factors that could causes tub bed off or ked roots (Anupama *et al.*, 2020)

Carrots arecropthatareableto adequately extractnutrientfromthesoildueto their deep-rooting nature. It is necessary for soil test to be carried out before planting and throughout development to measure 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 arethe 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 15thand 16thcenturies in central Europe. The reason for popularity of orange carrots was because it was observedtocontainhighProVitamineA.ThemajorAntitoxidantfoundincarrotsareCarote noids 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 prevention. Carotene level gradually increases with growth and is more concentrated at the corticlethanthecore.Carrotshavehighnutritionalvalue.Itisagoodsourceof dietaryfiberandoftracemineralsmolybdenum(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 immunoenhancers. 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 wellmakestheproductsmoreconvenienttobeconsumedbyconsumers.Consumers requested for high quality, a fresh, nutritiveandconvenientlypreparedvegetablehasincreasedsomuchintherecentyears.Thi shas ledtothedevelopment oflightlyprocessedvegetables.Preparation oflightlyprocessedcarrotsis done by peeling the epidermal layer of the carrot roots; this is one of the most popular products thatareavailablein the UnitedStates.Oneof the disadvantagesof thisprocessing methodisthat it makes carrots susceptibleto different physiological changesthat 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 whiteblush is

developedwhentheepidermallayerispeeledoffandthisresultindehydrationand lignification on the carrots surface (Bolin andHuxsoll,1991).

Thoughcarrots are important sources of nourishment to human beings (Kaur*et al.*, 2017),specifically vitamins, and could serve as an important ingredient in enhancing healthandproperdiets.However,theyarenotablesourcesofchemicalandmicrobial 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 othervegetables are consumed for their enormous nutritional benefits without thoughts of possiblecontamination 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 spoiltfruits and vegetables has been on a rising side in recent years, thus, a quest to isolate andidentify these pathogens, in particular fungi that causes spoilage shouldbe recommended as a control measure.

1.2 StatementofProblem

Increase in awareness of the health benefits of carrots has resulted in an increase in consumption.Manyvegetablesareconsumedrawtoretainthenaturaltasteandheat labile nutrients. It is claimed that Microbes are found all over the globe with some few exceptions, including sterilized surfaces.They include normal flora that is non pathogenic,which contribute to the larger percentage and pathogenic 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 identificationoffungiresponsibleforspoilageoffreshcarrot,toalsoknowpossible food borne fungi pathogen on carrots (Anupama*et al.*, 2020).

1.3 Aim

[

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

1.4 Objectives

Themainobjectiveofthisstudyistoisolateandidentifyfungiresponsibleforthe spoilage of carrots.

Specifically,thisresearchwilldothefollowing:

i. IsolateandidentifypossiblepathogenicfungioncarrotssoldinIpata Market, Ilorin.
ii. Determineantifugalsusceptibilitypatternsofthepathogensfromcarrotssold in Ipata Market, Ilorin.

1.5 ResearchQuestions

i. What method was used to isolate and identify possible pathogenic fungi oncarrots sold in Ipata Market, Ilorin?
ii. Whataretheantifugalsusceptibilitypatternsofthepathogensfromcarrotssold in Ipata Market, Ilorin?

1.6 SignificanceoftheStudy

Carrots are root vegetables that are highly consumed in every family. It is essential to health because ofitshigh nutritional value.It providesnutrientssuchasvitaminsand mineralsandalso is of medical important. Carrots are liable to contamination from various sources such as soil, man, water, air, and insects(Yong,2014).Therefore, Isolationandidentificationofpathogenicbacteriafromfreshcarrotsisnecessary,to enlighten consumer of various ways of hygienic practices that leads to reduction of microbial load and a determination of the antifungal susceptibility patterns of the isolates in case of food borne outbreak inthe country (Anupama*et al.*, 2020).

CHAPTERTWO

LITERATUREREVIEW

2.1 Carrot

2.1.1 OriginandDomestication

The Carrot (*Daucuscarota)* is a root vegetable, usually orange in color, 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. Eastern carrots tend to have less deeply divided leaflets with heavy leaf pubescence in some cultivars. For any carrot production,early flowering is unsatisfactory, eastern carrots have a greater tendency toward early floweringthanwesterncarrots,likelyduetothesomewhatwarmerclimatesoverthe easternproductionrange.Beyondtheyellow,purple,andorangerootcolors,eastern carrots have long included red-rooted types while western carrotsincluded white-rootedtypes. Carrot use hasalso varied across production areas, with a more predominant use as animal forage in the east but largely human use as a root vegetablein the west (Philipp *et al.,* 2020).

CarrotisthemostwidelygrownmemberoftheApiaceaeorUmbelliferae.They area domesticated form ofthe wild carrot, *Daucuscarota*a native to Europe and Southwestern Asia.This diverse and complex plant familyincludes 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). Underlyingvarietaldistinctionsbaseduponstoragerootcolorandshapeis

adaptation to cool versus warm growing temperatures. Carrot is categorized as a cool-seasonvegetableandthemajorityofeffortoncarrotbreedinghasbeentowards 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 excessiveheatcanretardplantgrowth,inhibitrootcolordevelopment,andstimulate 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 climatesforsubtropicaltypes(Philipp*etal.*,2020).Subtropicalcarrotstendtogrow 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 rootproductionandfloweringsothatthesamecultivartheoreticallycouldbegrown anywhere in the world, if temperature requirements are met.

Infact,manycarrotcultivarsarewidelyadaptedandcanbegrownoversuch

extremeproductiontemperaturesasrepresentedbynorthoftheArcticCircletohighland 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 3000 to 5000 years ago in Switzerland and Germany(Laufer,1919).Thisseedisthoughttobefromwildcarrotusedforflavoror 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),butsuchestimateshavelittlereliabledatatoconfirmthemand truevalueislikelymuchmore.Thedevelopmentofcultivarsadaptedforcultivation in both summer and winter seasons on all continents has allowed a year-round availability of carrot products 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 consumerneedsareaddressedbypublicandprivatecarrotbreedersthatincorporate modern technologies into the classical breeding process (Philipp *et al.,* 2020).

Thegeneticimprovementofcarrothasbeenanongoingeffortthroughoutits cultivation and domestication. Before the 20thcentury, carrot production was small scale in family or community gardens.A portion of the crop was likely protected in thefieldoverwinterwithmulch,orthebestrootssavedincellarswerereplantedthe subsequent spring to produce a seed crop. There is no written record of what traitswereevaluatedoranyotherdetailoftheselectionprocessinthisperiod,butall domesticatedcarrotdiffersfromitswildprogenitorsinforminglarger,smoother storageroots,soitisclearthatthesetraitsalsowereimprovedthroughregularselection.Sele ction 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 saythatcolorandflavorwereprimaryselectioncriteriasincetheywerethetraits 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

Carrotswerethecolorsofthefirstdomesticatedcarrots.Theseweretheonlycolors recordeduntilthe16thto17thcenturywhenorangecarrotswerefirstdescribedand sooncametobepreferredin boththeeasternand westernproduction areas

(Rubatzky et al. 1999, Simon, 2000). Banga compiled an extensive list of comments about carrots over history and while purple carrots were usually (butnotalways) regarded as better flavored than yellow, the darkstainstheyleftonhands, cookware,andincookingwaterraisednegativecommentsbysomeauthors.Wedonotknow why early carrot breeders shifted their preference to orange types, but thispreferencehashad asignificanteffectinprovidingarichsourceofvitaminA, fromalpha- andbeta-carotene,tocarrot consumersever since. Soonafter orangecarrotsbecame popular,thefirstnamedcarrotcultivarscametobedescribedintermsofshape,size, color, and flavor, and the first commercially sold carrot seed included reference to this growing list of distinguishing traits.

2.1.2 DiseaseResistance

Disease and pests limit carrot production to some extent in all carrot production regions. Leaf blights caused by *Alternariadauci*, *Cercosporacarotae*, and *Xanthomonascampestris*pv.*carotae*,powderymildew(*Erisipheheraclei*),carrotfly (*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 isregular disease occurrence to make progressin

selectingforgeneticresistanceformostdiseases.Oftenhighlysusceptiblecultivars

or inbreeds are interspersed among entries to be tested in the field and in some casesnaturalinoculationissupplementedwithinoculumfromartificiallyinfested plants. This approach has been used in selecting for resistance to *Alternaria* leaf blight (Boiteux*etal.*,1993;Simon andStrandberg,1998),andaster yellows (Gabelman*etal.*,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*etal*.,2000)in controlled environments such as a greenhouse or growth chambers have also been developed.

2.1.3 ConsumerQuality

Selectionforuniformorangecolorhasbeenexercisedbycarrotbreedersforthelast century.The nutritional quality conferred by the provitamin. A carotenoid that account for the orange color of carrots has received the attention of carrot breeders since the 1960s beginning with extensive efforts of W.H. Gabelmanand his students(Umiel*et al* 1972;Buishand*et al* 1979).As a result, selection has raised

provitamin carotene content in typical U.S. carrot varieties by70% between 1970 and 1992 (Simon, 1992). Yellow, purple, red, and white carrots have received a renewedlevelofinterestinrecentyearsasgrowerslookfornewnichemarketsand consumers become more aware of the nutritional benefits of pigments. To support selection 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 carrotcolorarecausedbycarotenoidsluteinandlycopene,respectively,andpurple carrot color is caused by anthocyanins (Surles*et al.*, 2004). When no pigments accumulate, carrots are white. The commercialinterestin carrotsofunusualcolorshasstimulatedresearchtodeterminethegenetics 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,aSCARmarkerdevelopedfor*Y2*(BradeenandSimon,1998),and20QTL 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 observed for purple color,as a wide range of pigmentation patterns occur,andatleastoneothergene,*P2*,influencespigmentationinaerialplantparts (Simon,1996).Todevelopbreedingstockwithpotentialcommercialapplication,

carrot breeders utilize traditional regional carrots and long- ignored heirloom cultivarswithunusualcolorsincrosseswithadapted,good-flavoredorangecarrots tocombineunusualcolorwithacceptableflavorformodernconsumers(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 incompletedominancesothatlow-nitrateparentsarenecessarytoobtainlow–nitrate hybrids. In fact, while heterosis has significant positive effects upon manycarrot production attributes, it is not observedforcarotenoid or nutrient content, as mid parent values are observed in the majority of hybrids (Philipp *et al*., 2020).

Carrot flavor isa very important variable influencing consumer decisions. Flavor differenceswerenotedbetweenpurpleandyellowcarrotshundredsofyearsagoand among modern orange carrot root types today, sweet and juicy flavor can be found in a wide range oftypes 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 ofharsh or turpentine flavor, caused by volatile terpenoids is the primary flavor component evaluatedinselectingforimprovedflavorsincehighlevelsinharshcarrotscan

mask sweet flavor. Laboratory –facilitated selection is sometimes used for sweetness,usingrefractiveindex,colorimetric,orHPLCmethodstoquantify 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 patternsofinheritancearecomplex.Sweetflavor,notsurprisingly,isassociatedwith higher sugar content which is polygenic, although as in glemajorgene, *Rs*,determines whether reducing sugars glucose and fructose, or sucrose, are theprimary 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,thebrittlenessthataccompaniescrisptexturetendstohave anegativeimpactonthe“durability”ofcarrotsinmechanicalharvestingandwashing (Philipp *et al.,* 2020).

2.2 NutritionalValueofCarrot

2.2.1 Bioavalaibilityofβ-Carotene

DeficiencyinVitaminAremainsamajornutritionalprobleminmosteconomically

disadvantaged areas of the world (Olson 1994a, Sommer*et al.*,1996), this makes the populationtorelyondietarysourcesofprovitamin.Acarotenoidtomeettheneedof vitamin A. It has been considered that the most appropriate solution to this problem is the strategies developed by Public health which enhanced the increased intake of carotenoid rich vegetables and fruits (Solomonand Bulux 1993). Various factors affect the bioavailability of carotenoids, such as characteristics ofthefood source, interaction withother dietaryfactors 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 feedingof processedvs.rawvegetablesthepercentagechangesinplasmaofcis-β-caroteneand α-carotene concentration remains the same. Daily consumption of processed carrots within4weekswillresultproductionofplasmaβ-caroteneresponsecomparetothe 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-caroteneisomers.Resultfromstudieshavealsomadeasuggestionthatis omersofcis-β-carotenehavelessofprovitamineAactivitythanthatofall-trans-β-

carotene, and lower bioavailability may also be explained bysome absorption and discriminationofisomers(Erdman*etal*.,1993,Gaziano*etal.*,1995,dePee*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 CalciumTransportActivityinCarrot

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 bemore 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 levelsofbetacarotene(theprecursortoVitaminA)andothervitaminsandminerals; however, like many vegetables, they are a poor source of dietary calcium. By engineeringcarrotsandothervegetablestocontainincreasedcalciumlevels,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 high-level expression of a deregulated Arabidopsis calcium transporter. An Arabidopsis vacuolar calcium anti porter, termed Cation exchanger 1 (CAX1), contains an N-terminal auto inhibitory domain. Expression of N- terminaltruncationsofCAX1 (sCAX1)inplants such as potatoes, tomatoes ,and carrots increase the calciumcontent in the edible portion of these foods. Presumably, these sCAX1-expressing plantshaveheightenedsequestrationofcalciumintothelargecentralplantvacuoles.(Roger *etal.,* 2007.Modificationofcarrotsto express increased levels of a plant calcium transporter(sCAX1), and these plants contain higher calcium content in the edible portions of the carrots, helps to improve the bioavailable calcium content of a staple food;when applied to a wide variety of fruits and vegetables, this strategy could lead to more calcium consumption in the diet. By this means one could rid of low intake of calcium in a deficient population. (Roger *et al.*, 2007)

2.3 StorageandPreservationofCarrots

Garden vegetables lose their physiochemical and organoleptic properties in a few days after harvesting especially when they are stored in ambient conditions(Caron *etal.*,2003).In carrots,mass loss and the incidence of disease in the root are the principal causes of post harvest loss during storage and commercialization (Oliveira *etal.*,2001).Inmostvegetables,masslosses of5%orhighercanproducewrinkling

and a consequent decline in consumer acceptance. This is due to high rates of transpiration, which affects the product's appearance by wrinkling and altering the texture of its skin, among other effects (Caron *et al.*, 2003). The water content of carrot roots varies from 85to90%, a largepart of which is lost through transpiration. Transpiration is a consequence of vapor pressure deficit (VPD), which results from the difference between the humidity at the surface of the product and the humidityof the surrounding air (Chitarra, 2005). Devraj (2001) emphasizes that 25-30% ofthe production of fruits and vegetables are wasted due to the lack of proper postharvest handlingand storage. Carrot is well-storable vegetable species (Valšíková*et al.*, 2009). The shelf life of carrot quality is ranged from 3 to 6 month at the temperature from - 0,5°C to+1,5°C (Valšíková*et al.*, 2002), Uher*et al.*, 2009) indicate that carrot designed for storage requires high relative air humidity because its anatomical structure does not allow preventing to waterlosseseffectively.Carrot should bestoredatrelativelyhumidityof98-99%.Theusefullife of product, e. g. carrot can be extended by using flexible plastic film that acts as modified atmosphere packaging. The aim of plastic film is to reduce the respiration, defend to the weight loss and microbial growth rates, as well as delay enzymatic deterioration, with the end effect of prolonging shelf life (Kumar *et al*., 1999), (Caron *et al.*,2003) also stated that package is very important factor affecting to the weight loss and storage period of carrot roots. (Oliveira 2001) found that the most suitable package material, fromaspectofweightloss,isPVCfilm.Ontheotherside,(Ayub*etal*.,2010)observed

ahigherpercentageofcarrotrootssproutingwhenstoredwrappedinPVCfilm.

(Koraddiand*et al.,* 2011)examined the effect of various types of packing materialswithseveralvegetablespeciesinrefrigerator.Theyalsoconfirmedtheimportant role of package from aspect of weight loss and shelflife of stored products (Philipp*et al.,* 2020).

2.4 Fungi

Fungi are eukaryotic microorganisms. Fungi can occur as yeasts, molds, or as a combination of both forms. Some fungi are capable of causing superficial, cutaneous, subcutaneous, systemic or allergic diseases. Yeasts are microscopic fungi consisting of solitary cells that reproduce by budding. Molds, in contrast, occur in long filaments known as hyphae, which grow by apical extension. Hyphae can be sparsely septate to regularly septate and possess a variable number of nuclei. Regardless of their shape or size, fungi are all heterotrophic and digest their food externally by releasing hydrolytic enzymes into their immediate surroundings (absorptive nutrition). Other characteristics of fungi are the ability to synthesize lysine by the L-α-adipic acid biosynthetic pathway and possession of a chitinous cell wall, plasma membranes containing the sterol ergosterol, 80S rRNA, and microtubules composed of tubulin.

2.4.1 Physiology

Fungi can use a number of different carbon sources to meet their carbon needs for the synthesisofcarbohydrates,lipids,nucleicacids,andproteins.Oxidationofsugars,

alcohols, proteins, lipids, and polysaccharides provides them with a source of energy. Differences in their ability to utilize different carbon sources, such as simple sugars, sugar acids, and sugar alcohols, are used, along with morphology, to differentiate the various yeasts. Fungi require a source of nitrogen for synthesis of amino acids for proteins, purines and pyrimidines for nucleic acids, glucosamine for chitin, and various vitamins. Depending on the fungus, nitrogen may be obtained in the form of nitrate, nitrite, ammonium, or organic nitrogen; no fungus can fix nitrogen. Most fungi use nitrate, which is reduced first to nitrite (with the aid of nitrate reductase)and then to ammonia.

Nonfungal organisms, including bacteria, synthesize the amino acid lysine by the *meso*-α,ε-diaminopimelic acid pathway (DAP pathway), whereas fungi synthesize lysine by only the L-α-adipic acid pathway (AAA pathway). Use of the DAP pathway is one of the reasons microorganisms previously considered to be fungi, such as the myxomycetes, oomycetes, and hyphochytrids, are no longer classified as fungi. The DAP and AAA biosynthetic pathways for lysine synthesis represent dichotomous evolution.

2.4.2 MeaningofIsolationandIdentification

In microbiology, the term isolation refers to the separation of a strain from a natural, mixed populationoflivingmicrobes,aspresentintheenvironment,forexampleinwater orsoil floraor from living beings with skin flora, oral flora or gut flora, in order to identify the microbe(s) of interest. Historically, the laboratory techniques ofisolation firstdeveloped in thefield of bacteriology and parasitology (during the19th century), before those in virology during the 20thcentury (Wikipedia 2021). Identification: Bacteria are classified and identified to distinguish among strains and togroupthembycriteriaofinteresttomicrobiologistsandotherscientists(Baron,

1996).

CHAPTERTHREE

MATERIALANDMETHODS

3.1 StudyArea

TheresearchwasdoneinIpataMarket,IlorinareaOfKwaraState.

3.2 Collectionofsamples

Ten (10) carrot samples with soft rot symptoms were purchased from Ipata Market, Ilorin. They were kept in sterile polythene bags before transporting to microbiology laboratory at Kwara State Polytechnic Ilorin where analysis was done.The carrots were washed with clean running water which was followed by cutting of fat the margin of rotted tissue segments (1g)with a sterilized knife and grinded with mortar and pestle.

3.3 Isolationofcoliforms

Coliforms were isolated by membrane filtration technique through a membrane filtration funnelwith a 50ml capacity.The membrane filtration funnelwaspositionedatafixedportionattachedtoavacuumpumpallowing passageofwaterintoporousandsterilizedmembranefilter(0.45µm).Withan aid of sterile forceps ,the filters were positioned on Mac Conkey agar plates after influx of 100ml of carrot samples. The media was prepared and was followed by autoclaving at 121OC for 15mins at 15Ib prior inoculation with the filters.

3.4Fungalcharacterization

Ten-foldserialdilutionswithdilutionfactorof10-3platedoutwith1mlof

samples inoculated into prepared and solidified potato dextrose agar (PDA) plates. The PDA consists of 30 mg/lof chloramphenicol which hindersbacteria growth. Incubation was done for two (2) days at roomtemperature.All fungal isolates were characterized based on macroscopic and microscopic examination.

3.5 SusceptibilityTestProcedure

Sterile Petri dishes with Muller Hinton Agar was prepared.A pinch of the isolates was picked using sterile wire loop and dipped into sterile normal saline;the turbidity was compared with 0.5Macfarland standard. A sterile cotton swap was dippedintotheinoculumandgentlystreakstheentiresurfaceofthemediumuntil evenly distributed to have a confluent growth on the petriplate. The inoculums were allowed to dry for 5 minutes along with lid in place. Thediscs wereappliedapartusingasepticتechnique.Itwasthenincubatedat35°Cfor24hrsafter allowingthe discto diffusewithinforsometimes.The plateswereexaminedfor zonesof inhibition (Barth *et al.,* 2009).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 RESULTS

The coliform count ranged from1.0±0.26to4.8±0.37x103CFU/g for samples CAGandCAD.The fungal counts ranged from 0.8±0.22to5.5±0.40x103CFU/g for samplesCAHandCAArespectively.Thefollowingfungiwereisolatedasshown in Table2:*Aspergillus niger*, *Rhizopus* sp. ,*Fusarium* sp. ,*Cladosporium* sp.and *Mucor* sp.*Aspergillus niger*(40%) was highest in the order of dominance while *Mucor* sp. (9%) had least occurrence as represented inTable3.

Table1:Microbialcounts(CFU/g)ofthecarrotsamples(×103)

Samples	Totalcoliformcount(CFU/g)	Totalfungalcount(CFU/g)
CAA	2.5±0.11	5.5±0.40
CAB	2.4±0.02	1.0±0.32
CAC	3.4±0.18	3.8±0.38
CAD	4.8±0.37	2.1±0.55
CAE	1.2±0.22	3.1±0.18
CAF	2.7±0.41	2.1±0.09
CAG	1.0±0.26	1.8±0.14
CAH	2.4±0.13	0.8±0.22
CAI	2.3±0.19	2.1±0.10
CAJ	1.5±0.16	1.5±0.17

Keys:CAA–CAJ=CarrotsamplesA–J

Table2:MicroscopicandMacroscopiccharacterizationoffungalisolates

Culturalmorphology	Microscopiccharacteristics	Fungalspecies
Presence of numerous black dots	Dichotomousbranching.Septateandhyalinedetected.Long,smoothconidiophores with hyaline, usually darkerat theapex.Numerous blackspores.	*Aspergillusniger*
Appeared whitish to cream coloration, turned bluish brown with presence of sporodochia	Short and multi-branched. Septate hyphae.Cylindrical,fusiform,curvedshapepedicellate foot cell, blunt and short	*Fusarium*sp

	apicalcell.Appearedinpairsorsinglewithglobose,hyaline,smoothandroughwalled.	
Colonies appeared olive-green to brown or black colonies	Branchedchains.Septatewithbrownhyphae. Conidiophores are erect and darkpigmented.Conidiaappearedcylindricalinshape.Fragile spore chains	*Cladosporium*sp
White to grey and fast-growing.Older colonies appeared grey tobrown	Branched. Non septate.Smooth, short withgreencolorationofconidiophores.Appeared simple,branched which formsanapical,globular sporangiasupportedandelevatedbyacolu mn-shapedcolumella	*Mucor*sp
Appeared dense with aerial mycelium. Previouslywhitebefore turning to grey	Branched.Nonseptatewithstolons.Greyishblack,flattenedandglobosesporangia,appearedpowderywith numerousspores	*Rhizopus*sp

Table3.Frequencyby occurrenceoffungalspeciesfromsampled carrots

Fungi	%
Aspergillusniger	40
*Rhizopus*sp	20
*Fusarium*sp.	16
*Cladosporium*sp	15
*Mucor*sp	9

4.2 DISCUSSION

Five(5)fungalspecieswerereportedlyisolatedfromthestudywhichincluded:

Aspergillus niger, *Rhizopus* sp., *Mucor* sp., *Cladosporium*sp. and *Fusarium*sp. The fungi species weresimilarly identified by Adebayo-Tayo*et al.* (2012), Iniekong*et al.* (2015) and Onuorah*et al.*(2016) who isolated similar fungal groups from carrots and other vegetables sold in themarket. Many of these fungi isolates linked vegetables and fruits have shown to causespoilage. Theseincluded*Fusarium*sp.,*Aspergillus*sp.,and*Cladosporium*sp.(Harding*etal.* ,2017).

Usually, spoilage fungi are also known to be toxigenic or pathogenic and they havebeenreportedlyisolatedfromvegetablesorfruits.Atthetimeofstorageand refrigeration, certain moulds may harbor mycotoxins which are injurious to human and animal health.Fungi pathogens could also cause allergies.*Aspergillus niger*(40%),which had the highest percentage occurrence in this study arenotable producers of different toxic metabolites, like naphthopyrones and malformins. Ochratoxins whichis also produced by *Aspergillusniger*, is a mycotoxin which causes hazard to man and otheranimalshealth.

CHAPTER FIVE

CONCLUSIONANDRECOMMENDATION

5.1 CONCLUSION

Thisstudyrevealedthatcarrothasaplethoraoffungiwhichcausespoilageandarealsopathogenictohumanhealth.Thereis,therefore,needtoensurethatcareistakeninhandling,washingandprocessingcarrotsbeforeconsumptionsoastopreventfoodspoilagethatmightleadtoinfectionsand food-borne diseases caused by fungi. It is also expedient to control food spoilage microorganism in order to reduce economic loss due to food spoilage.

5.2 RECOMMENDATIONS

I. Inordertoavoidfood-bornediseaserisk,specialattentionmustbepaidto improvementandcontrolofthehygienicqualityoffreshcarrotssuchas:Hand washing, epidermal scrapping, thorough washing should be practiced by both the seller and the consumer;these will reduce the fungi load on carrots to minimal.

II. Thebuyerandtheconsumershouldbeeducatedonthevarioussourcesof fungi contamination of carrots and the effect of using polluted water to wash vegetable or not washing at all before eating and the use of unclean packaging materials and the need for proper sanitation of the surroundings where carrots are sold.

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