ISOLATION AND IDENTIFICATION OF FUNGI RESPONSIBLE FOR SPOILAGE OF CARROTS

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CERTIFICATION

This is to certify that this project topic **"ISOLATION AND IDENTIFICATION OF FUNGI RESPONSIBLE FOR SPOILAGE OF CARROTS''** was carried out by **USMAN, Hassanat Motunrayo** with matriculation number **HND/23/SLT/FT/0180**, a student of Science Laboratory Technology, Institute of Applied Sciences (IAS), Kwara State Polytechnic , Ilorin.

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DEDICATION

I dedicated this project work to Almighty Allah, my Creator , who gave me the privilege to finish this my work successfully , and to my parents Mr. and Mrs. Usman that support me financially and also with prayers.

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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 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 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 Mucorsp. (9%). Results from this study affirmed that spoilage fungi are present in carrots, therefore care must be taken in handling, washing band processing carrots before consumption so as to prevent spoilage that might lead to infections and food-borne outbreaks due to fungi.

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

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CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Carrots (*Daucuscarota*) is a biennial herbaceous species, it is part of the Apiaceae Family. 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 are the orange carrots and 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 has adequate 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 under full sun exposure, this is opposite to very clay-like or wet, chalky soil. Tillage of soil is done to loosen 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 pebble sand stones in the soil. Pythium root die back, nematodes, are exposure to frost and other factors that could causes tubbed of forked roots (Anupama*etal.*,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 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 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 15^{th} and 16^{th} centuries in central Europe. The reason for popularity of orange carrots was because it was observed to contain high Pro Vitamine A. The major Antitoxidant found in carrots are Carotenoids and Anthocyanin. Yellow carrots are highly rich in Alpha and Beta carotene and rich in Pro Vitamine A (daSilva Dias 2014). 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 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, poly acetylenes, vitamins and 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 well makes the products more convenient to be consumed by consumers. Consumers requested for high quality, a fresh, nutritive and conveniently prepared vegetable has increased so much in the recent years. 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 (Peiyin and Barth 1998). A new protective layer called white blush is developed when the epidermal layer is peeled off and this result in dehydration and lignifications 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 illnesses arising 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 notable 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 raw to retain the natural taste and heat 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 identification of fungi 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 pathogenic 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 responsible for the spoilage of carrots.

Specifically, this research will do the following:

i. Isolate and identify possible pathogenic fungi on carrots sold in Ipata Market, Ilorin.
ii. Determine antifugal susceptibility patterns of the pathogens from carrots sold in Ipata Market, Ilorin.

1.5 Research Questions

i. What method was used to isolate and identify possible pathogenic 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 family. It is essential to health because of its high nutritional value. It provides nutrients such as vitamins and minerals and also is of medical important. Carrots are liable to contamination from various sources such as soil, man, water, air, and insects (Yong,2014). Therefore, Isolation and identification of pathogenic bacteria from fresh carrots is necessary, 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 in the 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 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 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, *Daucuscarota*a native to Europe and Southwestern Asia. This diverse and complex plant family includes several other vegetables, such as parsnip, fennel, celery, root parsley, celeriac, arracacha, and many herbs and 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, in contrast to a greater emphasis on the ability to produce a marketable crop in warm climates for subtropical types (Philipp *et al.,* 2020). 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 mancrops, 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 high land 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. In fact, carrot seed was found in early human habitation sites as long as 3000 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; Fonte sand Vilela, 2003; Vilela, 2004). In 2005, world production approached 24 Mton1.1 million 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 products with relatively stable prices to 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 breeders that 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 that 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 (but not always) regarded as better flavored than yellow, the dark stains they left on hands, cook ware, and in cooking water raised negative comments by some authors. We do not know why early carrot breeders shifted their preference to orange types, but this preference has had a significant effect in providing a rich source of vitamin A, from alpha –and beta-carotene, to carrot consumer sever since. Soon after orange carrots became popular, the first named carrot cultivars came to be described in terms of shape, size, color, and flavor, and the first commercially 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 carrot production regions. Leaf blights caused by *Alternariadauci*, *Cercosporacarotae*, 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 in breeds 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*etal.*,1993; Simon andStrandberg,1998),and aster yellows (Gabelman*etal.*,1994). For soil borne disease and pests, heavily infested disease evaluation plots have been established for *Meloidogyneincognita, M. javanica* (Vieira*etal.*,2003), Methods for

evaluating resistance to *Alternaria* leaf blight (Simon and Strand berg, 1998; Paweleck*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 Consumer Quality

Selection for uniform orange color has been exercised by carrot breeders for the last 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. Gabelman and 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 renewed level of interest in recent years as growers look for new niche markets and 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 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 interest in carrots of unusual 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 observed for purple color, as a wide range of pigmentation patterns

occur, and atleast 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 heterosis 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, improve draw 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 of 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 a single major gene, *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 is a convenient time to evaluate quality attributes. Unfortunately, the brittleness that accompanies crisp texture tends to have a negative impact on the "durability" of carrots in mechanical harvesting and 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 nutritional problem in most economically disadvantaged areas of the world (Olson 1994a, Sommer*et al.,*1996), this makes the population to rely on dietary sources of provitamin. A carotenoid to meet the need of 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 (Solomon and Bulux 1993). Various factors affect the bioavailability of carotenoids, such as characteristics of the food source, interaction with other dietary factors and various subject characteristics (Bowen *et al.,* 1993, Erdman *et al*., 1993, Olson1994b, Parker 1996), Size of the particle, the location 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 bio availability 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 studies have also made a suggestion that is omersofcis-β-carotene have less of provitamine A activity than that of all- trans- β-carotene, and lower bio availability 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 serum β-caroteneorretinol 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 States and 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 uptake and 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- terminal truncations of CAX1 (sCAX1) in plants such as potatoes, tomatoes, and carrots increase the calcium content in the edible portion of these foods. Presumably, these sCAX1- expressing plants have heightened sequest ration of calcium into the large central plant vacuoles. (Roger *etal.,* 2007. Modification of carrots to 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 bio available 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 Storage and Preservation of Carrots

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 *et al.,* 2001). In most vegetables, mass losses of5% or higher can produce wrinkling 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 85 to 90%, a large part 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 humidity of the surrounding air (Chitarra, 2005). Devraj (2001) emphasizes that 25-30% of the production of fruits and vegetables are wasted due to the lack of proper postharvest handling and 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 water losses effectively. Carrot should be stored at relatively humidity of 98-99%. The useful life 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, from aspect of weight loss, is PVC film. On the other side, (Ayub*etal.*,2010) observed a higher percentage of carrot roots sprouting when stored wrapped in PVC film. (Koraddi and *et al.,* 2011) examined the effect of various types of packing materials with several vegetable species in refrigerator. They also confirmed the important role of package from aspect of weight loss and shelf life 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 synthesis of carbohydrates, lipids, nucleic acids, and proteins. Oxidation of sugars, 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.

Non fungal 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 Meaning of Isolation and Identification

In microbiology, the term isolation refers to the separation of a strain from a natural, mixed population of living microbes, as present in the environment, for example in water or soil flora or from living beings with skin flora, oral flora or gut flora, in order to identify the microbe(s) of interest. Historically, the laboratory techniques of isolation first developed in the field of bacteriology and parasitology (during the 19th century), before those in virology during the 20th century (Wikipedia 2021). Identification: Bacteria are classified and identified to distinguish among strains and to group them by criteria of interest to microbiologists and other scientists (Baron,

1996).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study Area

The research was done in Ipata Market, Ilorin area Of Kwara State .

3.2 Collection of samples

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 Isolation of coliforms

Coliforms were isolated by membrane filtration technique through a membrane filtration funnel with a 50ml capacity. The membrane filtration funnel was positioned at a fixed portion attached to a vacuum pump allowing passage of water into porous and sterilized membrane filter (0.45µm). With an aid of sterile forceps, the filters were positioned on MacConkeyagar plates after influx of 100ml of carrot samples. The media was prepared and was followed by autoclaving at $121^{O}C$ for 15 mins at 15 Ib prior inoculation with the filters.

3.4 Fungal characterization

Ten- fold serial dilutions with dilution factor of 10^{-3} plated out with 1ml of samples inoculated into prepared and solidified potato dextrose agar (PDA) plates. The PDA consists of 30 mg/l of chloraphenicol which hinders bacteria growth. Incubation was done for two (2) days at room temperature. All fungal isolates were characterized based on macroscopic and microscopic examination.

3.5 Susceptibility Test Procedure

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 0.5 Mac far land standard. A sterile cotton swap was dipped into the inoculum and gently streaks the entire surface of the medium until evenly distributed to have a confluent growth on the petri plate. The inoculums were allowed to dry for 5 minutes along with lid in place. The discs were applied apart using aseptic technique. It was then incubated at 35°C for 24 hrs after allowing the disc to diffuse with in for sometimes. The plates were examined for zones of inhibition (Barth *et al.*, 2009).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 RESULTS

The coliform count ranged from 1.0±0.26to4.8±0.37x10^3CFU/g for samples CAG and CAD. The fungal counts ranged from 0.8±0.22to5.5±0.40x10^3CFU/g for samples CAH and CAA respectively. The following fungi were isolated as shown in Table 2: *Aspergillusniger*, *Rhizopus*sp., *Fusarium*sp., *Cladosporium*sp. And *Mucor*sp. *Aspergillusniger* (40%) was highest in the order of dominance while *Mucor*sp. (9%) had least occurrence as represented in Table 3.

Table1: Microbialcounts(CFU/g)ofthecarrotsamples(×10^3)

Samples	Total coliform count (CFU/g)	Total fungal count (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= Carrot samples A –J

Table2: Microscopic and Macroscopic characterization of fungal isolates

Cultural morphology	**Microscopic characteristics**	**Fungal species**
Presence of numerous black dots	Dichotomous branching. Septate and hyaline detected. Long, smooth conidiophores with hyaline, usually darker at the apex. Numerous black spores.	*Aspergillusniger*

Appeared whitish to cream coloration, turned bluish brown with presence of sporodochia	Short and multi-branched. Septatehyphae. Cylindrical, offusiform, curved shape pedicellate foot cell, blunt and short apical cell. Appeared in pairs or single with globose, hyaline, smooth and rough walled.	*Fusarium*sp
Colonies appeared olive-green to brown or black colonies	Branched chains. Septate with brown hyphae. Conidiophores are erect and dark pigmented. Conidia appeared cylindricalin shape. Fragile spore chains	*Cladosporium*sp
White to grey and fast-growing. Older colonies appeared grey to brown	Branched. Non septate. Smooth, short with green coloration of conidiophores. Appeared simple, branched which forms anapical, globularsporangia supported and elevated by acolumn-shaped columella	*Mucor*sp
Appeared dense with aerialmycelium. Previously white before turning to grey	Branched. Nonseptate with stolons. Grey ishblack, flattened and globosesporangia, appeared powdery with Numerous spores	*Rhizopus*sp

Table3. Frequency by occurrence of fungal species from sampled carrots

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

4.2 DISCUSSION

Five (5) fungal species were reportedly isolated from the study which included: *Aspergillusniger*, *Rhizopus*sp., *Mucor*sp., *Cladosporium*sp. And *Fusarium*sp. The fungi species were similarly 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 the market. Many of these fungi isolates linked vegetables and fruits have shown to cause spoilage. These included *Fusarium*sp. *Aspergillus*sp., and *Cladosporium*sp. (Harding *et al.*,2017).

Usually, spoilage fungi are also known to be toxigenic or pathogenic and they have been reportedly isolated from vegetables or fruits. At the time of storage and refrigeration, certain moulds may harbor mycotoxins which are injurious to human and animal health. Fungi pathogens could also cause allergies. *Aspergillusniger* (40%), which had the highest percentage occurrence in this study are notable producers of different toxicmetabolites, like naphthopyrones and malformins. Ochratoxins which is also produced by *Aspergillusniger*, is a mycotoxin which causes hazard to man and other animals health.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This study revealed that carrot has aplethora of fungi which cause spoilage and are also pathogenic to human health. There is, therefore, need to ensure that care is taken in handling, washing and processing carrots before consumption so as to prevent food spoilage that might lead to infections and 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. In order to avoid food-borne disease risk, special attention must be paid to improvement and control of the hygienic quality of fresh carrots such as: H and 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. The buyer and the consumer should be educated on the various sources of 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.

REFERENCES

Adams, M.R., Hartley, A.D. and Cox, L.J. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Journal of Food Microbiology* 6: 69–77.

Albrecht,J.A., Hamouz, F.L., Sumner, S.S. and Melch, V.1995. Microbial evaluation of vegetable ingredients in salad bars. *Journal of Food Protection* 58: 683–685.

Anupama Sapkota. Indoletest principle, media, procedure, types, result, uses. Microbe notes (online microbiology and biology study notes) October 23, 2020.

Ayub, P.A., Gioppo, M., Reghin, M.Y. 2010. Evaluation of the use of plastic film of polyvinyl chloride (PVC) in the storage of carrots. Semina, vol. 31, 2010,no.4, p.959-966.ISSN 1679-0359.

Baron EJ. classification. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.

Bennik, M.H.J., Vorstman, W., Smid, E.J. and Gorris, L.G.M. 1998. The influence of oxygen.

Beuchat, L.R., Nail, B.V., Adler, B.B. and Clavero, M.R.S.1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. Journal of Food Protection 61: 1305–1311.

Brackett,R.E.1992.Shelfstabilityandsafetyoffreshproduceasinfluencedbysanitationand disinfection. Journal of Food Protection 55: 808–814.

Bradeen, J.M., and Simon, P.W.1998.Conversion of an AFLP fragment linked to the carrot Y2 locus to a simple, codominant PCR-based marker form. Theor. Appl. Genet. 97:960-967.

Breton, D., C. Béasse, F. Montfort and Villeneuve, F.2003. Focus on the recent evolution of soil-borne diseases of carrots in France. Proc. 30th Intl. Carrot Conf. Sept 7-10, 2003, USA.

Buish and, J.G., and Gabelman, W.H. 1979. Investigations on the inheritance of colour and carotenoid content in phloem and xylem of carrot roots (DaucuscarotaL.). Euphytica28:611-632.\

Bulux,J., deSerrano, J., Giuliano, A., Perez, R., Lopez, Y., Rivera, C., Solomons,

N.W. & Canfield, L.M. (1994) Plasma response of children to short-term chronicb-carotene supplementation. Am. J. Clin. Nutr. 59: 1369–1375.

Busayo R. Adegun, Anthonia O. Oluduro, Oladipupo A. Aregbesola. 2019 Isolation and molecular characterization of *citrobacter* species in fruits and vegetables sold for consumption in ILE-IFE, Nigeria. journal homepage: www.elsevier.com/locate/sciaf

Caron, V.N., Jacomino, A.P., Kluge, P.A. 2003. Storage of 'Brasilia' carrot treated with waxes. Horticultura Brasileira, vo.21, 2003, no. 4,p.597-600.ISSN1806-9991.

Carbon dioxide on the growth of the prevalent Enter obacteriaceae and Pseudomonas species isolated from fresh and controlled-atmosphere-stored vegetables. Food Microbiology 15:459–469.

De Pee, S., West, C.E., Muhilal, Karyadi, D. & Hautvast, J. G.(1995) Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 346: 75–81.

Devraj,T.S.2001. Drying and shelf life of fresh cauli flower. Indian Food Packer, vol. 40,2001, no. 6, p. 7-11. ISSN 0019-4808.

Erdman,J.W., Bierer, T.L. & Gugger, E.T. (1993) Absorption and transport of carotenoids. Ann. N.Y. Acad. Sci.691:76–85.

Fontes,R.R., and Vilela, N.J.2003. The current status of Brazilian crops and future opportunities. Acta Hort. 607:135-141.

GadafiIddrisu Balali, Denis DekugmenYar, Vera Gobe AfuaDela, Priscilla Adjei-Kusi, "Microbial Contamination, an Increasing Threat to the Consumption of Fresh Fruits and Vegetables in Today's World", International Journal of Microbiology, vol. 2020, Article ID 3029295, 13 pages, 2020. https://doi.org/10.1155/2020/3029295

Gaziano,J.M., Johnson,E.J., Russell,R.M., Manson,J.E., Stampfer,M.J., Ridker,P.M., Frei,B., Hennekens,C.H. & Krinsky, N.I. (1995) Discrimination in absorption or Transport of β-caroteneisomers after oral supplementa-tion with either all trans-or9-cis- β-carotene. Am. J. Clin. Nutr. 61: 1248–1252.

Geldreich, E.E., Huf, C.B., Bordner, R.H., Kabler, P.W. and Clark, H.F. 1962. The faecal coli- aerogenes flora of soils from various geographical areas. Journal of Applied Bacteriology 25: 87-93.

JWWilson, MJSchurr, CLLeBlanc, RRamamurthy, KLBuchanan, CANickerson2002 Mechanisms of bacterial pathogenicity Postgrad Med J 2002;78:216–224

Koraddiand,V.,Devendrappa,S.2011.Analysis of physiological loss of weight of vegetables under refrigerated conditions. Journal of Farm Sciences, vol.1, 2011,no. 1, p.61-68.ISSN2250-0499.

Kumar,J.,Mangal,J.I.,Tewatta,A.S.1999. Effect of storage conditions and packing materials on shelf life of carrot cv. Hisar Gairic. Vegetable Science, vol. 26, 1999, no. 2, p. 196-197.ISSN0970-6585.

Lisle,J.T., Broadaway,S.C., Prescott,A.M.,Pyle,B.H., Fricker,C. and McFeters, G.A.1998. Effects of starvation on physiological activity and chlorinedis infection resistance in Escherichia coli O157:H7. Applied Environmental Microbiology 64: 4658–4662.

Lopes,A.C.D.S., Rodrigues, J.F.and Morais, M.A.D. 2005. Molecular typing of Klebsiella pneumonia isolates from public hospitals in Recife, Brazil. Microbiological Research 160:37-46.

Olson,J.A.(1994a) Hypo vitaminosis A: contemporary scientific issues. J. Nutr. 124 (suppl.): 1461S–1466S.

Oliveira, V. P.,Gianasi, L., Mascarenhas, M.H. T., Pires, N. M., Viana, M. C. M.2001. Packaging carrots cv. Brasilia with pvc. film. Ciênciaeagro tecnologia, vol.25, 2001, no.6,p. 1321-1329.ISSN1981-1829.

Parker,R.S.(1996) Absorption, metabolism and transport of carotenoids. FASEBJ.10:542– 551.

Pawelec A.,C. Dubourg, and Briard, M.2006. Evaluation of carrot resistance to Alternaria Leaf Blight in controlled environments. Plant Path. 55:68-72.

Poor, C. L., Bierer, T. L., Merchen, N. R., Fahey, G. C. & Erdman, J. W. (1993) The accumulation of a-and b-carotene inserum and tissues of predominant calvesfed raw and steamed carrot slurries. J. Nutr. 123: 1296–1304.

Ponniah,J.,Tunung,R.,Margaret,S.P.,Son,R.,Farinazleen,M.G.,Cheah,Y.K.,Nishibuch i, M., Nakaguchi, Y. and Malakar, P.K. 2010. *Listeria monocytogenes*in raw salad vegetables sold at retail level in Malaysia. Food Control 21: 774-778.

Public Health England (2019), Preparation of samples and dilutions, plating and sub-

culture. National Infection Service. Food, Water & Environmental Microbiology Standard Method FNES26 (F2); Version 4.

Puspanadan,S., Afsah-Hejri, L., Loo,Y.Y, Nillian, E., Kuan, C.H.,Goh, S.G., Chang, W.S., Lye, Y.L., John, Y.H.T., Rukayadi, Y., Yoshitsugu, N, Nishibuchi, M. and Son, R. 2012. Detection of *Klebsiellapneumoniae*in raw vegetables using Most Probable Number- Polymerase Chain Reaction (MPN-PCR). International Food Research *Journal* 19(4): 1757-1762 (2012).

Rao,D.V. and Rao,K. R.G. 1983. Some characteristics of Klebsiellastrains isolated from foods and water. Journal of Food Science and Technology 20: 269-272.

Reynolds,G.,Mekras,C.,Perry,R.andGraham,N.1989.Alternativedisinfectantchemicalsfor trihalomethane—a review. Environmental Technology (Letters) 10: 591–595.

Rubatzky,V.E.,C.F.Quiros,andSimon,P.W.1999.CarrotsandrelatedvegetableUmbelliferae. CABIPubl.,NewYork.

Ryan,K.J.andRay,C.G.2004.Sherris Medical Microbiology (4thed.). McGraw Hill. ISBN 08385-8529-9.

Sahilah, A.M., TuanSuraya, T.S.,Noraida., I., Ahmad Azuhairi, A., Chai, L.C.andSon,R.2010.Detection of virulencegenes and enterobacterial repetitive intergenic consensus- PCR (ERIC-PCR) analysis among raw vegetable isolates of Campylobacter jejuni. International Food Research Journal17:681-690.

Sagar Aryal. catalase test-principle, uses, procedure, result, interpretation with precautions. Micro biology info. com. june 11,2018.

Sagar Aryal (2018). Coagulase Test Principle, Procedure, Types, Interpretation and Examples. https://microbiology info.com/coagulase-test-principal-procedure-types-interpretation-and-examples/

Sagar Aryal (2018). Methyl Red (MR) Test-Principle, Procedure and Result Interpretation. https://microbiology info.com/methyl-red-mr-test-principle-procedure-and-result- interpretation/

Sagar Aryal. Oxidesetest principle, procedure and result. Micro be notes (onlinemicrobiologyand biology study study notes)march 22,2021. https://microbenotes.com/oxidase-test-principle-procedure-and-results/

Sagar Aryal (2018). Voges Proskauer (VP) Test https://microbenotes.com/voges-proskauer-vp-test/

Simon,P.W.2000.Domestication,historicaldevelopment,andmodernbreedingofcarrot. PlantBreed.Rev. 19:157-190.

Simon,P.W.,andStrandberg,J.O.1998.DiallelanalysisofresistanceincarrottoAlternarial eaf blight. J. Amer. Soc. Hort. Sci. 123:412-415.

Simon,P.W.1996.Inheritanceandexpressionofpurpleandyellowstoragerootcolorincarro t.J.Hered.87:63-66.

Simon,P.W.1992.Geneticimprovementofvegetablecarotenecontent.In:Biotechnologya nd nutrition: Proc. Third Int. Symp. D.D. Billsand S.-D. Kung (eds.), Butterworth- Heinemann, London. pp 291-300.

Simon, P.W.,C.E. Peterson, and Lindsay, R.C.1982.Genotype, soil ,and climate effects on sensory and objective components of carrot flavour. J.Amer.Soc. Hort.Sci.107:644- 648.

Solomons,N.W. & Bulux,J.(1993) Plant sources of provitamin A and human nutriture.Nutr.Rev.51:199–204.

Sommer,A.&West,K.P.(1996)VitaminADeficiency.Health,Survival,andVision.Oxfor d University Press, New York, NY.

Stomme l,J. R., and Simon, P.W.1989. Phenotypic recurrent selection and heritability estimates for total dissolved solids and sugar type in carrot. J.Amer.Soc. Hort. Sci.114:695-699.

Surles, R.L., Ning Weng, P.W. Simon, and Tanumihardjo, S.A. 2004. Carotenoid profiles and consumer sensory evaluation of specialty carrots (DaucuscarotaL.) of various colours.J. Agric. Food Chem. 52:3417-3421.

Tunung, R., Ghazali, F.M., Noranizan, M.A., Haresh, K.K., Lesley, M.B., Nakaguchi, Y., Nishibuchi, M.andSon, R.2011. Rapid detection and enumeration of pathogenic Vibrio parahaemolyticus in raw vegetables from retail outlets. International Food Research Journal 18: 67-78.

Tunung, R., Margaret, S.P., Jeyaletchumi, P., Chai, L.C., Zainazor,T.C.,Ghazali, F.M., Nakaghuchi,Y., Nishibuchi,M. and Son, R.2010. Prevalence and quantification of *Vibrio*in raw vegetable satretaillevel. Journal of Microbiology and Biotechnology 20(2): 391-396.

Uher,A.,Kóňa,J.,Valšíková,M.,Andrejiová,A.2009.Zelenina –poľnépestovanie.Nitra. SPU,2009.212s.ISBN978-80-552-0199-3.

Umiel,N.,andGabelman,W.H.1972.Inheritanceofrootcolourandcarotenoid synthesisin carrot, Daucuscarota, L.: Orange vs. red. J. Amer. Soc. Hort. Sci. 97:453-460.

Usha,M.R.,Tunung,R.,Chai,L.C.,Ghazali,F.M.,Cheah,Y.K.,Nishibuchi,M.andSon, R.2010.Astudyon*Campylobacterjejuni*cross-contaminationduringchilledbroiler preparation. International Food Research *Journal* 17: 107-115.

Valšíková,M.,Kopec,K.Pozberovátechnológiazáhradníckychplodín.Nitra:SPU,2009,158s.ISBN978-80-552-0313-3.

VieiraJ.V.,F.A.S.Aragão, and Boiteux,L.S.2003.Heritability and gain from selection for field resistance against multiple root-knot nematode species (Meloidogyne incognita race 1 and M. javanica) in carrot. Euphytica 130:11-16.

VilelaN.J.2004.Cenoura:umaliment on obrenamesa popular. Horticultura Brasileira 22: cover article.

Wang,M.,andGoldman,I.1996.Resistance to root knotnematode (Meloidogynehapla Chitwood) in carrot is controlled by two recessive genes. J. Hered. 87:119-123.

XianzhouNie, Rudra P. Singh,Chapter 33 - Viroid Detection and Identification by Bioassay,Editor(s): Ahmed Hadidi, Ricardo Flores, John W. Randles, Peter Palukaitis,Viroids and Satellites,Academic Press,2017,Pages 347-356.

Yang,Z.,Jiao,X.,Cao,G.,Fang,W.andGu,R.2008.Isolationandmolecularcharacterization of *Vibrio parahaemolyticus*from fresh, low-temperature preserved, dried and salted seafood productsin two coastal areas of eastern China. International *Journal* of Food Microbiology 125: 279-285.

Zhou, J. R., Gugger, E. T. & Erdman, J. W. (1996) The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of a-and b-carotene in the ferret model. J. Am. Coll. Nutr. 15: 84–91