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Proximate Analysis, Isolation and Identification of Fungal Flora from Fresh and Dried Tomatoes obtained from Rimi Market, Nigeria

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Abstract

Tomato is a widely consumed fruit both in raw and processed forms. It is rich in vitamin A and vitamin C, Carbohydrate, protein, fat and fibers. It is rich in lycopene, which has many beneficial health effects. This study was conducted to identify the fungal flora associated with fresh and dried tomato and also to compare the proximate analysis of fresh and dried tomato. The samples were obtained from Rimi market in Kano. The samples were cultured using Potato Dextrose Agar (PDA). Pure culture of the resulting fungal colonies were obtained from primary plate and identified by using morphological and microscopic characterizations. Two fungi were isolated from fresh tomato: *Aspergillus species,* and *Mucorspecies*. *Aspergillus species* has the highest occurrence in fresh tomato and five fungi were isolated from dried tomato: *Mucorspecies, Fusariumspecies*. *Aspergillus species, Yeast and Rhizopusspecies. Rhizopus species* have the highest number of occurrence in dried tomato. Carbohydrate, fat, crude fibre and ash content were found to be higher in dried tomato than the fresh tomato. The result of the study shows that fresh tomato has less fungi isolate as compared to the dried. Therefore it is recommended to use fresh tomato instead of dried.

Keywords: Tomato, fungi, proximate analysis

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are a berry, annual, short-lived herbaceous plant of the family *Solanceae*. Tomato is a widely consumed fruit in both raw and processed forms. It is rich in vitamin A and vitamin C, carbohydrate, protein, fat and fibers. Large amount of water in tomato makes it susceptible to spoilage by the action of microorganisms. Low sugar content

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of Tomato makes it sweetless (Onurah *et al*., 2015).Tomato has antioxidant activity and some bioactive compounds(Carotenoids, flavonoids, phenolic acids).Lycopene was also found in tomato, it is an important carotenoid in human plasma, but unlike ß-Carotene it does not have the activity of vitamin A. Lycopene has the ability to prevent oxidative reactions. Oxidative processes of cell metabolism generate toxic compounds which can be neutralize by lycopene, thus protecting certain biomolecules (lipids, proteins and DNA). Lycopene has the ability to reduce cholesterol in blood. (Cernisev and Galima, 2007). Drying is the oldest preservation method, requiring only the sun's energy over several days. This involves removal of water from the fruit, leaving all the vitamins, minerals and flavor. In order to sun- dry tomatoes in the traditional way, you need ideal weather conditions - long, hot, sunny summer days with a gentle breeze and low humidity. For those with less than ideal weather conditions, tomatoes may be dried in an oven or a dehydrator. A 100% vegetable product contains virtually no fat and no cholesterol, low in sodium and naturally low in calories. Vitamin A is a compound with antioxidant property which was found in Sun dried Tomatoes .It protect the cell from damage and also lower the risk of cancer. Fungal are an ancient group not as old as bacteria, which fossil evidence suggests may be 3.5billion years old but the earliest fungal fossils are from Ordovician, 460 to 455million years old. (Lorri *et al*., 2012).It has the ability to digest and absorb nutrient directly through its cell walls. They are used in production of food and spirits, including cheese, bread, and beer. Some fungi has bioactive compounds, therefore can be used to synthesize drugs. Due to economic hardship,most people go for the sun dried tomatoes. Despite the health hazards associated with the consumption of the sun dried tomatoes. There is need to educate the public on risk of fungi present in the tomatoes and to compare the nutritional values of fresh and dried tomatoes. Some of these fungal diseases could be systematic and opportunistic pathogens and has increased substantially during the past several decades. Most of these fungal isolates have mycotoxin, which is very harmful to human health

This research was aimed to isolate and identify fungi associated with fresh and dried tomatoes fruits and to also compare the proximate analysis of the fresh and sun dried tomatoes.

MATERIAL AND METHODS

Sample Collection Area

A total of twenty samples each of both the fresh and dried tomatoes were randomly collected from Rimi market in Kano. Rimi market is directly opposite first bank and it is located in Kano state.Its popularly known as kasuwar Rimi. It is a sprawling open- air market particularly known for discounted household item, including foodstuff, fruits, vegetables etc.

SAMPLE COLLECTION AND CULTURE MEDIA PREPARATION

The two samples were placed in a clean plastic bag and labeled respectively. The sample was then carried to the laboratory and it was worked on immediately. Potato dextrose agar (PDA) containing chloramphenicol (30mg/l) was used. The culture media was prepared according to manufacturer's recommendations. The quantities of the appropriate medium or base medium were weighed. This was followed by dissolving the weighed amount of media in 400ml of distilled water. The media was heated to boil over bunser flame until agar melts. The molten agar media was allowed to cool to between 45 degree to 50 degree and the pH adjusted according to the manufacturer's recommendation. The media was cotton plugged and wrapped with aluminum foil and was autoclaved at 121 degree at the pressure of 15 pound per square inch for 15 minutes. After sterilization, the media was aseptically dispensed in

20ml aliquots sterile petri dishes and was allowed to set on the flat, then aseptically dispensed into sterile petri dishes. The petri dishes were labeled appropriately.

INNOCULATION

Isolation of storage fungi from each of the dried and fresh tomatoes were carried out by using the method of Ali *et al.,* (2006). A section of both the fresh and dried tomatoes were cut and place on different plates of potato dextrose agar (PDA). The plates were incubated at ambient room temperature (25 -30) degree for 3 days. After incubation, colonies of different shape and colours were observed on the plates. From the inoculated plates the different fungal isolate with different coloration observed such as: white, black, pink and cream which signifies different fungal colonies. A pure culture of each colony type on each plate were obtained and maintained. This was done by subculturing each of the different colonies onto PDA slant in McCartney bottles and incubated at room temperature for another 3 days(Ali *et al.,* 2006).

IDENTIFICATION OF FUNGAL ISOLATE

The identification of isolated fungi was done according to the method of James and Natalie(2001). The fungi isolated were identified on the basis of cultural and morphological features such as colony growth pattern, conidial morphology. The microscopic characterization was done using wet mount in which, a drop of lactophenol blue stain was placed on a clean grease-free sterile glass slide after which a sterile inoculating wire loop was used to pick the fungal mycelium and placed unto the glass slide then cover with a cover slip and then viewed under the microscope using 10 and ×40 magnification. The microscopic examination of actively growing mold was on the basis of structure bearing spores, hyphae (whether septate or non septate). The fungi species were observed, identified and recorded using a manual for identification of fungi by Sigurd Funder.

PROXIMATE ANALYSIS FOR FRESH AND DRIED

The moisture, protein, crude fiber, fat, ash and carbohydrate (by difference) contents were analyzed according to the AOAC method.Ash content was determined by incinerating 5 g of the samples in a muffle furnace at 550^{o}C for 4 h, cooling in a dessicator and weighing. Fat content was determined by Soxhlet extraction method in ethyl ether. Crude fiber was determined by the enzymatic-gravimetric method.Moisture was determined by drying the samples in a hot air oven at 150 ^{o}C for 3 h until a constant weight was determined. Protein content was by Kjeldahl method (total % Nitrogen×6.25 conversion factor). Crude fiber was determined by the enzymatic-gravimetric method. All the proximate analyses were carried out in triplicates and calculated in percentage.

Statistical Analysis

Triplicate results were subjected to SPSS, version 4.

RESULTS AND DISCUSSION

The result of fungal flora isolated from and dried tomato and the proximate analysis of fresh and dried tomato are presented in tables 1-5, plate 1-8 and fig. 1-2

Table 1: Morphological characteristic of fungi isolated from Fresh Tomato Rimi market

SAMPLES	MORPHOLOGICAL CHARACTERISTICS IN CULTURE
1FT	White, heavy, wooly, fluffy and growth covering entire plate.
2FT	Black, cotton like structure, heavy and growth covering entire plate.
3FT	White, heavy, wooly, fluffy and growth covering entire plate.
4FT	White, heavy, wooly, fluffy, growth covering entire plate.
5FT	White, heavy, wooly, fluffy and growth covering entire plate.
6FT	White, heavy, wooly, fluffy and growth covering entire plate.
7FT	White, heavy, wooly, fluffy and growth covering entire plate.
8FT	White, heavy, wooly, fluffy and growth covering entire plate.
9FT	White, heavy, wooly, fluffy and growth covering entire plate.
10FT	White, heavy, wooly, fluffy and growth covering entire plate.
11FT	Pinkish, cotton like and fluffy.
12FT	White, heavy, wooly, fluffy and growth covering entire plate.
13FT	Black, cotton like structure, heavy and growth covering entire plate.
14FT	Black, cotton like structure, heavy and growth covering entire plate.
15FT	White, heavy, wooly, fluffy and growth covering entire plate.
16FT	White, heavy, wooly, fluffy, growth covering entire plate.
17FT	White, heavy, wooly, fluffy and growth covering entire plate.
18FT	White, heavy, wooly, fluffy and growth covering entire plate.
19FT	White, heavy, wooly, fluffy and growth covering entire plate.
20FT	White, heavy, wooly, fluffy and growth covering entire plate.

Key: FT –fresh tomato

1-20-: Number of plates

Table 2: Morphological characteristics of fungi isolated from Dried Tomato

Samples	Morphological characteristics in culture
1DT	White, heavy, wooly, fluffy and growth covering entire plate.
2DT	White, heavy, wooly, fluffy and growth covering entire plate.
3DT	Pinkish and white cotton like growth
4DT	Flat white cotton like growth.
5DT	Heavy cotton like growth with black spot.
6DT	Pinkish and white cotton like growth.
7DT	White, heavy, cotton like growth.
8DT	White, cotton like growth, covering the entire plate.
9DT	White, heavy cotton like growth.
10DT	Flat , white, cotton like growth.
11DT	Flat, white, cotton like growth.
12DT	White and cotton like growth.
13DT	White and cotton like growth.
14DT	White, heavy, cotton like growth.
15DT	Pinkish and white cotton like growth.
16DT	White, creamy, cotton like growth.
17DT	White, creamy, cotton like growth.
18DT	White, heavy and cotton like.
19DT	Black, fluffy, cotton like.
20DT	Black, fluffy and cotton like.

KEY: DT- dried tomato

1-20: number of plates

Table 3: Microscopic Characteristics of fungi isolated from fresh Tomato from Rimi Market Kano.

Samples	Microscopic characteristics	Fungi identified
1FT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus Niger*
2FT	Septate hyphae with conidiospores borne on conidia.	*Aspergillus spp.*
3FT	Thick septate hyphae	*Aspergillus spp.*
4FT	Conidiospores with septate hypha.	*Aspergillus spp.*
5FT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp*
6FT	Unseptate hyphae are irregular in size and ribbon like.	*Mucor spp.*
7FT	Thick septate hyphae with conidia borne.	*Aspergillus spp.*
8FT	Thick septate with conidia borne	*Aspergillus spp.*
9FT	Thick septate hyphae with conidia borne in chains from the sterigmata	*Aspergillus spp.*
10FT	Thick septate hyphae with conidia borne in chains from the sterigmata	*Aspergillus spp.*

Table 4: Microscopic characteristics of fungal isolate from from Rimi market Kano.

Sample	microscopic characteristics	fungi
11FT	Thick septate hyphae with conidia borne in chains from the sterigmata	*Aspergillus spp.*
12FT	Septaehypae with conidiospore borne on conidia	*Aspergillus spp.*

13FT	Thick septate hyphae with conidia borne in chains from the sterigmata	*Aspergillus spp.*
14FT	Thick septate hyphae with conidia borne in chains from the sterigmata	*Aspergillus spp.*
15FT	Septaehypae with conidiospore borne on conidia	*Aspergillus spp.*
16FT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*
17FT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*
18FT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*
19FT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*
20FT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*

Key: FT- fresh tomato

Table 5: Microscopic characteristics of fungi isolated from Dried Tomato from Rimi market

Samples	Microscope characteristic	Fungi identified
1DT	Hyphae is irregular in size and septate with black sporangium	*Mucor spp.*
2DT	Hyphae is irregular in size and septate with black sporangium	*Mucor spp.*
3DT	Oval micronidia and branched conidiospore.	*Fusarium spp.*
4DT	Hyphae is irregular in size and septate with black sporangium	*Mucor spp.*
5DT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*
6DT	Oval micronidia and branched conidiospore	*Fusarium spp.*
7DT	Oval micronidia and brancedcondiospore.	
8DT	Irregular size and small hypae, sepate.	*Fusarium spp.*
9DT	Non-septate hyphae and irregular in size.	*Rhizopus spp.*
10DT	Irregular size and small hyphae, sepate.	*Fusarium spp.*
11DT	Non-septate hyphae and irregular in size.	*Rhizopus spp.*
12DT	Non-septate hyphae and irregular in size.	*Rhizopus spp.*
13DT	Non septate hyphae and irregular in size.	*Rhizopus spp.*
14DT	Unsepate hyphae are irregular in size and ribbon like.	*Aspergillus spp.*

Table 6: microscopic characteristics of fungal from dried tomato(DT) from Rimi market Kano.

Samples	microscopic characteristics	fungi identified
15DT	Irregular size and small hypae, sepate.	*Fusarium spp.*
16DT	Spherical and irregular size cell with budding attached	*Yeast spp.*
17DT	Spherical and irregular size cell with budding attached	*Yeast spp.*
18DT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*
19DT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*
20DT	Thick septate hyphae with conidia borne in chains from the sterigmata.	*Aspergillus spp.*

Key: DT- dried tomato

1-20: number of plates

Table 7: Proximate composition of Fresh and Dried Tomato from Rimi Market, Kano

	FRESH TOMATOE	**DRIED TOMATOE**
MOISTURE	89.14 ± 1.65	8.84 ± 0.23
FAT CONTENT	1.78 ± 0.28	5.39 ± 0.15
ASH CONTENT	0.80 ± 0.02	9.88 ± 0.03
PROTEIN CONTENT	2.18 ± 0.08	4.50 ± 2.08
CRUDE FIBER	2.73 ± 0.08	33.79 ± 0.65
CARBOHYDRATE	3.35 ± 0.14	37.89 ± 0.62

Values are mean ± standard deviation

DISCUSSION

Tomatoes gotten from Rimi market were found to harbor some fungi .This might be due to the high population density as the market are located where virtually anyone from all classes can be found to carry out economic activities. Two fungi were isolated from fresh tomatoes namely :*Aspergillus spp.*and*Mucor spp.* Four fungi were isolated from dried tomatoes namely: *Mucor spp., Fusarium spp., Aspergillus spp., Rhizopus spp. Which* Were associated with spoilage of fresh and dried tomato from Rimi market. This study shows that *Aspergillus niger* has the highest number of occurrence in fresh tomato. This support the work of Onuorah *et al.,*(2015)and Ibrahim *et al*.,(2011) who reported that *Aspergillus niger* is the major causes of spoilage in tomato. *Rhizopus spp.*has the highest number of occurrence in dried tomato obtained from Rimi market. *A.flavus, A. niger, A. parasiticus*, were also isolated in dried tomatoes. These agree with the findings of Samuel *et al*., (2017).Contamination of tomato by fungi are some of the major factors that affect its storage and preservation (Aliyu *et al*.,2018). High water content of tomato, condition of storage, mode of handling, as well as the quality of the fruit could be the determinant of spoilage by fungal: thus, a great source of mycotoxin contamination. The huge loss of tomato across the globe is due to microbial spoilage, since it is composed of mostly of water, microbes grow best using it as its substrate (Abhinaba, 2009).

The moisture content of the present study was higher in fresh tomato than dried tomato, these occur as a result of removal of water content of the dried tomato by sun drying. Moisture content of food is very important on nutrient density and shelf life of agricultural produce. Concentration of nutrients increase due to removal of moisture (Morris *et al.,*2004). Therefore, for vegetables and fruits to be preserved for a long time before use, the moisture content has to be reduced (Kolawole *et al.,* 2011). Dried food substances, especially food crops with high moisture content will favor the growth of microorganism at a high growth rate and moisture content of >15% and said to promote enzymatic reactions and interactions of other constituents of the dried product leading to loss of vitamins (Ugwu *et al*., 2014).The moisture content of the solar and open sun dried vegetables can be said to be within the acceptable moisture level for dried vegetables. The fat content of the dried tomato was higher than fresh tomato. The ash content of the dried tomato was higher than fresh tomato. Ash content indicates mineral content of food substance the variation in ash content might be attributed to the type of vegetable used, soil variation and maturity level of vegetables.

Protein content was found to be higher in dried tomato than the fresh tomato. This may be attributed to the controlled and relatively higher temperature of the sun drying during the drying process (Ismail *et al*., 2016).The crude fibre of the dried tomato was higher than fresh tomato. Dietary fibre in vegetables increases bulk and reduces food transit time in the alimentary canal and the incidence of constipation and other related diseases. Fibre is useful for maintaining bulk, motility and increasing intestinal tract. It is also necessary for healthy condition, curing of nutritional disorders and food digestion (Ifon *et al*.,2009).Carbohydrate content is higher in dried tomato than fresh tomato. Vegetables in their fresh state have been noted to be poor sources of carbohydrate. However, after drying, the carbohydrate content of vegetables increases (Kolawole *et al*., 2011). Low carbohydrate content of fresh vegetables showed that they supply little or no energy when consumed except when supplanted with other foods (Rossello *et al*., 2000).

CONCLUSION

Tomato fruit is the world's most popular home garden fruit and the second most consumed vegetable after potato in the world. Tomato is highly used in stew and sauce making in Nigeria. As most Nigerians cannot do without taking stew in their daily meals and diets, the presence of potentially pathogenic organism in tomato can leave to great economic and health hazard. This research has revealed that fresh tomato has lesser fungal isolate than dried tomato. The work also revealed that fresh tomato has some pathogenic fungal. It also differentiate the types of fungal present in fresh and dry tomato. The proximate analysis revealed a significant change in moisture, protein, fat, carbohydrate, crude fibre and Ash.

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