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INVESTIGATION OF FUNGAL CONTAMINATION IN FRESH RAISIN JUICE IN MOSUL CITY AND ITS SUBURBS

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Abstract: Elected(40) a random sample of raisin juice from both sides of Mosul city (right and left) and its suburbs, which included the areas of Bashiqa, Al-Hamdaniya, Bartella, Al-Shekhan, Aqra and Dohuk, for the period from January 2023 to April 2024, to detect the extent of contamination of these samples with fungi and yeasts using the culture media Potato Dextrose Agar and Sabouraud Sucrose Agar. The results of the culture of the samples showed that there were fungi that grew on the two media and others that grew on one media. All isolates were diagnosed based on their morphological and biochemical characteristics and diagnosis using the planting medium. (121) fungal isolates of different species were obtained with a contamination rate of (100%) distributed as follows: The highest contamination rate for fungal species 33/121 (27.3%). Candida spp. and (27.3%)33/121 Apergillus spp., respectively, while I reached Contamination rate of raisin juice with Wickerhamomycesanomalus15/121(12.39%),Saccaromyces cervisiae14/121(11.57%), Rhotodorula spp.14/121(8.26%), and Penicillium ssp. and Exophiala dermatitidis (3.3%) each. Four fungal isolates wereFor each of them, followed by the following fungal species: Geotrichum spp. and Fusarium spp. and Epidermophytes floccosum by percentage pollution reached(0.82%) respectively but Pollution rate by Mushrooms(4/121(2.47% Cladosporiumoxysporu and (1.65%)2/121 Trichophyton mentagrophytonThe results of the study showed that (26)A sample of raisin juice was heavily contaminated with filamentous fungi and yeasts.By (65%)And it isR1, R3, R4, R5, R7, R9, R10, R11, R12, R14, R15, R16, R17, R19, R20, R21, R22, R26, R28, R29, R30, R35, R36, R38, R39, and R40. The other samples were contaminated with fungi at a low rate. And I ranto be sureDiagnosisfor fungal isolationUsing molecular methods to compare nitrogenous base sequences at the National Center for Biotechnology InformationNCBI using BLAST And it was recordedstrainnewAndCandida oleophilastrain MAYKAIY and sequenced as PP961930.1 in GenBankGlobal.

Keywords: Fresh Raisin Juice , Fungal contamination, Mosul City And Its Suburbs

Introduction

Raisins are dried grapes Grape is a berry fruit that generally grows in a group of deciduous woody vines belonging to the Vitaceae family. (Venkitasamy et al., 2019). Grapes are an important and economically well-known fruit all over the world (Kui et al., 2020). Grape production is estimated at more than 74.5 million tons per year, based on a cultivation area of 7.12 million hectares. China,

the United States, Italy, Spain, France, Turkey, Argentina, India and Iran are the largest producers of grapes (Anjum et al., 2020). It is mainly consumed as food such as raisins, juice and wine and as fresh fruit or used in various products such as vinegar. It is considered a rich source of vitamins, minerals, carbohydrates, dietary fiber and substances chemical vegetarian like polyphenol and promotes general health (2023,.Jaiswal et al). It is also considered antibacterial, antioxidant and anti-inflammatory due to its content of Polyphenol (Imran et al., 2017). Black grapes have large amounts of antioxidants and grapes are considered a seasonal fruit that grows abundantly and thus they were dried and converted into raisins for storage and use throughout the year, either by the old method of drying under the sun or in the air (2015,.Wang et al). As for modern drying using microwaves, ovens and drying under infrared rays (Wang et al.,2016 and Khiari et al., 2018a). Old and new drying techniques greatly affect changes in the volatile organic compounds that contribute to the aroma of raisins.((Torres et al., 2015; Wang et al., 2017). Raisins have benefits for blood pressure and cholesterol low-density lipoproteins, triglycerides, and fatty acids oxidizing in the blood and cytokines that cause inflammation, this indicates the possibility that raisins greatly reduce the risk of developing type 2 diabetes.) (T2DM, cardiovascular disease, cancer, and gut health, indicating that raisins consumed as part of a healthy diet are rich in nutrients (Das, 2008 and Restani et al., 2016).

Raisin juice is considered one of the popular juices it is used as a healthy alternative to caffeinated drinks such as coffee, tea and soft drinks. It has many benefits such as antioxidants, anti-cancer and anti-diabetic properties, and vitamins and minerals play an effective role in preventing a cancer, heart disease, diabetes and high cholesterol (Madappa et al., 2018). It contains 70% of its weight sugar and 2% of its weight protein. It is also rich in elements, especially K, Na, P, Mg, and Ca+ and vitamins A, B3, C. there are many contaminated according to the type of storage until consumption and thus affects the production of juice and wine (Welke, 2019) . This results in contamination of raisin juice with mycotoxins (Pena et al.,2010).Studies have shown that fungal contamination attributed to climate conditions and physiochemical and for grapes and wine and its effects on production (Freire et al., 2017).

Methods

(40) samples of fresh raisin juice were collected from Mosul city to the right and left and its suburbs, including the areas of Bashiqa, Al-Hamdaniya, Bartella, Al-Shekhan, Aqra and Douw, with 50 ml of each sample, and placed in special sterile boxes to avoid contamination and take information about each sample in terms of the source of the raisins, the date of making the juice, the method of storing it and the components of the juice. The sample collection period was from January 2023 to April 2024, and the samples were cultured using the dilution method on the media (PDA) Potato Dextrose Agar and Sabouraud Sucrose Agar (SSA) (Ronald et al., 1959 and Marth, 1978). To determine the extent of fungal contamination, it was diagnosed morphologically by relying on the color of the colony in the dish from both sides, the diameter of the colony, the presence and color of secretions, and the appearance of the fungal hyphae. Microscopically, a small piece of the colony was taken with a small amount of the medium on a glass slide, then mixed with a drop of lactophenol dye or crystal violet dye, then the cover glass was placed on it and examined under a microscope at a magnification of 40x (de-hoog and Gurro,1997 ; Pitt and Hocking ,1997 ; Samson and Frisvad ,2004).Yeasts were diagnosed using differential media based on the taxonomic key (Pitt and Hocking, 1997), where 7 differential media were used, including MEA25, MEA37, MEA28, Malt Extract Agar, Czapek Agar (CA), Malt Acetice Agar (MAA), Malt Extract yeast 50% Glucose Agar (MY 50G), and Extract Yeast Malt 5% or 10% salt 12% Glucose Agar (MY10-12). The isolates were cultured

after purification on SSA medium for two days until diagnostic tests were performed, and the general characteristics were recorded in terms of colony color, texture, and size. The diagnosis of the fungal isolate was confirmed using molecular methods, where DNA was isolated from two-day-old yeast, its purity was checked, PCR was performed, and DNA was isolated by electrophoresis and sequencing. The result was then sent to the fungal isolate for the purpose of conducting a nitrogenous base sequence determination test after the sample was diagnosed using ITS primers in the United States of America.

Results and Discussion

Isolated and identified the contaminating fungi for (40) sample of natural raisin juice sold in the markets of Mosul and its suburbs from December 2023 to April 2024. Elected(28)sample from shops randomly on the side in Mosul right and left it included all of the shops in Al-Muthanna district, Al-Zahour district, Al-Majmoua Al-Thaqafiya district, Mosul University, Al-Darkazliyya, Al-Masarif district, three samples from Bashiqa district, two samples from Sheikhan district, two samples from Bartella, and one sample from Al-Hamdaniya and two samples from Aqra district and four samples from Dohuk governorate, where the location of the shop and the source of the raisins were taken, as well as the date of making the raisin juice from all the selected shops. The fresh juice was composed of (dried raisins, sugar, and a dried mint leaves and a Synthetic dye). The results of the study showed pollution for large samples-Mold and Yeast ,there were fungi growing on both media SSA, PDA and fungi grown on the only one medium. Sample R1, R2 and R5 isolated from Bashiqa stores contained on a mold and yeast in both media SSA, SDP which was the source of raisins from the perfumers of Mosul from it C.oleophila one types of Candida which was registered as a new genotype in NCBI named Candida strain MAYKAIY and we have shown Aspergillus niger and Saccaromyces cerevisiae and P.atramentosum was found in the plate containing SSA medium and not in SDP medium. The fungus C.tropicalis was one of the most contaminated fungi in these samples.

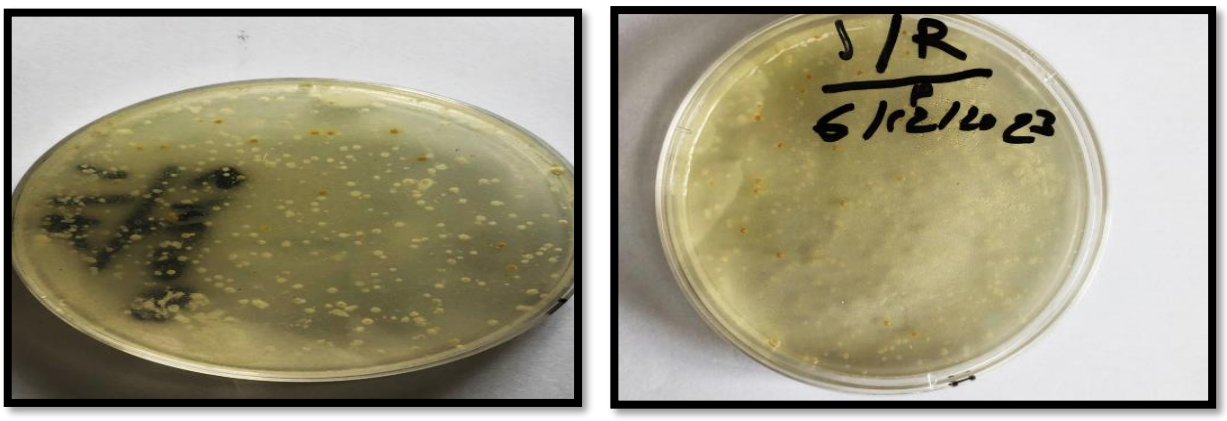
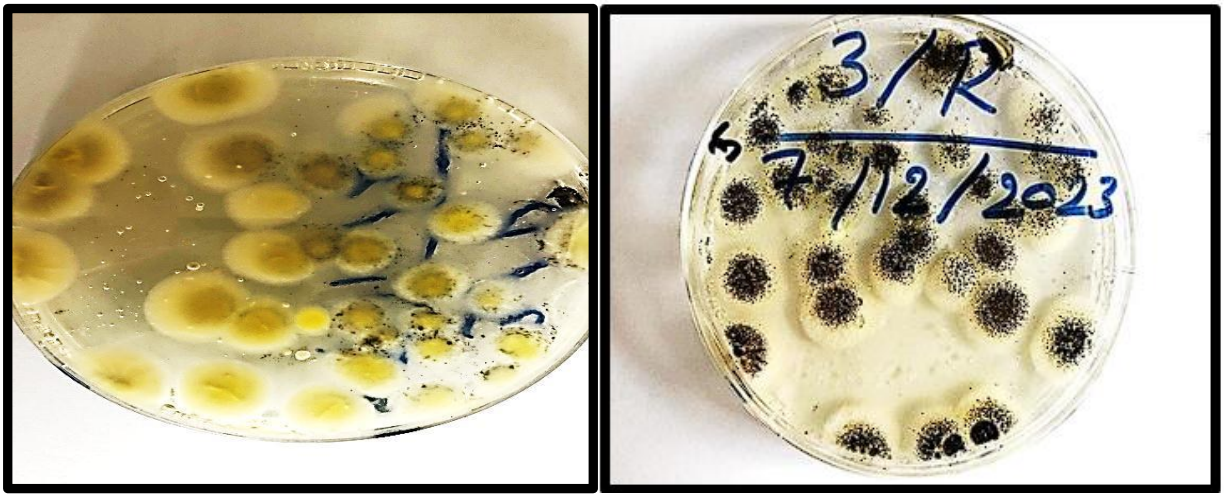


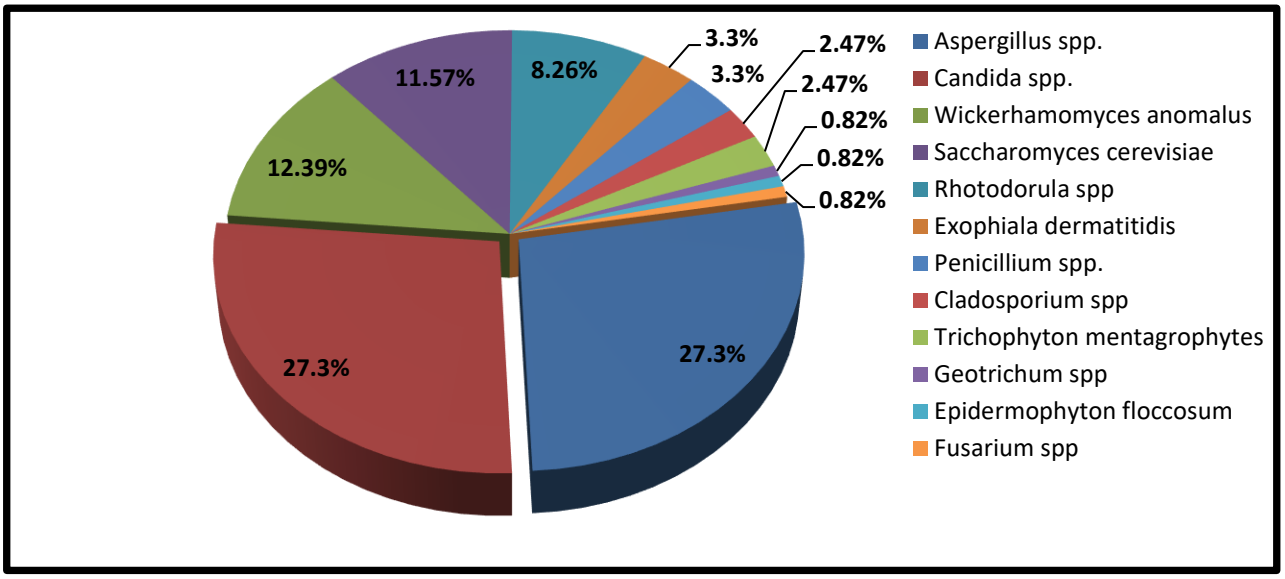
Figure (3): C.tropical yeast

As for the samples taken from Aqrah district, it was found that:R27 contaminated onlyWith A.nigerand R28 contaminated with A.niger, Rhodotorula and Wickerhamomyces anomula andC.parapsilosis, where we grew upRhodotorulaOn each ofPDA did not grow on SSA, but the most common species that appeared between the two samples was A.niger, whiletheSamplesR3andR13TakenFrom the judiciarytheTwo old men grew mushrooms A.niger, C. oleophila, Saccharomyces cerevisiae in both SDP The most frequent fungus in both samples A.niger Figure (4): A. niger on the SSA medium

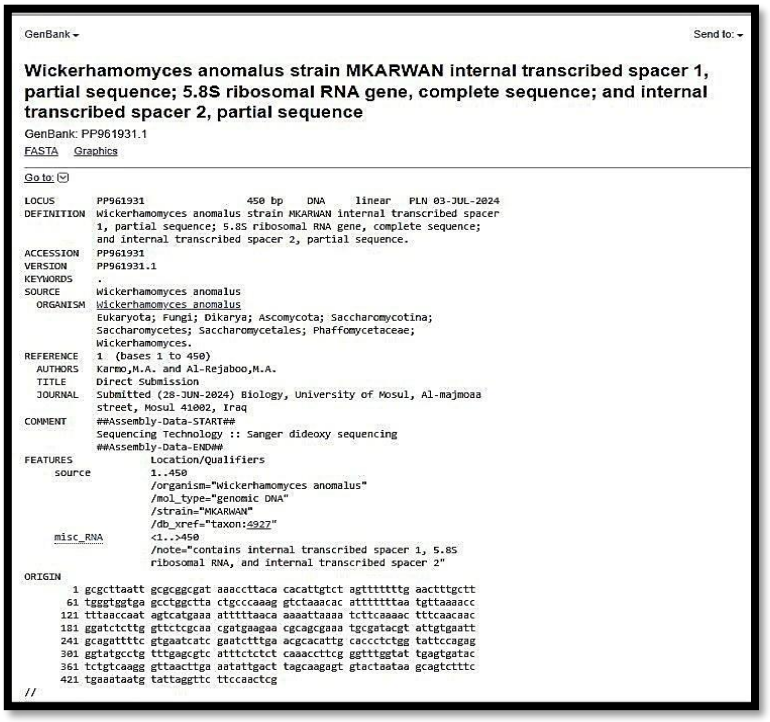


the two samples R10, R11 from Bartella area were sourced in Mosul grew both Cladosporium cladosporioides, A. niger, Saccaromyces cerevisiae, C. oleophila, C. tropicalis, Rhodoroula, although both samples were taken that from two different places and also it was A.niger more visible in both samples and the sample R12 from area the Hamdaniya was the source of raisins from northern Iraq contaminated with- Penicillium, C. parapsilosis , A.niger , Saccaromycetes services and one sample of Rhodotorula , the most common fungi are: A.niger collect four samples of R18,R23,R24,R7 from Dohuk was the source of raisins taken from scattered areas in northern Iraq contaminated with- C.tropicalise and C. oleaphila, Wickerhamomyces anomula, Rhodotorula, and a species of Aspergillus spp. While C. tropicalis was abundant, its proportion was large. As for the samples of Mosul city on the left side, they were:R14, ,8R4,R6,R9,R19,R20,R25,R26,R27,R30,31R, R32,R33,R34 R35, R36, R37,R38, R39,R40 most of all the raisin samples were from Aqra district and northern Iraq. All kinds of fungi grew A. niger, C.oleaphila, Cladosporium cladosporioides, Rhodotorula, C.krusei, C.albican, C.parapsilosis, C.tropicalis and species of Penicillium. The contamination rate was high in some samples and low in others . As for the right side of the city of Mosul, the samples wereR15, R16, R17, R18 are contaminated with Aspergillus sp and C.tropicalis and Saccaromyces cereivsiae and Wickerhamomyces anomalus and C. parapsilosis and Rhodotorula and C.krusei and C.albican , AThe more contaminated the samples were Rhotorula it was found that all samples of raisin juice were contaminated to varying degrees with fungi, with the contamination rate being- Aspergillus sp. 27.3% While she was Candida sp Contaminated by 27.3% and12.39% Contaminated with- Wickerhamomyces anomalus followed by other fungi at a rate of Say and it was kindAspergillus niger andCandida spFrom moreTypesContamination and spread in samples.

The shape (5):Shows the percentage of contamination of the study samples with fungal and yeast species..



It was found that the most highly contaminated samples with fungi were:R1, R3, R4, R5, R7, R9, R10, R11, R12, R14, R15, R16, R17, R19, R20, R21, R22, R26, R28, R29, R30, R35, R36, R38, R39, R40 either samples are less contaminated it is:R2, R6, R8, R13, R18, R18, R23, R24, R25, R27, R31, R32, R34, R40. In general, all samples contained yeasts and mold. The results that appeared to us were consistent with what the scientist mentioned (Alomari et al., 2014) found that Aspergillus niger was dominant in some dried fruits during isolation and also observed different species of Penicillum spp. It was agreed results the current study with (Iamanaka et al., 2005), Aspergillus niger is the most prevalent in raisin samples in the Brazil , (Allam et al., 2020) it was found that the genus Aspergillus spp is dominant by 27.3% over the rest of the other genera yeasts and other filamentous fungi, this study agreed with our study.



GenBank ▾ Send to: ▾

Wickerhamomyces anomalus strain MKARWAN internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: PP961931.1

FASTA Graphics

Go to: ⊻

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VERSION     PP961931.1
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            Wickerhamomyces.
REFERENCE   1  (bases 1 to 450)
  AUTHORS   Karmo,M.A. and Al-Rejaboo,M.A.
  TITLE     Direct Submission
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      421 tgaaataatg tattaggttc ttccaactcg
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Figure(6):New mushroom pattern registration Candida oleophila strain MAYKAIYAnd give it an ID number PP961930 in NCBL.

Conclusion

The study revealed significant contamination of raisin juice samples collected from various regions of Mosul and its suburbs with a variety of fungal and yeast species. Among the 121 fungal isolates, the most prevalent contaminants were Aspergillus niger and Candida spp., each accounting for 27.3% of the total contamination, while other species such as Wickerhamomyces anomalus, Saccharomyces cerevisiae, and Penicillium spp. were also identified. Notably, a new strain of Candida oleophila (MAYKAIY) was identified and registered in the NCBI database. The findings underscore the potential health risks associated with consuming contaminated raisin juice, particularly with regard to mycotoxin production. These results have broader implications for food safety in regions with similar climatic conditions and warrant further research into improved handling, storage, and contamination prevention methods. Molecular diagnostic tools should also be leveraged for more precise identification and monitoring of fungal contamination in future studies.

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