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The use of nanohybrid curcumin to inhibit some types of local yeasts Isolate

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***Abstract*---**The objective of this study was to use nanohybrid Curcumin Cur-ZnO and Cur-MgO as well as free curcumin to detect, isolate, characterize, and inhibit the development of certain yeasts locally isolated from some spoilage foods. Yeasts responsible for spoiling were recovered in 21 different colonies from cheese, cooked cheese, pickles, fig jam, apple jam, dates, rice, canned chickpeas, and dried figs. Nine yeast isolates were chosen for further morphological and biochemical investigation using VITK 2. Six different genera and species of yeast were found, with three of them, all members of the genus Candida (Candida inconspicua/lambica, Candida krusei, and Candida famata), being the most common. It was determined that between one and two isolations belonged to the genus Zygosccharomyces SPP, and that one isolation belonged to each of the races Trichosporon asahii, Malassezia furfur, Kloekera SPP, and Rhodotorula, with probabilities ranging from.87 – 96%. The results showed that the concentration-dependent inhibition was greatest for the Cur-ZnO nanohybrid, next for the Cur-MgO nanohybrid, and finally for the free curcumin.

***Keywords*---**yeastsisolate, nanohybrid Curcumin, VITK 2 identification

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Introduction

As a result of rapid technological advancement in many spheres, including food processing and the addition of chemical additives to make food last for longer, the percentage of food pollution has doubled in the past fifty years, which is extremely worrisome for the health of the consumer (Leyva Salas et al., 2017). According to Fisher et al. (2018), although there are many factors that contribute to the worldwide food waste crisis, microbial damage of any kind (bacteria, yeasts, fungi, parasites, primitives, viruses) and its impact on the quality and properties of the product is the most dangerous and widespread.

Food corruption is the deterioration of the quality of the product or food by changing it into another form that is undesirable and unfit for human consumption and is harmful to health, while food spoilage refers to a change in physicochemical qualities that affects its acceptability by the consumer without leading to health harm (Majumdar et al., 2018).

According to Ojha et al. (2018), Bacteria, molds, and yeasts are all examples of microorganisms that can cause food to become bad. Putrefaction, caused by microbiology producing proteolytic enzymes in protein-rich foods, and rancidity, caused by microbiology producing the enzyme lipase in fatty foods, may alter the appearance, flavor, and odor of the foods, respectively (Amit et al., 2017). or harm occurs during fermentation as microorganisms break down carbohydrates to release organic acids, alcohols, and gases (Majumdar et al., 2019).

Many scientists and engineers in the twenty-first century have come to recognize the importance of nanotechnology in the field of food industries, but this use of nanotechnology in agriculture and food is still relatively new in comparison to its use in the field of pharmaceuticals and pharmaceuticals. Nanosensors for food and nanocomponents added to food are expected to enter this field to maintain food quality and safety, and while successful applications of nanotechnology in foods are still limited, some important foundations and concepts of this technology have been developed in the field of food engineering (Ravichandran, 2010). Shailendiran et al. (2011) used ultrasound, organic solvents like alcohol, and polar solvents like water to find different forms of curcumin nanoparticles; they were detected using spectral methods and the transmission electron microscopy; and they tested the nanoparticles' antimicrobial activity. Although curcumin has unusual medicinal properties, the challenge is still the lack of bioavailability of the compound due to its poor solubility. The efficiency of the manufactured nanotechnology was compared to that of free curcumin by adding the created amounts of the material to tubes containing gram-positive and gram-negative pathogenic bacteria of the gram dye. Curcumin's conversion to the nanoform improved its bioavailability and water solubility, showing promise for a solution to the compound's low solubility in water. Curcumin was also synthesized in a number of different compounds by Siviero et al. (2015) to enhance its bioavailability.

Materials and Methods

1. **Yeast isolates sources** The sample of yeast isolates were collected on November of 2022 from different sources as shown below:

   cheese, cooked cheese, pickles, fig jam, apple jam, dates, rice, canned chickpeas and dried figs from local market's in Baghdad.

2. **Culture media**

   Potato Dextrose Agar (PDA) The PDA media was prepared as described by the manufacturing company. The media was autoclaved at 121^{o} C and 15 pound/inch^{2} for 15 min.

   Potato Dextrose broth (PDB) The media was prepared as described by the manufacturing company. The media was autoclaved and used to fungi isolated growth.

Methodology

Isolating and purifying Many isolated samples were collected from various locations and sources as shown at (1), and They were transferred to the laboratory in clean polyethylene container. The power plate method as described by APHA, (1978) was used after the Decimal dilution procedure for food samples. Then put 1 ml of each dilution in a dish and the PDA medium was poured into the dishes and the dishes were incubated after hardening at a temperature of 37 ° C for 72 hours. The purification of the insulation obtained by repeated transfer on the PDA medium was carried out by planting them by the Streaking method and the dishes were incubated at a temperature of 37° C for 72 hours until the appearance of pure colonies and similar in appearance (Ahmed, 2019) and microscopic examination using the blue methyl dye (Kurtzman and Fell, 1998).

Identification of yeast isolates

Cultures- Microscopic characteristics

The Cultures characteristics of yeast colonies on the center of the PDA have been studied in terms of the colony's color, texture, surface, edges, and shape as reported in Barnett *et al.*, 2000. Microscopic diagnosis of the isolates was carried out using the blue methylene dye to confirm their shape and size.

Isolates preservation :

The pure isolates were grown on Slants from the PDA medium at 37°C for 72 hours, kept in the refrigerator at 5°C and used for daily use, while the isolates themselves grew on the sterile PDB medium in tubes and added sterile glycerol and frozen for long-term use (Kreager- Vanrij, 1984).

Vitek 2 YST identification:

The identification was made using the Vitek 2 system Version: 08.01 in the Central Health Laboratories / Ministry of Health according to the instructions of the manufacturer (BioMerieux – France) according to the method described by (AL-Abedi., 2020).

Evaluation of inhibition effect of Nanohybrid Curcumin (Cur-ZnO, Cur-MgO) against some types of yeast:

The diffusion method using drilling as described by Patil *et al*, 2021 was used to detect the inhibitory efficacy of hybrid nanopreservatives after being dissolved in Dimethyl sulfoxide (DMSO).

the inhibitory efficacy of Nanohybrid Curcumin (Cur-ZnO , Cur-MgO) and Cur-free against *Candida albicans, Candida famata, Candida krusei, Candida inconspicua/ lambica, Kloekera* SPP, *Trichosporon asahii , Malassezia furfur, Rhodotorula Zygosccharomyces* SPP , was tested according to the Well Diffusion Assay method according to (Patil *et al*, 2021). Using concentrations of the Cur-ZnO and Cur-free (0.02, 0.05, 0.07, 1, 5, 10, 15 and 20 mg / ml) and Zinc oxides at a concentration of (20) mg/ml as a control treatment after being dissolved in Dimethyl sulfoxide (DMSO).

Results and Discussion:

1) Yeast isolation:

A total of 21 fungal isolates were collected from spoiled foods such as soft cheese, cooked cheese, pickles, fig jam, apple jam, dates, rice, canned chickpeas, dried figs, and other stale foods. Sources, symbols, numbers, and figures (Table 1) (4-1) Quantity of Yeast Isolates, two damaged pickle models yielded 8 yeast isolates (38%), one damaged date model yielded 5 isolates (24%), and one cooked fig model yielded 1 isolate (4.7%). Yeasts that have been cultivated in isolation on the PDA medium and have been transferred several times have achieved complete purity.

2) Cultures- Microscopic characteristics of yeast isolates:

Within three days of incubation at 37°C, a study examined the morphological and microscopic properties of farms. Yeast colonies are bigger than bacterial colonies, some are oval or spindle, others are spherical, and some are rectangular; they have no whips or other movable parts, and can only be distinguished from bacterial colonies by using a microscope. consistent with Al-(2009) Ani's description of yeasts, which list several shapes (sphere, oval, pear, cylindrical, triangle, and rectangle) and states that some yeasts appear as real or false mycelium because of differences in size and the presence or absence of a real nucleus, as well as the fact that most colonies of young yeasts are wet and sticky and can look either delicate and white or creamy or pink, and that some colonies change slightly with age. From a total of 21 yeast samples, 9 were chosen after removing all duplicates and those from the same origin.

Tab. 1: Distribution of isolated yeasts by sources of their isolation and percentage of their presence

Source of insulation	Symbol of isolation	Number	Percentage of isolates
spoiled cooked cheese1	A1	-	0%
spoiled cooked cheese2	A2	1	4.7%
spoiled cheese1	A3	1	4.7%
spoiled cheese2	A4	1	4.7%
spoiled pickles 1	A5(a,b,c,d,e)	5	24%
spoiled pickles 2	A6(a,b,c)	3	14%
figs Jam	A7	-	0%
Apple Jam	A8	-	0%
spoiled date	A9(a,b,c,d,e)	5	24%
spoiled rice	A10	-	0%
spoiled Canned chickpeas	A11	-	0%
Dried fig	A12(a,b,c,d,e)	5	24%

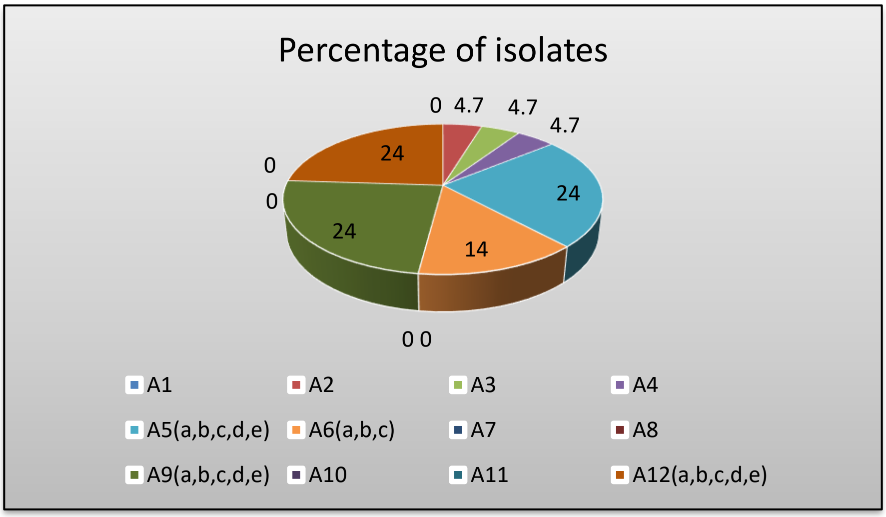

Fig. 1: Percentage of yeast isolates

3) Vitek 2 YST identification for isolates yeast:

Soliman and Aly (2011) noted that the Vitek 2 approach was used to identify the isolates because of its superior accuracy and speed relative to more traditional biochemical assays, which require extensive sample preparation and vast volumes of substances Table (2). There were three isolates from the genus *Candida* included *Candida inconspicua/lambica, Candida krusei* and *Candida famata* while it was found that one isolation belonged to the races *Trichosporon asahii, Malassezia furfur, Kloekera* SPP and *Rhodotorula*, as well as About two isolation related to the genus *Zygosccharomyces* SPP with probability ratios ranging between (87-96)%.

Tab. 2: identification results of the Vitek 2 system of isolated yeasts in the study

Genus & Spp.	Yeast cod	Probability ratio	identification
Malassezia furfur	**A5d**	**95%**	**Very good**
Candida inconspicua/ lambica	**A5c**	**95%**	**Very good**
Trichosporon asahii	**A12**	**96%**	**Excellent**
Kloekera SPP	**A9a**	**90%**	**good**
Candida krusei	**A5b**	**95%**	**Very good**
Candida famata	**A3**	**87%**	**Low**
Rhodotorula glutinis/ mucilaginosa/(Crypto.laurentii)	**A4**	**96%**	**Excellent**
Zygosccharomyces SPP	**A6a**	**96%**	**Excellent**
Zygosccharomyces SPP	**A5a**	**96%**	**Excellent**

1- Evaluation of inhibition effect of Nanohybrid Curcumin Cur-ZnO against some types of yeast:

The development of resistance against antifungal drugs to yeast types such as *Candida* has become a critical problem facing therapeutic protocols and therefore new solutions must be found to combat them (Neelofar, et al., 2011). The inhibitory effectiveness of Nanohybrid Curcumin (Cur-ZnO, Cur-MgO) and free curcumin towards 10 isolates of pathogenic and food- spoilage yeast was studied, it is noted through the results of the current study the Nanohybrid Curcumin (Cur-ZnO) and (Cur-MgO) in concentrations (0.1, 0.2, 0.3, 0.4, 1.9, 3.8, 5.8, 7.7) , (0.05، 4.1 ، 2.7 ، 1.4 ، 0.3 ، 0.2 ، 0.1 ، 5.5) mg/ml respectively and free curcumin in concentrations (0.2, 0.5, 0.7, 1, 5, 10, 15, 20) mg/ml, has an inhibitory effect for the types of yeasts used in the experiment as shown in Table (3), as it is noted that the Nanohybrid Curcumin (Cur-ZnO) at highest concentration of 7.7 mg/ml gave a high inhibitory activity towards yeast *Candida albicans* with an inhibition diameter of 43 mm followed by yeasts *Rhodotorula glutinis/mucila -ginosa/(Crypto.laurentii)* and *Zygosccharomyces* SPP with an inhibition diameter of 40 mm followed by *Zygosccharomyces* SPP A8 with an inhibition diameter of 39 mm followed by yeasts (*Candida famata, Candida krusei, Malassezia furfur, Candida inconspicua/lambica, Kloekera SPP and Trichosporon asahi*) with inhibition diameters (39, 37, 36, 35, 34, 33, 31) mm respectively. It was also noted that the hybrid nanopreservative (Cur-MgO) with its highest concentration of 5.5 gave the highest inhibitory effectiveness towards yeast *Candida albicans* with a diameter of 40 mm followed by yeast *Rhodotorula glutinis/mucila -ginosa/*(*Crypto.laurentii*) with an inhibition diameter of 35 mm, followed by isolation *Zygosccharomyces* SPP with an inhibition diameter of 34 mm, followed by *Zygosccharomyces* SPP A8 with an inhibition diameter of 33 mm, followed by yeast isolates *Malassezia* *furfur, Candida inconspicua/lambica, Candida famat, Candida krusei, Kloekera* SPP and *Trichosporon asahii* where they reached Inhibition

diameters (32, 31, 31, 30, 28, 28) mm respectively While free curcumin at a concentration of 20 mg/ml was observed to give the highest inhibitory effectiveness towards isolation *Candida albicans* with an inhibition diameter of 38 mm followed by isolates *Rhodotorula glutinis/mucila*

-ginosa/(*Crypto.laurentii*) and *Zygosccharomyces* SPP with an inhibition diameter of 32 mm followed by the two isolates *Zygosccharomyces* SPP A8 and *Malassezia furfur* with an inhibition diameter of 30 mm followed by isolates (*Candida inconspicua/lambica, Candida famat, Candida krusei*) and with a 29 mm inhibition fungus, followed by isolates (*Trichosporon asahii, Kloekera* SPP) and with inhibition diameter (24, 20) mm and straight Fig. 2.

Another study demonstrated that curcumin possessed activity against 38 different strains of Candida, including some fluconazole-resistant strains such as C. albicans, C. glabrata, C. krusei, C. tropicalis, and C. guilliermondiii as the 90% microinhibitory dose (MIC90) was a sensitive and antifungal-resistant species between 250-650 µg/mL (Khan, et al., 2012).

Tab.3: inhibition effect of Nanohybrid Curcumin Cur-ZnO and Cur-free against some types of yeast

Yeast isolates	Clear zone of preservatives (mm)																							
	Nanohybrid Curcumin (Cur-ZnO) concentration mg/ml								Nanohybrid Curcumin (Cur-MgO) concentration mg/ml								Free curcumin (Cur) concentration mg/ml							
	0.2	0.5	0.7	1	5	10	15	20	0.2	0.5	0.7	1	5	10	15	20	0.2	0.5	0.7	1	5	10	15	20
	Actual concentration of Nano preservative																							
	0.1	0.2	0.3	0.4	1.9	3.8	5.8	7.7	0.05	0.1	0.2	0.3	1.4	2.7	4.1	5.5	-	-	-	-	-	-	-	-
Candida albicans	17	17	18	20	26	31	37	43	16	17	18	19	23	28	35	40	15	15	16	16	21	26	32	38
Malassezia furfur	20	21	21	22	26	29	32	35	17	17	18	18	22	26	29	32	11	12	13	13	16	21	26	30
Candida inconspicua/ lambica	13	13	14	15	19	24	29	34	16	17	17	18	21	25	28	31	13	13	14	14	18	22	26	29
Trichosporon asahii	17	17	18	19	22	25	28	31	15	15	16	16	19	22	25	28	12	12	13	13	16	19	21	24
Kloekera SPP	17	18	19	20	23	26	30	33	13	14	15	15	18	21	26	28	6	7	7	8	11	14	17	20
Candida krusei	16	17	18	19	24	28	32	36	15	15	16	17	20	24	27	30	15	16	17	17	20	23	26	29
Candida famata	18	19	20	21	25	29	33	37	16	16	17	17	21	25	28	31	14	15	16	16	20	24	26	29
Rhodotorula glutinis/ mucila -ginosa	18	19	20	21	26	32	36	40	17	17	18	19	23	27	31	35	16	17	18	18	21	25	29	32
Zygosccharomyces SPP	18	19	20	24	28	32	36	40	17	18	18	19	22	26	30	34	15	17	17	18	20	24	28	32
Zygosccharomyces SPP A8	18	18	19	23	26	31	35	39	15	16	17	18	21	25	29	33	14	15	16	16	21	24	26	30

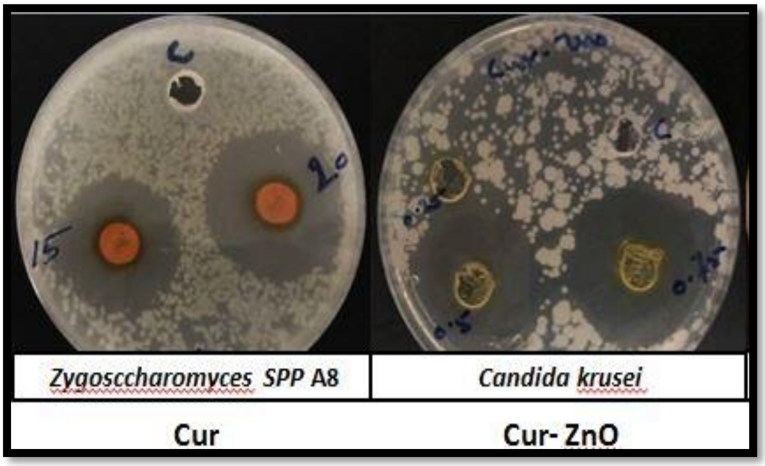

Fig. 2: the inhibitory activity of Nanohybrid Curcumin (Cur-ZnO) and free curcumin (Cur) against some types of yeast.

Nelson, et al., (2017) noted that curcumin nanoparticles offer a high probability of being used as a therapeutic material against fungi and pathological bacteria and also noted that is generally recognized as safe (GRAS). Neelofar, et al., (2011) also pointed out that the decrease in the production of protease enzymes and the alteration of the associated membrane leads to a change in the activity and properties of the enzyme ATPase, which are critical factors of the fungal cell that give curcumin antifungal activity.

References

Leyva Salas, M., Mounier, J., Valence, F., Coton, M., Thierry, A., & Coton, E. (2017). Antifungal microbial agents for food biopreservation—A review. *Microorganisms*, *5*(3), 37.

Ahmed, N. S., & Al–Shamary, E. I. (2019). Antioxidant production from local fungal isolate. *The Iraqi Journal of Agricultural Science*, *50*(1), 398-408.

Fisher, M. C., Hawkins, N. J., Sanglard, D., & Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, *360*(6390), 739-742.

Majumdar, A., Pradhan, N., Sadasivan, J., Acharya, A., Ojha, N., Babu, S., & Bose, S. (2018). Food degradation and foodborne diseases:
159 a microbial approach. In *Microbial contamination and food degradation* (pp. 109-148). Academic Press.

Amit, S. K., Uddin, M., Rahman, R., Islam, S. M., & Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture & Food Security*, *6*(1), 1-22.

Majumdar, A., Pradhan, N., sadasivan, J., Acharya, A., Ojha, N., Babu, S.(2019). Microbial Contamination & Food Degradation', Handbook of Food Bioengineering, Elsevier.Pp.127-130.

Ravichandran, R. (2010). Nanotechnology applications in food and food processing: innovative green approaches, opportunities and uncertainties for global market. International Journal of Green Nanotechnology: Physics and Chemistry, 1(2), P72-P96.

Shailendiran, D., Pawar, N., Chanchal, A., Pandey, R. P., Bohidar, H. B., & Verma, A. K. (2011, December). Characterization and antimicrobial activity of nanocurcumin and curcumin. In *2011 International Conference on Nanoscience, Technology and Societal Implications* (pp. 1-7). IEEE.

Siviero, A., Gallo, E., Maggini, V., Gori, L., Mugelli, A.,Firenzuoli, F., & Vannacci, A. (2015). Curcumin, a golden spice with a low bioavailability. *Journal of Herbal Medicine*, *5*(2), 57-70.

Patil, N., Bhaskar, R., Vyavhare, V., Dhadge, R., Khair, V. and Patil, Y. (2021). Overview on methods of sysnthesis of nanoparticals International Journal of Current Pharmaceutical Research. 13 (2): P. 11-16.

Ojha, K. S., Tiwari, B. K., & O'Donnell, C. P. (2018). Effect of ultrasound technology on food and nutritional quality. In *Advances in food and nutrition research* (Vol. 84, pp. 207-240). Academic Press.

APHA: American Public Health Association. (1978). Standard methods for the examination of dairy products. 14th Ed. Washingtion, DC, USA. 449 p.

Kurtzman, C. P. and Fell, J. W. (1998). "The yeasts, a taxonomic study", 4th Edition, Elsevier, Amsterdam,77-102.

Barnett, J. A.; Payne, R. W. and Yarrow, D. (2000). Yeasts: Characteristics and identification, 3rd ed. Cambridge .University Press. England.

Kreager, Vanrij, N. J. W. (1984). A toxonomic study in the yeasts. 3th ed.; Elsevier Science Publisher. Netherland.

AL-Abedi, S.F. (2020).Comparative study for Candida SPP isolated and identification from goats milk with mastitis between API Candida chromogenic agar and VITEK 2 system. Biochem. Cell. Arch. Vol. 20, No. 2, pp. 5329-5332

AL-Ani, winner. (2009). Microbiology in Food and Modern Techniques in its Detection. Al-Manhaj House for Publishing and Distribution. pp. 34-64.

Soliman, N.S. and Aly, S.A.(2011). Occurrence and identification of yeast species isolated from Egyptian Karish cheese. Journal of Yeast and Fungal Research Vol. 2(4).: 59 – 64.

Neelofar, K., Shreaz, S., Rimple, B., Muralidhar, S., Nikhat, M., & Khan, L. A. (2011). Curcumin as a promising anticandidal of clinical interest. *Canadian Journal of Microbiology*, *57*(3), 204-210.

Khan, N., Shreaz, S., Bhatia, R., Ahmad, S. I., Muralidhar, S., Manzoor, N., & Khan, L. A. (2012). Anticandidal activity of curcumin and methyl cinnamaldehyde. *Fitoterapia*, *83*(3), 434-440.

- Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., & Walters, M. A. (2017). The essential medicinal chemistry of curcumin: miniperspective. *Journal of medicinal chemistry*, *60*(5), 1620-1637.