Egypt. J. Chem. **Vol. 66,** No. **SI 13, 973-982** (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Preparation of Chitosan/Ginger and Chitosan/Garlic microcapsules by using Emulsion Cross-linking Method

Rasha A. I. El-Desokey [1*], Nagwa A. Abdallah[2], Halla A. Hussein[1], Shaimaa M. Fahmy and Ali M. Saeed[2]

1Radiation Microbiology Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority

2 Microbiology Department, Faculty of Science, Ain- Shams University, Cairo, Egypt.

Abstract

Microcapsulation is an essential technique to stabilise and protect active ingredients of nutraceuticals during various manufacturing processes. This study aimed to control bacterial contamination of nutraceutical raw materials and protection of nutraceutical active ingredients using chitosan microcapsules. Thirteen bacterial isolates were isolated from ginger GI and garlic G. Thermal resistance isolates (GI 7, GI 3& G2) were exposed to different gamma radiation doses; their lethal doses were 8.0 KGy for GI 3, and 9.0 KGy for G 2 and GI 7. The GI 3 and GI 7 isolates showed relative resistance against chitosan, they were identified by 16S rRNA gene sequencing as *Enterococcus faceium* and *Enterococcus durans*, respectively. A microcapsule preparation was formed using the emulsion cross-linking method by incorporating ginger or garlic with chitosan. The results revealed that the chitosan concentration of 4% and 20 ml of GST achieved 92.5% and 87.5% encapsulation yields for chitosan/ginger and chitosan/garlic microcapsules, respectively. Chitosan-ginger formed microcapsules were thermally stable while Chitosan-garlic microcapsules were partially unstable.

Keywords: nutraceuticals, chitosan, microbial contamination, microcapsulation, emulsion cross-linking method.

1.Introduction

Nutraceuticals are natural, chemical, or bioactive substances that illustrate biological activities and physiological benefits to elevate the body's natural recovery. Studying health topics involves exploring bioactive components from different food sources. Functional foods are considered nutraceuticals if they support the protection and management of diseases [1]. Nutraceuticals can be used as food and pharmaceutical ingredients with great potential against disease targets [2]. Moreover, there are a few challenges to defining the health benefits of certain foods, improving immune function, avoiding certain diseases, and decreasing the side effects and healthcare costs of nutraceuticals and dietary supplements [3]. Several phytochemicals and bioactive components exist in some herbs and medical plants that ensure their medical importance in modern functional and nutraceutical foods. Ginger, which is considered a nutraceutical, has high nutraceutical potential against many physiological threats due to the presence of 6-gingerol [4]. Also, garlic is a common spice with several health benefits due to its varied bioactive compounds, such as saponins, phenolic compounds, and polysaccharides [5]. Furthermore, ginger and garlic contain active ingredients with several health benefits [6]. Several studies have shown important biological functions of garlic, including antioxidant, anti-inflammatory, immunomodulatory, anti-diabetic, and antibacterial properties [7]. The use of natural bioactive compounds as food ingredients or therapeutic agents has some limitations because their bioactivity is affected by processing and storage conditions [8] since these compounds are degraded with high temperature [9,10]. Physical and chemical transformations are other problems that affect their stability and bioactivity [11].

Also, Cross-contamination may occur during the manufacture, processing, storage, or transport of nutraceuticals [12]. Microbial contamination of nutraceutical products is clinically harmful. It can

*Corresponding author e-mail:(rashaeldesokey82@gmail.com), (R.A. El-Desokey)
Receive Date: 08 March 2023, Revise Date: 26 April 2023, Accept Date: 30 April 2023
DOI: 10.21608/EJCHEM.2023.198896.7704

cause active drug ingredients to be metabolised into less effective or chemically inactive forms [13]. Microbial spoilage of nutraceutical products can be caused by bacteria, yeasts, or fungi, which are all extremely versatile in their metabolic activities. *Enterococcus* spp. is Gram-positive, catalase-negative, non-spore-forming, and facultatively anaerobic bacteria. They are responsible for serious infections; Enterococcus *faecium* is most commonly recovered from humans, and *Enterococcus durans* is found in the intestines of animals and is responsible for sporadic cases of endocarditis in humans. The presence of *Enterococcus* spp. in some foods may indicate contamination or poor hygienic conditions and increase the risks to public health [14].

Chitosan, a natural antimicrobial nontoxic polymer, is considered an alternative to antibiotics. Chitosan is a cationic polysaccharide with several benefits, including excellent biological activity, good biocompatibility and biodegradability, and pH sensitivity for acid-triggered gastric delivery. Chitosan was prepared as a novel material for many applications as nanoparticles, microspheres, and the preparation of single-layer nonwoven fabric treated with chitosan nanoparticles and its utilisation in gas filtration [15, 16, 17,18]. Chitosan has become a promising natural polymer for the preparation of microcapsules it has important role in the control of nutraceutical microbial contamination and the protection of nutraceutical active ingredients. Chitosan is highly soluble and able to bind using its functional groups; certain bioactive compounds and nutraceuticals should be supported against environmental factors. Encapsulation can be employed as a method to preserve these compounds; due to its biodegradability and biocompatibility, chitosan is an ideal ingredient for the encapsulation and controlled release of bioactive compounds [19]. Microcapsules have been widely used for the transfer and release of materials in the pharmaceutical, cosmetic, and food industries. The encapsulation of active nutraceutical compounds in core-shell microcapsules exhibits great interest for taste and odour masking and the controlled release of drugs. In pharmaceutics, encapsulation of medicines, nutrients, and living cells can be used for protection by a solid biocompatible shell [20]. A number of techniques are available for the microencapsulation of natural bioactive compounds (core materials) and can be broadly divided into three types: chemical, physicochemical, and physical methods [11]. These techniques vary according to the core materials types and their further application in the food system. Different types of delivery systems that can be created based on emulsion technique are conventional emulsions (oil-in-water), multiple emulsions (water-in-oil-in-water), It has the advantages of compatibility with the food matrix, economic production, higher loading capacity**.**

2. Materials &Methods

2.1. Sample collection and bacterial isolation

Two ginger and one garlic raw sample were collected in sterile containers from a pharmaceutical factory in Egypt on the 10th of Ramadan and carried to the laboratory for bacterial isolation. The samples were 10-fold diluted in sterilised saline solution. Then 1 ml from each dilution was inoculated individually on Baird Parker agar medium plates. The plates were incubated at 37 $^{\circ}$C for 24 hours. One typical colony was picked from each selective agar plate and purified.

2.2. Study of thermal death time for bacterial isolates

Raw materials were sterilised by autoclaving for 15 minutes and then artificially contaminated by the isolated bacteria. The artificially contaminated raw materials were incubated in the oven at 60, 70, and 80 $^{\circ}$C for 0, 5, 10, 15, and 20 min, then inoculated on nutrient agar to evaluate bacterial growth [21].

2.3. Effect of different gamma irradiation doses on the viability of bacterial isolates

The selected bacterial isolates were cultivated on 100 ml nutrient broth for 24 h at 37°C in a shaking incubator (200 rpm). The bacterial cells were collected by centrifugation at 8000 rpm for 10 min and re-suspended in 10 ml sterile saline solution. The bacterial solutions were exposed to different gamma irradiation doses (1, 2, 3, 4, 5, 6, 7, 8, and 9 kGy) at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. Using Cobalt-60 model Russian gamma cell (Issledodovate) with dose rate 1.208 kGy/h. Irradiated cells and control (non-irradiated cells) were serially diluted with sterile saline, then plated on nutrient agar media, and incubated at 37ºC for 24 h [22].

2.4. Antimicrobial activity of different concentrations of chitosan against selected bacterial isolates

The agar well diffusion method determined the antimicrobial activity of different concentrations of chitosan. An inoculum from each bacterial isolate (1.0×10^3 Cfu/ml) was separately seeded in a nutrient agar medium. A volume of 100µL of different chitosan concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, and 0.4%) was added to the 7.1 mm well. The plates were

incubated for 1 h at 4ºC for diffusion and then incubated at 37 ˚C for 24 h. The antimicrobial activity was detected by measuring the inhibition zones diameter (mm). Amoxicillin & clavulanic acid (AMC: 20/10 mcg), vancomycin (VA: 30 mcg), Rifampin (RF: 30 µg), Cefuroxime (CXM: 30 µg) were used as positive control [23].

2.5. Molecular Identification

The total DNA of the selected bacterial isolates was extracted as illustrated by the instruction manual with DNA extraction kits (Thermos, Fisher Scientific, USA) and kept frozen at – 20 °C until the PCR reaction was completed. A pair of universal bacterial primers, 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) & 1492R (5′-GGT TAC CTT GTT ACG ACT T- 3′), was used for amplifying the target gene. Two microliters of the bacterial DNA were used as a model for the PCR reaction. PCR was carried out by Premix Taq (My Taq, Bio line, UK), consistent with the instruction manual. PCR had been completed in genius model FGENO2TD thermal cycle (Techno, England). The PCR conditions were used for 5 min for primary denaturation at 94 °C, at that time 35 cycles of 1 min at 94 °C, 1 min at 54 °C, and 1 min at 72 °C, and finally 10 min at 72 °C for amplification of genes. The amplified genes were run on 1% agarose gel to determine the size and purity of the products. The amplified bands had been cleaned by a PCR product purification kit (Thermos, Fisher Scientific, USA). The forward directions of the partial 16S rRNA gene were sequenced in Macro gen, Korea. The BLAST search program identified the sequence, National Center for Biotechnology Information (NCBI), and National Library of Medicine, USA [24]. Alignments Sequencing was completed by Crustal W 1.83 XP software. A phylogenetic tree was made using the neighbor-joining method by MEGA 6 software. Then, the sequence was submitted by the Ban kit tool **(NCBI website; www.ncbi.nlm.nih.gov)** to get the accession number.

2.6. Preparation of microcapsules by emulsion cross-linking method

A modified method of [25] was used for microcapsule preparation**.** Two grams of ginger and garlic were added to different concentrations of chitosan solution (2.0%, 3.0%, and 4.0%). A homogenizer homogenized the mixtures for 30 min to produce an emulsion of oil in water. The emulsion was added to 150 ml of corn oil to form the oil medium, then was agitated for 1 h to form oil/water/oil emulsion. Glutaraldehyde saturated toluene (GST) solution was formed and added drop by drop to the emulsions every 15 min. The mixture was stirred for 15 min, then 2 ml of glutaraldehyde 25% were added and Shaked for 2 h. Microcapsules were collected by centrifugation for 10 minutes. The microcapsule product was cleaned with petroleum ether and n-hexane and dried at 70ºC.

2.7. Encapsulation yield

Encapsulation yield is the ratio between the formed microcapsules weight after drying and the total weight of the core material.

2.8. Scanning electron microscopy (SEM)

JEOLJSM-6510LV Scanning Electron Microscope (SEM) at the National Center for Radiation Research and Technology (NCRRT), Egypt, examined the morphology of the formed microcapsules.

2.9. Thermal gravimetric analysis (TGA)

The stability study was evaluated by thermal gravimetric analysis (STPT 1000) at a heating rate of 10ºC each min. with a continuous nitrogen rate of 20 ml per min. at the National Center for Radiation Research and Technology (NCRRT), Egypt [25].

2.10. Fourier-transform infrared spectroscopy (FTIR)

study of Functional groups and chemical structure of chitosan, ginger, garlic and formed microcapsules was performed by FTIR spectroscopy at the National Center for Radiation Research and Technology (NCRRT), Egypt [18].

2.11. Statistical analysis

All experiments were triplicated, and the mean ± standard error was calculated. The data were analyzed by one-way analysis of variance (ANOVA), and the difference in means was established with Tukey's multiple range test with a P-value of less than 0.05 was considered significant.

3. Results

3.1. Isolation of bacteria from the nutraceutical raw materials

Thirteen black bacterial isolates were selected from garlic and ginger raw materials grown on Baird parker (photo.1) The isolates GI 1, GI 2, GI 3, GI 4, GI 5, GI 6, and GI 7 from ginger, while isolates G 1, G 2, G 3, G 4, G 5, and G 6 from garlic.

Photo.1: black bacterial colonies on Baird Parker agar medium.

3.2. Determination of thermal death time for bacterial isolates

Thirteen bacterial isolates were isolated from ginger and garlic powders only 3 isolates were heat resistant to temperatures (60, 70, and 80ºC) for the selected times. The lethal time for isolate G 2 was 10 min at 80ºC while isolates GI 3& GI 7 were 15 min at 80ºC (Table.1).

Table (1): Study of Thermal death time for the bacterial isolates**.**

Resistant isolates	Death time at different temperatures		
	60 ºC	**70** ºC	**80** ºC
G2	15 min	15 min	10 min
GI3	20 min	20 min	15 min
GI7	20 min	20 min	15 min

3.3. Effect of different gamma irradiation doses on the viability of bacterial isolates

The selected bacterial isolates (G 2, GI 3, and GI 7) were exposed to different gamma radiation doses ranging from 1.0 to 9.0 KGy, and the count was recorded. The count of GI 3, GI 7, and G 2 isolates was decreased by increasing the dose of gamma radiation. The lethal dose of gamma radiation was 8 KGy for GI 3 and 9 KGy for G 2 and GI 7. There was a significant difference at $p \leq 0.05$ (Fig.1).

3.4. Antimicrobial activity of different concentrations of chitosan on bacterial isolates from row material.

The sensitivity of the (GI3 and GI7) selected isolates was increase with increasing chitosan concentrations (0.2 to 4.0%) using well diffusion method. the inhibition zones of GI3 ranged from 15 mm to 42 mm while GI7 zones ranged from 15 mm to 44 mm when chitosan concentration increased from 0.2 to 4.0 %. Garlic isolate G2 showed complete sensitivity to low concentrations of chitosan no inhibition zones were detected (Fig.2).

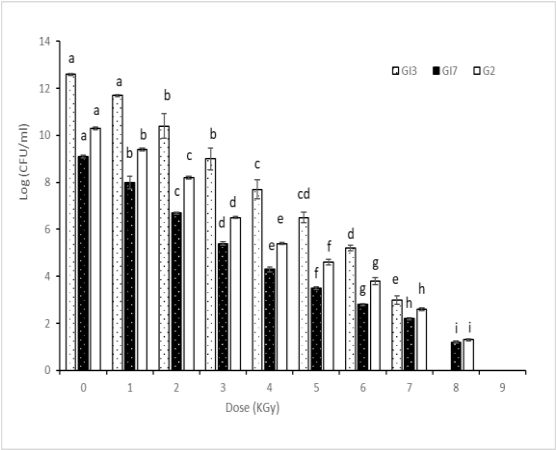

Fig (1): Effect of increasing Gamma irradiation doses on the viability of bacterial isolates. [a-i] values in the same column followed by different letters indicate a significant difference ($p < 0.05$).

In this experiment four antibiotics were used also to determine the antibiotic resistant isolates and as positive control to measure chitosan efficiency, they were Amoxicillin & Clavulanic acid (20/10 µg), Vancomycin (30 µg), Rifampin (30 µg), and Cefuroxime30 µg. Results in table 4 showed that generally the inhibition zones diameter occurred by different chitosan concentrations were rising by increasing chitosan concentrations. Conc. 4.0% had the most efficient killing ability (44mm) against GI7. Its inhibition zone was greater the most powerful antibiotic (AMC: 42mm), More over the efficiency of chitosan conc 1.0 % equal to and CXM inhibition zones both of them recorded 30mm against GI7, also the efficiency of chitosan conc. 3.0% equal to AMC its inhibition zone both of them recorded 42 mm against GI7. Otherwise, 1.0% chitosan conc. efficiency was equal to CXM (28mm) against GI3 while rest of chitosan concentrations efficiencies were approximately the same antibiotics efficiencies. [a-h] values in the same column followed by different letters indicate a significant difference $p < 0.05$ (Fig. 2).

3.5. Molecular Identification of the selected bacterial isolates

Two isolates (GI 3 and GI 7) were selected for their resistance to different concentrations of chitosan ranging from 0.2 to 4%. The 16s rRNA gene identified

them as *Enterococcus faecium* and *Enterococcus durans* (sequence similarity 99%).

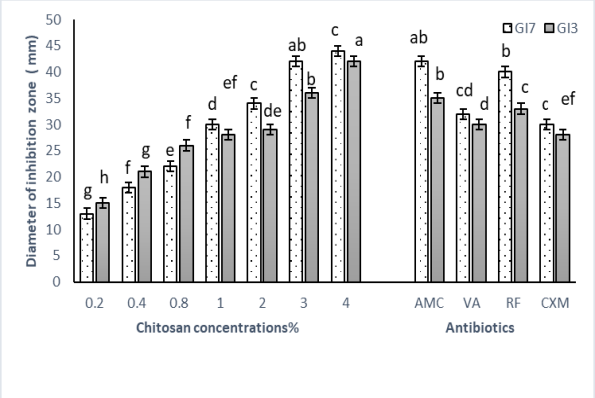

Fig (2): Antimicrobial activity of different concentrations of chitosan and antibiotics was used as a standard positive control against ginger isolates

The nucleotide sequence was submitted to GenBank as *E. faecium* strain R2 and *E. durans* strain R1 under accession numbers OK605881 and OK605880, respectively. Neighbor-joining phylogenetic tree with the alignment of 16S rRNA gene revealed that *E. faecium* strain R2 was placed in the phylogenetic cluster with *E. faecium* strain 4427 while *E. durans* strain R1 in the cluster with *Enterococcus* strains. The topology of the phylogenetic tree confirmed the NCBI-BLAST results and the belonging of our strains to the genus *Enterococcus* (Fig.3).

Fig. (3): Neighbor-joining phylogenetic tree of 16S rRNA genes. The numbers at the nodes are bootstrap values recovered from 100 trees.

3.6. Chitosan microcapsules coating raw materials Preparation by Emulsion cross-linking methods

According to the results in fig. (2), chitosan concentrations (2.0, 3.0, and 4.0%) were chosen to form microcapsules. The recorded data indicated that increasing chitosan concentration and GST volume increased the encapsulation yield of chitosan/ginger and chitosan/garlic microcapsules, as recorded in table (1). The encapsulation yield was 92.5%& 87.5% for chitosan/ginger and chitosan/garlic microcapsules, respectively, using 4% chitosan concentration and 20 ml of GST.

Table (2): Preparation of chitosan microcapsules coating ginger or garlic using Emulsion crosslinking methods.

Chitosan conc. (%w/v)	Volume of GST ml	Encapsulation Yield %	
		Chitosan-Ginger microcapsules	Chitosan-Garlic microcapsules
2%	10	42.5	33.2
	20	45.0	37.5
3%	10	83.3	70.5
	20	85.2	73.4
4%	10	91.0	83.2
	20	92.5	87.5

3.7. Characterization of the formed microcapsules

3.7.1 Scanning electron microscopy:

The morphology of the produced chitosan-ginger and chitosan-garlic microcapsules were examined by SEM analysis. Both microcapsules had a spherical shape. Chitosan-garlic microcapsules have a smoothly rigid surface (Fig 4a), while chitosan-ginger have a slightly irregularly rigid surface (Fig 4b)

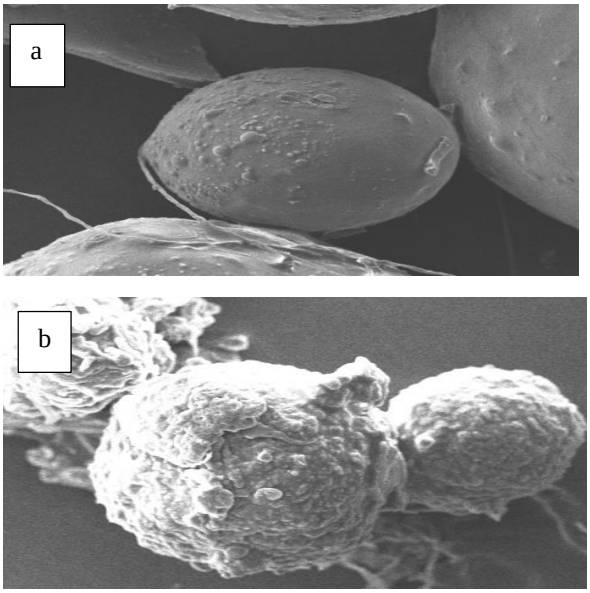

Fig (4): SEM for chitosan-garlic microcapsule (a) and chitosan-ginger microcapsule (b).

3.7.2. Thermogravimetric analysis for the prepared microcapsule:

TGA analysis of microcapsules was completed by plotting the microcapsules mass reduction against the increased temperature according to TGA graphs. Firstly, the water was evaporated at temperatures between 30ºC and 230ºC. Then melting of microcapsule at temperatures ranging from 230ºC and 450ºC was selected according to saccharide rings dehydration, depolymerization, and decomposition of the acetylated and deacetylated unit of the polymer. In the case of chitosan-ginger microcapsules, the mass was reduced to half at 375 ˚C using a chitosan concentration of 4 % and 20 ml of GST (Fig. 5a). While in the case of chitosan-garlic microcapsules, the mass reduced to half at 132 ˚C using chitosan concentration 4% with 10 or 20 ml of GST. Results indicated that chitosan-garlic microcapsules are less thermal stable than chitosan-ginger microcapsules (Fig. 5b).

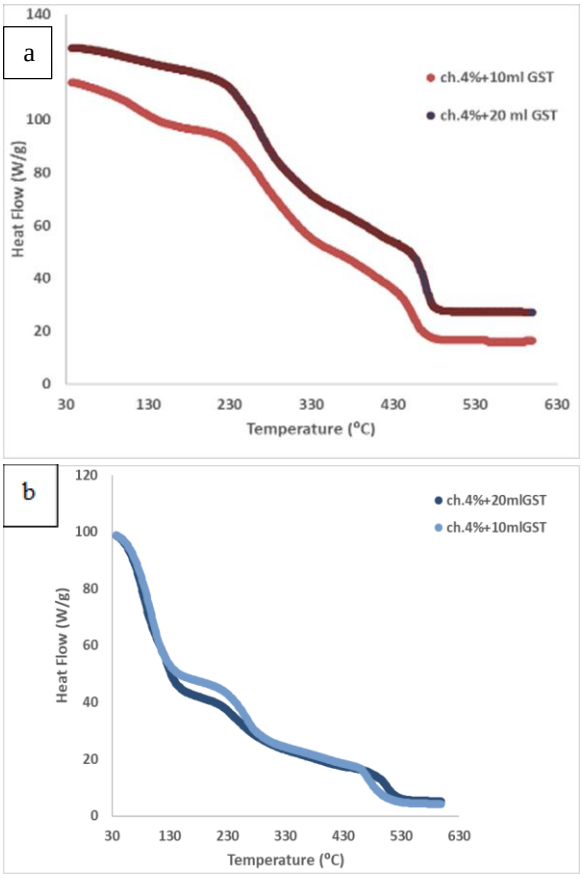

Fig. (5): TGA analysis for chitosan-ginger microcapsules (a) and chitosan-garlic microcapsules (b).

3.7.3. Fourier-transform infrared spectroscopy (FTIR)

Ginger particles results were depicted in (Fig. 6,7). The spectral region focused on 500 and 4000 cm−1, which contained more appropriate information on the chief chemical functional groups. Also, The main characteristic peaks of standard chitosan were at 3379 (–OH stretch), 2880 (C–H stretch), 1632 (N–H bend), 1378 (bridge O stretch), and 1076 cm-1(C–O stretch).The complexity of the IR spectra of chitosan come from the complicated and specific network of H-bonds in which the OH, C-O and NH groups are involved for each polymorphic form, and to the typical broadness of the IR bands of natural polymers. The results showed some similarity of values of FTIR between ginger powder and ginger in microcapsule (Fig.6.,7) which mean there is slight change in the chemical structure of both. The beak at 3329.76 for O-H Stretch and H- bond in wavenumber 2880 and 2974.02 cm-1 assigned for C-H Stretch, while 1376.3 represents C-H bending. As we can see in FTIR analysis charts that the microcapsules did not show new peaks, only a change in the peak width and slightly shifting the frequency, this indicates the absence of any significant interaction between the microcapsule's contents. While The FTIR bands of the garlic microcapsules were different from IR spectrogram of garlic (Fig.6.,7). Those spectrograms revealed both characteristics of garlic and chitosan with some wavelength shift, and also showed some new bands which indicated the complex formation of chitosan and garlic. The microcapsules exhibited functional group, chemical structure and characteristics of both original substances. The difference between IR spectra of garlic powder and microcapsules were found at 3400–2700 and 1800–500 cm^{-1} indicated alkyl group-hydroxy or possibly amino substituent, hydroxyl or amino compound general and aliphatic alcohol with carbonyl substitution.

4. Discussion

The microcapsulation of active nutraceutical compounds in core-shell microcapsules-controlled release of drugs, control microbial contamination and protect the active ingredients from degradation by a solid biocompatible shell. Encapsulants that are suitable for food applications must be biodegradable, and stable during processing, storage and consumption. The greatest suitable encapsulants are natural polymers such as chitosan, [17].

In this study collected two ginger and one raw garlic sample for bacterial isolation on selective media (Baird Parker). Thirteen different black colonies were selected and purified. [26] used Baird Parker medium for the *staphylococci* and *enterococci* isolation from food and pharmaceuticals.

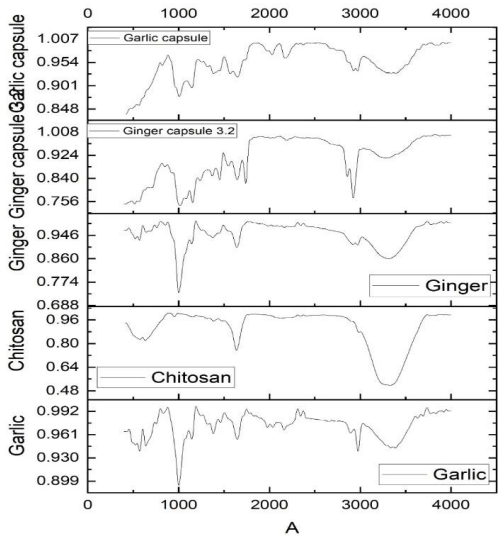

Fig. (6): FTIR analysis By Multiple panels.

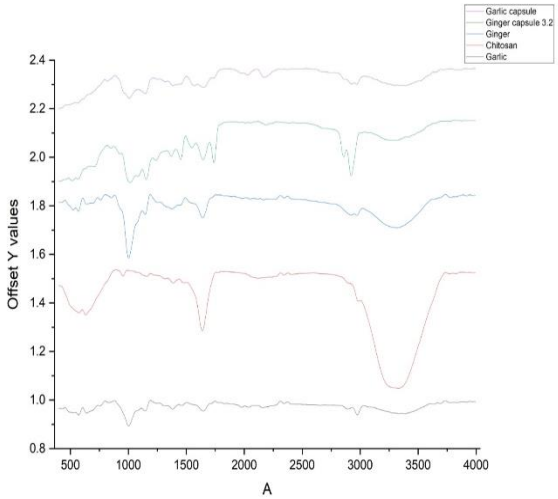

Fig. (7): FTIR analysis Stacked lines by y offsets.

The thermal stability of the selected isolates was evaluated, and the most thermal-resistant isolates (G 2, GI 3, and GI 7) exposed to 60, 70, and 80 ˚C were selected. The lethal time was 10 min at 80 ˚C for isolate G 2 and 15 min at 80 ˚C for GI 3 and GI 7 isolates. Similarly, [27] reported that *Enterococcus sp.* grows at high temperatures this may be due to the formation of cellular aggregations during heat treatment.

The selected bacterial isolates were exposed to gamma irradiation doses ranging from 1.0 to 9.0 KGy. All the isolates' counts were decreased by increasing the dose of gamma radiation. The isolate GI 3 was killed at 8.0 KGy, while the isolates G 2 and GI 7 were killed at 9.0 KGy. [28] showed a crucial positive connection between the killing of pathogenic bacteria and gamma irradiation. Also, [29] revealed that a dose of 7 kGy was sufficient for total inactivation of pathogenic bacteria. Microorganisms DNA is the most target for irradiation, bases of DNA molecules are highly susceptible to radiation with result of cleavage of phosphodiester bonds of DNA, then damage to DNA would cause bacterial cells to loss their ability for replication, and lead to bacterial cell's death [30].

In this study, the antimicrobial activity of different concentrations of chitosan (0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0 and 4.0%) against the3 selected isolates were determined using well diffusion method. Generally, garlic isolate was very sensitive to chitosan, 0.02% chitosan concentration was sufficient to kill this isolate. while ginger isolates were more resistant. their inhibition zones were ranged from 15 mm to 42 mm for GI_3 and 15 mm to 44 mm for GI_7 when chitosan concentrations ranged from 0.2 to 4.0 % respectively. [31] reported that chitosan has antibacterial /antifungal activities. they added that chitosan is useful in pharmaceutical and other applications.

In this study four antibiotics were used also to determine the antibiotic resistant isolates and as positive control to measure chitosan efficiency, they were Amoxicillin & Clavulanic acid (20/10 µg), Vancomycin (30 µg), Rifampin (30 µg), and Cefuroxime30 µg. Results showed that Chitosan concentration 4.0% had the most efficient killing ability (44 mm) against GI7. Its inhibition zone was greater to the most powerful antibiotic (AMC: 42 mm), while the efficiency of chitosan concentration 1.0 % equal to CXM inhibition zones both of them recorded 30 mm against GI_7, also the efficiency of chitosan conc. 3.0% equal to AMC. Both of them had inhibition zone 42 mm against GI_7. This was similar to the findings of [32] they revealed that chitosan can be used as an alternative antimicrobial agent for the bacterial infection's treatment.

The antibacterial activity of chitosan may be due to disrupting the cell membrane/cell wall, forming a dense polymer film on the cell's surface and blocking the exchange of nutrients, interaction with microbial DNA, and/or chelation of nutrients by chitosan [33]. Also, chitosan can inhibit microbes by neutralizing negative charges on the microbial surface [34].

The isolates GI 3 and GI 7 were selected and identified by 16S rRNA gene as *Enterococcus faceium* R2 (OK605881) & *Enterococcus durans* R1 (OK605880), respectively. As similar, [35] isolated *Enterococcus faecium* and other Enterococcus species from plants. Moreover, [26] isolated *E. faecium* on Baird-Parker agar medium.

Obtained results revealed that the chitosan concentrations that showed maximum efficient killing for GI3 & GI7 isolates were selected for microcapsules preparation. Increasing the concentration of chitosan

and GST volume, the encapsulation yield of chitosan/ginger and chitosan/garlic microcapsules increased.

In present study chitosan used as a wall material for coating the core material (ginger or garlic) in small droplets, then crosslinking agent was added to form a crosslink between amine groups in chitosan with aldehyde groups (toluene saturated glutaraldehyde) to produce microcapsules similarly to [34].

The chitosan concentration of 4.0% and 20 ml of GST produce 92.5% and 87.5% of encapsulation yield for chitosan/ginger and chitosan/garlic microcapsules, respectively. [35] illustrated that the chitosan microcapsules yield between 81.35% and 98.93%. The microcapsules yield decreased when GST volume decreased at the same chitosan concentration. Also, the highest yield of chitosan microcapsules was 99.19%, achieved by 25 ml of GST.

The thermal stability of all prepared chitosan microcapsules was estimated by thermal gravimetric analysis similarly to [35]. The chitosan/ginger and chitosan/garlic microcapsules (chitosan conc. 4%+ 20 ml GST) reached to half of their mass at temperature 375 ^{o}C and 132 ^{o}C, respectively. This mean that chitosan/ginger microcapsules have more thermal stability than chitosan/garlic microcapsules.

In the present study, FTIR was used to test the stability of the raw material's functional groups. the formed chitosan/ginger microcapsules did not show new peaks, only a change in the peak width and slightly shifting the frequency. This indicates the absence of any significant interaction between the microcapsule's contents. Similarly, to [36]. While The FTIR bands of the chitosan/garlic microcapsules were different from IR spectrogram of garlic and chitosan. Those spectrograms revealed both characteristics of garlic and chitosan with some wavelength shift, and also showed some new bands which indicated the complex formation of chitosan and garlic.

5.Conclusion

Chitosan, a natural biopolymer with a wide variety of physicochemical and biological properties has numerous applications in the fields of agriculture, textile, nutritional enhancement and food processing, waste water management, cosmetics, drug delivery and many other medical and pharmaceutical applications. The chitosan concentration of 4% was used as an effective antimicrobial agent against bacterial isolates isolated from ginger and garlic. The ginger or garlic microcapsules were prepared by cross-linking emulsion on chitosan coating. The prepared microcapsules were spherical with a smooth surface. The encapsulation yield of chitosan/ginger and chitosan/garlic microcapsules were 92.5 and 87.5%, respectively, using 4% chitosan concentration and 20 ml of GST. Chitosan-ginger microcapsules were thermally stable while Chitosan-garlic microcapsules were partially unstable.

6.Conflict of interest

The authors declare no conflicts of interest.

7. Acknowledgments

I would like to thank everyone who helped me in carrying out this research.

8.References

1- Wildman, R. E., Wildman, R., & Wallace, T. C. (2020). *Handbook of nutraceuticals and functional foods*. CRC press.

2- Manzoor, M., Singh, J., & Gani, A. (2022). Exploration of bioactive peptides from various origin as promising nutraceutical treasures: In vitro, in silico and in vivo studies. *Food Chemistry*, *373*, 131395.

3- Gupta, R. C., Lall, R., & Srivastava, A. (Eds.). (2021). *Nutraceuticals: efficacy, safety and toxicity*. Academic Press.

4- Shoaib, M., Shehzad, A., Omar, M., Rakha, A., Raza, H., Sharif, H. R., Shakeel, A., Ansari, A., ... & Niazi, S. (2016). Inulin: Properties, health benefits and food applications. *Carbohydrate polymers*, *147*, 444-454.

5- Szychowski, K. A., Rybczynska-Tkaczyk, K., Gawel-Beben, K., Swieca, M., Karas, M., Jakuczyk, A., Matysiak M. , Binduga U. E . & Gminski, J. (2018). Characterization of active compounds of different garlic (Allium sativum L.) cultivars. *Polish Journal of Food and Nutrition Sciences*, *68*(1).

6- Ban, Z., Zhang, J., Li, L., Luo, Z., Wang, Y., Yuan, Q., Zhou, B., Liu,H. & Liu, H. (2020). Ginger essential oil-based microencapsulation as an efficient delivery system for the improvement of Jujube (Ziziphus jujuba Mill.) fruit quality. *Food chemistry*, *306*, 125628.

7- Yun, H. M., Ban, J. O., Park, K. R., Lee, C. K., Jeong, H. S., Han, S. B., & Hong, J. T. (2014). Potential therapeutic effects of functionally active compounds isolated from garlic. *Pharmacology & therapeutics*, *142*(2), 183-195.

8- Zhao, L.; Temelli, F.; Curtis, J.M.; Chen, L. Encapsulation of Lutein in Liposomes Using Supercritical Carbon Dioxide. *Food Res.Int.* **2017**, *100*, 168–179.

9- Cheng, Y.S.; Lu, P.M.; Huang, C.Y.; Wu, J.J.Encapsulation of Lycopene with Lecithin and α-Tocopherol by Supercritical Antisolvent Process for Stability Enhancement. *J. Supercrit. Fluids* **2017**, *130*, 246–252.

10- Cittadini, A.; Munekata, P.E.S.; Pateiro, M.; Sarriés, M.V.; Domínguez, R.; Lorenzo, J.M. Encapsulation Techniques to Increase Lipid Stability. In *Food Lipids Sources, Health Implications, and Future Trends*; Lorenzo, J.M., Munekata, P.E.S., Barba, F.J., Pateiro, M., Domínguez, R., Eds.; Academic Press: Oxford, UK, 2022; pp. 1–46; ISBN 9780128233719.

11- Domínguez, R.; Pateiro, M.; Munekata, P.E.S.; McClements, D.J.; Lorenzo, J.M. Encapsulation of Bioactive Phytochemicals in Plant-Based Matrices and Application as Additives in Meat and Meat Products. *Molecules* **2021**, *26*, 3984. https://doi.org/10.3390/MOLECULES26133984

12- Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S. & Peixe, L. (2021). Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 1: Methodology, general data gaps and uncertainties. *EFSA Journal*, *19*(10), e06852.

13- Sadgrove, N. J. (2022). Honest nutraceuticals, cosmetics, therapies, and foods (NCTFs): standardization and safety of natural products. *Critical Reviews in Food Science and Nutrition*, *62*(16), 4326-4341.

14- Bouymajane, A., Filali, F. R., Oulghazi, S., Ed-Dra, A., Benhallam, F., El Allaoui, A., Anissi. J., Sendide, K., Ouhmidou, B. & Moumni, M. (2018). Occurrence, molecular and antimicrobial resistance of Enterococcus spp. isolated from raw cow's milk trade by street trading in Meknes city, Morocco. *Germs*, *8*(2), 77.

15- Ibrahim, H. M., Saad, M. M., & Aly, N. M. (2016). Preparation of single layer nonwoven fabric treated with chitosan nanoparticles and its utilization in gas filtration. *International Journal of ChemTech Research*, *9*(6), 1-16.

16- Yan, D., Li, Y., Liu, Y., Li, N., Zhang, X. and Yan, C. (2021). Antimicrobial Properties of Chitosan and Chitosan Derivatives in the Treatment of Enteric Infections. *Molecules*, 26**,** 7136.

17- Wang, W., Meng, Q., Li, Q., Liu, J., Zhou, M., Jin, Z., & Zhao, K. (2020). Chitosan derivatives and their application in biomedicine. *International journal of molecular sciences*, *21*(2), 487.

18- Abou-Okeil, A., Refaei, R., Khalil, E. M., Moustafa, S. E., & Ibrahim, H. M. (2022). Fabrication of novel green magnetic electrospun nanofibers based on Fe3O4 nanoparticles/PVA/chitosan/collagen. *Egyptian Journal of Chemistry*, *65*(8), 285-298.

19- Maleki, G., Woltering, E. J., & Mozafari, M. R. (2022). Applications of chitosan-based carrier as an encapsulating agent in food industry. *Trends in Food Science & Technology*.

20- Georges, Ye, Q., Selomulya, N., & C. (2018). Microencapsulation of active ingredients in functional foods: From research stage to commercial food products. *Trends in Food Science & Technology*, *78*, 167-179.

21- Martinez, S., López, M., & Bernardo, A. (2003). Thermal inactivation of Enterococcus faecium: effect of growth temperature and physiological state of microbial cells. *Letters in applied microbiology*, *37*(6), 475-481.

22- Binte Atique, F., Ahmed, K. T., Asaduzzaman, S. M., & Hasan, K. N. (2013). Effects of gamma irradiation on bacterial microflora associated with human amniotic membrane. *BioMed research international*, *2013*.

23- Shah, A. H., & Korejo, N. A. (2012). Antimicrobial resistance profile of Salmonella serovars isolated from chicken meat. *J. Vet. Anim. Sci*, *2*(1), 40-46.

24- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research*, *25*(17), 3389-3402.

25- Fahrurrozi, M., & Wirawan, S. K. (2019). Antioxidant activity and controlled release analysis of red ginger oleoresin (Zingiber officinale var rubrum) encapsulated in chitosan cross-linked by glutaraldehyde saturated toluene. *Sustainable Chemistry and Pharmacy*, *12*, 100132.

26- Vandera, E., Tsirka, G., Kakouri, A., Koukkou, A. I., & Samelis, J. (2018). Approaches for enhancing in situ detection of enterocin genes in thermized milk, and selective isolation of enterocin-producing Enterococcus faecium from Baird-Parker agar. *International journal of food microbiology*, *281*, 23-31.

27 Liu, S., Tang, J., Tadapaneni, R. K., Yang, R., & Zhu, M. J. (2018). Exponentially increased thermal resistance of Salmonella spp. and Enterococcus faecium at reduced water activity. *Applied and Environmental Microbiology*, *84*(8), e02742-17.

28- Ebrahim, H., Abou ElNour, S., Hammad, A. A., Abouzeid, M., & Abdou, D. (2022). Comparative effect of gamma and electron beam irradiation on some food borne pathogenic bacteria contaminating meat products. *Egyptian Journal of Pure and Applied Science*, *60*(1), 62-72.

29- Jebri, S., Yahya, M., Rahmani, F., Amri, I., Hamdi, M., & Hmaied, F. (2022). Inactivation of biohazards in healthcare wastewater by E-Beam and Gamma irradiation: a comparative study. *Environmental Science and Pollution Research*, *29*(50), 75575-75586.

30- Elsonbaty, S. M., & Ismail, A. F. (2020). Nicotine encourages oxidative stress and impairment of rats' brain mitigated by Spirulina platensis lipopolysaccharides and low-dose ionizing radiation. *Archives of biochemistry and biophysics*, *689*, 108382.

31- Qin, Y., Li, P., & Guo, Z. (2020). Cationic chitosan derivatives as potential antifungals: A review of structural optimization and applications. *Carbohydrate polymers*, *236*, 116002.

32- Jeon, S. J., Oh, M., Yeo, W. S., Galvao, K. N., & Jeong, K. C. (2014). Underlying mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases. *PLoS One*, *9*(3), e92723.

33- Matica, M. A., Aachmann, F. L., Tøndervik, A., Sletta, H., & Ostafe, V. (2019). Chitosan as a wound dressing starting material: Antimicrobial properties and mode of action. *International journal of molecular sciences*, *20*(23), 5889.

34- Fahrurrozi, M., & Wirawan, S. K. (2016). Microencapsulation technology of ginger oleoresin with chitosan as wall material: a review. *Journal of Applied Pharmaceutical Science*, *6*(12), 209-223..

35- Jayanudin, Fahrurrozi, M., Wirawan, S. K., & Rochmadi. (2019). Preparation of chitosan microcapsules containing red ginger oleoresin using emulsion crosslinking method. *Journal of Applied Biomaterials & Functional Materials*, *17*(1), 2280800018809917.

36- Wirjosentono, B., & Marpaung, L. (2020). Microencapsulation of ginger-based essential oil (Zingiber cassumunar roxb) with chitosan and oil palm trunk waste fiber prepared by spray-drying method. *Case Studies in Thermal Engineering*, *18*, 100606.