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وحماية المستهلك

PRODUCTION OF NANO-BETAGLUCANS FROM BAKING YEAST *Saccharomyces cerevisiae* AND STUDYING SOME OF ITS PROPERTIES AND ITS APPLICATION IN THE MANUFACTURE OF SOFT CHEESE

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ABSTRACT

The inhibitory activity of the extracted and nano-betaglucan compound was estimated against pathogenic bacteria, as the average diameter of the inhibition zone at a concentration of 200 mg/mL gave the highest inhibitory activity for all types of isolates with significant differences at a probability level (P≤0.05), of which we singled out of Gram-positive bacteria when converting it into a nanocomposite. The results showed that there was no growth inhibition zone for lactic acid bacteria. The cytotoxicity of betaglucan nanocomposite was estimated, as it was observed in the Microculture Tetrazolium Test (MTT) that there were significant differences between the viability of cells in the Human dermal Fibroblast cell line (HdFn) (control treatment) and the concentrations of betaglucan nanocomposite at a probability level of 0.05, as the number of cells decreased. The presence of anticancer activities of the nanocomposite betaglucan produced in this study in the human breast cancer cell line Michigan Canner Foundation-7 (MCF-7), whose concentrations increase, the number of cells decreases, starting from (0) to (200) µg/mL. The nanocomposite betaglucan was added to the curd of soft cheese in proportions of 0.1, 0.25 and 0.5%. All these treatments were subjected to chemical, microbial and sensory tests. The results were: A significant decrease in the percentage of moisture compared to the comparison treatment, the chemical indicators of soft cheese were monitored, which included (fat, protein, acidity and pH). Soft cheese models with added nano-betaglucan manufactured in general were characterized by a decrease in the number of bacteria, yeasts and molds contaminating the cheese compared to the comparison treatment during the storage period. The aforementioned results were generally reflected in the sensory evaluation results, as the highest scores were given for taste, flavor, texture, color, and appearance. on soft cheese samples (treatments T2, T3, and T4) respectively, compared to the control treatment (T1).

Keywords: Nano betaglucan, Soft cheese, *Saccharomyces cerevisiae.*

[*]**The article is taken from the master's thesis of the first researcher.**

إنتاج البيتاكلوكان النانوي من خميرة الخبز ودراسة بعض صفاته وتطبيقه في صناعة الجبن الطري

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الخلاصة

قدرت الفعالية التثبيطية لمركب البيتاكلوكان المستخلص والنانوي ضد البكتريا المرضية اذ كان معدل قطر هالة التثبيط وبتركيز 200 ملغرام مللتر قد اعطى اعلى فعالية تثبيطية لانواع العزلات جميعها وبفروقات معنوية عند مستوى احتمالية (P≤0.05) ازداد قطر هالة التثبيط لنمو البكتريا الموجبة لصبغة كرام عند تحويل البيتاكلوكان المستخلص الى مركب نانوي، اظهرت النتائج عدم وجود منطقة تثبيط نمو لبكتريا حامض اللاكتيك. قدرت السمية الخلوية لمركب البيتاكلوكان النانوي اذ لوحظ في فحص Microculture Tetrazolium Test (MTT) وجود فروق معنوية مابين عيوشية viability الخلايا في خط الخلايا الليفية الجلدية للانسان HdFn (معاملة السيطرة) ومعاملات تراكيز مركب البيتاكلوكان النانوي عند مستوى احتمالية 0.05) اذ قلت اعداد الخلايا الحية بزيادة التركيزمما يدل على عدم احداث أي آثار سمية لمركب البيتاكلوكان النانوي وكذلك للخلايا السرطانية وجود فعالية مضادة للسرطان anticancer activities لمركب البيتاكلوكان النانوي المنتج في هذه الدراسة في خط خلايا سرطان الثدي للانسان MCF-7 والذي بزيادة تراكيزه تتناقص اعداد الخلايا بدءا من (0) الى (200) مايكروغرام/ مللتر. اضيف مركب البيتاكلوكان النانوي الى خثرة الجبن الطري بالنسب 0.1 ، 0.25 و 0.5 % ، خضعت هذه المعاملات جميعا للفحوصات الكيمائية والمايكروبية والحسية كانت النتائج: انخفاض ملحوظ في نسبة الرطوبة المفقودة من معاملات الجبن مقارنة بمعاملة المقارنة (الدهن، البروتين ،الحموضة والاس الهيدروجيني) حسب عدد الأحياء المجهرية في الجبن الطري في المعاملات المختلفة وقد تميزت نماذج الجبن الطري المضاف له البيتاكلوكان النانوي المصنعة بشكل عام بانخفاض معدل أعداد البكتريا والخمائر والأعفان الملوثة للجبن مقارنة بمعاملة المقارنة خلال مدة الخزن ، وإنعكست مجمل النتائج المذكورة آنفاً على نتائج التقييم الحسي، إذ أعطيت أعلى الدرجات المتعلقة بالطعم والنكهة والقوام والنسجة واللون والمظهر على نماذج الجبن الطري (المعاملات T2 وT3 وT4) على التوالي مقارنة بمعاملة المقارنة (T1).

الكلمات المفتاحية: البيتاكلوكان النانوي، الجبن الطري، خميرة الخبز.

INTRODUCTION

Oral administration of betaglucan nanocapsules at levels of 100 and 200 mg for 4 consecutive days leads to a hepatoprotective factor (**Mahmoud *et al.*, 2015**; **Al-Jumaiee *et al.*, 2021)** and it has recently been proven that they are used to deliver drugs that target pathological microorganisms. Yeast is also abundant in operations. Different types of bacteria, including fermentation, where betaglucan derived from yeast help the host's defense against infection through their antibacterial effect on pathogenic bacteria and microorganisms. Nanotechnology is concerned with the development of new techniques and methods that are measured in nanometers, which are a fraction of million. This technology has many applications in a variety of fields, including food applications such as: food processing, food preservation systems and application technology. Others use nanotubes that reduce oxygen intake and keep the product moist. There are sensors to detect contamination of food products at the same time **(Al-Hadedee *et al.*, 2019)**. studied the antitumor efficacy of nanoparticles against human breast cancer in MCF-7 cancer cells through fibrillation and composition inhibitory uptake experiments and found anticancer drug doxorubicin in nanoparticles to establish drug delivery systems **(Huang *et al.*, 2020)**.

MATERIALS AND METHODS

The beta-glucan nanocomposite was prepared according to **(Anusuya & Sathlyabama, 2014)**. The nanoparticle size of beta-glucan was determined by **Ashraf *et al.* (2020)**, the field emission scanning electron microscopy was estimated by **Lee *et al.* (2020)**, the X-ray diffraction was estimated by **Kadhum & Hussein (2020)**. The zeta potential was estimated by **Elnagar *et al.* (2021)**, diagnosis of nano-beta-glucan using infrared spectroscopy by **Hussein & Muslim (2019)**, ultraviolet spectrum measurement by **Al-Hadedee (2016)**. Three genera of pathogenic bacteria *Staphylococcus aureus, Psedoumonas aeurginosa* and *Eschericha coli* and two genera of lactic acid bacteria were used for the two isolates *Lb.bulgaricus* and *St.thermophilus* as a single starter and *St.thermophilus (Lb.bulgaricus)* as a mixed culture **(Balouiri *et al.*, 2016)**. The experiment was conducted at the Natural Products Research Center and Drug Discovery/ College of Medicine/ University of Malaysia to detect the cytotoxic effect of a series of concentrations of the nano-betaglucan compound on the human dermal fibroblast normal cell line (HdFn) and the breast cancer cell line. (MCF-7) in vitro using the microculture tetrazolium test (MTT), and the steps for tissue culture were carried out according to the method described by **(Al-Moussawi, 2021)**. Four treatments of soft cheese were made, Treatment T1 included the manufacture of soft cheese without adding (control treatment), T2 added 0.1% of the nano-betaglucan to the soft cheese curds, while T3 included adding 0.25% of the nano-betaglucan to the soft cheese curds, and T4 added 0.50% of the soft cheese. Nano beta-glucan to soft cheese curds **Awda *et al.* (2019)**. All of these treatments were subjected to chemical, microbial, and sensory tests, as follows: pH and Fat of cheese according to the method mentioned by **Ling (2008)**. Acidity percentage and Moisture were estimated according to the mentioned method by **Association of Official Analytical Chemists (2010)**, the percentage of protein in processed cheese was estimated using the microcalcidal method, according to **Joslyn (1970)**. Microbiological tests (Total Count, Coliform, Yeasts and Molds and Psychrophilic bacteria) according to **American Public Health Association (1978)**. The sensory evaluation of soft cheese samples was carried out by specialized professors in the Department of Food Sciences - College of Agricultural Engineering Sciences - University of Baghdad, and a sensory evaluation form for soft cheese developed by **Al-Dahan (1977)** was used. The Statistical Analysis System -**SAS (2018)** program was used to analyze the data to study the effect of different treatments on the studied traits according to a complete randomized design (CRD-completely randomized design), and the significant differences between the averages were compared with the Least significant difference test (LSD).

RESULTS AND DISSCUSION

Study of the physical properties of nanoscale beta-glucan including:

nanoparticles of beta-glucan: It is noted from Figure (1) that the average size of nanoparticles of beta-glucan the Size Analyzer was 189.4 nm. It is higher than what was obtained by **Parthasarathy *et al. (*2021)** as the betaglucan nanoparticles had a length of 88.91 nm.

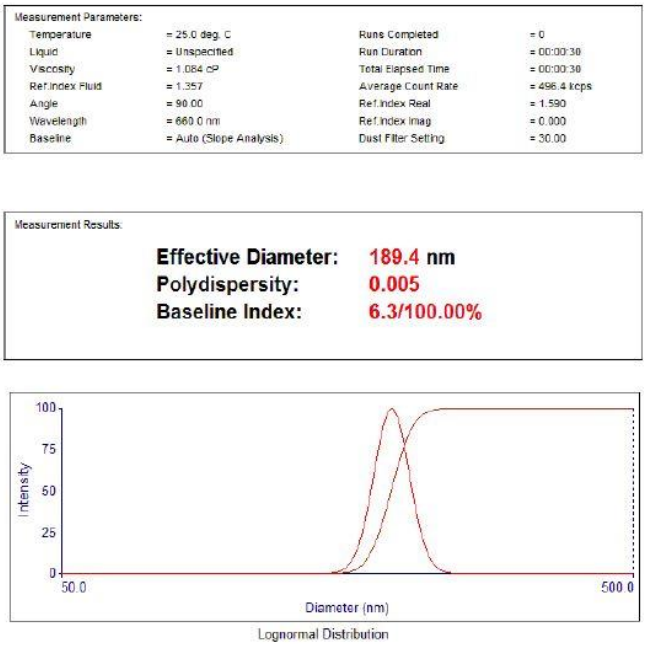

Figure (1): The average size of nanoparticles of beta-glucan.

Figure (2) shows Field Emission Scanning Electron Microscope (FESEM) images of different spectra of nanoparticles of beta-glucan. The diameters of nanoparticles of beta-glucan ranged between (25.37-74.14). These results are consistent with what was found by **Elnagar *et al.* (2021)** when comparing treated bacteria in the complex consisting of silver nanoparticles and beta glucan with untreated bacteria after 0, 5 and 10 hours, as the bacteria treated with the complex after 5 hours had normal cells and a smooth structure with no evidence of deformations or decomposition but after 10 hours or more, the membranes began to appear, and the membranes began to release their internal components.

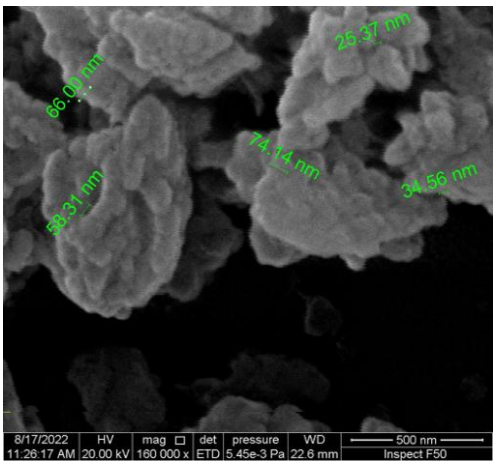

Figure (2): the surface of nanoscale beta-glucan using the FESEM device.

Figure (3) shows using the x-ray diffraction (XRD) device, as we note the emergence of three peaks at 22.12°, 20.18°, and 16.15° for the beta-glucan nanocomposite. These results agree with what was found by **Parthasarathy *et al.* (2021)** There were three peaks (23.08°, 34.5°, 45.2°), which were crystalline in nature from GluNPs.

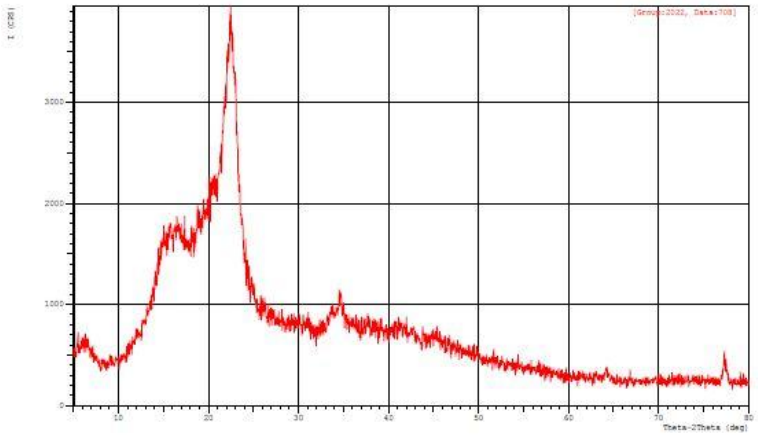

Figure (3): XRD spectrum analysis of beta-glucan nanoparticles.

Zeta Potential: To study the electrostatic properties of the nano-betaglucan solution, the zeta potential was calculated in Table (1) for the nano-beta-glucan - 22.80 mV. This may be due to the fact that its particles are extremely small and within the nano level, and this leads to a high electrical potential difference on its surface, and these results were in line with what was found by **Elnagar *et al.* (2021)** as the zeta potential values for silver particles were -27.4 mV, for beta-glucan -21.3 mV, and for the complex consisting of beta-glucan and silver nanoparticles. -24.8 mV.

Table (1): The zeta potential (mV) of beta-glucan*****

Type or extract	Zeta potential (mV)
β-glucan nanocomplex	-22.80

The result showed UltraViolet-Visible spectroscopy (UV-Vis) absorbance at five wavelengths (278, 664, 697, 754 and 779) (0.699, 0.821, 0.862, 0.906 and 0.913) nm for the beta-glucan nanoparticles, as shown in Figure (4). These results were in agreement with **Parthasarathy *et al.* (2021)** as the UV-Vis spectroscopy showed the absorbance at 2.2 at the wavelength of 389 nm for the betaglucan nanoparticles.

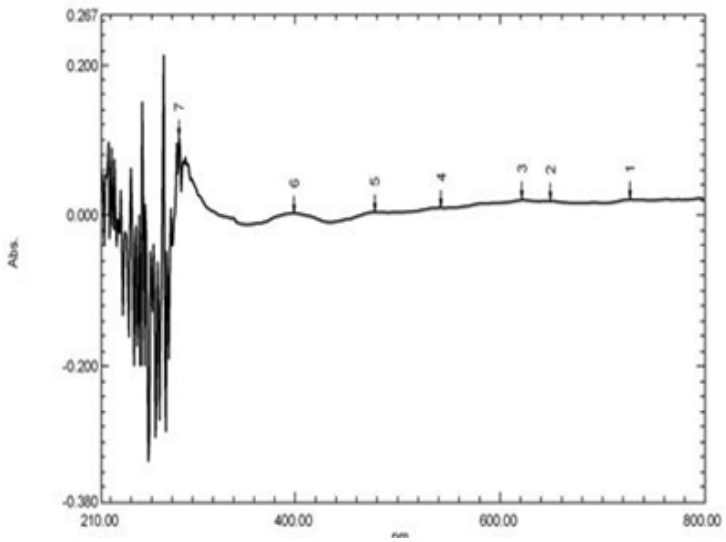

Figure (4): UV-Vis absorption spectrum of the beta-glucan nanomodel.

Fourier Transformed Infrared (FTIR) infrared spectrum

Fourier Transformed Infrared (FTIR) infrared measurement was used to confirm the presence of active groups on the beta-glucan extract extracted from *S.cerevisiae* yeast for the nano-betaglucan. Figure (5) shows the spectrum of the groups determined for the beta-glucan nanocomposite using Fourier Transform Infrared spectroscopy, as they were 1111 and 1429. and 3417 cm^{-1}. When referring to the I.R. tables, it was found that the absorption spectra belong to the active groups C-O-C, C-H, and O-H, respectively. The absorption spectrum of free hydroxyl groups and nano- betaglucan is 2922 cm^{-1}, and these results agree with what was found by **Atta-Allah *et al.* (2023)** showed that the carboxyl groups of the beta-glucan were 1013.67 cm-1 for the nano-betaglucan. The absorption spectrum of the free hydroxyl groups was 3367 cm^{-1} for the nanoparticles of betaglucan.

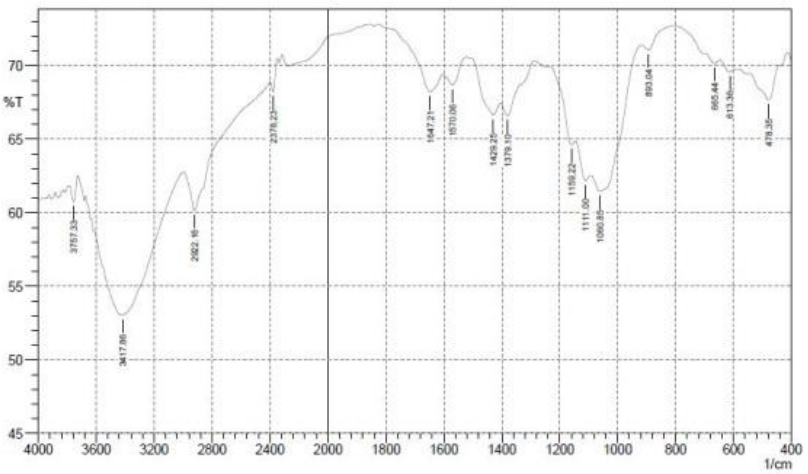

Figure (5): FTIR spectrum analysis of nanoscale beta-glucan.

the results showed in Table (2) that the beta-glucan solution extracted from *Sacchraomyces cerevisiae* at a concentration of (50,100,150and 200) mg/ml increased the diameter of the inhibition for the growth of Gram-positive bacteria when it was converted to Nanocompound, *Staphylococcus aureus* had 23 mm and Gram-negative bacteria inhibited it also, as *E. coli* and *Pseudomonas aeuroginosa* had 23 and 21 mm respectively. The effect of betaglucan in inhibiting the growth of Gram-positive bacteria is due to its effectiveness in breaking the glycosidic bond of the type $\mathcal{B}$ -1- 4 that links the building blocks N-acetyl muramic acids and N-acetyl glucose amine constituting the peptidoglycan layer, which leads to breaking and destroying the cell wall. And then inhibiting or stopping the growth of these bacteria and these results came in agreement with what **Parthasarathy *et al.* (2021)**. The inhibitory activity of nanoscale beta-glucan against the growth of lactic acid bacteria was estimated, and the results showed that there were no zones of growth inhibition for lactic acid bacteria *Lb.bulgaricus*, *St.thermophilus*, and (*St.thermophilus & Lb.bulgaricus*) (mixed culture). Use of betaglucan in fermented milk products therapeutic foods as a support or as a texture stabilizer or thickener without having any effect on these intestinal bacteria **(Pal Singh & Bhardwaj, 2023)**.

Table (2): The effect of beta-glucan on the activity of microorganisms**.***

Type	Name	Average Halo Diameter (mm) nano beta glucan				LSD Value
		50	100	150	200	
Gram-negative bacteria	*Eschericia coli*	12	18	20	23	5.02 *
	Pseudomonas aeuroginosa	11	17	19	21	4.81 *
Gram positive bacteria	*Staphylococcus aureus*	12	15	17	23	5.37 *
LSD Value		2.17 NS	3.05 NS	3.19 NS	2.85 NS	---
* (P<0.05), NS: no significant.						

Examination of the cytotoxicity assay of nanoscale beta-glucan in HdFn cell line and anticancer efficacy in McF-7 cell line using MTT assay

This experiment aimed to evaluate the toxicity of beta glucan nanocomposite at different concentrations ranging from (0-200) µg/mL and with a difference of 6.25 µg/mL between one concentration and another in the natural human dermal fibroblast cell line HdFn within 24 hours using the MTT. Which is one of the colorimetric diagnostic methods that do not need a long time. This test depends on measuring the activity of living cells that 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) is in turn dehydrogenated by mitochondrial dehydrogenases that convert soluble yellow tetrazolium dye into violet insoluble formazan crystals. The method of reduction (**Abdel-Fattah *et al.*, 2012**) and changing the cell number, increasing or decreasing, leads to a change in the amount of formed formazan crystals, which indicates the degree of toxicity causing the tested substance. Figure (6a) shows the presence of significant differences between the viability of cells in the line Human dermal

fibroblast skin HdFn (control treatment) and concentrations of betaglucan nanocomposite at a probability level of (0.05) and according to Graphpad prism version 9.4 (Graphpad Software Inc, Jolla CA) as the number of living cells decreased with increasing concentration, which indicates that no toxic effects of the compound nanoparticle betaglucan. The results show in Figure (6b) the presence of anticancer activities of the nanoparticle beta glucan produced in this study in the human breast cancer cell line MCF-7, whose concentration increases, the number of cells decreases, starting from (0) to (200)µg / ml, and this It is consistent with what was mentioned by **Atta-Allah *et al.* (2023)** when they measured the cytotoxicity of different concentrations of beta-glucan, as nanoparticles (15.62-1000 µg / ml) were determined using hepatic pollutants, normal human hepatocytes and cancer cell lines (HepG2).

Studies of the properties of soft cheese manufactured by adding the nanocomposite beta-glucan

Moisture content: The results shown in Table (3) show the moisture content of the different soft cheese treatments during the storage period of 14 days, where the percentage of moisture at zero age was 70.84, 66.83, 67.43, and 69.72% for the T1, T2, T3, and T4 treatments respectively. These percentages are within the limits of the moisture percentage specified by the Iraqi standard specification **(2020)** for soft cheeses, which is more than 67%, and they agree with what I found (**Al-Rubaee, 2009**; **Al-Azzawi, 2018**) Which mentioned that the moisture content of mozzarella cheese produced from fresh milk and added betaglucan from oats at a rate of 0.2% is 55.5%, The percentage at the end of the storage period was 66.53, 62.81, 65.42, and 65.72% for the treatments T1, T2, T3, and T4 respectively. The reason is attributed to the fact that the entire betaglucan compound contains the amount of moisture and prevents it, which increases the clarity of the cheese for storage **Alnemr *et al.*, (2013)**.

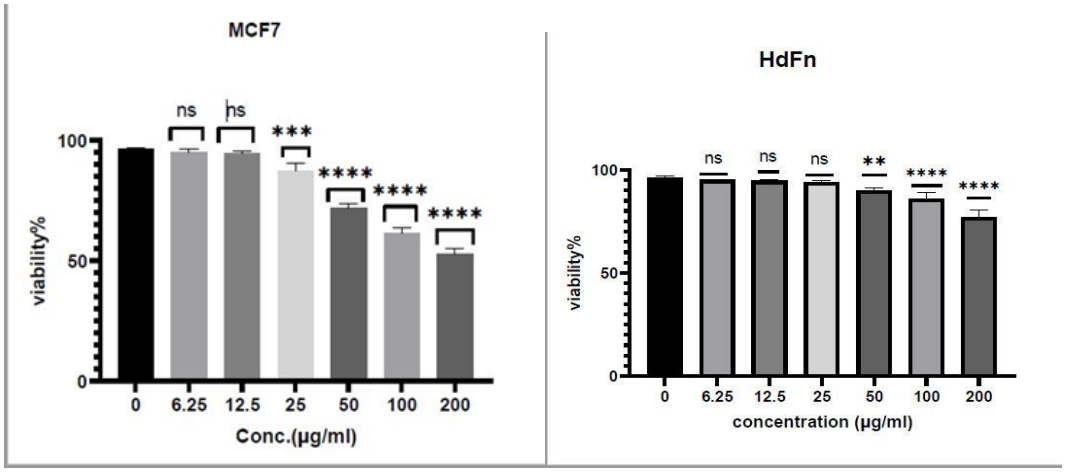

Figure (6): Cytotoxicity assay of betaglucan nanocomposite produced from *S.cerevisiae* yeast in HdFn cell line and anticancer in MCF-7 cell line - using the MTT assay, denoted (a) normal human dermal fibroblast line, (b) MCF-7 cancer cell line NS: no significant differences, ** $p \leq 0.05$: significant differences.

Table (3): Humidity percentages in the treatments of soft cheese manufactured by adding nano-betaglucan during the storage period of 14 days at 4°C ±2*.

Treatments	% moisture in soft cheese treatments during storage ages (days)					LSD Value
	0	3	7	9	14	
T1	70.84	67.73	67.09	66.77	66.53	4.05*
T2	66.83	66.25	63.40	63.36	62.81	3.96*
T3	67.43	67.39	66.87	65.50	65.42	2.91NS
T4	69.72	68.64	67.39	66.54	65.72	4.18*
LSD Value	* 4.71	* 4.06	* 4.96	3.81 NS	3.79 NS	--------
* (P<0.05), NS: no significant.						

*** The numbers in the table represent an average of two repeaters.**

The percentage of fat

The results in Table (4) show the percentage of fat for the processed cheese under study T1, T2, T3 and T4 during the storage period of 14 days, as the readings show that the percentage of fat in all treatments of soft cheese was close after the end of the manufacturing process (zero age), and it ranged between 3.45% -3.78% The reason for the difference in the increase in the percentage of fat between the treatments may be due to the difference in the moisture content between these treatments and the result of the loss of moisture in them. When comparing these results with what other researchers found, we notice that they are consistent with what I found **(Al-Azzawi, 2018)**, which stated that the percentage of fat increased as the storage period progressed, which was accompanied by a decrease in the percentage of moisture in this cheese. The percentage of fat was immediately after manufacturing for the cheese fortified with betaglucan.

Table (4): Percentages of fat in the treatments of soft cheese manufactured by adding the nanocomposite betaglucan during the storage period of 14 days at 4°C ±2*.

Treatments	% fat in soft cheese treatments during storage ages (days)					LSD Value
	0	3	7	9	14	
T1	3.70	2.96	3.08	3.46	2.48	0.677*
T2	3.78	3.54	2.78	2.62	2.72	0.703*
T3	3.65	3.60	3.28	3.25	2.84	0.638*
T4	3.45	3.70	2.45	3.58	2.78	0.762*
LSD Value	0.369 NS	0.605 *	0.537 *	0.614 *	0.391 NS	--------
* (P<0.05), NS: no significant.						

*** The numbers in the table represent an average of two repeaters.**

Protein percentage

Table (5) shows the percentages of protein in soft cheese for all treatments, as the percentages of protein at zero time were 24.00%, 27.00%, 25.00% and 28.00% for treatments T1, T2, T3 and T4, respectively. These results are close. As found by **Al-Azzawi (2018)**, who attributed the difference in the percentage of protein between the treatments to the difference in moisture content, and at the age of 14 days, the percentage of protein reached 21.00% and 25.00% in the two treatments T1 and T2, for the treatment T3 21.00%, and for the treatment T4 20.00%.

Table (5): Percentages of protein in soft cheese treatments manufactured by adding nano-betaglucan during the storage period of 14 days at 4°C ±2*.

Treatments	% protein in soft cheese treatments during storage ages (days)					LSD Value
	0	3	7	9	14	
T1	24.00	24.00	23.00	23.00	21.00	2.96*
T2	27.00	26.00	26.00	25.00	25.00	2.57NS
T3	25.00	25.00	22.00	22.00	21.00	3.02*
T4	28.00	26.00	26.00	24.00	20.00	4.15*
LSD Value	3.17*	2.08 NS	3.59*	2.81*	2.97*	--------
* (P<0.05), NS: no significant.						

*** The numbers in the table represent an average of two repeaters.**

Acidity and pH ratio

The acidity shares in balance with other compounds to give the taste and flavor of the cheese and thus influence the degree of consumer acceptance of the cheese **(Fox & Wallace, 1997)**. Nano beta glucan at a rate of 0.25% (T3) from 0.17 to 0.30% and in soft cheese manufactured by adding the compound beta-glucan nanoparticles at a rate of 0.50% (T4) from 0.18 to 0.28% after 14 days of the ripening period, while in soft cheese (T1) it increased from 0.16 to 0.36% and in soft cheese manufactured by adding the nanocomposite beta-glucan at a rate of 0.1% (T2), it increased from 0.16 to 0.35%. These results agree with what was found by **Al-Azzawi (2018)** who found that the acidity values of processed cheese increased from 0.30% to 1.8% during the storage period of 14 days. The results of the statistical analysis showed that there was a significant difference ($P < 0.05$) in the acidity values at the age of 0 and 14 days between the soft cheese manufactured by adding the beta-glucan nanoparticles at 0.25% and 0.50% (T3 and T4) and the control treatment (T1). The results of estimating the pH showed a significant decrease in its values during the storage stages of soft cheese and for all treatments, as it was noted from Table (6) that a decrease was observed The pH in treatment T1 ranged from 6.40 to 5.39, in treatment T2 from 6.40 to 5.56, in treatment T3 from 6.40 to 5.62, and in treatment T4 from 6.40 to 5.88 after 14 days of storage. The rate of increase in the percentage of acidity in cheese during the storage period **(Sudiana *et al*., 2022)**.

Table (6): Percentages of acidity and pH in the treatments of soft cheese manufactured by adding the nanocomposite betaglucan during the storage period of 14 days at 4°C ±2*.

Treatments	Soft cheese treatments for storage ages (days)									
	Acidity (%)					pH				
	0	3	7	9	14	0	3	7	9	14
T1	0.16	0.19	0.22	0.29	0.36	6.40	6.17	5.81	5.46	5.39
T2	0.16	0.20	0.24	0.31	0.35	6.40	6.34	6.31	5.75	5.56
T3	0.17	0.21	0.26	0.30	0.30	6.40	6.35	6.32	5.77	5.62
T4	0.18	0.23	0.28	0.28	0.28	6.40	6.37	6.34	5.79	5.88
LSD Value	0.052 NS	0.056 NS	0.062 NS	0.067 NS	0.0.62 NS	0.392 NS	0.305 NS	0.318 NS	0.451 NS	0.566 NS
NS: no significant.										

*** The numbers in the table represent an average of two repeaters.**

Microbiological examinations

Table (7) shows a decrease in the total numbers of microorganisms in the cheeses treatment with anti-microbial agents after the 14-day storage period, when they were in the control treatment, from 3.0×10^4 to 3.0×10^7 CFU/g respectively, while It is noted that it decreased in the treatment of soft cheese manufactured by adding the nanocomposite beta-glucan at a rate of 0.1% from 3.5×10^3 to 8.5×10^5 CFU/g and in the treatment of soft cheese manufactured by adding the nanocomposite beta-glucan at a rate of 0.25% from 1.0×10^3 to

4.0 × 10^5 CFU/g and in the treatment of soft cheese manufactured by adding nanoparticle beta-glucan at a rate of 0.50% from 9.0 x 10^3 to 3.5 x 10^5 CFU/g. As for mold yeasts, no growth was observed during the storage period in the soft cheese treatments manufactured by adding the nanocomposite beta-glucan at 0.1%, 0.25% and 0.50% (T2, T 3 and T4 treatments) which indicates that the use of antimicrobial agents represented in By adding the nano-beta-glucan compound, it contributed to reducing the growth of yeasts and molds compared to the treatment that was not used with it Table (7), as the presence of beta-glucan in these processed cheeses played an important role, so it is widely used in food preservation, especially in the cheese industry, because it has the ability to inhibit or Destruction of molds by **Parthasarathy *et al.* (2021)**.The results indicate that the differences in the numbers of microorganisms between the treatments of soft cheese manufactured with the addition of the nanoparticle betaglucan (T2, T3, and T4) were slight to some extent and within the permissible limits in this type of cheese, which indicates the possibility of using This compound in the manufacture of soft cheese is nutritionally acceptable and hygienic. Conforms to **Iraqi Standard Specification No. (5/2270) for the year 2015** regarding microbial limits for milk and its products.

Table (7): The results of microbial tests, mL/gm, in the treatments of soft cheese manufactured by adding the nanocomposite beta-glucan during the storage period of 14 days at a temperature of 4°C ±2 *.

Treatments	age (day)	Total Count	*E. coli*	Yeasts & Molds	Psychrophilic
T1	0	3.0×10^4	Nill	Nill	Nill
	3	1.3×10^5	1.5×10^1	Nill	3.6×10^1
	7	4.5×10^5	5.5×10^1	Nill	4.9×10^1
	9	6.5×10^5	8.5×10^1	4.0×10^2	7.7×10^1
	14	3.0×10^7	1.2×10^2	4.0×10^3	9.0×10^1
T2	0	3.5×10^3	Nill	Nill	Nill
	3	1.0×10^5	1.1×10^1	Nill	Nill
	7	2.0×10^5	4.0×10^1	Nill	Nill
	9	4.5×10^5	7.2×10^1	Nill	Nill
	14	8.5×10^5	7.8×10^1	Nill	6.0×10^1
T3	0	1.0×10^3	Nill	Nill	Nill
	3	7.5×10^4	Nill	Nill	Nill
	7	3.0×10^5	Nill	Nill	Nill
	9	3.3×10^5	1.2×10^1	Nill	Nill
	14	4.0×10^5	2.4×10^1	Nill	4.0×10^1
T4	0	9.0×10^3	Nill	Nill	Nill
	3	6.5×10^3	Nill	Nill	Nill
	7	2.1×10^4	Nill	Nill	Nill
	9	1.1×10^5	1.0×10^1	Nill	Nill
	14	3.5×10^5	2.0×10^1	Nill	Nill
LSD value		27.63 *	16.55 *	0.00 NS	14.67 *

*** The numbers in the table represent an average of two repeaters.**

Sensory evaluation

Table (8) shows the results of the sensory evaluation, as it was noted that the average scores granted to the characteristic of taste and flavor ranged between 45 and 40 degrees from the average of 45 degrees for all studied treatments at the beginning of the storage period, which indicates that there are no significant differences between all these treatments, and with follow-up progress in The age of the cheese It was noted that the average scores granted for this characteristic in the treatments T2, T3 and T4 ranged between 8 and 9 degrees at the age of 3 days, while at the age of 14 days of storage ranged between 8 and 10 degrees, which indicates that the storage process in these samples was well carried out and prevented the appearance of strange tastes and odors. T1. Otherwise, the scores given to these characteristics were relatively high in Soft cheese added to the nanocomposite beta-glucan, i.e. treatments T2, T3 and T4, as the storage period progresses, The reason for this may be due to the role played by the betaglucan nanocomposite added to the manufactured soft cheese in retaining water and reducing moisture loss, thus providing an environment with water activity (aw) suitable for the work of starter bacteria, and this is consistent with what **Al-Azzawi (2018)** mentioned. These results indicate The possibility of using the nano-betaglucan compound added to the soft processed cheese and preserving the cheese during the storage period, as the two treatments T3 and T4 gave the best results, in addition to the antimicrobial role of the nano-betaglucan, which also contributes to preventing the growth of microorganisms,especially (obligated aerobic).

Table (8): Results of sensory evaluation of soft cheese parameters manufactured with the addition of nano-beta-glucan during a storage period of 14 days at a temperature of 4°C **±2*.**

Treatments	cheese age (day)	Taste and flavor (45)	texture and tissue (35)	color (10)	Appearance (10)	final score (100)
T1	0	45	35	10	9	99
	3	43	33	10	10	96
	7	40	30	8	9	87
	9	———	———	———	———	———
	14	———	———	———	———	———
T2	0	45	35	10	10	100
	3	45	33	10	10	98
	7	43	33	10	10	96
	9	43	32	9	9	93
	14	43	32	9	9	93
T3	0	45	35	10	10	100
	3	45	35	10	10	100
	7	45	35	10	10	100
	9	44	33	10	10	97
	14	43	33	10	9	97
T4	0	45	35	10	10	100
	3	45	35	10	10	100
	7	45	35	10	10	100
	9	45	35	10	10	100
	14	43	34	10	10	97
LSD value		4.07 *	3.26 NS	1.18 NS	1.06 NS	5.86 *

*** The numbers in the table represent an average of two repeaters.**

CONCLUSIONS

The extracted beta-glucan was converted into a nanocomposite by (base - acid) method. The physical, chemical and functional properties of the nanocomposite were determined. FESEM was used to detect the surface of the extracted and prepared nano-beta-glucan compound, which reached (182.5-256.6) nm (25.37-74.14) nm respectively. As for the XRD, UV-Vis. The inhibitory activity of the extracted and nano-betaclocan compound was estimated against pathogenic bacteria, as the average diameter of the inhibition ring at a concentration of 200 mg/mL gave the highest inhibitory activity for all types of isolates. The results showed that there was no growth inhibition zone for lactic acid bacteria.The cytotoxicity of betaglucan nanocomposite was estimated, as it was observed in the MTT that there were significant differences between the viability of cells in the HdFn cell line (control treatment) and the concentrations of betaglucan nanocomposite at a probability level of 0.05, as the number of cells decreased. The presence of anticancer activities of the nanocomposite betaglucan produced in this study in the human breast cancer cell line MCF-7, whose concentrations increase, the number of cells decreases, starting from (0) to (200) micrograms / milliliter.The nanocomposite beta-glucan was added to the curd of soft cheese in proportions of 0.1, 0.25 and 0.50%. All these treatments were subjected to chemical, microbial and sensory tests. The results were:A significant decrease in the percentage of moisture lost from the cheese treatments compared to the comparison treatment ,The chemical indicators of soft cheese were monitored, which included (fat, protein, ash, acidity and pH),According to the number of microorganisms in soft cheese in different treatments. Soft cheese models with added nano-betaglucans manufactured in general were characterized by a decrease in the number of bacteria, yeasts and molds contaminating the cheese compared to the comparison treatment during the storage period. The total number of bacteria decreased by approximately two logarithmic cycles in the end of the storage period in the soft cheese samples manufactured by adding nano-betaglucan compared to the comparison cheese. The aforementioned results were generally reflected in the sensory evaluation results, as the highest scores were given for taste, flavor, texture, texture, color, and appearance on soft cheese samples (treatments 2T, T3, and T4) respectively, compared to the control treatment (T1).

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