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The Effectiveness of Zinc Oxide Nanoparticles and Wormwood Extract in Prolonging the Preservation of Soft Cheese

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Abstract:

This study was conducted to evaluate the effectiveness of zinc oxide nanoscale and watery wormwood extract loaded on gelatin packaging on the microbial content of soft cheese during different storage periods, as five treatments were manufactured from cheese, which included control sample (T1) uncoated, gelatin-coated cheese sample without adding T2, cheese sample coated and added ZnONPs (T3), coated cheese sample and added to it watery wormwood extract (T4), cheese sample added to ZnONPs and wormwood extract ((T5) and the results showed an increase in the number of The total of bacteria until the end of the storage period, as T5 was the lowest among the parameters 4.19 WM/g compared to the control sample, and not all the transactions showed any growth in the lipolysis and proteolytic bacteria per day, and the growth began with the progress of the storage period and at the end of storage, the control sample was the highest in the two types of bacteria 4.16 and 4.69 FM/g, respectively, compared to the rest of the fewer treatments of these bacteria. Yeasts and molds did not show growth in all transactions during the first two weeks of storage except for T1 and T2, which recorded 1.40 and 1.28 FTM/g respectively.

Keywords: Zinc oxide nanoparticles, soft white cheese, microbial content of cheese, watery wormwood extract**.**

Introduction:

Soft white cheese is a favorable environment for the growth of many microorganisms due to its content of many key nutrients such as proteins, fats and vitamins in addition to its high moisture content, all of which were a major cause of microbial growth and thus pollution (Pintado et al., 2015). As a result of the production of soft cheese from raw or pasteurized milk by adding rennet to it and its high moisture content of more than 50% and its richness in important nutritional components and possession of pH close to neutral and therefore has become a suitable environment for microbial growth, especially pathological bacteria, which has become the cause of many pathological infections resulting from eating soft cheese It is worth noting that there are those who make soft cheese without any heat treatment of milk and therefore the microbial and chemical properties of cheese will increase the risks caused by Pathological microbes (Jawad, 2016 .(The microorganisms are the main axis in the process of developing the soft cheese industry for its role in giving color, flavor, taste and increasing shelf life, as for the presence of harmful microorganisms such as disease and toxin-producing and that enter milk in several ways such as contamination of milk collection utensils or the lack of cleanliness of workers or during manufacturing processes or after manufacturing and thus causing damage to soft cheese (Broadbent et al., 2011). The labor force during the manufacture, packaging and transportation of soft cheese is a cause of contamination, and the use of unhealthy water in cleaning the tools and utensils used in the

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manufacture of cheese are all sources of contamination of cheese (Verraes et al., 2015). Nanoparticles have received more care and attention and are used in many fields for their good properties as these particles provide solutions to environmental challenges due to the shape, size and distribution of these particles (Wu et al., 2019 .(The use of nanocarriers also has an important role in overcoming major challenges that may harm the food material by protecting and controlling the release of many active biological compounds, including natural antioxidants, as these products face many problems, including low life span and difficulty in packaging and handling, which results in economic losses due to the appearance of unwanted flavors, odors and baits (Maqsoudlou et al., 2020). It is worth noting that ZnONPs have good effectiveness against many microorganisms that cause food spoilage, as these particles are characterized by their wide uses compared to other nanoparticles, where their effectiveness is through the oxidative stress mechanism as a result of the formation of types of reactive oxygen and disruption of cell membranes as a result of the accumulation of ZnONPs (Souza et al., 2019 .(Medicinal plants and herbs around the world have been considered for centuries to be important in the treatment of various diseases for their effectiveness, availability and ease of use compared to chemical treatments (Yeung et al., 2020). Wormwood is one of the important medicinal plants and because of its effectiveness and frequent uses, there have been many studies on it, as it is used in spices and as an aromatic plant and a food preservative because of its activity against microbes and its natural antioxidants in addition to its therapeutic importance (De-Sment, 2017). Dairy products are one of the most consumed foods and have great importance for human health, but their main problem is that they are perishable, so the goal of this study was:

Production of edible gelatin casings and fortified with zinc oxide nanoparticles and aqueous wormwood extract and evaluation of their effectiveness on the microbial content of soft cheese samples to extend their shelf life and maintain their microbial qualities for a longer period.

Materials and methods of work:

Manufacture of soft white cheese:

Soft cheese manufacturing method: The cheese was prepared according to the Fox et al. (2017) method, where fresh cow's milk collected from one of the milk processors in Salah al-Din Governorate was pasteurized at a temperature of 63 ° C for 30 minutes and then cooled directly to a temperature of 37 ° C and microbial rennet (resonance enzyme) was added by (1 g / 25 liters of milk) according to the company's instructionsMeito Sangyo CO. LTD and calcium chloride was added by 0.02% and left for 45 minutes until the cheese was obtained. The required curd was placed in a piece of sterile gauze for the purpose of getting rid of the largest amount of whey and obtaining the pieces of caseins and the curd was cut and salt was added by 2% of the weight of the curd and packing in special molds and was kept in the refrigerator at a temperature of (5±2) º C until chemical, microbial and sensory tests are conducted during the storage period of 21 days.

Water wormwood extract: The aqueous extract of wormwood was prepared based on the method described by (Handa, 2008) by dissolving 25 g of wormwood powder with 250 ml of distilled water, i.e. a ratio of (10:1) and then stir the mixture by the vibrator (Shaker) for half an hour and at a speed of 150 cycles / minute, and leave the mixture in the refrigerator for 24 hours for the purpose of soaking, and then filtered by several layers of gauze to get rid of the remains of the plant parts are not dissolved and then filtered again using Filter papers (Whatmann No.1) Then the filtrate was

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taken to be dried using a lyophilyzer to obtain the extract in the form of a lyophilized powder for the purpose of preparing the concentrations required in the study and keeping the lyophilized powder in a tight and sterile container in the refrigerator at a temperature of 4 °C until use (Gowda et al., 2004). The process was repeated sequentially in the same steps and conditions until a sufficient amount of extract was obtained.

Nanoparticles: Zinc oxide nanoparticles processed by the American company (Research Nanomaterials) were used with the following specifications with a purity of 99.9% and a special surface area of more than 30 m 2 / g, and the size of the particles less than 50 nm and density 0.3 g / cm 2, which has a lamellar appearance that was in the form of odorless white powder.

Gelatin Film Preparation: Membrane solutions were prepared according to the De (De-Carvalho & Grosso, 2004) with a weight of 10 g of gelatin powder, dissolved in 80 mL of distilled water and mixed all the ingredients using a hot plate magnetic stirrer at a temperature of 60 °C for 15 minutes and then added the glycerol. By 30% of the weight of dry gelatin, complete the volume to 100 ml of distilled water and adjust the pH to 7, then add the extract and nanoparticles. To get rid of air bubbles in the membrane solution, use a vacuum pump for 10 minutes, then store the solution in the refrigerator under conditions Darkened to prevent oxidative stress.

The Microbial Tests for the Soft Cheese: The total bacterial numbers and the number of yeast and molds in the soft cheese samples were estimated using the spreading method, according to what (Frank & Yousef, 2004). Harrigan & McCence, (1976) were followed to estimate the numbers of proteolytic and lipolytic bacteria.

Statistical Analysis: The results of the experiments were analyzed using the Liner Model General within the ready-made statistical program (SAS, 2012) to study effect of factors on the complete random design of the CRD. Duncan test performed to determine the significance of the differences between the averages of the factors affecting the traits studied at (0.05) level (Duncan, 1955).

Results and discussion:

1- Total number of bacteria:

Table (1) shows the total number of bacteria for all soft cheese samples that are not coated or coated with gelatin casings alone or loaded with ZnONPs and watery wormwood extract, which showed an increase in the total number of bacteria with the progress of the storage period and until the end of the period of 21 days. As its numbers and for all transactions T1, T2, T3, T4, T5 on the first day 7.47, 6.58, 5.19, 4.88, 4.08 FD/g respectively, and with the progress of the storage period, the gradual increase of the total bacterial numbers of all transactions occurred, as they reached the highest numbers at the time 21 days, as the results of the statistical analysis showed that these transactions have obtained significant differences in the rate of increase in bacterial numbers, as the T1 and T2 transactions recorded the highest value, which is 9.12 and 8.31 FD/g on Respectively, the T5 treatment recorded a lower value of 4.19 TM/g due to the synergistic role played by zinc oxide nanoparticles and aqueous wormwood extract in inhibiting the growth of microorganisms.

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Table (14-4) Total number of bacteria (WTM/g) for soft cheese samples coated with gelatin films stored for 21 days at 5±2 ºC.

Impact of transactions	Effect of storage periods				Average impact of transactions
	24hours	7 days	14 days	21days	
T1	0.01 ± 7.47 D	0.01 ± 7.81 C	0.01 ± 8.45 A	0.01 ± 9.12 B	0.11 ± 8.21 A
T2	0.01 ± 6.58 H	0.01 ± 6.93 G	0.01 ± 7.27 E	0.01 ± 8.31 F	0.08 ± 6.97 B
T3	0.01 ± 5.19 M	0.01 ± 5.46 K	0.01 ± 5.63 I	0.01 ± 5.01 O	0.07 ± 5.32 C
T4	0.01 ± 4.88 P	0.01 ± 5.10 N	0.01 ± 5.24 L	0.01 ± 5.53 J	0.07 ± 5.19 D
T5	0.01 ± 4.08 T	0.01 ± 4.42 Q	0.01 ± 4.32 R	0.01 ± 4.19 S	0.04 ± 4.25 E
Average effect of storage periods	0.33 ± 5.64 D	0.33 ± 5.94 C	0.40 ± 6.18 A	0.41 ± 6.43 B	

*Different capital letters within one column indicate significant differences (p≤ 0.05) between the effect of the coefficients

*Different capital letters within the same row indicate significant differences (p≤ 0.05) between the effect of storage periods

*Different lowercase letters vertically and horizontally indicate significant differences (p≤ 0.05) between the effect of interference

T1 : Control T2 : Gelatin and cheese T3 : Zinc and gelatin T4 : Artemisia and gelatin T5 : Artemisia and zinc gelatin.

The decrease in the total number of bacteria in the treatment of soft cheese coated with gelatin fortified with zinc oxide nanoparticles can also be due to the antimicrobial effect of these particles through the production of free radicals on the surface of bacteria, and then damage Lipopolysaccharide in the cell membrane and the release of metal ions from the surface of the nanoparticles that bind to the cell membrane, causing the death of microbial cells, in addition to the occurrence of indirect antimicrobial effects, as well as cellular destruction due to electrostatic interaction and direct penetration. of Zno nanoparticles in the bacterial cell and thus these particles have been shown to enhance the antimicrobial encapsulation activity and extend the shelf life of the packaged product (Rojas et al., 2019 .(Bajpai et al. (2008) also pointed to the role of plant extracts such as medicinal herbs in reducing or preventing the growth of microorganisms due to their content of phenolic compounds, which in turn act as antimicrobial agents due to their ability to alter the permeability of microbial membranes. These results are consistent with the findings of Ahmed (2020), who pointed out the role played by nanoparticles loaded on gelatin shells in reducing the total number of bacteria compared to uncoated samples. It also agreed with the findings of Muhamad et al. (2023), who pointed out in their study the effectiveness of zinc nanoparticles loaded on the casings produced from whey proteins in preserving soft cheese samples preserved by cryological. It also converged with Fadel (2022), as he showed in his study that samples with added nanoparticles had less growth compared to the control treatment, due to the role of this acid in its ability to tear the outer shell of the microbial cell.

2. Fat-decomposing bacteria: Table (2) shows the number of lipolysis bacteria for all samples of soft cheese that are not coated or coated with gelatin casings alone or loaded with ZnONPs and water-based wormwood extract, as we note from the table that there is no growth and for all transactions on its first day, where growth began on the seventh day of storage and this growth continued to increase gradually with the progress of the storage period, We note from the table that the coefficients T1 and T2 were the highest, as they recorded on the seventh day 3.24 and 2.47 FM/g, compared to the samples T3, T4 and T5, which recorded 1.78, 1.92 and 1.49 FTM/g, respectively, as the results of the statistical analysis showed that all the transactions had significant differences in the rate of increase in bacterial numbers, as the T2 coefficients were recorded. T3, T4, T5 at 21 days 4.10, 2.15, 2.04 and 1.37 TMW/g, respectively, compared to T1 at 4.16 TMW/g The reason for the presence of numbers of lipolysis bacteria was due to the high percentage of fat resulting from the low moisture content, as well as the changes in soft cheese samples during storage, while T5 was the lowest, due to the joint role played by ZnO nanoparticles with the aqueous extract of wormwood in inhibiting microbes or reducing their growth.

Table (2) Preparation of lipolysis bacteria (WTM/g) for soft cheese samples coated with gelatin films stored for 21 days at 5±2 ºC.

Impact of transactions	Effect of storage periods				Average impact of transactions
	hours 24	7 days	14 days	days 21	
T1	0.00 ± 0.00 O	0.01 ± 3.24 I	0.01 ± 3.89 C	0.01 ± 4.16 A	0.84 ± 2.82 A
T2	0.00 ± 0.00 O	0.01 ± 2.47 K	0.01 ± 3.62 D	0.01 ± 4.10 B	0.79 ± 2.54 B
T3	0.00 ± 0.00 O	0.02 ± 1.78 L	0.01 ± 2.06 G	0.40 ± 2.15 E	0.51 ± 1.49 C
T4	0.00 ± 0.00 O	0.01 ± 1.92 M	0.01 ± 2.18 H	0.01 ± 2.04 F	0.50 ± 1.53 D
T5	0.00 ± 0.00 O	0.01 ± 1.49 N	0.01 ± 1.64 K	0.01 ± 1.37 J	0.35 ± 1.12 E
Average effect of storage periods	0.00 ± 0.00 D	0.15 ± 2.18 C	0.34 ± 2.67 B	0.42 ± 2.76 A	

*Different capital letters within one column indicate significant differences (p≤ 0.05) between the effect of the coefficients

*Different capital letters within the same row indicate significant differences (p≤ 0.05) between the effect of storage periods

*Different lowercase letters vertically and horizontally indicate significant differences ($p \leq 0.05$) between the effect of interference

T1 : Control T2 : Gelatin and cheese T3 : Zinc and gelatin T4 : Artemisia and gelatin T5 : Artemisia and zinc gelatin.

These results are consistent with the findings of Shihab et al. (2021) in their study, in which they showed the role of nanoparticles and awsaj extract in preserving the brick through the role of nanoparticles in inhibiting the growth of lipolysis bacteria, and attributed this to the fact that these particles carry a positive charge, while microorganisms have a negative charge on their outer wall, which results in electromagnetic attraction between microorganisms and nanoparticles, causing oxidation (oxidation) and thus eliminating them or by increasing their permeability. The membrane, which leads to the entry of substances from the external environment, which results in osmosis pressure, the destruction of the cytoplasmic content, and then cell death, as well as the role of the gossip plant in reducing the number of lipolysis bacteria due to the content of this plant of active compounds that inhibit the growth of microorganisms. I also agreed with Ahmed's findings (2020 .(Which indicated the lack of growth of lipolysis bacteria in soft cheese samples coated with gelatinous shells and fortified with nanoparticles and nisin due to the role played by these compounds in inhibiting microbial growth compared to non-coated samples. It agreed with the findings of Muhamad et al. (2023) in their study, in which they showed the effectiveness of zinc nanoparticles loaded on the membranes of whey proteins in inhibiting bacteria and their ability to preserve the soft cheese preserved in them. These results also agreed with what Albertos et al. (2017) pointed to the role of edible gelatin membranes with the addition of alcoholic olive leaf extract as an antimicrobiotic and antioxidant used in food preservation, as it significantly reduced the growth of lipolytic bacteria and increased their inhibitory effectiveness by increasing their concentration.

3- Proteolytic bacteria: Table (3) shows the number of proteolytic bacteria for all samples of soft cheese that are not coated or coated with gelatin casings alone or loaded with ZnONPs and watery wormwood extract, as we note from the table that there is no growth and for all transactions on its first day, where growth began on the seventh day of storage and this growth continued to increase gradually with the progress of the storage period, We note from the table that the coefficients T1 and T2 were the highest, as they recorded on the seventh day 3.15 and 2.63 FD/g, compared to the samples T3, T4 and T5, which recorded 1.56, 1.90 and 1.40 FM/g, respectively, as the results of the statistical analysis showed that all the transactions had significant differences in the rate of increase in bacterial numbers, as the coefficients T2 were recorded. T3, T4, T5 at 21 days 4.03, 1.93, 2.38 and 1.72 FTM/g respectively compared to T1 which recorded 4.69 FM/g and was the highest among all transactions while T5 was the lowest 1.72 FM/g The reason for the decrease in the rate of increase in the number of proteolytic bacteria is the role played by ZnONPs and wormwood extract, which supported gelatinous shells, in addition to the ability of the barrier membranes, including oxygen permeability, and thus showed excellence in reducing the growth rate of microorganisms. It is worth noting that the high protein content in soft cheese in conjunction with the decline in moisture content in addition to the occurrence of chemical changes in its composition can be a reason for the presence of protein-degrading bacteria.

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Table (3) Preparation of Proteolytic Bacteria (WTM/g) of Soft Cheese Samples Coated with Gelatin Films Stored for 21 Days at 5±2 ºC.

Impact of transactions	Effect of storage periods				Average impact of transactions
	24hours	7 days	14 days	21days	
T1	0.00 ± 0.00 P	0.01 ± 3.15 I	0.01 ± 4.07 C	0.01 ± 4.69 A	0.99 ± 2.97 A
T2	0.00 ± 0.00 P	0.01 ± 2.63 K	0.01 ± 3.48 D	0.01 ± 4.03 B	0.79 ± 2.53 B
T3	0.00 ± 0.00 P	0.02 ± 1.56 L	0.01 ± 1.72 G	0.40 ± 1.93 E	0.51 ± 1.30 C
T4	0.00 ± 0.00 P	0.01 ± 1.90 M	0.01 ± 2.27 H	0.01 ± 2.38 F	0.50 ± 1.63 D
T5	0.00 ± 0.00 P	0.01 ± 1.40 O	0.01 ± 1.58 N	0.01 ± 1.72 J	0.31 ± 1.17 E
Average effect of storage periods	0.00 ± 0.00 D	0.14 ± 2.12 C	0.35 ± 2.62 B	0.57 ± 2.95 A	

*Different capital letters within one column indicate significant differences (p≤ 0.05) between the effect of the coefficients

*Different capital letters within the same row indicate significant differences (p≤ 0.05) between the effect of storage periods

*Different lowercase letters vertically and horizontally indicate significant differences (p≤ 0.05) between the effect of interference

T1 : Control T2 : Gelatin and cheese T3 : Zinc and gelatin T4 : Artemisia and gelatin T5 : Artemisia and zinc gelatin.

These results are consistent with what Bonilla and Sobra (2019) find, showing that proteolytic bacteria showed no growth in the first four days of storage and showed growth after the tenth day. These results also converged with what El-Sisi et al. (2015) pointed out that the growth rates of proteolytic bacteria reached 1.5-6.4 TCM/g during the specified storage period, and the reason for the decrease in the rates of increase in the growth of proteolytic bacteria is due to the role of nanoparticles loaded on gelatinous membranes, which proved their role in inhibiting the growth of microorganisms. It also agreed with the findings of Ahmed (2020), as he pointed out in his study the role of zinc and silver nanoparticles and nisin compound in reducing growth rates in soft cheese samples coated with gelatin shells and fortified with these materials through these materials acting as preservatives and inhibitors of microbial growth. I also agreed with what Muhamad et al. (2023) pointed out, as they showed the high effectiveness of whey protein shells fortified with zinc nanoparticles in preserving soft cheese samples preserved by cryophilization, thus improving their microbial qualities and extending their shelf life.

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4- Yeasts and molds: Table (4) shows the preparation of yeasts and molds for all samples of soft cheese that are not coated or coated with gelatin casings alone or loaded with ZnONPs and watery wormwood extract and this examination is an indicator to assess the quality of soft cheese and its suitability for consumption, as we note from the table that there was no growth on the first and seventh day and for all transactions, while the T1 and T2 treatments began to grow on day 14, as they recorded 1.40 and 1.28 FM/g, respectively. However, the other transactions did not show growth, but at the end of the storage period of 21 days, growth began in the transactions T3, T4 and T5, but at lower rates than other transactions, as they recorded 0.53, 0.68, 0.49 FM/g, respectively, and the reason for this is due to the role of nanoparticles through their effect on the cell wall, increasing its permeability and stopping the replication of genetic material, as well as the plant extract and its active compounds, which were carried on gelatin shells and thus Inhibition of the growth of yeasts and molds and the possibility of extending the shelf life of soft cheese .Also, the process of pasteurization of milk before the manufacturing process and following the correct methods in the manufacture of soft cheese has a positive role in the microbial qualities of cheese and thus the product is clean and healthy, and the packaging process played an important role in creating a new environment for its importance in retaining gases, especially oxygen, because of its great impact on the breathing process.

Table (17-4) Preparation of yeasts and molds (WTM/g) for soft cheese samples coated with gelatin films stored for 21 days at 5±2 ºC.

Impact of transactions	Effect of storage periods				Average impact of transactions
	24hours	7 days	14 days	21days	
T1	0.00 ± 0.00 H	0.00 ± 0.00 H	0.01 ± 1.40 C	0.01 ± 2.53 A	0.32 ± 0.98 A
T2	0.00 ± 0.00 H	0.00 ± 0.00 H	0.01 ± 1.28 D	0.01 ± 1.62 B	0.22 ± 0.73 B
T3	0.00 ± 0.00 H	0.00 ± 0.00 H	0.00 ± 0.00 H	0.01 ± 0.53 F	0.07 ± 0.13 D
T4	0.00 ± 0.00 H	0.00 ± 0.00 H	0.00 ± 0.00 H	0.01 ± 0.68 E	0.08 ± 0.17 C
T5	0.00 ± 0.00 H	0.00 ± 0.00 H	0.00 ± 0.00 H	0.01 ± 0.49 G	0.06 ± 0.12 E
Average effect of storage periods	0.00 ± 0.00 C	0.00 ± 0.00 C	0.18 ± 0.54 B	0.21 ± 1.23 A	

*Different capital letters within one column indicate significant differences (p≤ 0.05) between the effect of the coefficients

*Different capital letters within the same row indicate significant differences (p≤ 0.05) between the effect of storage periods

*Different lowercase letters vertically and horizontally indicate significant differences (p≤ 0.05) between the effect of interference

T1 : Control T2 : Gelatin and cheese T3 : Zinc and gelatin T4 : Artemisia and gelatin T5 : Artemisia and zinc gelatin.

These findings are consistent with the findings of Muhamad et al. (2023), who showed in their study the role played by zinc nanoparticles added to whey protein shells used to preserve soft cheese samples and their effectiveness in inhibiting the growth of yeasts and molds during the specified storage period. It also agreed with the findings of Shihab et al. (2021), which showed the role of nanoparticles and awsaj extract loaded on gelatin packaging in preserving dairy products from contamination with yeasts and molds during the specified storage period. Gray et al. (2012) also pointed to the role played by nanoparticles in inhibiting the growth of yeasts and molds by affecting the cell wall and increasing its permeability, thus inhibiting respiratory enzymes, stopping the replication of genetic material, disrupting normal cell functions and thus cell death.

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