**Journal of Food and Dairy Sciences**

**Journal homepage & Available online at: www.jfds.journals.ekb.eg**

# Effect of Chia Seeds Nanoparticles as Natural Antimicrobial in Preserving Chicken Burger during Frozen Storage at -18°C for 6 months

**Amany Abd El-Halim[1*]; N. Hafez[1]; Kh. Khalil[1]; Rehab Mahmoud[2] and Samah Abd El- Tawab[1]**

Cross Mark

[1] Department of Food Science and Technology, Faculty. of Agriculture, Fayoum University.
[2] Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef 62511, Egypt.


**ABSTRACT**

The increasing demand for natural and safe food products has prompted research into alternative preservation methods that extend shelf life without compromising food quality. This study explores the use of natural antimicrobials chia seeds nanoparticles (CSNP), to preserve chicken burger during six months of frozen storage at -18°C. The study evaluated the microbial stability of chicken burger treated with varying concentrations of chia seeds nanoparticles (0.5, 1 and 2 %) by determinate total bacterial count and the presence of specific pathogens, including *Staphylococcus aureus, coliform bacteria, yeast, and molds*, during the frozen storage period at -18°C. The results indicate that all treatments significantly reduced microbial counts compared to the control, the control showed the highest bacterial counts, emphasizing the importance of effective preservation methods. This study found that (CSNP) significantly control *Staphylococcus aureus* growth in chicken burger during frozen storage at -18°C. All concentrations of CSNP varying degrees of antibacterial activity yeast and mold growth, with effectiveness increasing with higher concentrations. All CSNP treatments, especially CSNP 2%, demonstrate the potential of CSNP treatments to not only inhibit but also eliminate coliform bacteria during the frozen storage period at -18°C. This study highlights the potential of using natural antimicrobial agents as chia seeds nanoparticles (CSNP) in meat preservation, reducing reliance on synthetic additives, and supporting public health through safer food products.

***Keywords:*** meat products, *Staphylococcus aureus, coliform bacteria,* Chicken burger, nanoparticle.

## INTRODUCTION

The demand for safe and high-quality food products has never been more pressing. In an era where foodborne illnesses remain a significant public health concern, ensuring the microbial safety of perishable food items, such as chicken burgers, is crucial (Centers for Disease Control and Prevention (CDC) 2018). Traditional preservation methods, including refrigeration, freezing, and chemical preservatives, have been widely used to extend the shelf life of food products by inhibiting the growth of spoilage and pathogenic microorganisms (Davidson *et al.,* 2013). However, these methods are not without drawbacks. Chemical preservatives, while effective, have raised health concerns among consumers due to their potential links to allergies and other adverse health effects (Song *et al.,* 2019). Moreover, the use of synthetic additives is increasingly viewed as undesirable in the context of clean-label products, prompting a shift towards natural alternatives (Asioli *et al.,* 2017). Natural antimicrobials are gaining popularity as a safer and more acceptable means of food preservation. Derived from plants, these substances can inhibit the growth of a wide range of microorganisms, thereby enhancing food safety and extending shelf life without the need for synthetic chemicals (Burt 2004 and Gyawali and Ibrahim 2014). Among the various natural antimicrobials explored, chia seed nanoparticles have shown promise due to their potent bioactive compounds. Chia seeds are rich in omega-3 fatty acids, fiber, and antioxidants, which contribute to their antimicrobial (Kulczyński *et al.,* 2019). The potential of nanotechnology to enhance the effectiveness of natural antimicrobials has attracted considerable attention in recent years. Nanoparticles can be engineered to increase the surface area and bioavailability of antimicrobial agents, thereby enhancing their interaction with microbial cells and improving their efficacy (Duncan 2011). Chia seed nanoparticles offer an innovative approach to food preservation; this approach could lead to more efficient preservation methods, ensuring both food safety and quality over extended storage periods (McClements 2012).

Chia flour can enhance the health benefits and consumer acceptance of low-fat chicken patties. A study using 2% chia flour showed improved properties, but future research is needed to determine changes under specific storage conditions Kesemen and Akköse (2024).

By comparing the microbial stability of chicken burgers treated with this chia seed nanoparticle, this study will contribute to the growing body of knowledge on natural food preservation methods. The results could pave the way for the development of new preservation technologies that align with consumer preferences for natural and safe food products, ultimately supporting public health.

## MATERIALS AND METHODS

### 1. Chia seeds

Chia seeds (***Salvia hispanica* L**.) were purchased from the local market at Fayoum city, Egypt and stored in vacuum-sealed bags at -18°C until used.

### 2. Chicken meat

Fresh chicken and Spices were purchased from the local market at Fayoum, city, Egypt.

* **Corresponding author.**
**E-mail address: aaa45@fayoum.edu.eg**
**DOI:10.21608/jfds.2024.331067.1176**

**3. Preparation of chia seeds nanoparticle (CSNP)**

Chia seed was obtained through a top-down synthesis approach. The chia seed was subjected to a size-reduction process using the ball milling technique. Ball milling is a widely used top-down nanoparticle Korni *et al.,* (2021). The commercial chia was inserted into a photon ball stainless steel milling vessel. The used balls were fabricated from porcelain and the ball/natural zeolite mass ratio was 10:1. Ball diameter ranged from 1.5 to 1.8 cm, and vessel diameter was 7.5 cm for 16 hs at Faculty of Graduate Studies, Beni Suef University.

**4. Preparation of chicken burger**

Chicken burgers were formulated as indicated in Table 1 in Department of Food Science and Technology, Faculty of Agriculture, Fayoum University. Chicken burgers were formulated as indicated in Table 1 according to Heikal et al. (2019). Treatments were: control sample (not addition Chia seed nanoparticle), Chia seeds nanoparticles (1) (CSNP 0.5%), Chia seeds nanoparticles (2) (CSNP 1%), Chia seeds nanoparticles (3) (CSNP 2%), were placed in foam dishes, sealed with polyethylene bags, and they were kept at (-18 ± 1°C) until analysis.

**Table 1. Composition of chicken burger formulas**

| Ingredients | (%) |
|---|---|
| Chicken breast meat | 78 |
| Soy bean flour | 10 |
| Fresh onion | 10 |
| Sodium chloride | 1 |
| Black pepper | 0.5 |
| All spices | 0.5 |

**5. Determination of the proximate chemical composition of chia seeds**

Proximate chemical compositions: moisture, protein, fat, and carbohydrates 100 – (Moisture + protein + fat + ash) of chia seed were carried out by the methods of (A.O.A.C 2012) in Department of Food Science and Technology, Faculty of Agriculture, Fayoum University.

**6. Determination Size of chia seeds nanoparticles**

The procedure of sample preparation for size was explained According to the work described by Sayed et al. (2022) by Malvern instruments (Zetasizer Ver. 7.03 MAL1121994) in Faculty of Graduate Studies, Beni Suef University.

**7. Microbiological Examination**

The sample is sliced with sterilized scissors and forceps and carefully combined by a sterilized spoon. The mechanical shaker was used to homogenize aseptically 10 ml of prepared samples to 90 ml of sterile 0.1 ml percent peptone water for one minute. 1.0 ml of the previous homogenate was applied to 9 ml of sterilized diluents. The samples prepared were subjected to the following inspections ISO (2003a) in Department of Food Science and Technology, Faculty of Agriculture, Fayoum University.

**Total viable bacteria count**

Total viable bacteria count was assessed according to ISO (2003a) and the counts were expressed as (CFU/g). Duplicate plates of Standard Plate Count Agar were inoculated with 0.1 ml of the previously prepared decimal dilutions, evenly spread onto the surface of the plates and incubated at 30 °C for 72 hours.

**Total count of *Staphylococcus aureus***

From the previously prepared decimal dilutions, 0.1 ml was transferred onto the dry surface of duplicate plates of Baird-Parker medium supplemented with egg yolk tellurite (OXOID 2010) and spread with sterile bent glass spreader until the surface of the medium appears dry. The plates were incubated at 37ºC for 24-48 hours. Plates which contain a maximum of 150 typical and/ or atypical colonies were choosing to calculate the Staphylococci count. Typical colonies (circular, smooth, convex, moist, grey to jet black, shiny greater than one mm in diameter with or without white margined surrounded by clear zone extending to the opaque medium) were counted and recorded.

**Yeast and molds counts**

From each of the previously prepared sterile dilutions, 1ml aliquots were delivered into duplicate sets of petri dishes, previously inoculated with 10ml of sterile Potato Dextrose agar medium after solidification, inoculated as well as control plates were incubated at an inverted position at 3 for 5 days (OXOID 2010).

***Coli form group* count**

From each of the previously prepared sterile dilutions, 1ml aliquots were delivered into duplicate sets of petri dishes, previously inoculated with 10ml of sterile MacConkey Agar After solidification, inoculated as well as control plates were incubated at an inverted position at 37°C for 48 hours (OXOID 2010).

## RESULTS AND DISCUSSION

**1. The Chemical Composition of Chia Seeds (*Salvia hispanica* L.)**

Table (2) showed that proximate chemical composition of chia seeds. The results illustrated that the moisture content of chia seed is the moisture content of chia seeds is 6.20%, indicating that they have a relatively low water content, which contributes to their long shelf life and makes them suitable for storage. The ash content of 3.38% reflects the minerals content in chia seeds. Ash represents the inorganic residue remaining after combustion, which includes essential minerals such as calcium, magnesium, and phosphorus.

Chia seeds contain 20.32% protein, making them a good source of plant-based protein. This high protein content is beneficial for vegetarians and vegans looking to increase their protein intake. With a lipid content of 29.32%, chia seeds are rich in healthy fats, particularly omega-3 fatty acids (alpha-linolenic acid). Chia seeds have a carbohydrate content of 40.78%. Most of these carbohydrates come from dietary fibers, which aid in digestion this result nearby Da Silva Marineli *et al.* (2014).

**Table 2. The Chemical composition of chia seeds *(Salvia hispanica* L.*)***

| Chemical Composition | % |
|---|---|
| Moisture | 6.20 |
| Ash | 3.38 |
| Protein | 20.32 |
| Lipids | 29.32 |
| Carbohydrates | 40.78 |

**2. Size of Chia seeds nanoparticles (CSNP) by Malvern instruments (Zetasizer Ver. 7.03 MAL1121994).**

After grinding chia seeds in (a ball milling) for 16 hours, we measured the size of these particles using a Malvern Instruments (Zetasizer Ver. 7.03 MAL1121994). the size of the samples was 378.8 nm. This size increases the surface area, which increases the effectiveness of these particles as antimicrobials and their use in preserving chicken burger.

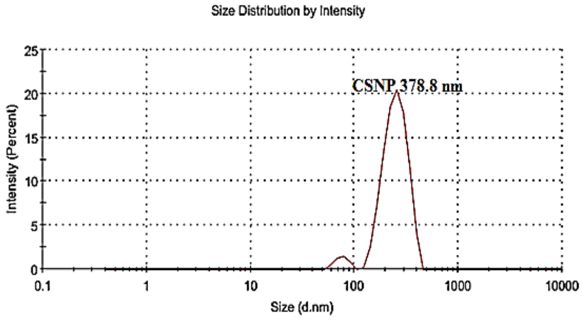


**Fig. 1. Size of Chia seeds nanoparticles by Malvern instruments (Zetasizer Ver. 7.03 MAL1121994)**

### 3. Effect of Chia seeds nanoparticles on the total bacterial count on chicken burger during frozen storage at -18 °C for 6 months

Data in Table (3) evaluated the effect of different concentrations of Chia Seeds Nanoparticles (CSNP) on the total bacterial count of chicken burgers during six months of frozen storage at -18 °C. The results indicate that the total bacterial count increased progressively over time in all treatments, as expected due to the extended storage period. However, the presence of CSNP significantly influenced the rate of bacterial growth. The control group, which did not contain any CSNP, showed the highest bacterial counts throughout the storage period. The bacterial count decreased from $8.5\times10^6$ to $1.8\times10^4$ because freezing is considered a way to preserve meat. In the CSNP1 treatment, the total bacterial count also decreased over time but at a lower rate compared to the control sample. The total count started at $8.4\times10^6$ and decreased to $7\times10^3$ on the end of the frozen storage period. The presence of 0.5% CSNP appears to slow down bacterial growth, suggesting a mild antimicrobial effect (Yemane *et al.*, 2023) they finding that the antimicrobial properties of Chia Seed Nanoparticle due to their bioactive compounds, including Chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol. The CSNP2 treatment, containing 1% CSNP, exhibited a slightly better reduction in bacterial growth compared to CSNP1. The bacterial count decreased from $8\times10^6$ to $6.8\times10^3$ during the six months the frozen storage period. The lower initial and final counts in this group compared to the CSNP1 group suggest that increasing the concentration of CSNP enhances their antimicrobial effect. This finding aligns with the dose-dependent antimicrobial activity observed in other natural preservative studies. The CSNP3 treatment, which had the highest concentration of CSNP (2%), demonstrated the most significant antimicrobial effect. The bacterial count has begun decreased from $8.3\times10^6$ to $6.3\times10^3$ after six months of storage. Despite the increase, this group consistently showed the lowest bacterial counts across all storage periods, indicating that a higher concentration of CSNP is more effective at inhibiting bacterial growth. This supports the hypothesis that CSNP can serve as a natural preservative, potentially extending the shelf life of chicken burgers during frozen storage. Comparing the results across the different treatments, it is evident that CSNP have a concentration-dependent antimicrobial effect on the total bacterial count in chicken burger. The 2% CSNP treatment (CSNP3) was the most effective, suggesting that higher concentrations of CSNP could be beneficial for enhancing the microbial safety of frozen chicken products. This results nearby (Zaki, 2018).

**Table 3. Effect of Chia seeds nanoparticles on the total bacterial count (CFU/g) on chicken burger during frozen storage at -18 °C for 6 months**

| Treatments | Total bacterial count (CFU/g) during frozen storage ( months) at -18 °C | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | $8.5\times10^6$ | $8.8\times10^5$ | $8\times10^5$ | $9.54\times10^4$ | $7.3\times10^4$ | $2.5\times10^4$ | $1.8\times10^4$ |
| CSNP1 | $8.4\times10^6$ | $7.6\times10^5$ | $6.6\times10^5$ | $8.9\times10^4$ | $6.8\times10^4$ | $9\times10^3$ | $7\times10^3$ |
| CSNP2 | $8\times10^6$ | $7.5\times10^5$ | $6.5\times10^5$ | $8.6\times10^4$ | $6.6\times10^4$ | $8\times10^3$ | $6.8\times10^3$ |
| CSNP3 | $8.3\times10^6$ | $7.1\times10^5$ | $6.9\times10^5$ | $8.4\times10^4$ | $6\times10^4$ | $7.9\times10^3$ | $6.3\times10^3$ |

**CSNP1: Chia seeds nanoparticles 0.5%** **CSNP2: Chia seeds nanoparticles1%** **CSNP3: Chia seeds nanoparticles 2%**

### 4. Effect of Chia Seeds Nanoparticles on *Staphylococcus aureus* (CFU/g) on chicken burgers during frozen storage at -18 °C for 6 months

Table (4) and Fig. (2) investigated that the effect of (CSNP) at various concentrations on the growth of *Staphylococcus aureus* in chicken burgers during a six-month frozen storage period at -18 °C. The results demonstrate that CSNP have a notable impact on controlling the proliferation of *Staphylococcus aureus*, suggesting their potential as a natural antimicrobial agent in food preservation. In the control group, where no CSNP were added, *Staphylococcus aureus* counts decreased steadily over the six-month storage period. The bacterial count began at 16 × 10 and, despite an initial fluctuation, showed a marked decline to zero on the sixth month of frozen storage. This reduction to zero could indicate that prolonged frozen storage alone might reduce *Staphylococcus aureus* viability, but only after extended periods.

All CSNP treatments CSNP1 (0.5), CSNP2 (1%) and CSNP3 (2%) demonstrated varying degrees of antibacterial activity against *Staphylococcus aureus*, with the effectiveness generally increasing with higher concentrations of CSNP. CSNP1 (0.5%): In this treatment, the *Staphylococcus aureus* count began at 14 × 10 at the start and decreased to zero during sixth month on frozen storage. The presence of 0.5% CSNP significantly slowed the bacterial growth compared to the control group, particularly evident in the first few months of storage. By the second month, the count was reduced to 7×10 and then continued to decline, ultimately reaching zero by the end of the study period. This result suggests that even at a lower concentration, CSNP can effectively inhibit *Staphylococcus aureus* over time. The bacterial count in the CSNP2 treatment followed a similar trend as CSNP1 but showed greater efficacy in reducing bacterial growth. Starting at 14 × 10, the bacterial count decreased more rapidly, reaching 9×10 by the first month and then declining to zero by the sixth month. The enhanced antimicrobial effect of the 1% CSNP treatment compared to the 0.5% CSNP treatment indicates a concentration dependent response, where increasing the CSNP concentration results in more effective bacterial inhibition. CSNP3 (2%) with the highest concentration showed the most substantial antimicrobial effect against *Staphylococcus aureus*. The bacterial count began at 12 × 10 and decreased more sharply than in the lower concentrations, reaching zero by the fourth month of frozen storage. This early elimination of *Staphylococcus aureus* suggests that higher concentrations of CSNP provide a more robust antimicrobial effect, effectively suppressing bacterial growth within a shorter period. The data indicates that CSNP have a significant inhibitory effect on *Staphylococcus aureus* in chicken burgers during frozen storage, with higher concentrations providing more rapid and pronounced effects. The reduction to zero bacterial counts on the end of the frozen storage period in all CSNP treatments, especially CSNP2 and CSNP3, demonstrates the potential of CSNP to not only inhibit but also eliminate *Staphylococcus aureus* over time. This is a crucial finding for food safety, as it suggests that CSNPs could be used to enhance the microbial safety of meat products during storage. Furthermore, the concentration-dependent response observed in this study implies that adjusting the CSNP concentration can tailor antimicrobial effectiveness to specific food preservation needs, providing flexibility in application based on the type of food and desired shelf life this results nearby León Madrazo and Segura Campos (2023).

**Table 4. Effect of Chia Seeds Nanoparticles on *Staphylococcus aureus* count (CFU/g) on chicken burger during frozen storage at -18 °C for 6 months**

| Treatments | *Staphylococcus aureus* count (CFU/g) during frozen storage ( months) at -18 °C | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | 16×10 | 13×10 | 11×10 | 12×10 | 9×10 | 9×10 | 0 |
| CSNP1 | 14×10 | 12×10 | 7×10 | 6×10 | 5×10 | 0 | 0 |
| CSNP2 | 14×10 | 9×10 | 6×10 | 8×10 | 8×10 | 0 | 0 |
| CSNP3 | 12×10 | 10×10 | 2×10 | 8×10 | 8×10 | 0 | 0 |

**CSNP1: Chia seeds nanoparticles 0.5%**
**CSNP2: Chia seeds nanoparticles1%**
**CSNP3: Chia seeds nanoparticles 2%**

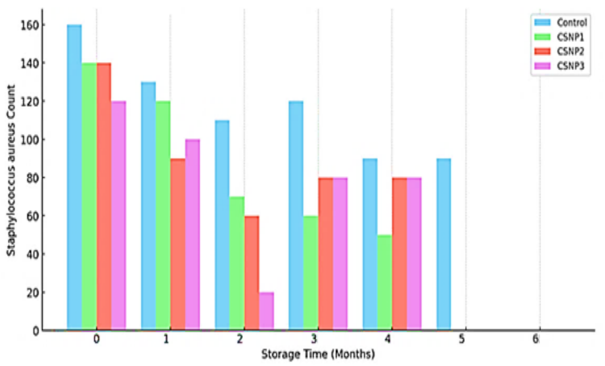


**Fig. 2. Effect of Chia Seeds Nanoparticles on *Staphylococcus aureus count* (CFU/g) in chicken burgers during frozen storage at -18 °C for 6 months**

## 5. Effect of Chia Seeds Nanoparticles on *coliform group* count (CFU/g) on chicken burgers during frozen storage at -18 °C for 6 months

Data in Table (5) and Fig. (3) indicated that effect of Chia Seeds Nanoparticles (CSNP) at various concentrations on the growth of coliform bacteria in chicken burger during six months of frozen storage at -18 °C. Coliforms are commonly used as indicators of food hygiene and safety, and their presence can signal potential contamination and spoilage (Auwerter 2021).The results indicated that CSNP significantly inhibit the growth of coliform bacteria, depending on the concentration used. In the control group, where no CSNP were added, the coliform bacterial count showed a notable decrease over the six-month storage period. The bacterial count started at 12 × 10 at the beginning of storage and reduced to 7 × 10 by the first month. By the second month, it further decreased to 3 × 10 and eventually reached zero on the third month of storage. This progressive decline suggests that extended frozen storage alone can reduce coliform viability in chicken burgers, though it does not immediately eliminate them. All CSNP treatments demonstrated a significant reduction in coliform growth compared to the control, with the effectiveness generally increasing with higher concentrations of CSNPs. CSNP1 (0.5%): In this treatment, the initial coliform count was 10 × 10 at the start of frozen storage at -18 °C. By the first month, the count decreased to 4 × 10 and reached zero on the second month. The presence of 0.5% CSNPs significantly slowed the growth of coliforms compared to the control group, particularly evident in the early months of storage. This result suggests that even at a lower concentration, CSNP can effectively inhibit coliform growth over time. CSNP2 (1%): The bacterial count in the CSNP2 treatment followed a similar trend as CSNP1 but showed slightly less effectiveness in reducing bacterial growth. Starting at 12 × 10, the bacterial count decreased to 5 × 10 by the first month and reached zero by the second month, indicating that the 1% CSNP treatment is effective, although slightly less so than the 0.5% concentration in terms of the rate of reduction observed. This suggests that the 1% concentration does not significantly enhance the antimicrobial effect compared to 0.5% under the tested conditions. CSNP3 (2%) with the highest concentration showed the most substantial antimicrobial effect against coliforms. The bacterial count began at 12 × 10 and decreased more sharply than in the lower concentrations, dropping to 2 × 10 on the first month and reaching zero by the second month of storage. This rapid elimination of coliform bacteria suggests that higher concentrations of CSNP provide a more robust antimicrobial effect, effectively suppressing bacterial growth within a shorter period. The data indicate that Chia Seed Nanoparticles have a significant inhibitory effect on coliform growth in chicken burgers during frozen storage, with higher concentrations providing more rapid and pronounced effects. This finding supports the hypothesis that CSNP can serve as an effective natural antimicrobial agent in food preservation, particularly against coliform bacteria, which are important indicators of food safety. The reduction to zero bacterial counts by the second month in all CSNP treatments, especially CSNP3, demonstrates the potential of CSNP to not only inhibit but also eliminate coliform bacteria over time. This is a crucial finding for food safety, as it suggests that CSNP could be used to enhance the microbial safety of meat products during storage. Samples are in accordance with EOS (2005) that does, allow the presence of *coliform group* in chicken burger, and the limit ($10^2$ CUF/g)

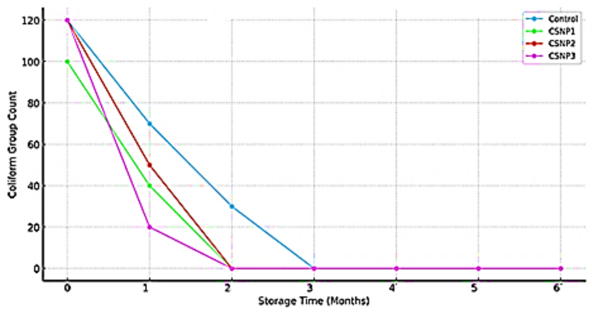


**Fig. 3. Effect of Chia Seeds Nanoparticles on coliform group growth (CFU/g) on chicken burgers during frozen storage at -18 °C for 6 months.**

**Table 5. Effect of Chia Seeds Nanoparticles on *coliform group* (CFU/g) growth on chicken burger during frozen storage at -18 °C for 6 months**

| Treatments | *coliform group* (CFU/g) frozen Storage time (months) at -18 °C | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | 12×10 | 7×10 | 3×10 | 0 | 0 | 0 | 0 |
| CSNP1 | 10×10 | 4×10 | 0 | 0 | 0 | 0 | 0 |
| CSNP2 | 12×10 | 5×10 | 0 | 0 | 0 | 0 | 0 |
| CSNP3 | 12×10 | 2×10 | 0 | 0 | 0 | 0 | 0 |

**CSNP1: Chia seeds nanoparticles 0.5%**
**CSNP2: Chia seeds nanoparticles 1%**
**CSNP3: Chia seeds nanoparticles 2%**

### 6. Effect of CSNPs in yeast and molds of chicken burgers during frozen storage at -18 °C for 6 months.

Data in Table (6) and Fig. (4) Evaluated the effect of Chia Seeds Nanoparticles (CSNPs) at different concentrations on the growth of yeasts and molds in chicken burger during six months of frozen storage at -18 °C. Yeasts and molds are significant microorganisms in food spoilage, and their presence in meat products can impact both safety and quality. The results indicated that CSNP have a notable inhibitory effect on the growth of yeasts and molds, with efficacy increasing with higher concentrations. In the control group, where no CSNP were added, the growth of yeasts and molds showed a fluctuating pattern over the six-month storage period at -18 °C. The initial count of yeasts and molds was $6 \times 10^2$, which increased to $13 \times 10^2$ by the first month. The count then decreased to 34 × 10 by the third month and further reduced to 15 × 10 by the fourth month, before reaching zero by the fifth month of storage at -18 °C. This reduction to zero on the fifth month suggests that prolonged frozen storage can eventually inhibit the growth of yeasts and molds, though initial fluctuations indicate some resistance to freezing conditions. All CSNP treatments (CSNP1, CSNP2, and CSNP3) demonstrated a significant reduction in yeast and mold growth compared to the control, with the effectiveness generally increasing with higher concentrations of CSNP. CSNP1 (0.5%) treatment, the initial count of yeasts and molds was $4.6 \times 10^2$, which increased to $10 \times 10^2$ by the first month. By the third month, the count had further decreased to 25 × 10, and on the fourth month, it reduced to 10 × 10. Yeasts and molds were completely eliminated by the fifth month. These results suggest that 0.5% CSNP have a substantial inhibitory effect on the growth of yeasts and molds, reducing their counts more effectively than freezing alone and achieving zero growth on the fifth month. The CSNP2 treatment showed a slightly better reduction in yeast and mold growth compared to CSNP1. Starting from an initial count of $4.0 \times 10^2$, the count increased to $5 \times 10^2$ on the first month and further to 33 × 10 by the third month. By the fourth month, the count was 13 × 10, reaching zero on the fifth month. The 1% concentration of CSNP provided a quicker reduction in yeast and mold growth compared to 0.5%, demonstrating a dose-dependent response where increased concentrations of CSNPs enhance antimicrobial efficacy. The CSNP3 treatment exhibited the most substantial reduction in yeast and mold growth among all treatments. The initial count was $3.2 \times 10^2$, which increased to $4.5 \times 10^2$ on the first month and further reduced to 14 × 10 on the third month. By the fourth month, the count had decreased to 9 × 10, reaching zero on the fifth month of storage. The rapid elimination of yeasts and molds suggests that a higher concentration of CSNP provides the most robust antimicrobial effect, effectively suppressing microbial growth faster than the lower concentrations. The data indicate that Chia Seed Nanoparticles significantly inhibit the growth of yeasts and molds in chicken burgers during frozen storage, with higher concentrations providing more rapid and pronounced effects these results nearby Mohamed and Safaa (2019).

**Table 6. Effect of Chia Seeds Nanoparticles on yeast and molds (CFU/g) on chicken burgers during frozen storage at -18 °C for 6 months**

| Treatments | yeast and molds (CFU/g) frozen Storage time (months) at -18 °C | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | $6\times10^2$ | $13\times10^2$ | 34×10 | 15×10 | 0 | 0 | 0 |
| CSNP1 | $4.6\times10^2$ | $10\times10^2$ | 25×10 | 10×10 | 0 | 0 | 0 |
| CSNP2 | $4.0\times10^2$ | $5\times10^2$ | 33×10 | 13×10 | 0 | 0 | 0 |
| CSNP3 | $3.2\times10^2$ | $4.5\times10^2$ | 14×10 | 9×10 | 0 | 0 | 0 |

**CSNP1: Chia seeds nanoparticles 0.5%**
**CSNP2: Chia seeds nanoparticles١ %**
**CSNP3: Chia seeds nanoparticles ٢%**

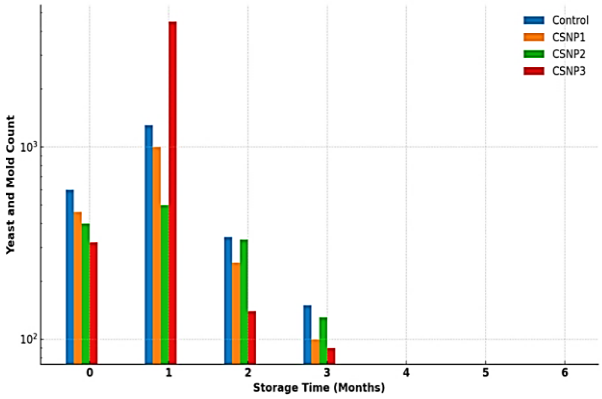


**Fig. 4. Effect of chia seed nanoparticle in yeast and molds (CFU/g) of chicken burgers during frozen storage at -18 °C for 6 months**

## CONCLUSION

This study explored the use of natural antimicrobial of chia seed nanoparticle (CSNP), for preserving chicken burgers during a six-month period of frozen storage at -18 °C. The results indicate that all treatments significantly inhibited microbial growth, including total bacteria, *Staphylococcus aureus*, coliform, yeast, and molds, compared to untreated samples control. Among the different treatments, CSNP 3 proved to be the most effective, achieving the lowest microbial counts by the end of the storage period. The study provides evidence those natural antimicrobials leveraging the benefits of nanotechnology to increase the surface area and bioavailability of active compounds. This enhanced effectiveness can improve food safety and quality, extending the shelf life of perishable products such as chicken burgers. Furthermore, the study suggests that using naturally derived antimicrobial agents can enhance food safety and promote clean-label products. This approach reduces the need for synthetic chemicals and provides effective microbial control, promoting a sustainable, health-conscious food system and reducing food waste.

## REFERENCES

AOAC (2012). Official Methods of Analysis of the Association of Analytical Chemists. 18th Ed., Washington, D.C., USA.

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., & Varela, P. (2017). Making sense of the 'clean label' trends: A review of consumer food choice behavior and discussion of industry implications. Food Research International, 99(1), 58-71.

Auwerter, J. P. (2021). The implications of switching from total coliform to Enterobacteriaceae as an indicator organism in a food manufacturing facility: a literature review.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods: A review. International Journal of Food Microbiology, 94(3), 223-253.

Centers for Disease Control and Prevention (CDC). (2018). Estimates of foodborne illness in the United States. CDC. https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

Da Silva Marineli, R., Moraes, É. A., Lenquiste, S. A., Godoy, A. T., Eberlin, M. N., & Maróstica Jr, M. R. (2014). Chemical characterization and antioxidant potential of Chilean chia seeds and oil (Salvia hispanica L.). LWT-Food Science and Technology, 59(2), 1304-1310

Davidson, P. M., Sofos, J. N., & Branen, A. L. (2013). Antimicrobials in food (3rd ed.). CRC Press.

Duncan, T. V. (2011). Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. Journal of Colloid and Interface Science, 363(1), 1-24.

EOS: Egyptian organization for specification and Quality control (2005). Standards for chicken burger. Ministy of Industry. Cairo Egyption EOS.

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. Food Control, 46, 412-429.

Heikal, Z., Youssef, M. K. E., Khalifa, A. H., Limam, S. A., & Mostafa, B. M. D. (2019). Improving the quality properties of chicken burger. *Journal of Food and Dairy Sciences*, *10*(6), 195-200.

ISO, International Standard Organization (2003a). ISO standard DIS 4833:2003(E). Microbiology of food and feeding stuff/Horizontal method for the enumeration of microorganisms/colony count technique at 30 oC.

Kulczyński, B., Kobus-Cisowska, J., Taczanowski, M., Kmiecik, D., & Gramza-Michałowska, A. (2019). The chemical composition and nutritional value of chia seeds—Current state of knowledge. Nutrients, 11(6), 1242.

Korni, F. M., El-Ela, F. I. A., Moawad, U. K., Mahmoud, R. K., & Gadelhak, Y. M. (2021). Prevention of Edwardsiellosis in Clarias gariepinus using ginger and its nanoparticles with a reference to histopathological alterations. Aquaculture, 539, 736603.

Kesemen, A. M., & Akköse, A. (2024). Evaluation of physicochemical, textural, and sensory properties of reduced-fat chicken patties produced with chia flour and κ-carrageenan. Italian Journal of Food Science/Rivista Italiana di Scienza degli Alimenti, 36(3).

León Madrazo, A., & Segura Campos, M. R. (2023). Antibacterial properties of peptides from chia (Salvia hispanica L.) applied to pork meat preservation. Journal of Food Science, 88(10), 4194-4217.

McClements, D. J. (2012). Nanoemulsions as potential delivery systems for bioactive components in functional foods. Annual Review of Food Science and Technology, 3, 345-371.

Mohamed, R. A., & Safaa, A. L. (2019). Effect of incorporation of chia seeds flour in chicken sausage on TBA values and microbial quality during cooling storage. Suez Canal University Journal of Food Sciences, 6(1), 97-103.

Oxoid, culture media, 2010.ingredients and other laboratory services.8th edition. Published by Oxoid LTD. London. Website http://www.oxoid.com

Sayed H., Mahmoud R., Mohamed H.F., Gaber Y., Shehata N., (2022), Co and Ni double substituted Zn-Fe layered double hydroxide as 2d nanoadsorbent for wastewater treatment. Key Engineering Materials 922, 193-213

Song, M., Huang, X., & He, X. (2019). Risk assessment and dietary exposure to synthetic food preservatives. Regulatory Toxicology and Pharmacology, 107, 104-111.

Yemane, H. G., Liudmilla, A. N., Tatyana, F. C., & Hifzur, R. (2023). Set.". Biological active substances based on chia seeds (Salvia Hspanica L.)." Polzunovskiy vestnik, 2, 136-146.

Zaki, E. F. (2018). Impact of adding chia seeds (Salvia hispanica) on the quality properties of camel burger "Camburger" during cold storage. International Journal of Current Microbiology and Applied Sciences, 7(3), 1356-1363.

## تأثير جسيمات بذور الشيا النانوية كمضاد طبيعي للميكروبات في حفظ برجرالدجاج اثناء التخزين المجمد علي -١٨م° لمدة سته اشهر

**أماني عبد الحليم[١]، نبيل حافظ[١]، خليل خليل[١]، رحاب محمود[٢] و سماح عبد التواب[١]**

[١] قسم علوم وتكنولوجيا الأغذية، كلية الزراعة، جامعة الفيوم.
[٢] قسم الكيمياء، كلية العلوم، جامعة بني سويف، بني سويف ٦٢٥١١، مصر.

### الملخص

يتزايد الطلب على المنتجات الغذائية الطبيعية والآمنة، مما حفّز البحث في أساليب الحفظ البديلة التي تُطيل مدة الصلاحية دون التأثير على جودة الطعام. اوضحت هذه الدراسة ان استخدام مضادات الميكروبات الطبيعية مثل جسيمات بذور الشيا النانوية (CSNP) في حفظ برجر الدجاج اثناء فترة التخزين المجمد لمدتة ستة أشهر. تم تقييم الاستقرار الميكروبي لبرجر الدجاج المعالج بتراكيز مختلفة من جسيمات بذور الشيا النانوية (٠,٥%، ١%، و٢%) من خلال تقدير إجمالي العد الكلي البكتيري ووجود بعض مسببات الأمراض المحددة، مثل بكتيريا *Staphylococcus aureus*، والبكتيريا القولونية، والخمائر، والفطريات، خلال فترة التخزين. أظهرت النتائج أن جميع المعاملات خفضت بشكل كبيرمن أعداد الميكروبات مقارنةً بالعينة الكنترول ، والتي سجلت أعلى معدل بكتيري، مما يؤكد أهمية استخدام أساليب حفظ فعالة. كما وُجد أن (CSNP) تتحكم بشكل ملحوظ في نمو بكتيريا *Staphylococcus aureus* في برجر الدجاج خلال فترة التخزين المجمد. وأظهرت جميع تركيزات CSNP درجات متفاوتة من النشاط المضاد للبكتيريا، ونمو الخمائر والفطريات، حيث تزايدت الفعالية مع زيادة التركيز. أثبتت جميع معالجاتCSNP ، وخصوصاً تركيز ٢%، قدرتها على تثبيط بل والقضاء علي البكتيريا القولونية مع مرور الوقت.الخلاصة اوضحت هذة الدراسة إمكانية استخدام المواد الطبيعية المضادة للميكروبات مثل جسيمات بذور الشيا النانوية (CSNP) في حفظ اللحوم، مما يقلل الاعتماد على الإضافات الاصطناعية ويدعم الصحة العامة من خلال منتجات غذائية أكثر أماناً.

***الكلمات الدالة:*** منتجات اللحوم، *Staphylococcus aureus*، البكتيريا القولونية، الخمائر، الفطريات، برجر الدجاج.