Effect of Cinnamon and Turmeric Nanoparticles Extract on microorganisms of Fresh Ground Beef During Cold Storage

Labeeb ahmed Al-Zubaidi, Amera Mohammed AL-Rubeii and Ahmed Sami Al-Salmany

1Department of Environment and Water, Ministry of Science and Technology, Iraq
2Department of animal production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
3Email: amera_alrubeii@coagri.uobaghdad.edu.iq

Abstract

This study aimed to evaluate the effect of cinnamon and turmeric nanoscale extracts on reducing the microbial load of ground beef stored under refrigerated temperature at 2 °C for 12 days. The study included six different treatments T1 positive control treatment (adding water), T2 negative control treatment (without addition) and T3, (Curcuma 248.3 ppm), T4 (Curcuma 496.9 ppm), T5 (Cinnamon 83.08 ppm) and T6 (Cinnamon 166.16 ppm). The treatments were kept with storage periods of 1, 4, 8 and 12 days, respectively. Some tests were performed to detect microorganisms. The results of the treatments recorded lower values for the total number of bacteria when compared with the two control treatments, and the treatment T6 recorded the lowest values. The results observed that there was a significant decrease (P< 0.05) in the numbers of cold-loving bacteria for the treatments to which the cinnamon and turmeric nanoparticles extract was added. Treatment T6 (cinnamon 166.16 ppm) recorded lowest number of cold-loving bacteria, reaching 4.47, 4.57 and 4.77, 5.22 bacterial units / gm. meat. The results of the added treatments showed a significant decrease (P <0.05) in the logarithm of the number of coliform bacteria compared to the positive control treatments (T1 and T2). In addition, T6 (cinnamon 166.16 ppm) reached lowest number of coliform bacteria and reached 3.69, 3.78, 3.87, 3.99 bacterial units / gm meat. This study concluded that the addition of cinnamon and turmeric nanoparticles extracts to fresh beef and stored by refrigerating for different storage periods. This antibacterial activity assured by a decrease in the logarithm of the total number of normal bacteria, cold-loving bacteria and coliform bacteria, compared with the control treatments for ground beef and cryogenic stock.

Keywords: Nanoparticles extracts, Cinnamon and turmeric nanoparticles, Microbial contamination, Ground beef.

1. Introduction

Meat and its products are characterized by their high nutritional value. They are a major source of the essential amino acids needed by the human body, as well as being a source of the B complex group of vitamins and some mineral elements such as iron [1]. Depending on the chemical and biological nature of the meat, They are susceptible to spoilage during storage as a result of fat oxidation and microbial growth, which are among the most influencing factors on food quality [2,3]. Microbial growth affects the incidence of food poisoning and economic losses as a result of spoiled meat [4]. Therefore, studies focused on the use of antioxidants in the meat industry, especially natural ones [5] , which are distinguished from industrial antioxidants as being safer to use and more acceptable by consumers and do not negatively affect human health [6]. The ever-increasing demand for meat products, increasing competition and health concerns have led the meat industry to adopt new and innovative methods. In general, the meat industry all over the world focuses on developing new production and manufacturing methods to meet consumer demand and thus the use of technologies such as nanotechnology can have a significant impact on the meat industry through its use in improving sensory receptivity and as an antimicrobial as well as the delivery of bioactive materials in a form Accurate to Target [7].

The diet naturally contains a variety of nano-sized elements that self-assemble and in a specific order to form structures of materials such as carbohydrates and fats. These materials differ from manufactured nanomaterials that can be used in nano-encapsulation, nanoeumulsion manufacturing and nanopolymers [8]. As nanotechnology refers to the new aspect of science, changing its physical and chemical properties and the main biological properties of materials, using new applications or increasing the benefit, and to maintain the pace of one from other industries. the meat industry has adopted this technology in many applications to improve the quality and safety of products such as improving the taste, flavor, texture, reducing the proportion of fat and salt in products, packaging techniques and improvement of the pathogen detection system [9].
2. Materials and Methods

This experiment was conducted in postgraduate laboratories at the College of Agricultural Engineering Sciences, University of Baghdad, and in the laboratories of the Ministry of Science and Technology. Local calf thigh meat was used, defatted, immediately after slaughtering. The meat was refrigerated at 2 °C for (12-10) hours, and it was cut with a sterile knife into small pieces with dimensions of 3-4 cm³ to facilitate the subsequent mincing process using sterile gloves. The meat was chopped using an electric grinding machine, and the pieces of meat were homogenized together to ensure that the components of the thigh muscles were evenly distributed, after that the weight of the meat was divided into six parts at a rate of 2.5 kg per part and each part was treated with the concentration of each substance according to the aforementioned ratios for each treatment. Each treatment was homogenized separately to obtain a homogeneous sample, and the experiment included six treatments according to the added concentrations of meat. The required checks were performed after the passage of 1, 4, 8 and 12 days respectively of refrigerated storage at a degree of 2 °C.

The number of colonies for TBC was calculated as stated in [10]. The cold-loving bacteria were calculated according to the method mentioned by [11]. And coliform bacteria according to the method of [12]. Turmeric nanoparticles (TAgNPs) and cinnamon nanoparticles (CAgNPs) were prepared using silver nitrate according to the method indicated by Krishnadhas [13] and Ojha [14] with some modifications. By taking a weight of 1 g of ethanolic extracts from cinnamon bay and turmeric, dissolved with 5 ml of solvent (THF), then added to 100 ml of silver nitrate mM AgNO3 (prepared with 0.169 g of AgNO3 solution to deionized water) separately at a flow rate of 0.2 ml / Accurate, with ultrasound power of 100 watts and a frequency of 42 kHz for 25 minutes, Then it was placed on a magnetic vibrator without heat for 20 minutes and then the samples were placed in a dark bottle and stored for 48 hours under dark conditions. Over a period of 48 hours the color of the solution was changed from yellowish orange to greenish yellow for TAgNPs and light brown to reddish brown for CAgNPs., This change in color indicates the formation of AgNPs nanoparticles. Then the reaction mixture was purified by centrifugation for 10 minutes at 10,000 rpm more than once to obtain a pure filtrate. An AFM was used to measure 3D visualization, qualitative and quantitative information, and many physical properties including the size, morphology, surface texture and roughness of nanoparticles [15]. SEM scanning electron microscopy was used to describe the size and morphology of the nanoparticles according to the method of [16].

3. Results and Discussion

The results of AFM analysis showed both the two and three dimensional views of CAgNPs and TAgNPs, respectively. They were spherical in shape, single or in aggregates, and the average size of particles were (42,32,28 nm) and (48,40,45 nm) for CAgNPs and TAgNPs, respectively (figures 1 and 2) [17]. These findings were agree with [18] that showed the biosynthesized silver nanoparticles were almost spherical, single (25–50 nm) or in aggregates (100nm).

3.1. Microbial tests for chilled ground beef

3.1.1. Total bacterial count

Table (1) shows the effect of the interaction between treatment and storage period on the total number of bacteria. Since the presence of a significant increase (P <0.05) in the total number of bacteria for the two control treatments as the storage period progressed, As the negative control treatment T1 recorded the highest values and were 6.82, 7.47, 9.10, and 9.89 bacterial units / g meat for storage periods 1, 4, 8 and 12 days, respectively. Whereas, the parameters to which the nanoscale extracts were added recorded lower values for the total number of bacteria when compared with the two control treatments. And treatment T6 recorded the lowest values, as the logarithm of the total number of bacteria was 5.10, 5.53, 5.69, 6.37 bacterial colony units / gm of meat for the same previous storage periods in a row, followed by treatment T4 (turmeric 496.9 ppm), where the logarithm of the total number of bacteria reached 46.5, 0.06, and 6.76 . 59.7 bacterial units / gm of meat for the same storage periods, respectively, compared with control treatment.It is worth noting that the levels of the total number of bacteria were within the acceptable level in the specifications of the quality control of frozen meat, which stipulates that the number of bacteria does not exceed 107 units forming a colony / gm of meat during meat storage. The content of spice and herbal extracts of phenolic compounds enables them to act as antimicrobial agents as a result of changing the permeability of microbial cell membranes [23]. Also, AgNPs nanoparticles have the ability to disrupt transport systems, including ion flow systems, which cause rapid accumulation of silver ions and thus inhibit cellular processes such as metabolism and respiration in target organisms [24]. In addition, AgNPs have the ability to penetrate the cell wall and affect the DNA and disrupt the ability to express ribosome proteins as well as some cellular proteins and enzymes necessary for the production of the energy complex ATP [25]. The change in the functions of cell membranes, such as the effect on electron transmission, food absorption, protein synthesis, enzymatic activity, and DNA synthesis, causes disturbances in the structure and functions of the cell, and this thus leads to its death [23]. [26] also indicated that the use of barley in ground beef stocked in refrigerator for 0,
3, 6 and 9 days led to a significant decrease (P < 0.01) in the logarithm of the total bacterial number compared to the control treatment.

Figure 1. (a) AFM image showed two dimentional of CAgNPs. (b) AFM image showed three dimentional of CAgNPs. (c) Showed column AFM digram of size range of CAgNPs.

Figure 2. (a) AFM image showed two dimentional of TNPs. (b) AFM image showed three dimentional of TNPs. (c) showed column AFM digram of size range of TNPs.
The results of SEM analysis showed that particles were spherical in shape, with nanometer in size for TAgNPs and CAgNPs (figures 3) [19]. Large nanoparticles were seen due to aggregation. This aggregation took place due to the presence of cell components on the surface of nanoparticles and acted as capping agent [20]. These results were agree with [21] SEM showed that determination formation of AgNPs, which were well dispersed and the aggregation of the particles were seen [22].

A: SEM image showed the shape and size of TAgNPs nanoparticles.  
B: SEM image showed the shape and size of CAgNPs nanoparticles.

Figure 3. SEM image.

Table 1. Effect of overlap between treatment and cold storage period in logarithm of total counts of normal bacteria and logarithms of total cryophilic count (colony forming unit / gm meat) ± standard error of chilled ground beef.

| Treatment       | Periods |
|-----------------|---------|
|                 | 1       | 4       | 8       | 12      |
| T1 positive control treatment (adding water) | 6.82±0.40 | 7.47±0.11 | 9.10±0.25 | 9.89±0.13 |
| T2 negative control (without addition) | 6.56±0.19 | 7.11±0.28 | 8.13±0.19 | 9.44±0.32 |
| T3 (turmeric 248.3 ppm) | 6.07±0.3 | 6.47±0.40 | 7.30±0.21 | 8.47±0.21 |
| T4 (curcuma 496.9 ppm) | 5.46±0.12 | 6.00±0.24 | 6.76±0.34 | 7.59±0.14 |
| T5 (cinnamon 83.08 ppm) | 5.90±0.24 | 6.29±0.20 | 6.64±0.12 | 7.95±0.20 |
| T6 (cinnamon 166.16 ppm) | 5.10±0.30 | 5.53±0.16 | 5.69±0.17 | 6.37±0.28 |

- The averages carrying different letters differ significantly between them at the level of (P <0.05).
- T1 positive control treatment (adding water), T2 negative control (without addition), T3 (turmeric 248.3 ppm), T4 (curcuma 496.9 ppm), T5 (cinnamon 83.08 ppm) and T6 (cinnamon 166.16 ppm).

As for the effect of the treatment on the logarithm of the total number of bacteria, it showed (Table 2) that there was a significant decrease (P<0.05) in the addition factors compared to the two control treatments, and its lowest value was in treatment T6 (166.16 ppm cinnamon), which recorded 5.67 bacterial units / gm of meat. Compared with the control treatments T1 and T2, which recorded the highest values, as they reached 8.32 and 7.81 bacterial units / gm meat for both treatments respectively, and this result is similar to what [27] found when he manufactured nanoscale extracts similar to the method of manufacturing the nanoscale extract in this experiment And using it as an antibacterial and antifungal, it had a greater effect on bacteria, especially the positive bacteria for the Gram stain .It is worth noting that the extracts in their nano state are more effective compared to the extracts of the same plant in its natural form [28]. These results are consistent with what [29] found when using nano extracts for both cinnamon and turmeric vegans manufactured in an environmentally friendly way, as cinnamon nan extract showed a greater effect against fungi compared to the nano extract of turmeric, and the reason was attributed to the role of effective plant compounds that are more quantitative and qualitatively in Cinnamon extract, which acts as a different anti-microbial.
Table 2. Main effect of treatment in bacterial tests ± standard error of cold-stored ground beef.

| The effect of treatment | Bacterial tests                      |
|-------------------------|--------------------------------------|
|                         | Logarithm of the total normal bacterial count | Logarithm of the total number of cryophilic bacteria | Logarithm of the total number of coliform bacteria |
| T1                      | 8.32±0.38 a                           | 7.16±0.41 a                                      | 4.81±0.26 a                                      |
| T2                      | 7.81±0.34 b                           | 6.81±0.37 b                                      | 4.73±0.25 b                                      |
| T3                      | 7.08±0.30 c                           | 6.12±0.26 c                                      | 4.37±0.16 c                                      |
| T4                      | 6.45±0.26 d                           | 5.46±0.20 d                                      | 4.06±0.11 b                                      |
| T5                      | 6.69±0.24 d                           | 5.64±0.19 d                                      | 4.12±0.11 b                                      |
| T6                      | 5.67±0.17 e                           | 4.76±0.13 e                                      | 3.83±0.10 e                                      |

Level of morale
- **significant at a significant level (p < 0.05).
- N.S was not significant at a significant level (p <0.05).

3.1.2. Psychrophilic bacteria count

Table (3) shows the effect of the interaction between the treatment and the storage period on the numbers of cold-loving bacteria, as a significant increase (P <0.05) was observed in favor of the positive control treatment (T1), which amounted to 5.72, 6.07, 7.79, 88.9 bacterial units / gm meat for periods Storage 1, 4, 8 and 12 days respectively. Negative control treatment (T2) was recorded as 5.56, 5.77, 7.43, and 8.89 bacterial units / gm meat, respectively, for the previously mentioned storage periods. Also, a significant decrease (0.05 P <) in the number of cold-loving bacteria was observed for the nanoparticles to which cinnamon and turmeric extract were added, and treatment T6 (cinnamon 166.16 ppm) recorded the lowest number of cold-loving bacteria, reaching 4.47, 4.57 and 4.77, 5.22 colony bacterial units / Gm of meat for all previous storage periods respectively. It was followed by T4 treatment (turmeric 496.9 ppm) and it reached 4.91, 4.14, 5.36, and 6.42 bacterial units / g meat for storage periods respectively. AgNPs have the ability to inactivate microbial enzymes and thus increase the production of reactive oxygen species (ROS) that are harmful to micro-cells [30]. Also, nanoparticles have the ability to inhibit and break down the cell walls of microorganisms and thus penetrate the cell and disrupt the functioning of its organelles [27]. As well as the synergistic role of the contents of plant extracts of biological compounds such as phenols that have the ability to inhibit the growth of microorganisms.

Table 3. Effect of interaction between treatment and cold storage period on logarithm of total cryophilic bacteria count (colony forming unit / gm meat) ± standard error of chilled ground beef.

| The studied adjective | Treatment | Storage period (day) |      |      |      |      |
|-----------------------|-----------|----------------------|------|------|------|------|
|                       |           | 1                    | 4    | 8    | 12   |      |
| Logarithm of total cryophilic count (ptc) (colony forming unit / gm meat) | T1 | 5.72±0.25 abcdefg | 6.07±0.11 abcdef | 7.79±0.13 bc | 8.89±0.40 a |      |
|                       | T2 | 5.56±0.19 abcdefg | 5.77±0.28 abcdef | 7.43±0.32 c | 4.02 8.45 |      |
|                       | T3 | 5.43±0.08 abcdefg | 5.42±0.40 abcdef | 6.29±0.21 edf | 7.36±0.31 c |      |
|                       | T4 | 4.91±0.34 abcdefg | 4.14±0.24 abcdef | 5.36±0.13 abcdef | 6.42±0.13 ed |      |
|                       | T5 | 5.08±0.12 abcdefg | 5.20±0.20 abcdef | 5.73±0.2 abcdef | 6.56±0.25 de |      |
|                       | T6 | 4.47±0.17 abcdefg | 4.57±0.16 abcdef | 4.77±0.2 abcdef | 5.22±0.3 abcdef |      |

- The averages carrying different letters differ significantly between them at the level of (P <0.05).
- T1 positive control treatment (adding water), T2 negative control (without addition), T3 (turmeric 248.3 ppm), T4 (curcuma 496.9 ppm), T5 (cinnamon 83.0 ppm) and T6 (cinnamon 166.16 ppm).

As for the effect of the treatment on the logarithm of the number of cold loving bacteria, Table (2) showed that there was a significant decrease (0.05 P <) in favor of treatment T6 (cinnamon 166.16 ppm), which recorded the lowest value if it amounted to 4.76 bacterial units / gm meat compared to the control treatments T1 and T2. Which recorded the highest values of 7.16 and 6.81 bacterial units / gm meat, respectively.
This result is similar to what [27], found when he manufactured nanoscale extracts similar to the method for making the nanoscale extract prepared in this experiment, and it had a significant effect on the positive bacteria for the Gram stain and an anti-fungal effect that was superior to conventional antifungals.

### 3.1.3. *Coliform bacteria*

Table (4) shows the effect of the interaction between treatment and storage period on the number of coliform bacteria. As a significant increase (P <0.05) was observed in the logarithm of coliform bacteria numbers in favor of the positive control treatments T1 and negative T2, which amounted to 4.00, 7.47, 4.85, 6.10 and 3.96, 4.25, 4.74, 96.5 units of bacterial colony / gm meat for both treatments respectively and for storage periods 1, 4, 8 and 12 days. While treatment T6 (cinnamon 166.16 ppm) recorded the lowest number of coliform bacteria and it was 3.69, 3.78, 3.87 and 3.99 bacterial units / g flesh for all storage periods respectively. The scientist, [31]. indicated that the nanomaterial manufactured from turmeric, which reached a size of 10 nanometers, had a clear effect by acting as an antimicrobial against coliform bacteria and some microorganisms, and this effect was greater than the effect of turmeric and cobalt separately. He also indicated that curcumin increases the binding of anti-microorganisms with these organisms, as well as a greater ability to penetrate the cell wall, and thus the possibility of introducing active substances into cells, especially metal ions toxic to microorganisms. The nanoscale extracts have an effective role in reducing microbial growth through the ability of AgNPs to disrupt transport systems, including ion flow systems, which causes the rapid accumulation of silver ions and inhibits cellular processes such as metabolism and respiration in target organisms [24]. Also, AgNPs were responsible for inactivating microbial enzymes, and thus they increase the production of reactive oxygen species (ROS) that are harmful to disease-causing micro-organisms [30].

Table 4. Effect of interaction between treatment and cold storage period on logarithm of total coliform count (colony forming unit / gm meat) ± standard error of chilled ground beef.

| Treatment | Storage period (day) |
|-----------|----------------------|
|           | 1                    | 4                    | 8                    | 12                   |
| T1        | 4.00±0.11<sup>ed</sup> | 7.47±0.40<sup>d</sup>  | 4.85±0.25<sup>eb</sup> | 6.10±0.13<sup>c</sup> |
| T2        | 3.96±0.28<sup>ed</sup> | 4.25±0.19<sup>e</sup>  | 4.74±0.19<sup>ebd</sup> | 5.96±0.32<sup>a</sup> |
| T3        | 3.90±0.40<sup>ed</sup> | 4.13±0.30<sup>c</sup>  | 4.47±0.08<sup>ebd</sup> | 4.97±0.21<sup>b</sup> |
| T4        | 3.83±0.24<sup>c</sup> | 3.95±0.12<sup>ed</sup> | 4.12±0.34<sup>c</sup>  | 4.33±0.14<sup>c</sup> |
| T5        | 3.81±0.20<sup>c</sup> | 3.97±0.24<sup>ed</sup> | 4.25±0.12<sup>c</sup>  | 4.47±0.20<sup>c</sup> |
| T6        | 3.69±0.16<sup>c</sup> | 3.78±0.30<sup>c</sup>  | 3.87±0.17<sup>c</sup>  | 3.99±0.28<sup>ed</sup> |

The averages carrying different letters differ significantly between them at the level of (P <0.05).

As for the effect of the treatment on the logarithm of the number of coliform bacteria, (Table 2) showed a significant superiority (0.05 P <) in the number of coliform bacteria in favor of the negative T1 and T2 positive control treatments, which reached 4.81 and 4.73 CFU / g meat for both treatments, respectively. While treatment T6 recorded the lowest values, and it was 3.83 bacterial units / g meat. The mechanism of action of nanoparticles in affecting microorganisms is their ability to inhibit and break down the cell walls of microorganisms and thus penetrate the cell and disrupt the functioning of its organelles [27].

### Conclusion

Through the results obtained from this study, we can conclude that adding cinnamon and turmeric extracts to fresh ground beef stored in cold storage contributed to reducing the logarithm of the total number of normal bacteria, cold-loving bacteria and coliform bacteria that cause spoilage and spoilage of meat.
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