Efficacy of Antimicrobial Moringa- Oil to Increase Shelf -Life of Table Eggs During Storage

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Abstract

The study aimed to evaluate the effectiveness of using different levels of moringa seed oil as a coating on specific qualities of table eggs stored at 20°C for 28 days, in addition to studying its antimicrobial growth properties. Five treatments(T1,T2,T3,T4,T5) were prepared with different ratios of oil to ethyl alcohol 70% (98-2, 96-4, 94-6, 92-8, 90-10) ml oil/ml ethyl alcohol, respectively), along with a control sample T0 (without addition). The results showed the effectiveness of moringa oil in maintaining the qualitative characteristics and prolonging the shelf life of table eggs during the storage period. A significant decrease ($P \leq 0.05$) was noted in egg samples with the control treatment T0 (without addition) compared to other treatments when studying the qualitative characteristics of eggs during the storage period. The control sample exhibited the highest percentage of weight loss, reaching 5.70%, and recorded the lowest values of the yolk index and Haugh unit, which were 0.255 and 56.99, respectively. Additionally, there was a rise in the pH of albumin, reaching 9.45 at the end of the storage period. Conversely, T5 treatment yielded the best results compared to the other treatments at the end of the storage period. Also, the findings showed the effectiveness of moringa seed oil against microbial contamination. It contributed to significant a reduction increased in the number of bacteria (total bacterial count, coliform ssp bacteria, fungi) present on the surface of the eggshells of all coated samples compared to the control sample (without addition) during the 28-day storage period, while no growth of Clostridium spp. bacteria was observed in any of the egg samples studied.

Keywords: Moringa oil, qualitative characteristics, microbial contamination, Haugh unit, yolk index, pH albumin.

Introduction

Eggs are considered to be food products with high nutritional value, as they contain proteins, essential and non-essential fatty acids, and many vitamins and minerals. They are extensively used in the field of food processing due to their unique characteristics such as emulsification, gelatinization, and the ability to be foam. The surface of the eggshell contains over 100,000 small openings called pores, which facilitate gas exchange (moisture and carbon dioxide) between the egg and its external environment [24]. This exchange causes changes in the viscosity of albumin, deterioration in yolk quality, and weight loss during storage, leading to significant economic losses. Furthermore, microorganisms such as Salmonella E.Coli bacteria, and other pathogenic microbes can penetrate through these pores, causing contamination [22]. Therefore, there is a need for techniques to reduce egg deterioration during storage by closing or reducing the permeability of these pores. Egg packaging techniques help reduce moisture and carbon dioxide loss and inhibit bacterial growth. [17[

Natural polymers such as complex carbohydrates and proteins, as well as waxes

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and oils, are widely used as edible packaging materials. [14] Research [4] has shown the effectiveness of egg packaging with chitosan in maintaining qualitative characteristics of eggs during storage compared to control samples (without packaging) when stored at 36°C for 36 days. [17] Other studies have examined the effects of packaging with various materials, such as oil, wax, paraffin, soy protein, and whey protein, on the physical, morphological, and chemical properties of fresh eggs, These studies indicated that these materials effectively preserved the physicochemical properties of the encapsulated eggs when stored at refrigerated temperatures compared to control samples (without packaging). Further research investigated the effect of encapsulating fresh eggs with soybean oil and butter on the qualitative characteristics during storage [15 .[Moringa seed oil contains multiple peptides that act as bacterial inhibitors against various bacterial types. [20] It is a powerful antioxidant with good moisture and gas retention properties. [2] Alcoholic moringa extract has been noted for its high ability to inhibit the growth of coliform bacteria and staphylococci. It also helps maintain the stability of the physical and chemical properties of beef stored for 10 days at 4°C [7]. In addition, research [18] has found that adding 5% moringa leaf extract reduces microbial content and fat oxidation when added to beef

Purpose of the Study

This study aims to evaluate the effectiveness of the alcoholic extract of Moringa seeds in extending the shelf-life of table eggs.

Material and Methods

2.1Oil Extract Preparation

The oil extract of Moringa seeds was prepared using the cold press extraction method. According to [9], mature and dry seeds with a diameter of 1 cm and a weight of 0.3-0.4 g were selected, The husks were removed, and the pulp was dried for one week at room temperature,Eight kilograms of seeds were placed in an extraction device, and hydraulic pressure was gradually applied. The extracted oil was collected in an opaque polyethylene container and left for three days at room temperature, away from light, to purify it from impurities such as proteins and mucous polymers through sedimentation by gravity. The oil was then filtered using cloths with 5-6 micron pores and transferred to a special glass container for refrigeration until use.

2.2Egg Wrapping

Fresh eggs of uniform size were purchased from local markets in the city of Kut (180 km south of Baghdad). The samples were randomly divided into five groups, with 60 eggs per group. A 70% ethyl alcohol solution was used to create the following dilutions:

- T1: 2 ml oil in 98 ml ethyl alcohol
- T2: 4 ml oil in 96 ml ethyl alcohol
- T3: 6 ml oil in 94 ml ethyl alcohol
- T4: 8 ml oil in 92 ml ethyl alcohol
- T5: 10 ml oil in 90 ml ethyl alcohol

In addition to the control treatment T0 (without packaging), samples were washed with tap water and dried with cotton wipes Spray the eggs with the extract so that it covers the eggs completely using a hand sprayer to ensure that the entire surface of the eggs was covered with the wrapping solution, then left to dry at 25°C for 20 minutes. Finally, the samples were stored at 20°C for 28 days, with readings taken every seven days,

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in triplicate, for each treatment following the methodology in [22.[

3.2Estimating the Percentage of Weight Loss During Storage

The percentage of weight loss for all treatments was estimated according to [18]. The eggs were weighed before storage (W1) for each treatment separately, and the weights of the same eggs were recorded at different storage periods (W2). The percentage of weight loss during storage was calculated using the following equation:

}Weight Loss} = {W1 - W2}/{W1} * 100

4.2[Measuring the Value of the Haugh Unit

The Haugh unit is a value that relates the weight of the egg to the height of the albumen and is calculated using the following equation, according to [18:[

) HU= 100 log(H-1.7×W0.37+7.6(

Here, (H) represents the height of the albumen (mm), and (W) is the weight of the whole egg. According to USDA (2000), eggs are classified into three groups: AA (Haugh values above 72), A (61 to 71), and B (below 60.(

5.2Calculating the Value of the Yolk Index

The yolk index is calculated as the ratio of the height of the yolk to its diameter. The height and diameter of the yolk are measured manually using the following equation, as stated in [17:[

Yolk index= yolk height/ yolk diameter

6.2Estimating the pH Value of Albumen

For measuring the pH value, 10 ml of both thick and thin albumen was taken and mixed well with a spoon, then placed in a 25 ml glass carafe. The pH value was measured using a pH meter, according to [5.[

7.2Coliform Bacteria Count and Total Bacterial Number

The number of coliform bacteria and total bacterial count was estimated according to [7] with some modifications. One gram of eggshells from treated eggs was taken, and 9 ml of sterile peptone water was added, mixed well, and then 1 ml of the mixture was used to create serial dilutions. One ml from each dilution was added to a petri dish using the pour plate method with Brilliance Agar media to estimate the number of coliform bacteria. These appear as pink colonies when incubated at 37°C for 24 hours.

For the total bacterial count, 1 ml from each dilution was poured into a petri dish with Plate Count Nutrient Agar and incubated at 37°C for 48 hours. Readings were taken using the following equation:

Bacteria number = colony number× 1 /dillution factor

8.2Enumeration of Clostridium spp. Bacteria

The total number of Clostridium bacteria was estimated according to the method used by [13], with slight modifications. One gram of eggshells was taken and sterile phosphate-buffered saline (PBS) was added at dilution rates of 1:100, 1:1000, and 1:10000. Then, 0.1 ml of each dilution was added to a culture medium consisting of sheep blood agar and Tryptose Sulfite-Cycloserine (TSC) agar with egg yolk. The samples were incubated under anaerobic conditions at 37°C for 24 hours, after which Clostridium bacteria appeared as black colonies. The bacteria were counted directly using the dilution equation, representing the total number of live bacteria per gram.

9.2Total Number of Fungi

The total number of fungi was estimated following the method described in [6]. One gram of eggshells was added to 9 ml of sterile peptone water (0.1%). Then, 1 ml of this suspension was added to 9 ml of peptone water. This process was repeated until

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reaching serial dilutions. From each dilution, 0.5 ml was placed in a petri dish containing Potato Dextrose Agar (PDA) and incubated at 25°C for five days. The number of colonies was determined using the Most Probable Number (MPN) method

10.2Statistical Analysis

The statistical program [21] was used for data analysis to study the effect of different treatments on the traits examined, based on a completely randomized design (CRD). The significant differences between averages were compared using the Least Significant Difference (LSD) test with a significance level of $P \leq 0.05$.

Results and Discussion

1.3Percentage of Weight Loss

Table 1.3 shows that all samples experienced weight loss during the storage period due to moisture loss through the pores on the eggshell surface, as indicated by [16]. There was a significant increase in the percentage of weight loss in the control samples (without packaging) compared to the other treatments, reaching 5.70% at the end of the storage period. This increased loss was attributed to the absence of a protective coating. Conversely, the Moringa oil coating played a crucial role in reducing moisture evaporation during storage. The T5 treatment samples showed the lowest percentage of weight loss, reaching only 3.95% at the end of the storage period. These findings are consistent with [22], which highlighted the role of oil coatings (such as rice, coconut, dates, and soybeans) in reducing weight loss compared to control samples without packaging when storing eggs at 20°C for five weeks.

Table 1.3 Effect of Addition of Oil Extract of Moringa Seeds on Percentage of Weight Loss

Tret	7 Day	14Day	21Day	28 Day
Cont	1.77	3.33	4.99	5.70
T1	0.97	2.99	4.35	4.88
T2	1.05	2.87	3.99	4.50
T3	1.11	2.90	3.70	4.29
T4	0.85	2.72	3.65	4.11
T5	0.87	2.74	3.50	3.95
LSD	0.299	0.268	0.393	0.331
* ($P \leq 0.05$).				

2.3Yolk Index

Table 2.3 illustrates the effect of encapsulation with Moringa oil on the yolk index. It is evident that all treatment samples experienced a decrease in the yolk index value as storage continued. This decrease can be attributed to the potential transfer of moisture from the albumen (high concentration) to the yolk (low concentration) due to the gradual breakdown of the membrane separating the two regions. This membrane acts as a barrier to moisture transfer, and its deterioration over time contributes to these changes, in line with the findings of [10.[

Furthermore, there was a significant decrease in the yolk index for the control samples (without packaging) compared to the other

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treatments by the end of the storage period, reaching a value of 0.255. In contrast, the T5 treatment displayed a non-significant increase compared to the other treatments, except for the control sample, and recorded a value of 0.343 by the end of the storage period. This improvement is likely due to the protective role of the oil membrane, which helps prevent yolk deterioration by limiting gas exchange between the egg interior and its external environment. These findings are consistent with [3], which reported that using whey protein to wrap eggs contributed to reducing yolk index deterioration and displayed significant differences from the control samples (without wrapping) during a six-week storage period at 25°C.

Table 2.3 Effect of Adding Oil Extract of Moringa Seeds on the Yolk Index

Tret	7 Day	14 Day	21 Day	28 Day
Cont	0.466	0.388	0.285	0.255
T1	0.467	0.433	0.374	0.316
T2	0.468	0.439	0.371	0.310
T3	0.466	0.443	0.383	0. 328
T4	0.468	0.439	0.381	0.336
T5	0.467	0.436	0.384	0.343
LSD	0.0236	0.0198	0.0251	0.0322
* (P≤0.05).				

3.3Haugh Unit

The results presented in Table 3.3 demonstrate that all samples experienced a decrease in Haugh unit values during the storage period. This decrease is attributed to the evaporation of carbon dioxide gas through the eggshell, leading to the dissolution of carbonic acid naturally present in the egg albumen, which plays a key role in maintaining albumen viscosity, as noted in [10]. The control treatment (without packaging) recorded a significant decrease in Haugh unit values compared to the other treatments, dropping from class AA at the beginning of storage to class B by the end, with a final value of 56.99 . This decline can be attributed to the absence of a protective coating, whereas the use of Moringa seed oil provided a hydrophobic barrier reducing moisture and carbon dioxide gas penetration. This aligns with findings by [18], which showed that using paraffin and carboxymethylcellulose membranes maintained egg quality during a four-week storage period at 20°C compared to uncoated samples.

Moreover, eggs treated with T5 exhibited significantly better results than other treatments, achieving a Haugh unit value of 73.56 by the end of the storage period. This is due to the high concentration of oil used in the T5 treatment (10ml/100ml ethyl alcohol), which effectively reduced moisture and carbon dioxide permeation and preserved egg quality, keeping them in class AA. This result is consistent with [1], which found that using 3% black seed oil yielded better outcomes

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compared to concentrations of 1% and 2% during a six-week storage period at 25°C.

Table 3.3 Effect of Adding Oil Extract of Moringa Seeds on Haugh Unit (HU(

Tret	day7	day14	day21	day28
Cont.	84.50	69.11	65.90	56.99
T1	85.21	72.70	69.50	62.64
T2	85.05	74.65	71.44	64.89
T3	85.88	74 .66	72.82	67.95
T4	86.89	74.33	73.75	69.58
T5	85.76	75.59	74.10	73.56
LSD	0.989	2.429	1.884	2.101
* (P≤0.05).				

4.3Albumin pH

Table 4.3 demonstrates that all treatments experienced an increase in albumin pH due to the evaporation of CO2 gas through the eggshell, leading to the gradual breakdown of carbonic acid naturally present in egg albumin and a subsequent reduction in acidity during storage. This observation is consistent with [3]. There was a significant increase in the pH of albumin in the control treatment (without packaging) compared to other treatments by the end of the storage period, reaching 9.45, which contributes to decreased albumin.

The effectiveness of Moringa oil in reducing CO2 evaporation played a crucial role in controlling pH increases during storage. This finding aligns with [14]which noted ability of oil from different sources to reduced in PH value of eggs during storage compared with control treatment, Conversely, the T5 treatment yielded the best results with a significant difference from other treatments, recording a pH of 8.99 at the end of the storage period viscosity.

Table 4.3 Effect of Adding Moringa Seed Oil Extract on Albumin pH

Tret	day7	day14	day21	day28
Cont.	8.55	9.18	9.33	9.45
T1	8.61	8.78	8.91	8.90
T2	8.55	8.90	8.97	8.96
T3	8.63	8.93	8.96	9.09
T4	8.68	8.89	8.94	9.15
T5	8.80	8.87	8.95	8.99
LSD	0.237	0.407	0.401	0.331
* (P≤0.05).				

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5.3Microbial Analysis

The results presented in Tables 5.3 and 6.3 show a significant increase in the numbers of total aerobic bacteria, coliform bacteria, and fungi for the control treatment (without packaging) compared to the other treatments, reaching (1.05 \times 10^5), (5.5 \times 10^4), and (4.0 \times 10^3), respectively, by the end of the storage period. This increase is attributed to the absence of the inhibitory effect of Moringa oil, which significantly contributed to reducing microbial growth during egg storage, consistent with findings reported by [2]. However, as noted in Table 7.3, we did not observe any growth of Clostridium bacteria across all treatments, likely due to their absence in the eggs used in the experiment.

Table 5.3 Effect of Adding Moringa Seed Oil Extract on the Total Number of Aerobic Bacteria log (CFU(

Tret	Day 7	Day14	day21	day28
Cont.	6.54 ± 0.01^{a}	7.41 ± 0.08^{a}	8.82 ± 0.07^{a}	9.55 ± 0.07^{a}
T1	6.43 ± 0.04^{ab}	6.55 ± 0.08^{b}	6.76 ± 0.06^{b}	7.80 ± 0.04^{b}
T2	6.38 ± 0.05^{b}	6.45 ± 0.04^{b}	6.90 ± 0.05^{b}	7.55 ± 0.03^{bc}
T3	5.90 ± 0.06^{c}	6.35 ± 0.07^{bc}	6.70 ± 0.04^{c}	7.40 ± 0.03^{c}
T4	5.79 ± 0.07^{c}	6.33 ± 0.02^{c}	6.55 ± 0.03^{c}	7.215 ± 0.02^{c}
T5	5.35 ± 0.08^{d}	5.85 ± 0.02^{d}	6.35 ± 0.01^{d}	6.95 ± 0.02^{d}

Table ((3-6) Effect of Adding Moringa Seed Oil Extract on the Total Number of coliform bacteria Log (CFU(

Tret.	Day 7	Day14	day21	day28
Cont.	1.98 ± 0.04^{a}	3.15 ± 0.05^{a}	4.23 ± 0.04^{a}	6.79 ± 0.07^{a}
T1	1.66 ± 0.06^{b}	2.39 ± 0.06^{b}	3.93 ± 0.05^{b}	4.93 ± 0.08^{b}
T2	1.45 ± 0.06^{c}	2.17 ± 0.08^{c}	3.60 ± 0.03^{c}	4.16 ± 0.03^{c}
T3	-	2.10 ± 0.09^{cd}	3.35 ± 0.06^{cd}	4.15 ± 0.05^{c}
T4	-	$2.15\pm0.06cd^{c}$	3.09 ± 0.07^{d}	3.85 ± 0.03^{d}
T5	-	2.02 ± 0.07^{d}	3.55 ± 0.03^{e}	3.90 ± 0.04^{d}

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Table (7-3): - The effect of adding the extract to the moronka seeds on the total number of Clostridium bacteria Log (CFU(

Tret	Day7	Day14	Day21	Day28
Control	-	-	-	-
T1	-	-	-	-
T2	-	-	-	-
T3	-	-	-	-
T4	-	-	-	-
T5	-	-	-	-

Table (8-3): - The effect of adding the oil extract of moringa seeds on the total number of molds and yeasts Log (CFU(

Tret.	Day7	Day14	Day21	Day28
Control	1.77 ± 0.09 ^{a}	2.85 ± 0.03^{a}	3.80 ±0.02^{a}	4.96 ± 0.02^{a}
T1	1.64 ±0.05^{b}	2.60±0.04^{ab}	3.65 ±0.03^{b}	3.62 ±0.03^{b}
T2	1.59 ±0.04^{b}	2.40 ±0.02^{b}	3.64 ±0.04^{b}	3.46 ±0.04^{b}
T3	1.45 ±0.06^{c}	2.32 ±0.03^{b}	3.59±0.06^{bc}	3.25±0.03^{bc}
T4	-	2.15 ±0.01^{c}	3.40 ±0.04^{c}	3.16 ±0.01^{c}
T5	-	2.18±0.02^{c}	3.44 ±0.01^{c}	3.21 ±0.02^{c}

Conclusions

- Moringa seed oil extracted by cold pressing has protective properties against moisture and gas penetration, making it suitable as an edible wrapper.
- Moringa oil possesses inhibitory properties against certain bacterial pathogens and contributes effectively to controlling microbial contamination.
- Using Moringa oil as a wrapper for table eggs helps maintain their qualitative properties during storage

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