Coating chicken carcasses or meatballs with collagen protein fortified with lsoenzyme and cinnamon oil, and studying its effect on the bacterial content when stored by freezing

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Summary

This study was conducted in the Department of Animal Production/ Faculty of Agriculture/University of Basrah and for the period 5 months to prepare collagen proteins fortified with lysozyme protein and cinnamon oil in the laboratory in the packaging of broiler carcasses (chest) by immersion method and study their microbial qualities when stored by freezing. Collagen extracted took 60 breast pieces of broilers obtained from local markets in Basra and are fresh. If these pieces were divided into four coefficients for each transaction three repeaters and each repeater consists of 5 pieces of the chest, these pieces were immersed in the prepared collagen proteins and according to the following coefficients:

T0 - Treatment of control coated polyethylene bags only.

T1 - treatment of the second chest cut-off coated with collagen only.

T2 - Third Treatment Breast Cuts Coated with Collagen Enriched with cinnamon oil.

T3- The fourth treatment of chest cuttings coated with collagen fortified with the enzyme lysozaem.

The transactions were stored by freezing at a temperature of 18-C at (0, 15, 30, 45) days and microbial tests were studied the total count of bacteria, Psychrophilic bacteria, Proteolytic bacteria and Lipolyt bacteria and the results showed a decrease in the number of Psychrophilic bacteria and Lipolyt bacteria in the third and fourth treatment compared to the control treatment.

Keywords: Collagen, covering, cinnamon oil, lysozyme protein

Introduction

The packaging process is an important part of the food processing processes for the purpose of containing and facilitating the transportation and sale of food products comfortably and maintaining their qualitative characteristics, plastics represented 70 percent of the total packaging materials used in the packaging of food products and that most plastics are not biodegradable and derived from non-renewable materials and that the durability characteristic of them made them useful but their presence in the environment has become continuous and the difficulty of disposing of their waste, which is released annually and at the rate of Thousands of tons of major problems that threaten the environment due to pollution caused by it due to its not decomposition easily as well as some of these substances have a negative impact on human health (1).

Many modern trends have emerged in packaging systems, namely the use of effective backing and means a packaging system that has characteristics that go beyond the reservation functions of moisture, gases, dissolved substances and others by introducing effective ingredients or materials within the

packaging system and to maintain the quality of the product and increase its shelf life (3). It also includes the displacement or expulsion of oxygen or carbon dioxide scavenging oxygen and scavenging carbon dioxide and as a moisture control agent and packaging antimicrobial technologies (11). The recycling and reuse of waste in some industries has become a feature of progress in many countries of the world to preserve the environment, and collagen proteins, which are the result of the waste of poultry slaughterhouses, especially their legs, have received wide attention as they have been used as one of the components of functional food and are considered one of the modern trends in the field of product manufacturing, not their uses for the production of New polymeric materials that are edible and biodegradable for their great ability to react and form clots and gels. Interest in collagen proteins has increased due to their health and environmental benefits and their availability in large quantities as waste, in addition to their in cheapness, high nutritional value and suitability in packaging and protecting the product from damage (5). **The study aimed to:**

This study aims to use collagen protein fortified with cinnamon oil and lysozyme protein as natural antimicrobials to prolong the storage period and improve the quality characteristics of chicken carcasses when stored in freezing as economically inexpensive as well as protect the environment from pollution in case of accumulation as unused waste for collagen.

Materials and methods

This study was conducted in the Department of Animal Production/ Faculty of Agriculture / University of Basra and for the period from 19/12/2021 to 10/5/2022, which aims to prepare collagen proteins fortified with lysozyme protein and cinnamon oil in the laboratory and use them in different concentrations in the packaging of broiler carcasses by immersion method and study their microbial qualities when stored by freezing.

Extract collagen from chicken legs

Gelatin is extracted according to the method of (13) from the legs of the chicken after washing the legs well with water and then the legs are cut by a sharp knife after which the skin is removed from the bone and then boil the legs with water for an hour at a temperature of 80 ° C to get rid of the fat and then filter the extract from the water after which it is treated with sodium hydroxide at a concentration of 0.2 % for 60 A minute in the refrigerator and then filter and wash the rest well with water then soak the extract with acetic acid at a concentration of 0.05% for 18 hours , the acidic solution is disposed of and washed with water and add sodium hydroxide until the confirmation of pH 11 degree and put it in a water bath at a temperature of 90 ° C for 6 hours and then filter and put in Autoclave for an hour and then cool it the layer of fat is removed and then dried in the normal oven at a temperature 55 ° C.

Birds used in the experiment: I took 60 pieces of breast of broilers obtained from the local markets in Basra and are fresh. If these pieces were divided into four laboratories for each transaction three repeaters and each repeater consists of 5 pieces of chest, these pieces were immersed in the prepared collagen proteins and according to the following coefficients:

1 - Treatment of control coated polyethylene bags only.

2 - The second treatment of chest cuts coated with collagen only.

3 - Third treatment chest cuts Coated with Collagen Enriched with Cinnamon Oil.

4 - The fourth treatment of chest cuttings coated with collagen fortified with the enzyme lysozyme.

The transactions were stored by freezing at a temperature of -18- ° C at (0, 15,

30, 45) days and microbial tests were studied (Total count of bacteria, Psychrophilic bacteria, Proteolytic bacteria and Lipolyt bacteria).

Microbial tests

The microbial numbers were estimated by preparing the decimal dilution by taking 1 g of the studied samples and adding to 9 ml a sterile peptone water solution 0.1% and mixed well to prepare the first dilution -110 and from it the rest of the decimal dilution was prepared in sterile test tubes each containing 9 ml of dilution solution to obtain appropriate dilution from the samples up to 5-10 Then the method of pouring dishes was used Pour plate method for all microbial tests, as 1 ml of each dilution was transferred to empty and sterile petri dishes and the nutrient medium was added to a degree Temperature 45 °C and mix the model with the culture medium in the dishes well and quietly and then leave until hardening then put the dishes in the incubator upside down and at a temperature of 35° C for 24-48 hours and 7 ° C for 5 - 7 days for cold -loving bacteria (8) The number of microbes growing in the unit Colony forming unit (cfu/g) was expressed as a colony formation unit and multiplied the number of colonies × inverted dilution, where the nutrient agar was used for macro bacteria, and to isolate the degrading bacteria, For protein, I use Nutrient Agar with 10% sorting milk, and Nutrient Agar with 1%, Nutrient Agar with 1% Tween 80 was used for the purpose of isolating lipolytic bacteria, according to the method described in (4).

Statistical analysis

The statistical analysis of the results was carried out using the CRDC random design within the SPSS program and compared the results with the test of the lowest significant difference L.S.D. ($P\leq 0.05$) at a significant level p) according to (2).

Results and Discussion

Effect of encapsulation with fortified collagen proteins and duration of freezing storage on microbial tests:

1 - Total count of bacteria

The results of Table (1) showed the effect of the segmental encapsulation of chicken carcasses with fortified collagen proteins and their effect on the total count of bacteria stored by freezing for different storage periods, as the results at the period of storage of zero showed no significant differences in the preparation of the total bacteria between the transactions. At the storage period of only 15 days, the T0 transaction showed a significant increase in $P \leq 0.05$ in the preparation of the total bacteria on the T1 transaction, which showed an increase with the nuclei ($P\leq 0.05$) in the total number of bacteria on the coefficients T2, T3 where their values reached (51.39×10^3, 37.00×10^3, 23.33×10^3,34.35×10^3) respectively. Either at the storage period of 30 and 45 days, the T0 transaction showed a significant increase ($P\leq 0.05$) in the preparation of the total bacteria on the rest of the transactions, and the T1 transaction showed a significant increase on the transactions T2, T3 No significant differences were observed between the total number of bacteria between T2, T3 with values (300.46, 310.96) (290.00, 270.53) at a storage time of 30 and 45 days. It is noted from the transactions that the best treatment that caused a good inhibition of microbial growth at the end of the storage period was the treatment T2 and T3 and these results agreed with (6) and (14) as the films of the compound gelatin loaded with curcumin NE showed antimicrobial activity against both *Salmonella typhimurium* and *Escherichia coli*).

This may be due to the protein lysozyme, which works to break down the cell wall of bacteria, especially positively charged, and thus its presence inhibits the growth of bacteria and works less on negatively charged bacterial cells. When encapsulating with fortified collagen proteins that contain vital compounds in cinnamon such as Synemledisd which inhibits the growth of bacteria (16).

Table (1) Effect of Cutting Chest Packaging of Chicken Carcasses with Fortified Collagen Proteins and Their Effect on the Total Number of Bacteria Stored by Freezing for Different Storage Periods (Average ± Standard Error)

Storage Periods/Day				Transactions
45	**30**	**15**	**Zero**	
$45.60^{a} \times 10^{3} \pm 3.38$	$50.46^{a} \times 10^{3} \pm 10.10$	$51.39^{a} \times 10^{3} \pm 18.49$	$45.53 \times 10^{3} \pm 12.77$	**T0**
$35.33^{b} \times 10^{3} \pm 4.33$	$34.33^{b} \times 10^{3} \pm 3.64$	$37.00^{b} \times 10^{3} \pm 2.08$	$40.00 \times 10^{3} \pm 4.16$	**T1**
$29.00^{c} \times 10^{3} \pm 3.00$	$30.46^{c} \times 10^{3} \pm 2.19$	$32.33^{c} \times 10^{3} \pm 3.17$	$43.26 \times 10^{3} \pm 2.84$	**T2**
$27.53^{c} \times 10^{3} \pm 2.59$	$31.96^{c} \times 10^{3} \pm 1.68$	$34.35^{c} \times 10^{3} \pm 1.45$	$41.00 \times 10^{3} \pm 15.30$	**T3**
*	**	**	N.S	**Level of morale**

N.S: means no significant differences ** means there are significant differences

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between the coefficients at the level of 0.05 transactions (T0) control treatment (T1) Collagen protein encapsulation (T2) encapsulation with chamber oil fortified collagen protein (T3) encapsulation with collagen protein fortified with lysozyme protein.

2 – bacteria Psychrophilic

Results of Table (2) The effect of the packaging of the cut-breasted carcasses of chicken carcasses with fortified collagen proteins and their effect on cold-loving bacteria stored by freezing for different storage periods as the results showed at a storage period zero the treatment T0 significantly exceeds ($P \leq 0.05$) on all transactions in the preparation of Psychrophilic bacteria as it reached (31.00) while the treatment T1 showed a significant superiority ($P \leq 0.0\ 5$) in the preparation of Psychrophilic bacteria over the two treatments, T2, T3 with a number of (24.60). At the storage period of 15 days, the T0 treatment showed a significant increase in the preparation of cold-loving bacteria on the T1 treatment, which showed a significant increase in ($P \leq 0.05$) in the number of Psychrophilic bacteria on the T2 and T3 coefficients as their values reached, (29.66, 20.35, 15.56, 17.00) respectively. Either at a storage period of 30 and 45 days, the T0 treatment showed a significant increase $P \leq 0.05$ in the numbers of Psychrophilic bacteria on the rest of the transactions, and the T1 treatment showed a significant increase on the T3 treatment and no significant differences were observed between the numbers of cold-loving bacteria between T1, T2 their values (14.00, 13.35) at a storage duration of 30 days. It is noted from the transactions that the best treatment that caused a good inhibition of microbial growth at the end of the storage period was the use of encapsulation with collagen proteins fortified with lysozyme protein and cinnamon oil, and this may be due to the action of lysozyme protein, which dismantles the cell wall and water enters the inside of the cell, and this leads to the explosion and death of the cell. The presence of cinnamaldehyde in cinnamon also works to highly inhibit microbes, and can inhibit the growth of Listeria and Escherichia coli bacteria in food products, thus increasing their shelf life (7) and (16).

Table (2) Effect of Cutting Chest Packaging of Chicken Carcasses with Fortified Collagen Proteins and Their Effect on the Total Number of Psychrophilic Bacteria Frozen Stored for Different Storage Periods (Average ± Standard Error)

Storage Periods/Day				Transactions
45	**30**	**15**	**Zero**	
24.00^{a} ×10² ± 3.07	26. 70^{a} ×10² ±2.69	29.66^{a} ×10² ± 2.18	31.00^{a} ×10² ±3.11	**T0**
15.00^{b}×10² ± 1.81	14.00 ^{b} ×10² ± 3.05	20.35^{b} ×10² ± 2.28	24.60 ^{b} ×10² ± 2.66	**T1**
12.00^{c} ×10² ± 2.56	13.35^{b} ×10² ± 2.02	17.00^{c} ×10² ± 1.60	19.30^{c} ×10² ± 3.48	**T2**
14.60^{c} ×10² ± 2.40	11. 20^{c} ×10² ± 0.33	15.56^{c} ×10² ± 1.45	17. 46^{c} ×10² ± 1.20	**T3**
**	**	**	**	**Level of morale**

N.S: means no significant differences ** means significant differences between transactions at the level of ($P \geq 0.05$) Transactions (T0) Control Transaction (T1) Collagen Protein Encapsulation (T2) Collagen Protein Enrichment with Leaf Oil (T3) Encapsulation with Collagen Protein Fortified by lysozyme protein**.**

3 – Lipolytic bacteria

The results of Table (3) showed the effect of the categorical packaging of the repellent of chicken carcasses with fortified collagen proteins and their effect on the frosted fat decomposing bacteria stored by freezing for different storage periods as the results at the zero storage period showed no significant differences between the transactions. When the storage period was 15 days by freezing, the T0 transaction showed a significant superiority ($P \leq 0.05$) The T1 transaction, which in turn significantly outperformed ($P \leq 0.05$) over the two transactions T2, T3amounted to a value of (6.60, 4.25, 3.95, 3.91) respectively. As for the storage period of 30 days, the transaction T0 significantly exceeded ($P \leq 0.05$) over the transactions T1, T2, T3 ay with a value of (7.50, 3.51, 3.40, 3.36) respectively. While the duration of the storage is 45 days did not differ from the storage period of 30 days as the T0 transaction showed a forced superiority of ($P \leq 0.05$) over other transactions. It is noted from the results that the general Lat T3, T2 and T1are the lowest numbers of bacteria compared to the control treatment T0with the progress of storage periods by freezing because the protein lysozyme reduced the number of bacteria, and the results of the analysis of the antibacterial potential using cinnamon-fortified packaging, which in turn may enhance the shelf life of food. Therefore, the use of chamber oil and lysozyme protein is necessary to protect food products from damage as well as from biological damage to meat as a result of storage, and the results have shown the absence of inhibitory activity on microorganisms when using packaging only, and therefore cinnamon oil should be added as an antimicrobial and the higher the concentration the greater the inhibition rate (15, 9).

Table (3) Effect of Cutting Chest Packaging of Chicken Sacrifices with Fortified Collagen Proteins and Their Effect on the Preparation of Freeze-Stored Lipolytic bacteria for Different Storage Periods (Average ± Standard Error)

Storage Periods/Day				**Transactions**
45	**30**	**15**	**Zero**	
7.6.66^{a} ×10² ± 1.76	7.50^{a} ×10²± 13.01	6.60^{a} ×10² ± 9.53	6.00 ×10²± 8.58	**T0**
3.6.00^{b}×10² ± 7.00	3.51^{b}×10² ± 2.08	4.25 ^{b} ×10²± 5.20	5.50×10²± 10.47	**T1**
3.2.33^{b}×10² ± 7.12	3.40^{b}×10² ± 3.78	3.95 ^{b} ×10²± 10.33	5.16×10²± 9.24	**T2**
3.4.60^{b}×10² ± 4.91	3.36^{b} ×10² ± 1.20	3.91^{b} ×10²± 1.85	5.70 ×10²± 3.60	**T3**
**	**	**	N.S	**Level of morale**

N.S: means no significant differences ** means significant differences between transactions at the level of (P≥ 0.05) Transactions (T0) Control Transaction (T1) Collagen Protein Encapsulation (T2) Collagen Protein Enrichment with Leaf Oil (T3) Encapsulation with Collagen Protein Fortified by lysozyme protein.

4 –Proteolytic bacteria

The results of Table (4) showed the effect of the categorical encapsulation of the repellent of chicken carcasses with fortified collagen proteins and their effect on the protein decomposer stored by freezing for different storage periods, as the results at the zero storage period showed no significant differences between the transactions. When the storage period was 15 days by freezing, the T0 transaction showed a significant superiority ($P \leq 0.05$) on the T1 transaction which in turn significantly outperformed ($P \leq 0.05$) on the two transactions T2, T3amounted to a value of (43.50, 36.30, 29.20, 26.29) respectively. At the storage period of 30 and 45 days, the T0 transaction showed a significant superiority $P \leq 0.0\ 5$ over the T1 transaction, which in turn significantly outperformed ($P \leq 0.0\ 5$) over the two transactions T2, T3wasvalued at 45 days' storage time (55.13, 39.36, 33.66, 30.30) and (56.30, 45. 00, 34.60, 33.46) respectively. It is noted from the results that the general Lat T3, T2, T1is the lowest number of bacteria compared to the control treatment T0 because the packaging, lysozyme protein and cinnamon oil reduced the number of bacteria, as the results of the analysis of the antibacterial potential using packaging which in turn may enhance the shelf life of foods (10). Therefore, the use of chamber oil and lysozyme protein is necessary to protect food products from damage as well as from biological damage to meat as a result of storage, and the results have shown the presence of inhibitory activity on microorganisms when using packaging, especially packaging support, and therefore preferably Add cinnamon oil as an antimicrobial and the higher the concentration the greater the inhibition rate (15 , 9).

Table (4) Effect of Cutting Chest Packaging of Chicken Carcasses with Fortified Collagen Proteins and Their Effect on the Preparation of proteolytic bacteria Stored by Freezing for Different Storage Periods (Average ± Standard Error)

Storage Periods/Day				Transactions
45	**30**	**15**	**Zero**	
56.30^{a} ×10²± 3.39	55. 13^{a} ×10²± 8.23	43.50^{a} ×10²± 1.76	30.63 ×10²± 4.00	**T0**
45.00^{b}×10² ± 7.00	39.36^{b}×10²± 4.63	36.30^{b} ×10²±1.76	27. 43×10² ± 3.71	**T1**
34.60 ^{c} ×10²± 3.84	33.66 ^{c} ×10²± 4.63	29.20^{c} ×10² ± 3.08	27. 16×10²± 2.33	**T2**
33.46^{c} ×10² ± 0.88	30.30 ^{c} ×10²± 3.17	26.29^{c} ×10²± 2.51	26.00×10²± 3.11	**T3**
**	**	**	N.S	**Level of morale**

N.S: means no significant differences ** means significant differences between transactions at the level of (P≥0.05) Transactions (T0) Control Transaction (T1) Collagen Protein Encapsulation (T2) Collagen Protein Enrichment with Leaf Oil (T3) Collagen Protein Enrichment Ayem.

Conclusions

The results showed that the use of collagen packaging fortified with cinnamon oil and lysozyme protein reduced the total number of bacteria in frozen breast segmented meat and extended its storage life.

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