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Estimate the efficiency of Lactoferrin incorporated with Calcium alginate as packaging film in improving the quality of frozen minced meat

Masoud AS, Asmaa, E. Hassan; Ebtisam MA, Dalia,Y.Youssef

Reference Lab for Safety Analysis of Food of Animal Origin, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Giza, Egypt.

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ABSTRACT:

The rising demand for processed foods has an impact on food quality and safety. Maintaining high standards and increased safety can be achieved through proper packaging. Antimicrobial packaging considered the next generation of packaging characterized by its microbial inhibition and traditional barrier properties. The purpose of this study is to assess how frozen minced meat quality is affected by the incorporation of lactoferrin (LF) with calcium alginate packaging film. The experimental groups were designed as follow: Group (1) Control negative (minced meat packed with normal packing material without treatment.), group (2) Treated group (minced meat packed with calcium alginate film with lactoferrin incorporation). Chemical properties of examined samples showed that TVB-N of treated samples remains within the accepted limit (18.2+0.42) till the end of the experimental period (105 days) while for control samples; it was recoded 25.2 mg/100g exceeding the permissible limit (20 mg/100g) at the same period. Meaning, the samples remained sound till 90th day only. Meanwhile, TBA samples of both groups remained within the accepted limit till the 75th day of storage only. Moreover, LF incorporated with alginate film has a significant antimicrobial effect on Aerobic Mesophilic count, with a discernible decline in the APC percentage between the treatment and control groups. In addition, the sensory attributes of both groups are nearly the same value. Therefore, it can be said that packaging technologies could have important role in extending shelf-life of minced meat, reduce the risk from pathogens and finally improve the quality of frozen minced meat.

INTRODUCTION:

Numerous techniques for food preservation have been developed, increasing food safety and prolonging food shelf life. Food products can be preserved using a variety of techniques, such as freezing, heating, high pressure processing, irradiation, adding preservatives directly, using cold plasma, pulsed electric field processing, and so on. Other techniques involve incorporating natural additives into packaging materials, which can be just as effective as the well-known traditional meth-

Corresponding author: Masoud, A. S. Reference Lab for Safety Analysis of Food of Animal Origin, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt..
E-mail: dramasoud12@gmail.com
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ods of packaging food **(Tavman et al. 2019).**

An inventive method for preserving or extending the shelf life of food items while guaranteeing their integrity, safety, and quality is active packaging. Active packaging is defined as "packaging systems that interact with the food in such a way as to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food" **(European Commission, 2009)** in accordance with European regulation (EC) No 450/2009.

Alginates are a type of bio-based polymer that can be produced by microorganisms. They are obtained from the cell walls of brown algae, including *Laminaria digtata* and *Ascophyllum nodosum*, where they exist as the sodium, calcium, and magnesium salts of alginic acid. The most advantageous characteristic of alginate, a linear, anionic, water-soluble polysaccharide, is that it can react with polyvalent metal cations to generate strong gels or polymers with limited solubility **(Alboofetileh et al. 2014; Younes et al. 2017).**

Rather than being indirectly added to food, active substances like natural antibacterial agents have evolved in recent years to be added to active packaging materials. Because the activity of an active ingredient may be diminished or inhibited when added directly to food due to interactions between the active ingredient and food ingredients and/or during food processing, adding an active ingredient through active packaging may be more effective than adding it to the food directly **(Yildirim et al. 2018).**

Lactoferrin (LF), Due to its presence in milk and other external mucosa, lactoferrin (LF), a multifunctional protein, plays a crucial role in newborns and acts as a defense protein for innate immunity. In recent decades, there has been a rise in the use of cow's milk fat in cosmetics, various functional items, and newborn formula (**Franco et al. 2018**).

Lactoferrin's bactericidal action has been shown to suppress a wide variety of bacteria and parasites, including *Toxoplasma gondii* and *Eimeria stiedai sporozoites* **(Omata et al. 2001).** Certain DNA and RNA viruses can be bound by LF (**Yi et al. 1997 and Anderson et al. 2001**). Moreover, it has high bactericidal and bacteriostatic effect on enteroinvasive *E. coli* HB 101, *Listeria monocytogenes, Streptococcus pyogenes,* and *Staphylococcus aureus and shigella flexnerri* **(Orsi, 2004; Valenti and Antonini, 2005).** In addition to its bacterial activity, LF has antifungal effect on some *Candida* spp. especially *Candida albicans* **(Samaranayake et al. 2001)**

This perspective informs the current study's coverage of cutting-edge technologies that can be applied to incorporate lactoferrin into calcium alginate-infused active packaging film, thereby enhancing the quality of frozen minced meat.

MATERIAL and METHODS

2.1 Preparation of packaging film

A slightly modified version of **Wang et al. (2018)**'s approach was used to prepare a 2% (w/v) Na-alginate solution in distilled water, stirring until it entirely dissolved at 75 ^{0}C. After stirring the solutions for 30 minutes, 25% of the dry mass of Na-alginate was added as plasticizer, namely glycerol. A 0.5% concentration of lactoferrin (Jarrows formula, USA; Multiapex accompany) was added. The mixture was allowed to degas for the entire night at 4 ^{0}C. Petri dishes (d=90mm) were filled with homogeneous film-forming solution, which was then allowed to dry for six hours at room temperature. The film was peeled, then allowed to soak in a 2% (w/v) CaCl2 solution for 20 minutes to solidify. After being taken out of the CaCl2 solution, the obtained disks were twice rinsed with distilled water, magnetically agitated for 30 minutes, and then allowed to dry at room temperature. Lastly, before being utilized, it is UV sterilized.

2.2 Minced meat manufacture and experimental design:

The standards set by the Egyptian Organization for Standardization and Quality Control, **ES 1694 (2005),** were followed in the production of minced meat. The meat was blended by blender then divided into two groups: group (1) control group (minced meat packed in normal packing material without any treatment), group (2) treated group (minced meat packed by prepared calcium alginate film incorporated with lactoferrin film). Each group consist of 100 g for each storage period. All control and treated samples were stored at -18^{0} C required and examined every 15 days for sensory attributes, chemical and microbiological criteria until spoilage occurred.

2.3 Sensory evaluation:

Sensory attributes for raw (texture, appearance and odor) and cooked minced meat (taste) samples were examined according to the scheme adopted by **(ISO 16779:2015)** using the 5-point assessment score according to the following scheme: 5= very good, 4= good, 3 = accepted, 2= bad and 1= very bad.

2.4 Microbiological analysis:

Microbiological analysis was performed for minced meat samples, to investigate the mean value of Total Aerobic Mesophilic count, Staph. aureus count, detection of Salmonella spp. and detection of Shigella spp. Preparation of the test samples as well as the initial suspensions and the decimal dilutions were carried out according to **ISO (6887-1/2017)**.

Subsequent enumeration and detection were conducted with the following methods:

2.4.1 Total Aerobic Mesophilic (APC): ISO 4833-2-2013 Cor1:2014 Amd. 1:2022

Standard Plate Count Agar (PCA, Oxoid) incubated at 30°C for 72 hours.

The microbial reduction percentages were calculated according to the following formula: Microbial reduction percentage (%) = (control CFU − treated CFU)/control CFU) × 100. In addition, the logarithmic scale reduction factor (Log10) was calculated using the formula RF = Log10 (A) − Log10 (B), where A is the number of colonies from (control) and B is the number of colonies (treated).

2.4.2 Coagulase positive Staphylococci: (ISO 6888-1, 2021).

Using Baird Parker agar (Oxoid) incubated at 34-38°C for 24- 48 hours **2.4.3 Detection of Shigella spp. (ISO 21567:2004)**

Using Hektoen enteric agar. (HE, Oxoid) incubated at (37 ± 1) °C for between 20 h and 24 h.

2.4.4 Detection of Salmonella spp. (ISO 6579 -1:2017 (E)) Amd. 1:2020

Using Xylose Lysine Deoxycholate agar (XLD agar, Oxoid) incubated at 37 °C and examined after 24 hours

2.5 Chemical examination:

2.5.1 Total volatile basic nitrogen (TVB-N): (ES 63-9/ 2006)

Determination of TVB-N according to method described by the Egyptian standard method. TVB-N value was calculated as mg/100g sample.

2.5.2Thiobarbituric acid values: (ES 63-10/2006).

Determination of TBA was according to Egyptian standard method. TBA value was calculated as mg malonaldehyde (Mal)/Kg sample.

2.6 Statistical analysis:

To perform statistical comparisons, the independent t test was employed. Three iterations of the experiment were carried out. Data were logarithmically transformed for bacteriological count, and then the mixed technique from SPSS software (version 20, IBM CO) was used to analyze the data. Fisher's least significant difference test was used to separate the means, and significance was assessed at α = 0.05 (P<0.05), which revealed the presence of a significant difference between the means.

RESULTS

Table 1. Sensory characteristics (mean ± SD) of control and treated group of frozen minced meat samples during storage at-18˚C. (1-5 scores)

Day	Group	Texture	Odor	Taste	Appearance	overall
Zero	control	5.0±0.00	5.0±0.00	5.0^{A}±0.00	5.0^{A}±0.00	5.0^{A}±0.00
	Treated	5.0±0.00	5.0±0.00	4.2^{a}±0.29	4.2^{a}±0.29	4.2^{a}±0.29
15th	control	5.0±0.00	5.0±0.00	5.0^{A}±0.00	4.2±0.29	4.2±0.29
day	Treated	5.0±0.00	5.0±0.00	4.2^{a}±0.29	4.2±0.29	4.2±0.29
30th	control	5.0±0.00	4.0±0.00	4.0±0.00	4.2±0.29	4.0±0.00
day	Treated	5.0±0.29	4.2±0.29	4.0±0.50	4.2±0.29	4.2±0.29
45th	control	4.0±0.50	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.50
day	Treated	4.0±0.00	4.2±0.29	4.0±0.50	4.0±0.50	4.2±0.29
60th	control	4.0±0.50	3.2^{A}±0.29	4.0±00	4.0±0.50	3.2^{A}±0.29
day	Treated	4.0±0.00	4.0^{a}±0.50	4.0±0.50	4.0±0.50	4.0^{a}±0.50
75th	control	3.0±0.50	3.0^{A}±0.50	3.0±0.50	3.0±0.50	3.0^{A}±0.00
day	Treated	3.2±0.29	4.0^{a}±0.00	3.0±0.00	3.0±0.00	4.0^{a}±0.00
90th	control	2.7±0.29	3.0±0.00	3.0±0.50	3.0±0.50	3.0±0.50
day	Treated	3.0±0.00	3.2±0.288	3.0±0.00	3.0±0.00	3.2±0.29
105th	control			Spoiled		
day	Treated	3.0±0.00	3.0±0.00	3.0±0.00	3.0±0.00	3.0±0.00

There are significance differences ($P<0.05$) between means having the same capital and small letter in the same column in the same inspection time.

Table 2. Total Aerobic Mesophilic count (APC) mean $\log_{10}$ cfu/g± SD) of control and treated group samples.

Day	Control	Treated
zero day	$3.71±0.03^{A}$	$3.17±0.20^{a}$
15th day	$3.87±0.01^{A}$	$3.36±0.06^{a}$
30th day	$4.13±0.14^{A}$	$3.85±0.14^{a}$
45th day	$4.72±0.02^{A}$	$3.98±0.02^{a}$
60th day	$5.34±0.04^{A}$	$4.25±0.29^{a}$
75th day	$5.87±0.06^{A}$	$4.52±0.466^{a}$
90th day	$6.00±0.012^{A}$	$5.11±0.10^{a}$
105th day	spoiled	5.77±0.20

There are significances differences ($P<0.05$) between means having the same capital and small letters in the same raw

Table 3. Log_{10} reduction of APC between the control and treated group samples

Examined day	APC reduction	
	Log_{10}cfu/g reduction	Reduction percentage
zero day	0.54	14. 56%
15th day	0 .1	13.18%
30th day	0.28	6.78%
45th day	0.74	15.68%
60th day	1.09	20.41%
75th day	1.35	23.00%
90th day	0.89	14.83%

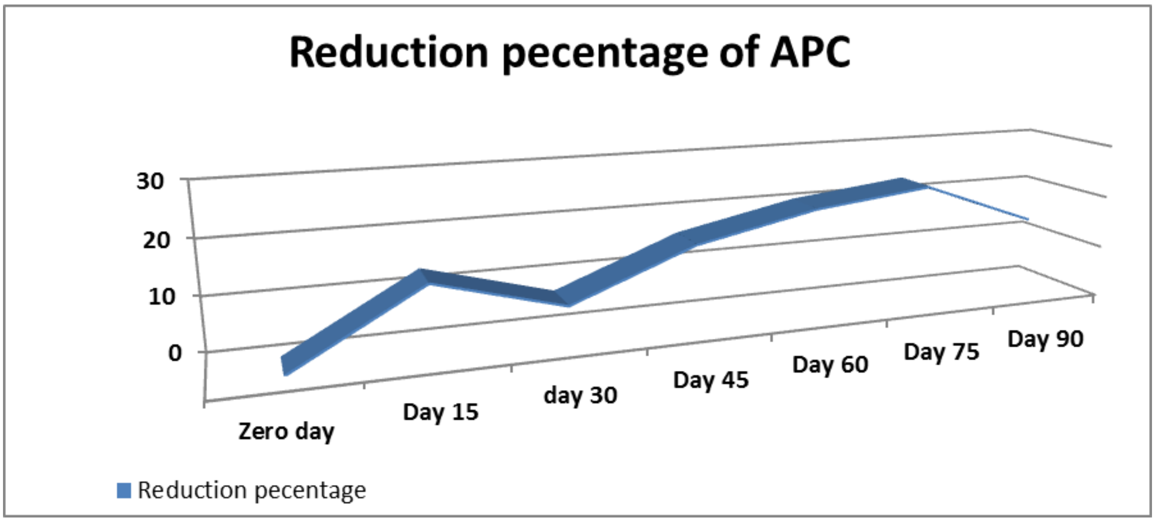

Fig 1. Log_{10} reduction of APC between the two group samples under test

Table 4. The mean values of total volatile basic nitrogen (mg/100g) of control and treated group samples.

Day/ treatment	Control	Treated
zero day	13.72±0.28	13.3± 0.14
15th day	14.0 ±0.00A	10.5 ± 0.04a
30th day	14.7±0.28A	11.2± 0.00a
45th day	16.8± 0.42A	14 .0± 0.00a
60th day	17.08±0.00A	14.7± 0.00a
75th day	18.9 ±0.28A	15.4 ±0.14a
90th day	19.6 ± 0.00A	16.8 ± 0.42a
105th day	25.2 ± 0.00A (Unaccepted)	18.2 ± 0.42a

There are significances differences (P<0.05) between means having the same capital and small letters in the same raw.

Table 5. The mean values of thiobarbituric acid content (mg/kg) of

day	Control	Treated
zero day	0.55± 0.08A	0.55± 0.04A
15th day	0.59±0.00A	0.59± 0.00A
30th day	0.62± 0.02A	0.55± 0.05a
45th day	0.70 ±0.03A	0.62± 0.02a
60th day	0.73± 0.00A	0.59± 0.09a
75th day	0.86± 0.00A	0.70± 0.00a
90th day	1.65± 0.05A Unaccepted	0.98± 0.08a Unaccepted
105th day	Unaccepted	1.01± 0.00

There are significances differences (P<0.05) between means having the same capital and small letters in the same raw.

DISCUSSION:

4.1. Sensory evaluation:

The results of the sensory scores (texture, odor, taste, appearance and overall acceptability) are shown in Table (1). For examined samples of both and control groups, the total organoleptic scores were high in the first week and then decreased gradually till the end of storage period. No significances differences (P>0.05) were observed between control and treated group from zero day till the 45th days of storage. While, the sensory scores of minced meats treated with lactoferrin was significantly higher (P < 0.05) than those of control one beginning from the day 60 of storage till the end of experimental periods. Such variants may be due to the significant difference between the treated group and control one in chemical and microbiological character till the end of storage period. **Tavassoli et al. (2016)** stated that alginate is a natural food grade polymer that can be used for the production of edible coatings and films used in packaging of food. In addition, **Wang et al. (2018)** concluded that alginates can be used to enhance the safety and quality of frozen chicken sausage without effect on their sensory characters.

Furthermore, **Montone et al. (2023)** showed that the hydroxyapatite/lactoferrin/quercetin (HA/LACTO-QUE) complexes loaded into active alginate were successful in preserving the color and flavor of fresh pork meat while maintaining overall acceptability until the end of the storage period.

4.2 Microbiological analysis:

Aerobic mesophilic count (APC) was recognized as an important parameter to evaluate the shelf-life stability. Therefore, the microbiological analysis of both control and treated groups were tested on days 0, 15, 30, 45, 60 and 90 and 105 days of storage at -18 °C for total aerobic mesophilic. While the microbiological analysis for Staphylococcus coagulase positive, detection of Salmonella spp. and, detection of Shigella spp. were performed only at day zero. as it is not detected for all samples during analysis. The result of APC shown in table (2) revealed that the mean log counts of control group started from 3.71±0.03 at the beginning of storage period (zero day) to 6.00 ±0.019 at the 90th day of storage while, treated group started from 3.17±0.2 at the beginning of storage period (zero day) and remained sound till the 105 day of storage (5.77±0.2) (end of storage period). There were significant differences (P < 0.05) between the groups incorporated with lactoferrin film and control one from starting of storage period till the end of the study. The results of control group were in accordance with the **Egyptian frozen minced meat standard 1694(2005**) until 90th day of storage while the results of lactoferrin film group was in accordance with ES until elapsing105 day of storage. Where, the APC was 10^6 (6 $\log_{10}$ cfu/g)) according to its basic requirements. These findings were almost identical to those of **Abad et al. (2021)** and **Montone et al. (2023**), who came to the conclusion that applying LF in edible and active films can be useful in preventing the growth of some pollutants and limiting the natural microbiota found in meat. Moreover, **(Orsi, 2004; Valentini and Antonini, 2005)**. High levels of bactericidal and bacteriostatic activity against invasive E. Coli HB 101, Listeria monocytogenes, Staphylococcus aureus, and Shigella flexneri may be demonstrated by LF.

Antimicrobial-equipped food packaging solutions can be used to manage spoiling microbes in addition to lowering pathogens **(Barros-Velazquez 2016)**. Without changing the food, itself, they can keep it away from harmful environments and stop microbes from growing **(Valencia- Chamorro et al., 2011).** Furthermore, antimicrobial packaging offers microbial suppression during storage and transit by allowing the integrated agent to release in regulated or prolonged ways (**Malhotra et al. 2015).**

Table (3) and Fig. (1) revealed that APC levels of reduction began to rise significantly starting from the 45th day of preservation (0.74 Log_{10}cfu/g, with a reduction percentage of 15.68%. Such reduction in APC was elevated more at 60th day recoding 1.09 Log_{10}cfu/g (20.41%), while the highest rate of APC reduction was 1.35 Log_{10} (23.00%). This could be due to the attachment of lactoferrin to the lipopolysaccharide of the bacterial cell wall and subsequently, prevent the pathogen from attaching to the host and eventually cause bacterial cell lysis. Lactoferrin has a well-established bacteriostatic impact on a variety of bacteria, including Gram-positive and gram-negative aerobes. Depriving the microbe of iron was the mechanism of action for its bacteriostatic activity. **Tara and Dupont (2019**) identified damage to the cell membrane as a second antibacterial activity. Contrarily, Lactoferricin (LF, 100 mg/g) and control ground beef samples did not significantly differ in APC, according to **Venkitanarayanan et al. (1999).**

4.3 Chemical analysis:

Total volatile basic nitrogen (TVB-N) is often used as a biomarker of protein and amine degradation, also as interpret of meat freshness **(Bekhit et al. 2021).** From the results showed in the table (4), the TVBN of the control group started from 13.72±0.28 mg/100g at the beginning of storage period (zero day) to 25.2 ± 0.0 which exceeded the limit at the end of storage period (105th day). On the other side, the TVB-N of LF group started from 13.3± 0.14 mg/100g at the beginning of storage period (zero day) to 18.2 ± 0.42 at the end of storage periods. There were significant differences ($P < 0.05$) in the TVBN between treated group incorporated LF and control one. The results of control group were in accordance with the **Egyptian minced meat standard 1694/2005** until 90th day of storage while, the results of LF film of treated group were in accordance with ES until 105th day of manufacture. Where, the TVB-N % was 20 mg/ 100g according to its basic requirements. These results were nearly similar to that concluded by **(Benito-Peña et al. 2016)** who stated that LF can enhance the physicochemical properties of food products. Moreover, **Hashem et al. (2022)** stated that sodium alginate improves the chemical quality of preserved chicken sausage.

Thiobarbituric acid (TBA) content through measuring the amount of Malonaldehyde (MDA) in food and food products, a major secondary by-product of lipid oxidation in a sample considered the common important quality index indicating fat oxidation, and it is the most widely used assay to quantify lipid oxidation products because of its simplicity and fastness, From the results showed in the table (5), the TBA of the control group started from 0.55± 0.08 at the beginning of storage period (zero day) to record 0.86 at 75 day which remains within the accepted limit (0.9 mg/Kg). The control group samples spoiled at the 90th day of storage recording (1.65± 0.05). In addition, the TBA value of the treated LF group started from 0.55± 0.04 at the beginning of storage period (zero day) and remained sound till the 75th day (0.70 ±00) and also spoiled at 90th day of storage (0.98± 0.08). Otherwise, the samples of both groups (control and treated) were spoiled at the same time (90 day of storage). There was significance difference ($P < 0.05$) in the TBA value between the group incorporated with LF packaging film and control one. These results were nearly similar to results concluded by **Hashem et al. 2022** who stated that sodium alginate improve the chemical quality of preserved chicken sausage and thiobarbituric acid (TBA) value were decreased significantly ($p<.0.05$) with different treatment levels but enhanced with the increase of days of intervals. In addition, **Abad et al. (2021)** and **Montone et al. (2023)** concluded that LF encapsulation improve the chemical quality of food products.

The study's findings indicate that lactoferrin packaging film is a useful tool for shielding food items from outside contamination. It can also stop chemical, physical, and biological changes, or deterioration, from occurring while the product is being stored or even being prepared.

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