Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

The effect of using edible coating fortified with lactoferrin on the shelf life of oriental sausage

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ARTICLE INFO

Keywords

Edible coating
Food Safety
Lactoferrin
Meat Products

Received 04/12/2023
Accepted 22/12 /2023
Available On-Line 31/12/2023

ABSTRACT

Meat and meat products are highly demanded with especial considerations of safety and health concerns; so, innovative preservation is essential in the acceptability of the meat products for the consumers. This study was conducted to evaluate the effect of carboxy-methyl-cellulose (CMC) fortified with lactoferrin (LF) (5%, 10% and 20%) edible coating in the sensory and microbial quality of home-made sausage. Lactoferrin was added directly on the minced meat; in addition, CMC-LF edible film was used as an external coating on the filled sausage samples. Samples were stored in refrigerator until examination. Results revealed significant enhancement in the overall acceptability of the treated samples with elongation in the shelf-life up to 12th, 15th and 18th days of storage in the treated samples with LF 5%, 10% and 20%, respectively; whereas, control untreated samples showed obvious signs of spoilage after the 6th day of storage. On the same line, microbiological count reductions were recorded revealing LF as a promising functional meat additive applied as an edible coating for improving sausage quality and shelf-life. Furthermore, the antimicrobial effect of LF appeared to be dose-time dependent, where higher concentrations gave more antimicrobial effects. So, usage of LF edible coating technique is recommended to be applied in the meat production for more safe meat products with longer shelf-life, as a low price and easily applied preservative technique.

1. INTRODUCTION

High biological value proteins and micronutrients found in red meat are all necessary for long-term healthy life. Therefore, a healthy, balanced diet is thought to include a reasonable amount of lean red meat (Giromini and Givens, 2022).
One of the most popular meat products is sausage, which is made from freshly minced meat, that may be furtherly processed using various recipes to produce specific sensory and storage qualities before being placed in a casing, including meat additives that are often used in sausages to enhance their quality, safety and shelf life (Dévi et al., 2020).
Because of ground high-fat content of oriental sausage, and oxygen-semi-permeable casing that promotes lipid oxidation, sausage is almost very perishable meat product. Numerous factors, such as the quality of the ingredients, the structure, content, processing, manufacturing conditions, and associated packaging system, affect the stability of food and extend its shelf life (Kumar et al., 2022).
Although, synthetic chemical preservatives have historically been widely used in food processing because of their inexpensive cost and strong antimicrobial action to prevent microbial contamination of meat products, a sizable portion of processed meat products are no longer in demand as consumer's preference of fresh, wholesome and nutrient-dense foods, and there is a growing global trend towards food free of chemical preservatives because of concerns about the potential negative health effects (Sridhar et al., 2021). Consequently, there is a greater need for natural substances as food preservatives due to the use of products made from plants, animals and microbes (Teshome et al., 2022). Microbial development can be inhibited and shelf life extended by natural preservatives (Yong et al., 2021). However, they can also have an impact on the food's acceptability as well as its flavor, taste, color, and texture. In order to counteract the growth of spoilage bacteria or food-borne illnesses, these natural preservatives may change cell metabolism, stop protein synthesis, and make microbial cell membranes more permeable (Baptista et al., 2020)
Many strategies have been studied to increase the utility of natural preservatives, such as combinations of multiple preservatives or food preservation methods including encapsulation and active packaging systems (Yu et al., 2021).
Although food oxidation and microbial growth can be controlled by cold storage, food packaging is thought to be an essential tool for maintaining the quality of meat products while they are being stored (Behbahani et al., 2021). One of the novel applications is active packaging owing to the migration of active compounds from edible coating to food, and sustains the safety and functionality of food (Majid et al., 2018). Edible coating economically decreases the cost of packaging by

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using less expensive packaging materials accompanied with overall enhancement of food quality (Nunes et al., 2023).

With its ability to be fortified with a variety of functional additives, the linear structure of cellulose in carboxymethyl cellulose sodium (CMC) produces a flexible, durable, transparent, and stable coating with numerous applications in the food industry (Gregorova et al., 2015). One of the innovative approaches to food preservation that improves quality, safety, and shelf life during storage is the incorporation of a functional substance, like lactoferrin, into an active edible coating (Lisitsyn et al., 2021).

The possible application of lactoferrin (LF), a major iron-glycoprotein present in milk from various mammals and having antibacterial properties against a range of Gram-positive and Gram-negative bacteria, fungi, and parasites, as a natural food preservative (Superti, 2020). It has a significant antibacterial effect, especially when it is iron-free; it binds to lipomannan, one of the bacterial cell wall components, or it binds to proteins on the surface of bacteria (Kell et al., 2020). LF also has antioxidant, antiviral, anti-inflammatory, immune-modulating, and anti-cancer properties; which have been confirmed by numerous studies that recorded the beneficial effects of LF on the intestinal epithelium through stimulation and developing of the intestinal epithelial cells, which optimizes the digestive processes and absorption of nutrients, and protects against the action of pathogens and food allergens (Weimer et al., 2020; El-Sherbeny and El-Shenawy, 2023).

Lactoferrin has antibacterial properties against both Gram-negative and Gram-positive bacteria, making it useful in the fight against foodborne pathogens through prevention of the biofilm formation by pathogenic bacteria such as Staphylococcus aureus or Pseudomonas aeruginosa, as well as direct inhibition or killing of microbial cells and immune system activation/inhibition (Jahani et al., 2015). Furthermore, because iron is bound by LF, its absence is associated with halting in the bacterial growth, which protects the body from infection (Zarzosa-Moreno et al., 2020).

Therefore, the current study explored the effect of using functional CMC edible coating fortified with lactoferrin on the sensory and bacteriological quality of oriental sausage during refrigeration storage.

2. MATERIAL AND METHODS

2.1. Materials

Raw fresh lean beef flank cut, fat, goat small intestine, salts and other seasoning ingredients were purchased from supermarkets located in Benha city, Qalubiya governorate. Carboxy methyl cellulose (CMC) and lactoferrin (LF) 20.000 IU/mg were obtained from Sigma-Aldrich, USA.

2.2. Preparation of experimental samples

Sausage was prepared according to Luvele (2023); where, 12 g of garlic powder, 35 g of NaCl salt, and 8 g ground pepper were added for each two kilograms of chopped meat (80% lean meat + 20% fat), mince, and mix well. Prepared mince was divided into four primary groups; where G1: control untreated group (minced meat only), G2: minced meat + 5% lactoferrin, G3: minced meat + 10% lactoferrin, and G4: minced meat + 20% lactoferrin. Followed by thorough mixing and stuffed in a pre-soaked natural casing in brine soln. (2%) and rinsed for eradication of excess salt.

Moreover, a blank and fortified working solution of CMC edible coating (Demitri et al., 2008); which were enriched with lactoferrin (5%, 10% and 20% conc.) (Hussein and El Sayed, 2022), followed by immersing of the filled sausage in it, and was left to dry in the room temperature representing outer edible coating.

Therefore, treated samples can be grouped as the following:

G1: Minced meat without any treatment (control)

G2: 5% treated minced meat + outer CMC-LF edible coat (5%)

G3: 10% treated minced meat + outer CMC-LF edible coat (10%)

G4: 20% treated minced meat + outer CMC-LF edible coat (20%)

Each group were subdivided into equal weights, and kept in the refrigerator (4±1^{O}C) for examination.

2.3. Sensory evaluation

Color, odor and texture of the samples were evaluated by five well-trained impartial investigators. Mean values of scores were recorded as overall sensory scores following Mörlein (2019) in scores (1 to 5), where ≤1- represented the worst while 5- represented the excellent mark.

2.4. Bacterial evaluations

Twenty-five grams of the sausage samples (from the core and intestine included) were prepared, and tenfold serial dilutions according to ISO 6887-1 (2017), counts of total bacteria, coliform, psychrotrophs, and total fungal counts were investigated according to ISO 4833-1 (2022), ISO 17410 (2019), ISO 4832 (2006), and ISO 21527-1 (2008), respectively.

Samples were examined according to clauses 2.3. and 2.4. at zero time (30 min. after dryness of the outer coat), and each three days until appearing of grossly spoilage signs (changes in color and/or odor and/or texture). Furthermore, the experiment was repeated in triplicate.

2.5. Statistical analysis

SPSS version 20 was used to analyze the data. The significance of the differences in the mean values of the groups under investigation was determined using ANOVA analysis and the Duncan posthoc value. A significance level of $p < 0.05$ was deemed significant.

3. RESULTS

The findings shown in Table (1) indicate that the treatment of oriental sausage with lactoferrin has a potential overall enhancement in the sensory quality of sausage samples, especially if used as fortified edible coating, where the treated samples showed a significant longer shelf life in comparison with the control samples which showed spoilage characters after the 6th day of storage. Higher lactoferrin concentration gave more acceptable sensory results revealing it concentration-time dependent treatment.

Table (1): Sensory profile of untreated and treated sausage samples in cold storage (4±1^{O}C).

Groups	Tested parameter	G1	G2	G3	G4
Zero day	Color	4.9±0.3	4.9±0.3	4.9±0.3	4.9±0.3
	Odor	4.7±0.3	4.7±0.3	4.7±0.3	4.7±0.3
	Texture	4.8±0.4	4.9±0.4	4.9±0.4	4.9±0.4
	Overall	4.8±0.1^{a}	4.8±0.1^{a}	4.8±0.1^{a}	4.8±0.1^{a}
3nd day	Color	3.0±0.2	4.7±0.4	4.7±0.1	4.6±0.5
	Odor	3.3±0.2	4.5±0.2	4.5±0.2	4.5±0.4
	Texture	3.5±0.3	4.8±0.3	4.9±0.3	4.7±0.3
	Overall	3.3±0.1^{b}	4.6±0.1^{a}	4.7±0.1^{a}	4.6±0.1^{a}
6th day	Color	2.0±0.1	4.0±0.1	4.5±0.1	4.4±0.2
	Odor	1.8±0.2	3.9±0.2	4.2±0.2	4.2±0.5
	Texture	2.5±0.3	4.1±0.3	4.6±0.3	4.2±0.3
	Overall	2.1±0.1^{c}	3.6±0.1^{b}	4.4±0.1^{a}	4.3±0.1^{a}
9th day	Color	<1	2.5±0.1	4.1±0.1	4.3±0.3
	Odor	<1	2.8±0.2	4.0±0.3	4.1±0.2
	Texture	<1	3.0±0.3	4.3±0.3	4.0±0.2
	Overall	Spoiled	2.8±0.1^{b}	4.1±0.1^{a}	4.1±0.1^{a}
12th day	Color	<1	<1	3.5±0.1	3.8±0.1
	Odor	<1	<1	3.2±0.3	3.5±0.3
	Texture	<1	<1	3.8±0.3	3.6±0.3
	Overall	Spoiled	Spoiled	3.5±0.2^{a}	3.6±0.2^{a}
15th day	Color	<1	<1	2.8±0.1	3.2±0.1
	Odor	<1	<1	2.5±0.3	2.8±0.3
	Texture	<1	<1	3.1±0.3	3.0±0.3
	Overall	Spoiled	Spoiled	2.8±0.2^{b}	3.0±0.2^{a}
18th day	Color	<1	<1	<1	2.7±0.1
	Odor	<1	<1	<1	2.3±0.3
	Texture	<1	<1	<1	2.2±0.3
	Overall	Spoiled	Spoiled	Spoiled	2.4±0.1

The values represent Mean ± SE of three experiments.
Means within the same row (abcd) followed by different superscript letters are significantly different (P ≤ 0.05). Zero time: 30 min. after outer coat dryness.
4.0-5.0 very good 3.1-3.9 good 2.1-3.0 Acceptable 1.1-2.0 Unacceptable 0.0-1.0 spoiled

As shown in Tables (2-5), a promising significant antimicrobial activity of the treatment technique was observed through significant reduction in the microbial counts and multiplication represented by their count/g of the treated samples. However, all of the examined additives showed antimicrobial activity, 20% lactoferrin treated group had higher inhibitory effect on the microbial growth.

Table (2): Aerobic plate count (log CFU/g) of untreated and treated sausage samples in cold storage (4±1^{O}C).

	Zero day	3rd day	6th day	9th day	12th day	15th day	18th day
G1	5.1± 0.3^{a}	6.4± 0.6^{a}	7.1± 0.2^{a}	Spoiled	Spoiled	Spoiled	Spoiled
G2	5.1± 0.2^{a}	5.0± 0.5^{b}	4.8± 0.6^{b}	5.6± 0.6^{a}	Spoiled	Spoiled	Spoiled
G3	5.1± 0.3^{a}	4.6± 0.4^{c}	4.3± 0.6^{c}	5.0± 0.6^{b}	5.8±0.6^{a}	6.1±0.6^{a}	Spoiled
G4	5.1± 0.3^{a}	4.3± 0.4^{d}	4.0± 0.5^{d}	3.8± 0.6^{c}	3.5± 0.7^{b}	4.2± 0.7^{b}	4.6± 0.7

Means within the same column (abcd) followed by different superscript letters are significantly different (P ≤ 0.05).

Table (3): Coliform count (log CFU/g) of untreated and treated sausage samples in cold storage (4±1^{O}C).

T	Zero day	3rd day	6th day	9th day	12th day	15th day	18th day
G1	2.6± 0.2^{a}	3.0± 0.4^{a}	3.7± 0.4^{a}	Spoiled	Spoiled	Spoiled	Spoiled
G2	2.6± 0.1^{a}	2.4± 0.3^{b}	2.6± 0.4^{b}	3.4± 0.4^{a}	Spoiled	Spoiled	Spoiled
G3	2.6± 0.3^{a}	2.1± 0.3^{c}	1.8± 0.3^{c}	1.4± 0.4^{b}	2.0± 0.1^{a}	2.4± 0.6^{a}	Spoiled
G4	2.6± 0.3^{b}	1.6± 0.2^{d}	1.4± 0.5^{d}	1.0± 0.4^{c}	1.2± 0.1^{b}	1.8± 0.4^{b}	2.5± 0.4

Means within the same column (abcd) followed by different superscript letters are significantly different (P ≤ 0.05).

Table (4): psychrotrophs count (log CFU/g) of untreated and treated sausage samples in cold storage (4±1^{O}C).

T	Zero day	3rd day	6th day	9th day	12th day	15th day	18th day
G1	2.1± 0.2^{a}	2.8± 0.3^{a}	3.3± 0.6^{a}	Spoiled	Spoiled	Spoiled	Spoiled
G2	2.0± 0.1^{a}	1.8± 0.2^{b}	1.5± 0.4^{b}	2.2± 0.4^{a}	Spoiled	Spoiled	Spoiled
G3	2.0± 0.3^{a}	1.6± 0.4^{c}	1.2± 0.3^{c}	1.0± 0.4^{b}	1.5± 0.4^{a}	1.8± 0.4^{a}	Spoiled
G4	2.0± 0.2^{a}	1.3± 0.3^{d}	1.0± 0.1^{d}	<1	1.1± 0.4^{b}	1.5± 0.2^{b}	2.5± 0.4

Means within the same column (abcd) followed by different superscript letters are significantly different (P ≤ 0.05).

Table (5): Mould and yeast count (log CFU/g) of untreated and treated sausage samples in cold storage (4±1^{O}C).

T	Zero day	3rd day	6th day	9th day	12th day	15th day	18th day
G1	2.2± 0.4^{a}	3.1± 0.3^{a}	4.4± 0.3^{a}	Spoiled	Spoiled	Spoiled	Spoiled
G2	2.2± 0.3^{a}	2.1± 0.1^{b}	2.0± 0.6^{b}	2.8± 0.6^{a}	Spoiled	Spoiled	Spoiled
G3	2.2± 0.3^{a}	2.0± 0.6^{c}	1.7± 0.5^{c}	1.5± 0.4^{b}	1.2±0.4^{a}	2.0±0.4^{a}	Spoiled
G4	2.2± 0.4^{a}	1.9± 0.2^{c}	1.5± 0.5^{d}	1.3± 0.4^{c}	1.0± 0.4^{b}	1.1± 0.4^{b}	1.5± 0.4

Means within the same column (abcd) followed by different superscript letters are significantly different (P ≤ 0.05).

4. DISCUSSION

Although meat and meat products are great sources of high-quality protein and fat supplements for consumers, they are also frequently contaminated, which creates an environment that is conducive to meat spoilage and even food poisoning (Yu et al., 2021).

Traditional sausages are considered stable meat products because of their pH levels and water activity. Still, special attention must be given to this type of product to avoid the growth of spoilage microbes. Oxygen exposure is the primary cause of this kind of product's quality decline because it causes oxidative rancidity, flavor and color changes, and the growth of aerobic bacteria and fungi. In this sense, it is imperative to stop or severely restrict microbial growth while it is still permissible (Barcenilla et al., 2022).

The shelf life of a food product is the length of time, when stored under recommended conditions, that it can be used safely without losing the necessary sensory, chemical, physical, microbiological, and functional qualities as stated on the label (Man, 2002).

Food quality, safety, and freshness are now highly prized. However, the main element affecting food safety is food packaging. Standard food packaging is frequently made of polymers like polyethylene, polypropylene, polystyrene, or polyethylene terephthalate without any additional functionality (Duncan, 2011).

Lactoferrin is a glycoprotein that can be found in milk and milk products, neutrophil granules, and exocrine secretions in mammals. Because of its capacity to bind iron inside microbial cells, lactoferrin has the potential to be used in functionally active food packaging (Lisitsyn et al., 2021). It is believed to be safe for human health because of their natural origins, and modern trends favor the use of natural preservatives rather than synthetic ones (Galié et al., 2018). Due to its ability to control free iron levels, LF has bacteriostatic and health-promoting qualities such as promoting bone formation, shielding the intestinal epithelium, and bolstering the immune system (Gomez-Estaca et al., 2010).

Referring to the current sensory evaluation results in Table (1), application of CMC-LF significantly enhanced the sensory profile of the treated sausage samples, which appeared to be dose-dependent where higher concentration gave better results, with significant enhancement in the overall acceptability up to 18th of storage in the treated sausage samples with 20% LF edible coating; whereas, control samples revealed spoilage after the 6th day of storage. The obtained findings may be referred to the tightly closed fortified CMC coating that provides favorable aroma and texture, accompanied as an oxygen and oil barrier properties to the product (Rad et al., 2021). Moreover, permission of LF migration through the casing into the product may delay the unfavorable changes that may occur (Hashhash, 2023). As was shown, the treated sausage samples with CMC fortified with LF coating extended the storage acceptability through the 12th, 15th and 18th day of storage in the treated groups with 5, 10 and 20% LF concentration, respectively. The obtained results came in line with the recorded results of Hussein and El Sayed (2022) and Hashhash (2023) who reported a significant enhancement in the physico-chemical characters of the treated meat products with LF; while, it does not agree with lee and chin (2008) who reported insignificant changes in the sensory characters of the treated sausage samples with LF, that may be attributed to the differences in the storage temperature, time of exposure, and the concentrations of the added LF.

Similar to transferrins, lactoferrin (LF) is a glycoprotein that binds iron, and functions as an essential component of mammals' innate host defense system and has antimicrobial properties against a variety of Gram-positive and Gram-negative bacteria (Jenssen and Hancock, 2009). In contrast to its bactericidal activity, which is believed to be mediated by binding to or modifying components of bacterial cell walls, such as lipopolysaccharide (LPS) molecules in Gram-negative bacteria and teichoic (TA) or lipoteichoic (LTA) acids in Gram-positive bacteria, LF has been described to exert a bacteriostatic effect based on its ability to sequester iron (Vorland et al., 1999); which results in depolarization, loss of membrane integrity, and the pH gradient.

Significant antimicrobial effect of the used CMC-LF bio-coating revealed obvious bactericidal effect appeared as significant reductions in the microbial counts, in the treated sausage samples, during first days of storage, as were recorded in Tables (2-5), which may be attributed to the ability of lactoferrin to consume the available free iron, which is essential for microbial growth and metabolism, in competitive manner with the microorganisms; moreover, it, also, can affect the microbial cell membrane permeability through lipopolysaccharide conjugation leading to cell membrane damage (Superti, 2020). On the other hand, a microbial counts re-elevated in various times of storage, that may be attributed to presence of lactoferrin-resistant microorganisms, as was recorded by Arnold et al. (1980) who quoted that, since organisms of the same species and even of the same strain can differ in susceptibility to lactoferrin, it appears that accessibility to the lactoferrin target site may account for differences in susceptibility. It appears that there may be a relation between virulence and resistance to lactoferrin. In addition, LF, also, can show bacteriostatic properties by holding iron at a lower pH, which is usually occurred in fresh healthy meats, which become of higher pH along with the storage time because of different effects of the enzymatic and microbial degradation of muscular nutrients, and the theory of oxidative stress (Niaz et al., 2019).

Apart from its antibacterial properties, lactoferrin and its derivatives have been found to possess a range of biological properties, such as anti-inflammatory, immunomodulatory, antiviral, and anti-carcinogenic effects. Since 2001, the Food and Drug Administration (FDA) has classified LF as Generally Recognized as Safe (GRAS). As a result, there has been a growing interest in using LF as a natural bioactive ingredient and preservative in food. These compounds can be sprayed onto carcasses or applied to the surface of beef before final packaging. However, the lack of information regarding these compounds' capacity to act and maintain activity in food, as well as their biodegradation, residual, and health effects, has limited their commercial applications (Naidu, 2001).

5. CONCLUSIONS

Addition of LF to the raw minced meat accompanied with Carboxy methyl cellulose fortified with lactoferrin (CMC-LF) edible coating proved to be a promising food keeping quality technique in the sausage production during refrigeration storage, with functional enhancing the sensory properties of the treated sausage samples; with controlling the microbial growth revealing the treated sausage safe, consumable meat product of almost long keeping quality. In addition, more investigations have to be conducted on their environmental, health concerns and safety in relation to residual ability and human daily dosage.

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