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Effect of Casein-Based Edible Coats Embodying Sorbic and Ascorbic Acids on the organoleptic, Physicochemical and Microbiological Quality of Frozen Beef Kofta

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ABSTRACT

Edible coats derived from a natural animal source and conveying natural active compounds to meat products may be the golden solution that sums up various preserving benefits. In this study, the main goal was to ascertain whether casein coat and casein coat enhanced with 1000 ppm Sorbic acid and 600 ppm ascorbic acid may be utilized to increase the acceptability of frozen beef kofta. In addition to the control trial, two coats were compared: a plain casein coat and a casein coat enhanced with 1000 ppm Sorbic acid and 600 ppm ascorbic acid. Organoleptic, bacteriological, proximate chemical analysis, pH, thiobarbituric acid reactive substances ("TBARS"), cooking characteristics, and instrumental colour evaluations were examined for each kofta treatment during three months of storage at -18°C. Results revealed that casein coats were able to boost several sensory attributes of raw and cooked kofta in addition to the overall acceptability of the raw product. Moreover, coats significantly decreased all tested bacterial counts and thiobarbituric acid reactive substance (TBARS) values in addition to maintaining compositional parameters from deteriorating during the storage period. As for cooking characteristics, they were all improved by applying casein coats when compared to the control. Casein coated with acid surpassed the plain casein coats in improving all parameters in addition to having the best colour scores for all three months of storage. It has been concluded that casein coats can be utilized to improve the quality of beef kofta without colour or flavour problems.

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INTRODUCTION

Meat products are palatable foods that are desired by different consumer classes but at the same time are highly perishable since they contain nourishing constituents such as protein, fat, fatty acids, vitamins, and minerals **(Balasubramanian *et al*., 2021).** This necessitates continuous searching for preservation methods that are satisfying to both consumers by being natural and complying with safety regulations and manufacturers by being applicable, effective, and relatively inexpensive **(Cutter, 2006).** Edible coating of food products is a hopeful direction in the sector of food preservation that includes various desirable techniques such as natural edible matrix that may contain natural additives to improve the shelf life and quality of the product **(Hashemi *et al*., 2020).** These coatings are considered smart solutions to several challenges that face meat product preservation and the extension of their shelf life.

Edible coatings originate from numerous natural biodegradable sources such as proteins, lipids, polysaccharides, or composites and therefore resolve the obstacle of plastic and synthetic wrappings by being eco-friendly **(Ju *et al*., 2019).** They are also functional matrices that have the convenient ability to express several additives and extend their release along the storage period, contrary to the instant action of directly adding those substances besides their inherent ability to enhance the microbiological, chemical, and physical wholesomeness of meat products (**Martín-Belloso *et al*., 2009).** Casein-based coats are practical types that form a clear, tasteless, and odourless edible layer around the product, acting as a physical barrier against the exchange of gases such as oxygen averting oxidative changes of the product and the migration of external contaminants that may affect sensory traits of the products, Their ability to prevent moisture loss is debatable since they are highly water permeable but form water resistible films when mixed with water and glycerol as a plasticizer **(Bonnaillie *et al*., 2014).** These advantageous qualities can be enhanced by incorporating additives such as organic acids that have both antimicrobial and antioxidant effects since these effects are limited by the application of casein film alone **(Bhagath and Manjula, 2019).**

Direct addition during the manufacturing of meat products is the most common form of organic acid application. However, there were several disadvantages reported by the direct application of organic acids, such as their interaction with other chemical additives or product constituents such as enzymes that may for instance have a negative impact on the sensory quality of the product **(Ben Braïek and Smaoui, 2021).** Therefore, the incorporation of organic acids into an edible coating may be a promising solution to overcome the problems arising from direct application, where the concentration of acids needed to be effective against microbial growth and lipid oxidation was reduced when combined into edible coatings **(Guillard *et al*., 2009).** Furthermore, this method of application allows the slow and controlled release of these acids to cover the whole storage period **(Quintavalla and Vicini, 2002).**

Ascorbic and Sorbic acids are meat products' most prominent organic acids. Both acids possess well-established antimicrobial and antioxidant properties besides the ascorbic acid’s ability to improve meat products' physicochemical and sensory traits **(De Jesus *et al*., 2021).** Thus, the aim of this study was to compare the conserving effects of both plain casein coat and casein coat enhanced with organic sorbic and ascorbic acids after being applied on beef kofta stored at -18 °C for three months through organoleptic, bacteriological, and physicochemical evaluations.

MATERIALS AND METHODS

Experimental design

A three-trial-based experiment was designed to investigate the individual effects of the application of casein coating alone and casein coating plus organic acids (sorbic and ascorbic acids) on the different quality attributes of frozen beef kofta immediately after processing (zero-time) and during the frozen storage period for three months against a negative control that did not receive any treatments. The impact of both treatments was assessed by organoleptic, bacteriological, and physicochemical quality parameter evaluations to determine any deviation that may have been caused by any of the treatments on the safety and acceptability of the product under study.

Raw materials

Brazilian frozen beef neck meat was provided from local stores within its stated storage life. Fresh mesenteric beef fat was obtained from a local slaughterhouse within one-hour post-slaughter, thoroughly rinsed with water, and kept frozen at -18 °C until processing. Sodium caseinate, glycerol, ascorbic, sorbic, and sodium tripolyphosphate were purchased from Avi-Chem laboratories, India. However, common salt and bread crumbs were supplied from local shops, in Cairo, Egypt.

Preparation of casein edible coats

A 2.5% aqueous solution of sodium caseinate was prepared by dispersing 150 g of caseinate powder in six litres of distilled water and stirring for three hours at room temperature, according to **Moreira *et al*. (2011).** After that, the glycerol was added to achieve a final glycerol/protein weight ratio of 0.28. Then the casein solution was divided into two baths, and to one of them scorbic and ascorbic acids were added to reach a final concentration of 1000 ppm and 600 ppm, respectively. The second bath was left without the addition of organic acids to evaluate the preservative effect of the casein coat alone.

Manufacturing of beef kofta and application of the coat

A base batter of beef kofta was formulated according to good manufacturing practise (GMP) using 60% beef meat, 14.0% added beef fat, 10% breadcrumbs, 13.5% cool iced water, 1.6% common salt, 0.33% sodium tripolyphosphate, and 0.05% spice mix essential for the flavour of Egyptian kofta. At the time of processing, both meat and fat were coarsely ground with a 5 mm blade (FAMA, Rimini-Italy). The minced beef and fat were transferred to a paddle mixer (Urgstallstraße, Germany), where the rest of the ingredients were added while mixing to a final batter temperature of -1 °C. Afterwards, the batter was transferred to the forming machine, where kofta fingers were evenly shaped into about six cm in circumference and about 11 cm in length. The formed kofta was then divided into three groups. The first group was the control, which received no treatment at all, and was packaged, labelled, and freeze-stored at -18 °C. The 2nd and 3rd groups were the ones coated by the casein coat alone and casein with organic acids, respectively. Before the application of coats, kofta fingers were stored at -18 °C overnight to maintain their shapes during coat application.

Coats were applied by dipping kofta fingers in the previously prepared baths for two minutes, followed by drying in a hot air oven using only forced airflow without heat, then dipping again for one minute and drying once more. Kofta fingers were weighed before and after dipping to determine the coating pick-up percentage, which was calculated to be 5.5%, and 4.12% for the casein coat alone, and the casein with acid coat, respectively. Afterwards, the coated kofta was returned to frozen storage.

Investigation of kofta

At each investigation time (at zero-time and monthly for three months), three samples from each kofta treatment were examined for organoleptic, bacteriological, proximate chemical analysis, pH, thiobarbituric acid reactive substances "TBARS", cooking characteristics, and instrumental colour evaluations.

Sensory evaluation

Fifteen well-trained panellists from the Food Hygiene Sector in the Animal Health Research Institute, Egypt, were asked to score raw kofta fingers from each treatment to assess the colour, odour, formation, and overall acceptability. After cooking, kofta was evaluated for flavour, juiciness, tenderness, and overall acceptability using a five-point scoring system, where one denoted the lowest score and five was the highest score. Before the main sensory analysis test, all panellists received several preparatory sessions for both raw and cooked kofta to become familiar with intensities of tested parameter.

Bacteriological examination

A tenfold decimal dilution was prepared according to **ISO (2017),** where 25 g of kofta fingers were homogenized with 225 ml of 0.1% peptone water. 0.1 ml of the selected concentrations were aseptically spread over the dried surface of double sets of plate count agar, where the 1st set was incubated at 35 °C for 48 h for the enumeration of the aerobic mesophilic count **(Morton, 2001)**, while the 2nd set was incubated at 4 °C for 10 days for the psychrotrophic bacterial count. Bacara agar plates were incubated at 30 °C for 24 h for enumeration of *Bacillus cereus* **(Tallent *et al*., 2020),** while for counting of *Staphylococcus aureus*, Baird Parker agar plates were incubated for 48 h at 37 °C **(Bennett and Ga 2016).** Concurrently, 1 ml from each of the first three previously prepared decimal dilutions was taken into nine tubes of lauryl sulphate, three tubes for each dilution, and then incubated at 35 °C for 48 h. Positive tubes showing gas and turbidity were recorded according to **FDA (2002),** and a loopful was transferred from each to *Escherichia coli* broth and incubated in a water bath at 44 °C for 48 h. Positive tubes, once again showing turbidity and gas were recorded as faecal coliforms.

Physicochemical analysis

Proximate chemical composition

The official methods established by the Association of Official Analytical Chemists **(AOAC) (2003)** were used for kofta sample preparation and proximate chemical analysis. Approximately 2 g of the prepared samples were weighed, spread in a glass dish, and dried in a mechanical convection oven at 125°C until constant weight was obtained to obtain the moisture content. The content of protein was determined in 0.5 g samples using the Kjeldahl method, and the protein percentage was obtained using the factor assigned to meat and meat products (6.25). For crude fat determination, a 10 g finely homogenized sample portion was dried for 1.5–2 h at 125°C, and then ether was extracted in the Soxhlet apparatus. 5g from each prepared sample were transferred to a muffle furnace and ignited at 500–600 °C to reach two successive constant weights to determine the ash content.

Thiobarbituric acid reactive substances (TBARS) and pH values

A 5 g sample was macerated with 97.5 ml of distilled water, and 2.5 ml of HCl 4N was used to bring the solution's pH to less than 1.5. A 50 ml extract was collected over 10 minutes of distillation. Equal volumes of the distillate and TBA reagent were heated for 35 minutes in a boiling water bath and then cooled. The absorbance of the sample was read against a blank at 538 nm **(Siripatrawan and Noipha, 2012).** The average of three readings was recorded as mg malondialdehyde/kg sample. The pH values of kofta were measured in the prepared sample solution using a digital pH metre **(Jenway model 3310).** Readings were taken three times, and the average was recorded.

Cooking characteristics

Frozen kofta fingers were left for a couple of minutes before being cooked for 2.5 minutes on each side to reach a 72°C core temperature using an electric grill. The weight, length, and diameter together with moisture and fat contents were measured before and after cooking to determine the cooking characteristics. The cooking yield was obtained by measuring the percentage of losses that occur after cooking. The moisture retention determined the amount of moisture retained after cooking per 100 g raw product **(El-Magoli *et al*., 1996),** while the fat retention was obtained by the equation established by **(Murphy *et al*., 1975).** And the diameter reduction measured the difference in kofta fingers diameter before and after cooking **(Piñero *et al*., 2008).**

Instrumental colour evaluation

The instrumental colour evaluation was estimated by measuring the Lightness (L^*), redness (a^*), and yellowness (b^*) values using a Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap **(Shin *et al*., 2008**).

Statistical analysis

A one-way ANOVA test of the IBM SPSS statistics software (version 20) was used to calculate the differences between means using the least significant difference (LSD) at ($P<0.05$). Results were compared in two directions. The first direction was to compare the different quality attributes among the different kofta treatments at zero-time and one-month intervals for three months. The second direction was to compare the differences that occurred in all examined parameters within the same treatment throughout the storage period.

RESULTS

Sensory attributes

The colour, forming, and overall acceptability of the raw product, in addition to the juiciness of the cooked product were significantly ($P<0.05$) enhanced in both the casein coated kofta compared to those of the control at zero-time and during the three-month storage trial. Moreover, kofta coated with casein alone showed the highest ($P<0.05$) odour and flavour scores, while cooked kofta coated with casein/acids showed the highest tenderness and overall acceptability scores. These results indicated that coating kofta with both casein coats did not alter the sensory characteristics of the products and preserved their flavour during storage (Table 1).

Table 1: Sensory attributes of raw and cooked kofta during storage at -18 °C for 3 months

	For raw kofta							
	Colour				Odour			
	0 time	1st M	2nd M	3rd M	0 time	1st M	2nd M	3rd M
Control	4.13± $0.13^{a,A}$	4.00± $0.26^{a,A}$	3.67± $0.19^{a,B}$	3.67± $0.33^{a,B}$	4.50± $0.58^{a,A}$	4.33± $0.17^{a,AB}$	4.17± $0.11^{a,B}$	4.17± $0.26^{a,B}$
Casein coat	4.50± $0.11^{b,A}$	4.27± $0.25^{b,AB}$	4.20± $0.22^{b,B}$	4.09± $0.16^{b,B}$	4.67± $0.33^{a,A}$	4.67± $0.33^{b,A}$	4.33± $0.42^{b,B}$	4.33± $0.19^{b,B}$
Casein+acids coat	4.67± $0.32^{b,A}$	4.33± $0.14^{b,AB}$	4.25± $0.28^{b,B}$	4.17± $0.11^{b,B}$	4.40± $0.26^{a,A}$	4.17± $0.13^{a,B}$	4.05± $0.23^{a,B}$	4.00± $0.37^{c,B}$
	Forming				Overall acceptability			
Control	4.00± $0.22^{a,A}$	3.86± $0.30^{a,A}$	3.67± $0.11^{a,A}$	3.67± $0.21^{a,A}$	4.00± $0.00^{a,A}$	4.00± $0.33^{a,A}$	3.93± $0.33^{a,A}$	3.90± $0.33^{a,A}$
Casein coat	4.60± $0.13^{b,A}$	4.43± $0.23^{b,A}$	4.39± $0.19^{b,A}$	4.33± $0.31^{b,A}$	4.40± $0.00^{b,A}$	4.40± $0.33^{b,A}$	4.33± $0.33^{b,A}$	4.25± $0.00^{b,A}$
Casein+acids coat	4.60± $0.16^{b,A}$	4.42± $0.11^{b,A}$	4.35± $0.18^{b,A}$	4.33± $0.30^{b,A}$	4.32± $0.33^{b,A}$	4.30± $0.33^{b,A}$	4.22± $0.33^{b,A}$	4.10± $0.33^{b,A}$
	For cooked kofta							
	Flavour				Juiciness			
	0 time	1st M	2nd M	3rd M	0 time	1st M	2nd M	3rd M
Control	4.50± $0.21^{a,A}$	4.23± $0.19^{a,B}$	4.23± $0.23^{a,B}$	4.20± $0.11^{a,B}$	4.50± $0.58^{a,A}$	4.33± $0.00^{a,AB}$	4.17± $0.33^{a,BC}$	4.03± $0.33^{a,C}$
Casein coat	4.67± $0.28^{a,A}$	4.50± $0.26^{b,A}$	4.41± $0.11^{a,,A}$	4.36± $0.13^{a,A}$	4.67± $0.33^{ab,A}$	4.53± $0.00^{b,AB}$	4.40± $0.33^{b,BC}$	4.27± $0.33^{b,AC}$
Casein+acids coat	4.33± $0.11^{a,A}$	4.22± $0.14^{a,A}$	4.20± $0.24^{a,A}$	4.18± $0.10^{a,,A}$	5.00± $0.00^{b,A}$	4.67± $0.33^{b,AB}$	4.50± $0.00^{b,B}$	4.43± $0.33^{b,B}$
	Tenderness				Overall acceptability			
Control	4.43± $0.15^{a,A}$	4.33± $0.28^{a,A}$	4.12± $0.31^{a,B}$	4.09± $0.16^{a,B}$	4.33± $0.23^{a,A}$	4.22± $0.25^{a,AB}$	4.15± $0.27^{a,B}$	4.00± $0.11^{a,C}$
Casein coat	4.67± $0.23^{a,A}$	4.51± $0.31^{a,A}$	4.19± $0.17^{a,B}$	4.17± $0.30^{a,B}$	4.53± $0.47^{b,A}$	4.35± $0.29^{a,B}$	4.21± $0.35^{a,BC}$	4.11± $0.24^{a,C}$
Casein+acids coat	5.00± $0.11^{b,A}$	4.67± $0.23^{b,A}$	4.33± $0.29^{b,B}$	4.33± $0.11^{b,B}$	4.71± $0.21^{b,A}$	4.67± $0.36^{b,A}$	4.57± $0.13^{a,A}$	4.45± $0.33^{b,B}$

*Values represent the mean of three independent replicates ± standard error

*$^{a-c}$: Values with different superscripts within the same column are significantly ($P < 0.05$) different

*$^{A-C}$: Values with different superscripts within the same row significantly ($P < 0.05$) different

Bacteriological analysis

Coating of kofta with different casein coats resulted in a significant ($P<0.05$) decline in all examined bacteria immediately after processing and throughout the three months of storage at -18 °C when compared to the control (Table 2). The results also revealed that the casein and acid coats exhibited an obvious ($P<0.05$) antibacterial effect over the casein coat alone, which resulted in a 15.67 and 8.49% reduction in the APC during the storage period respectively. Moreover, directly after the application of casein, acids, and casein coatings, the bacterial counts were reduced at a rate of 9.89, 6.5 for psychrotrophs, 20, 18.77 for *B. cereus*, and 16.17, 11.28% for faecal coliform, respectively, with the production of the product free from these bacteria at the end of the storage experiment. It is also clarified that *S. aureus* counts were below the detectable limit in coated kofta at zero time and throughout the storage period. In addition, frozen storage of different kofta treatments led to a significant ($P<0.05$) decline in all examined bacteria, however, the control group showed higher *S. aureus* counts than the permissible limit (two $\log_{10}$ CFU/g) stated by the Egyptian Organization for Specification and Quality Control for Frozen Balls **(EOS/1973 (2005)** at zero time and throughout the storage period**.**

Table 2: Bacteriological profile (log10 cfu/g) of control and coted kofta during storage at -18 °C for 3 months

	APC				Psychrotrophs			
	0 time	1st M	2nd M	3rd M	0 time	1st M	2nd M	3rd M
Control	6.66± $0.04^{a,A}$	4.48± $0.02^{a,B}$	4.33± $0.02^{a,C}$	3.95± $0.06^{a,D}$	3.84± $04^{a,A}$	<2.00± $0.00^{a,B}$	<2.00± $0.00^{a,B}$	<2.00± $0.00^{a,B}$
Casein coat	4.91± $0.02^{b,A}$	4.46± $0.09^{a,B}$	4.17± $0.03^{b,C}$	3.81± $0.02^{b,D}$	3.59± $0.06^{b,A}$	<2.00± $0.00^{a,B}$	<2.00± $0.00^{a,B}$	<2.00± $0.00^{a,B}$
Casein+acids coat	4.57± $0.02^{c,A}$	4.23± $0.03^{b,B}$	4.16± $0.01^{b,C}$	3.09± $0.02^{c,D}$	3.46± $0.09^{b,A}$	<2.00± $0.00^{a,B}$	<2.00± $0.00^{a,B}$	<2.00± $0.00^{a,B}$
	S. aureus				*B. cereus*			
Control	3.48± $0.05^{a,A}$	3.30± $0.02^{a,B}$	3.10± $0.10^{a,B}$	3.05± $0.00^{a,B}$	5.70± $0.02^{a,A}$	4.36± $0.01^{a,B}$	2.26± $0.14^{a,C}$	<2.00± $0.00^{a,D}$
Casein coat	<2.00± $0.00^{b,A}$	<2.00± $0.00^{b,A}$	<2.00± $0.00^{b,A}$	<2.00± $0.00^{b,A}$	4.63± $0.06^{b,A}$	4.30± $0.03^{a,B}$	<2.00± $0.00^{b,C}$	<2.00± $0.00^{a,C}$
Casein+acids coat	<2.00± $0.00^{b,A}$	<2.00± $0.00^{b,A}$	<2.00± $0.00^{b,A}$	<2.00± $0.00^{b,A}$	4.56± $0.05^{c,A}$	4.17± $0.03^{b,B}$	<2.00± $0.00^{b,C}$	<2.00± $0.00^{a,C}$
	Fecal coliforms							
Control	2.66± $0.00^{a,A}$	2.54± $0.07^{a,B}$	1.85± $0.02^{a,C}$	0.50± $0.03^{a,D}$				
Casein coat	2.36± $0.02^{b,A}$	2.01± $0.04^{b,B}$	1.37± $0.042^{b,C}$	<2.00± $0.00^{b,D}$				
Casein+acids coat	2.23± $0.05^{c,A}$	1.78± $0.07^{c,B}$	0.93± $0.06^{c,D}$	<2.00± $0.00^{b,D}$				

*Values represent the mean of three independent replicates ± standard error
*$^{a-c}$: Values with different superscripts within the same column are significantly (P < 0.05) different
*$^{A-D}$: Values with different superscripts within the same row are significantly (P < 0.05) different

Physicochemical analysis

Proximate chemical composition

Proximate chemical analysis showed that both casein-coated kofta had significantly ($P<0.05$) higher moisture, protein, and fat, and significantly ($P<0.05$) lower ash content than the control at zero-time and during the frozen storage (Table 3).

Thiobarbituric acid reactive substances (TBARS) and pH values

The mean values of TBARS of both casein-coated kofta were significantly (*P*<0.05) lower than those of the control at zero time and throughout the frozen storage (Table 3). The application of the casein coat led to a reduction in TBARS value by 33.10%, moreover; the addition of organic acids increased the antioxidant activity of the casein coat with an oxidation-reduction rate of about 41.29%. The results also clarified that TBARS values of the control exceeded the permissible limit 1mg/kg established by (Warriss, 2000) at the 2nd month of storage; however, those of coated kofta were lower than this limit until the end of the storage experiment. Both application of casein coat and frozen storage resulted in a significant reduction in pH values of kofta (Table 3).

Table 3: Physicochemical analysis kofta during storage at -18 °C for 3 months

	Protein				Fat			
	0 time	1st M	2nd M	3rd M	0 time	1st M	2nd M	3rd M
Control	13.97±0.07^{a,A}	14.36±0.05^{a,B}	14.87±0.05^{a,C}	15.33±0.20^{a,D}	11.95±0.03^{a,A}	12.90±0.05^{a,B}	14.68±0.02^{a,C}	14.84±0.24^{a,D}
Casein coat	14.51±0.13^{b,A}	14.87±0.07^{b,AB}	14.99±0.01^{a,AB}	15.28±0.27^{a,B}	12.11±0.06^{a,A}	13.35±0.12^{b,B}	14.62±0.07^{a,C}	15.37±0.10^{b,D}
Casein+acids coat	14.61±0.13^{b,A}	15.25±0.05^{c,B}	15.97±0.04^{b,C}	16.22±0.06^{b,C}	12.40±0.06^{b,A}	13.15±0.20^{b,B}	14.67±0.11^{a,C}	15.57±0.18^{b,D}
	Moisture				Ash			
Control	60.01±0.23^{a,A}	59.99±0.01^{a,A}	58.57±0.06^{a,B}	57.40±0.33^{a,C}	2.80±0.07^{a,A}	2.99±0.02^{a,BC}	3.15±0.04^{a,C}	3.89±0.13^{a,D}
Casein coat	62.79±0.16^{b,A}	62.39±0.08^{b,A}	61.79±0.12^{b,B}	61.40±0.14^{b,C}	2.30±0.12^{b,A}	2.42±0.04^{b,AB}	2.51±0.04^{b,AB}	2.58±0.05^{b,B}
Casein+acids coat	62.78±0.17^{b,A}	62.12±0.22^{b,B}	61.93±0.06^{b,B}	61.65±0.19^{b,B}	2.27±0.05^{c,A}	2.28±0.04^{c,A}	2.30±0.01^{c,A}	2.32±0.12^{b,A}
	TBARS				pH			
Control	0.73±0.02^{a,A}	0.80±0.01^{a,B}	1.05±0.06^{a,C}	1.60±0.02^{a,D}	6.11±0.01^{a,A}	6.11±0.01^{a,A}	6.00±0.06^{a,B}	5.99±0.01^{a,B}
Casein coat	0.34±0.01^{b,A}	0.64±0.04^{b,B}	0.80±0.03^{b,C}	0.91±0.01^{b,D}	6.09±0.03^{a,A}	6.10±0.03^{a,A}	6.11±0.01^{b,A}	6.15±0.03^{b,A}
Casein+acids coat	0.32±0.01^{b,A}	0.52±0.04^{c,B}	0.74±0.03^{c,C}	0.77±0.01^{c,D}	6.01±0.04^{b,A}	6.00±0.03^{b,A}	5.95±0.02^{c,AB}	5.90±0.02^{c,B}

*Values represent the mean of three independent replicates ± standard error
*$^{a-c}$: Values with different superscripts within the same column are significantly (P < 0.05) different
*$^{A-D}$: Values with different superscripts within the same row are significantly (P < 0.05) different

Cooking characteristics

The cooking characteristics revealed that there was a significant (*P*<0.05) increase in cooking yield, moisture, fat retention, and a significant (*P*<0.05) decrease in diameter reduction in the coated kofta than the uncoated one at each sampling time (Table 4). The results also indicated that the addition of organic acids to the casein coat improved its physical properties, which resulted in a higher yield, moisture and fat retention, and lower diameter reduction than those of kofta coated with casein alone.

Table 4 :Cooking characteristics of kofta during storage at -18°C for 3 months

	Cooking yield				Moisture retention			
	0 time	1st M	2nd M	3rd M	0 time	1st M	2nd M	3rd M
Control	81.33 ± 3.02^{a,A}	80.56 ± 2.10^{a,B}	77.48 ± 1.11^{a,C}	74.88± 2.04^{a,D}	80.32 ± 2.23^{a,A}	74.87 ± 3.10^{a,B}	72.10 ± 2.54^{a,C}	70.65 ± 2.72^{a,D}
Casein coat	82.38 ± 3.00^{b,A}	81.25 ± 4.17^{b,B}	80.86 ± 2.34^{b,C}	78.35 ± 3.02^{b,D}	76.86 ± 2.06^{b,A}	75.68 ± 4.16^{a,A}	73.46 ± 3.13^{b,B}	72.95 ± 2.68^{b,C}
Casein+acids coat	85.51 ± 2.03^{c,A}	83.90 ± 3.18^{c,B}	81.21 ± 1.54^{c,C}	81.16 ± 1.01^{c,D}	75.92 ± 4.09^{c,A}	79.38 ± 3.15^{b,B}	74.60 ± 4.60^{b,C}	73.12 ± 1.98^{b,D}
	Fat retention				Diameter reduction			
Control	67.23 ± 4.01^{a,A}	66.13 ± 2.34^{a,A}	64.33 ± 2.32^{a,B}	62.43 ± 2.58^{a,C}	17.89 ± 0.92^{a,A}	19.50 ± 0.40^{a,B}	20.90 ± 0.81^{ab,B}	21.43 ± 0.65^{a,C}
Casein coat	72.97 ± 2.21^{b,A}	71.21 ± 1.91^{b,B}	69.45 ± 3.21^{b,C}	65.51 ± 2.38^{b,D}	14.20 ± 0.42^{b,A}	16.50 ± 0.31^{b,B}	18.26 ± 0.44^{b,C}	19.19 ± 0.28^{b,C}
Casein+acids coat	74.89 ± 2.23^{c,A}	72.68 ± 2.51^{b,B}	71.86 ± 1.82^{c,B}	68.87 ± 2.17^{c,C}	13.12 ± 0.31^{c,A}	16.32 ± 0.22^{b,B}	17.86 ± 0.76^{b,B}	18.98 ± 0.72^{b,C}

*Values represent the mean of three independent replicates ± standard error
*$^{a-c}$: Values with different superscripts within the same column are significantly (P < 0.05) different
*$^{A-D}$: Values with different superscripts within the same row are significantly (P < 0.05) different

Instrumental colour

Coating of kofta by casein embodying acids resulted in significant (*P*<0.05) elevations of lightness (L*), redness (a*), and yellowness (b*) values when compared with other treatments at zero-time and throughout the frozen storage period (Table 5). Meanwhile, there were non-significant (*P*>0.05) differences in all colour indices among the kofta coated with casein alone and the control immediately after processing and during the storage life. The results also showed that the frozen storage of kofta resulted in a significant (*P*<0.05) decrease in (L*) and (a*) values, while a significant rise in (b*) values in all kofta treatments with the control showed the obvious changes.

Table 5: Instrumental colour values of kofta during storage at -18°C for 3 months

	0 time	1st M	2nd M	3rd M
	(L*)			
Control	52.64±0.11^{a,A}	51.20±0.09^{a,A}	50.93±0.07^{a,B}	50.40±0.28^{a,B}
Casein coat	52.42±0.07^{a,A}	51.76±0.06^{a,B}	50.83±0.08^{a,C}	50.12±0.08^{a,D}
Casein+acids coat	53.11±0.40^{b,A}	52.97±0.11^{b,A}	52.86±0.11^{b,A}	52.58±0.11^{b,A}
	(a*)			
Control	13.63±0.18^{a,A}	12.73±0.16^{a,B}	12.02±0.12^{a,C}	11.59±0.04^{a,C}
Casein coat	13.12±0.02^{a,A}	12.82±0.19^{a,A}	12.15±0.11^{b,A}	11.99±0.83^{a,A}
Casein+acids coat	15.13±0.35^{b,A}	14.53±0.15^{b,A}	13.71±0.28^{c,A}	13.45±0.86^{b,A}
	(b*)			
Control	12.66±0.26^{a,A}	12.94±0.11^{a,AB}	13.25±0.10^{a,BC}	13.64±0.17^{a,C}
Casein coat	13.22±0.01^{a,A}	13.24±0.08^{a,A}	13.53±0.09^{a,A}	13.67±0.30^{a,A}
Casein+acids coat	14.00±0.16^{b,A}	14.02±0.10^{b,A}	14.75±0.10^{c,B}	15.12±0.54^{b,B}

*Values represent the mean of three independent replicates ± standard error

*$^{a-c}$: Values with different superscripts within the same column are significantly ($P < 0.05$) different

*$^{A-C}$: Values with different superscripts within the same row are significantly ($P < 0.05$) different

DISCUSSION

Sensory attributes

The enhancement of sensory parameters of coated kofta may have originated from their higher moisture, protein, and fat contents which positively affected the tenderness and juiciness during the early and final storage times **(Choi *et al*., 2008).** Slow flavour deterioration of both coating treatments during the storage lifetime may be explained by the antioxidant effect of casein **(Umaraw and Verma, 2017)** and organic acids **(Amaral *et al*., 2018)** that prevent fat oxidation, which is the main factor that reduces the flavour of meat products. However, **Bhagath and Manjula, (2019)** reported that the application of casein films did not affect the sensory attributes of the product, where casein films are transparent with no taste or flavour.

Bacteriological analysis

The reduction of bacterial counts in coated kofta may be correlated with the coagulation of the casein coat on the product which forms a special structural matrix that acts as an excellent gas barrier and seals the product surface against the migration of exterior contaminants. Furthermore, the antibacterial effect of organic acids has been explained by the penetration of the un-dissociated part of these acids into the bacterial cell wall, which then dissociates inside of it, leading to its disruption and death **(Pisoschi *et al*., 2018).** On the other hand, the effect of freezing on bacteria in meat products was briefly studied, and the dramatic reduction of water activity during the freezing time was the main cause for the decrease in bacterial counts **(Coombs *et al*., 2017).**

Physicochemical analysis

Proximate chemical composition

The higher protein content of coated kofta may be originated from the addition of protein during the application of casein coats. It is known that casein is a functional protein that has good moisture and fat binding properties, in addition; the casein coat may be denatured during the frozen storage forming an insulating layer, which prevents moisture loss and preserves the chemical constituents of the product. Furthermore, the properties of the casein coat didn't show any deterioration by the addition of organic acids, where the casein coat had higher stability at different pH conditions **(Shendurse *et al*., 2018).** These coating properties may also explain the slower changes that occurred in the chemical composition of coated kofta throughout the freezing period than the control, where obvious reduction in moisture content and elevation in other constituents were noted in the control at the end of the storage time when compared to zero-time of processing.

Thiobarbituric acid reactive substances (TBARS) and pH values

The antioxidant properties of the casein coat may be explained by denaturation of the casein matrix during coating formation, which acts as a potent oxygen barrier and subsequently delays lipid oxidation **(Umaraw and Verma, 2017).** The antioxidant properties and lipid stability have been reported when casein films **(Calderón-Aguirre *et al*., 2015**) and organic acids **(Eniolorunda *et al*., 2014**) were applied in different meat products.

The addition of organic acids into the casein coat and increasing the growth of lactic acid bacteria may be the main reasons for the reduction in pH values by casein acid coat application and frozen storage, respectively **(Chenoll *et al*., 2007).**

Cooking characteristics

Improvement in cooking characteristics may be related to the physical properties of the casein coat such as good emulsifying properties, higher stability in various storage temperatures, the ability to adhere to the wet surface, higher flexibility, and finally lowered dehydration of the product **(Shendurse *et al*., 2018).** Many studies reported that the casein coats had higher water vapour permeability due to their hydrophilic nature and the addition of acids may reduce this feature by 36% **(Avena-Bustillos and Krochta, 1993).** This finding was in agreement with **(Farhan and Hani, 2017)** who found that the cooperative forces among the polar and non-polar amino acids of casein may cause shrinkage during the application of the coat and a higher dehydration rate of the coated product. Therefore, the modification of the casein coating by the addition of plasticizers **(de Kruif *et al*., 2015)** is necessary to improve the physical and functional properties of the casein coat.

Instrumental colour

Improving the colour of kofta in casein and acid coat may be explained by the combined antioxidant effects of both casein coat and organic acids. These observations were in harmony with de **Azeredo (2012),** who reported enhancement and lower colour deterioration of meat products by the application of casein film. Moreover, **(Sánchez-Escalante *et al*., 2003)** found that the direct addition of ascorbic acid at a rate of 500 ppm maintained a higher (a*) score by decreasing the met-myoglobin formation in beef patties kept in modified atmosphere packages at 2 ºC for 20 days.

CONCLUSION

Based on the results of this study, it was revealed that casein coatings have clear preserving and improving effects on both hygienic and sensory traits of beef kofta since they were able to decrease overall bacterial loads in addition to their remarkable reducing effect on specific indicator bacteria as faecal coliforms and *S. aureus*. Additionally, these bio-coatings kept kofta compositional fractions and decomposition markers together with the sensory attributes and colour indices from deterioration during storage. And as an extra privilege ascorbic and sorbic acids were found not only to be compatible to be embodied in casein-based edible coats but also upgraded several properties of the casein network in addition to their inherent antibacterial and antioxidant properties giving a final combination that had better results in all tested criteria presenting a promising natural and applicable preserving technique in the meat products sector.

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Conflicts of interest

The authors have declared no conflict of interest

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