THE USE OF ELECTROSPUN IRON OXIDE NANOFIBERS IN COATING FROZEN BEEFBURGER

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ABSTRACT

This study was conducted on the in coating of beef burger using electrospun iron oxide nanofibers at two concentrations6, 8 % , to improve the chemical and physical properties of the beef burger during a 3-month frozen storage period. The results of the study showed that the addition of electrospun nanofibers contributed to reducing the total bacterial count, as well as the count of psychrophilic bacteria and coliform bacteria, to 4.88* 10^3, 4.62* 10^3, and 3.15* 10^1, 3.64 *10^1, respectively, and 2.15* 10^3, 2.49* 10^3 colony-forming units (CFU/g) respectively, during the final storage period. The moisture, protein, fat, and ash percentages in the frozen beef burger were found to be 60.12%, 19.09%, 19.31%, and 1.28%, respectively. The addition of iron oxide nanofibers also improved the water holding capacity of the frozen beef burger, with values of 49.16% and 39.69%, respectively. Furthermore, the pH values remained constant at 5.68 and 5.63, respectively, during the final storage period. The addition of nanofibers also reduced the peroxide value to 6.77 and 6.62 milliequivalents per kilogram, respectively, during the final storage period.The aim of this study was to investigate the effects of adding electrospun nanofibers to biopolymers used in beef burger on their qualitative properties.

Keywords: meat products, nanofibers, meat processing, food safety

***Part on Ph.D.disseertaion of the 1st author**

استخدام الياف أوكسيد الحديد النانوي المغزولة كهربائيا في تغليف البيرغر البقري المجمد

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المستخلص

أجريت عملية تغليف البيركر البقري باستخدام الياف أوكسيد الحديد النانوية المغزولة كهربائيا بتركيزين 8,6% لتحسين الخصائص الكيميائية والفيزيائية للبيركر البقري خلال فترة خزن بالتجميد لمدة 3 أشهر، من خلال نتائج الدراسة ساهمت إضافة الالياف النانوية المغزولة الى خفض اعداد البكتيريا الكلية كذلك اعداد البكتيريا المحبة للبرودة وبكتيريا القولون الى 10^3× 4.88، 10^3× 4.62 و 10^1× 3.15، 10^1× 3.64 و 10^3× 2.15، 10^3× 2.49 وحدة مكونة للمستعمرة لكل غم (CFU/g) على التوالي خلال فترة الخزن الأخيرة. كانت النسبة المئوية للرطوبة والبروتين والدهن والرماد في البيركر البقري المجمد 60.12, 19.09 , 19.31, 1.28 % على التوالي . ايضا ساهم إضافة الالياف النانوية من أوكسيد الحديد الى تحسين قابلية حمل الماء في البيركر البقري المجمد 49.16% و39.69% على التوالي، والحفاظ على قيمة الاس الهيدروجيني pH 5.68 و5.63على التوالي في الفترة الأخيرة من الخزن. وحسنت إضافة الالياف النانوية من قيمة رقم البيروكسيد في الفترة الأخيرة من الخزن 6.77 و6.62 ملي مكافئ / كغم على التوالي. هدفت هذه الدراسة الى معرفة تأثير إضافة الالياف النانوية المغزولة كهربائيا الى البوليمرات الحيوية المستخدمة في تغليف الأغذية في خصائصها النوعية.

الكلمات المفتاحية: منتجات اللحم، الالياف النانوية، تصنيع اللحوم، الياف نانوية، الامن الغذائي

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INTRODUCTION

Ensuring food safety and "food for all" is currently a bottleneck issue. To guarantee food safety (31), one key element is the advancement and development of food packaging technology. With the continuous increase in international trade, the focus should be on achieving sufficient food production, immediate food safety and security after manufacturing or packaging, which is also essential to provide everyone with fresh, healthy, and uncontaminated food (2, 4, 24). Most food contamination and microbial spoilage occur during transportation. Thus, food packaging plays a vital role in extending the shelf life of the product without compromising its quality (6). In addition to protecting and providing safe and nutritious food, reducing food loss and preventing microbial growth are essential characteristics of good packaging materials (1, 3, 7, 23). In this regard, particularly in the past decade, there has been an increasing demand for extended shelf life, product safety, environmental issues, and cost efficiency. For this purpose, several different material systems have been developed and exploited to produce highly efficient food packaging materials. Recently, special attention has been given to the electrospinning technique for the preparation of nanoscale structured or surface-functionalized food packaging materials using electrospun functional nanofibers (24). Electrospun nanofiber materials with desired structural properties can be produced through the processing conditions used in the electrospinning process and the properties of the solution and due to their high surface-to-volume ratio and tunable structural properties, electrospun materials can offer several benefits in food packaging (10, 17, 28). Meat is one of the most perishable food products involved in packaging. In fact, meat serves as an ideal medium for the growth of harmful microorganisms. Contamination of food products with microbial growth can pose health risks to humans. The risk is particularly significant when meat products become contaminated with pathogenic microorganisms, as tissues derived from healthy animals are generally free from microorganisms (18). The increasing demand for meat products, coupled with growing competition and health concerns, has led to the adoption of new and innovative methods in the meat industry. Generally, the meat industry worldwide focuses on developing new production and manufacturing techniques to meet consumer demands. Therefore, the use of technologies such as nanotechnology can have a significant impact on the meat industry by improving sensory acceptance, acting as an antimicrobial agent, and precisely delivering bioactive materials to the target (13, 24).

MATERIALS AND METHODS

Iron Oxide Nanoparticles: Iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$) with a purity of 99%, Iron(III) chloride hexahydrate ($FeCl_3$) with a purity of 98%, and Sodium hydroxide (NaOH) with a purity of 98% were used. Iron oxide nanoparticles were prepared by the precipitation method (19).

Synthesis of Fe_2O_3 nanoparticles: Fe_2O_3 nanoparticles were prepared using the deposition method according to (19).

Preparation of whey proteins membrane and electrospun nanofibers: Prepare the membrane solution according to the method described previously (30) Using processed whey proteins from a company Bypro (USA).

Electrospinning process: In this method, 2 g of PVP K60 is dissolved in distilled water, and 0.06 g of Fe_2O_3 is added. The solvent is mixed by magnetic stirring at 100°C for two hours. To obtain a well-homogeneous solvent with good viscosity, the solvent is subjected to ultrasonic probe sonication for 30 minutes at a power of 70 W. After obtaining a highly homogeneous solution, it is injected into a syringe, and nanofibers are prepared by applying 15 kV voltage and a solution flow rate of about 50 μm/s for four hours (19). The nanofibers were prepared with two concentrations of 6% and 8% of iron oxide nanoparticles. The method described by (15) was followed for moisture, protein, fat, and ash analysis. The pH was determined according to the method described by (16). Peroxide value was estimated using the method provided by (15).

RESULTS AND DISCUSSION

Data in Table 1 shows the chemical analyses of the burger beef when iron oxide nanoparticles were added at concentrations of

6% and 8%. The results indicated significant differences between the burger patties treated with nanofibrous coatings compared to the control treatment during the storage periods. The protein (CP)precentage increased during the storage periods due to the decrease in moisture content. On the first day of both treatments, the cp precentage was 17.22% and 16.94%, respectively, while in the final period after three months, it was 19.09% and 19.31% when using nanofibrous packaging. In contrast, the protein content in the control treatment for both packaged and unpackaged patties was 17.12% and 17.36% in the initial period, respectively, and 19.41% and 19.28% in the final storage period. These results are consistent with the findings of (8, 9), where the protein precentage was 18.61% in refrigerated packaged beef burgers. The results also align with (25, 26), as cp precentage was higher in chicken meat coated with chitosan membranes compared to the non-coated control treatment, and this increase in protein precentage correlated with the decrease in moisture precentage in the different treatments.

Table 1. The chemical analyses (precentage) of frozen beef burger when adding coating with nanofiber biopolymers

Periods/treatment		*Moisture %*	*Protein%*	*Fat%*	ASH%
***hours* 24**	**Fe1**	**64.50 ± 0.26 A**	**17.22 ±0.19 h gf**	**16.43 ± 0.32 l**	**1.35 ± 0.06 bac**
hours24	**Fe2**	**63.96 ± 0.50 Ba**	**16.94 ± 0.20 gh**	**16.51 ± 0.44 l**	**1.78 ± 0.22 a**
Control 1		**63.93 ± 0.47 Ba**	**17.12 ± 0.38 gfh**	**17.18 ± 0.08 kl**	**1.28± 0.07 bc**
Control		**62.21± 0.27 c**	**17.36 ± 0.18 egdfh**	**18.38 ± 0.17 fhegi**	**1.47± 0.20 bac**
Month 1	**Fe1**	**62.17 ± 0.12 c**	**18.07 ± 0.22 egdfc**	**17.82 ± 0.15 khji**	**1.42 ± 0.08 bac**
Month1	**Fe2**	**62.008 ± 0.20 c**	**18.41 ± 0.38 bdac**	**17.56 ± 0.44 kji**	**1.43 ± 0.13 bac**
Control 1		**61.75 ± 0.44 dc**	**18.08 ± 0.29 egdfc**	**18.28 ± 0.21 fhjgi**	**1.249 ± 0.03 bc**
Control		**60.87± 0.06 fe**	**18.04± 0.06 egdfc**	**19.24 ± 0.09 cebd**	**1.37± 0.10 bac**
Month 2	**Fe1**	**60.40 ± 0.35 feg**	**18.80 ± 0.34 bac**	**18.74 ± 0.17 fhegd**	**1.42 ± 0.09 bac**
Month 2	**Fe2**	**61.008 ± 0.20 de**	**18.91 ± 0.36 bac**	**18.18 ± 0.45 hjgi**	**1.39 ± 0.013 bac**
Control 1		**60.25 ± 0.17 fheg**	**18.79 ± 0.25 bac**	**19.17 ± 0.10 fcebd**	**1.27± 0.06 bc**
Control		**59.84 ± 0.09 hg**	**18.39 ± 0.11 ebdac**	**19.60 ± 0.22 cbd**	**1.63± 0.06 ba**
Month 3	**Fe1**	**60.03 ± 0.01 fhg**	**19.31 ± 0.205 ab**	**19.16 ± 0.18 fcebd**	**1.34 ± 0.09 bac**
Month 3	**Fe2**	**60.12 ± 0.09 Fheg**	**19.09 ± 0.30 bac**	**19.31 ± 0.40 cebd**	**1.28± 0.12 bc**
Control 1		**59.38 ± 0.30 h**	**19.41 ± 0.23 a**	**19.76 ± 0.09 cb**	**1.33 ± 0.07 c**
Control		**57.43 ± 0.26 i**	**19.28 ± 0.32 ab**	**21.05 ± 0.33 a**	**1.68± 0.06 ba**

The averages, which bear different letters, differed significantly (0.05 & 0.01) among them,Fe1 6% addition, Fe2 8%, control treatment with coating Control 1, control treatment without coating

Also, Table 1 shows the percentage of moisture in frozen beef burgers. From the results, we can observe a decrease in moisture content in frozen burger patties with increasing storage period, with significant differences between the treatments. The highest moisture loss was observed in the control treatment without packaging, reaching 57.43% in the final storage period. On the other hand, the moisture percentage in nanofiber-wrapped patties was 60.03% and 60.12% in the final storage period. In the case of the control treatment with packaging, the moisture percentage was 59.38%. The decrease in moisture percentage can be attributed to the loss of free water from the unpackaged burger patties. Food packaging, especially for meat products, aims to reduce moisture loss from the food items. This has significant benefits in terms of controlling water transfer between the food material and the external environment, improving food quality, shelf life, minimizing food shrinkage, and reducing moisture loss, which can affect the physical and chemical properties of meat. The results of the study align with the findings of (20), who reported that packaging fish meat using shellac coatings contributed to reducing moisture loss. Furthermore, Table 1 demonstrates the fat content in beef burgers. From the results, we observe an increase in fat percentage in both the control treatment (unwrapped) and the control treatment with packaging, significantly higher than the nanofiber-wrapped burger patties. In the final storage period, the fat percentage in the control treatments was 21.05% and 19.76%, respectively, while it reached 19.16% and 19.31% in the nanofiber-wrapped burger. The higher fat content in the control treatments can be attributed to the high moisture loss. As the moisture loss was lower in the nanofiber-wrapped burger patties, the fat percentage was also lower compared to the other treatments. The results from Table 1 also indicate the effect of nanofiber packaging on the ash content in frozen beef burgers. The table demonstrates significant differences between the nanofiber-wrapped treatments and the control treatments. In the final storage period, the ash percentage was 1.34% and 1.28% in the nanofiber-wrapped treatments, respectively. On the other hand, the ash percentage in both control treatments during the final storage period was 1.33% and 1.68%, respectively, for the wrapped and unwrapped control treatments. The increase in ash content can be attributed to the decrease in moisture content during the storage period. These findings are consistent with the results of (12, 29), who observed an increase in ash percentage in the unwrapped control treatment, reaching 1.20%, while the ash content decreased to 1.10% when chicken meat was wrapped with chitosan coatings during storage.

Table 2 . The percentage Water holding capacity, Peroxide value and pH value of frozen beef burger when coating with nanofiber biopolymers

Periods/treatment		WHC	Peroxide	pH
hours 24	Fe1	48.85 ± 0.57 ba	3.35 ± 0.05 i	5.76 ± 0.02 ehdgf
hours 24	Fe2	49.16± 0.58 a	3.31 ± 0.12 i	5.78 ± 0.03 ebdcf
Control 1		48.70± 0.28 ba	4.93 ± 0.35 h	5.84 ± 0.02 Ba
Control		49.11 ± 0.26 a	5.66 ± 0.21 fg	5.84 ± 0.01 A
Month 1	Fe1	44.44 ± 0.18 d	4.90± 0.01 h	5.83 ± 0.02 bdac
Month 1	Fe2	46.26 ± 0.48 c	4.89 ± 0.01 h	5.80 ± 0.008 ebdac
Control 1		44.90± 0.11 d	6.02 ± 0.31 fe	5.78 ± 0.01 ebdacf
Control		44.24 ± 0.10 d	7.44 ± 0.33 c	5.77 ± 0.006 edgcf
Month 2	Fe1	41.61 ± 0.55 f	5.67 ± 0.03 fg	5.67 ± 0.03 kjl
Month 2	Fe2	42.87 ± 0.12 e	5.91 ± 0.01 f	5.75 ± 0.01 ehgf
Control 1		41.96 ± 0.27 fe	7.27 ± 0.35 dc	5.74 ± 0.01 ehgif
Control		40.99 ± 0.23 F	8.39 ± 0.32 b	5.70 ± 0.03 khjgi
Month 3	Fe1	40.16 ± 0.58 h	6.77 ± 0.01 dc	5.65 ± 0.02 Kl
Month 3	Fe2	39.69 ± 0.25 g	6.62 ± 0.03 de	5.70 ± 0.01 khji
Control 1		38.76± 0.65 hg	8.46 ± 0.60 b	5.68 ± 0.006 kjli
Control		36.36± 0.06 i	9.73 ± 0.32 a	5.63 ± 0.008 L

The averages, which bear different letters, differed significantly (0.05 & 0.01) among them,Fe1 6% addition, Fe2 8%, control treatment with coating Control 1, control treatment without coating

Results in Table **2** show the percentage Water holding capacity, Peroxide value and pH value of frozen beef burger. The results indicate the water-holding capacity of frozen burgers, showing significant differences between burgers coated with nanofibers and the control treatments. The water-holding capacity during the final storage period was found to be 49.16% and 39.69% for the coated burgers, while it was 38.76% and 36.36% for the control treatments. The significant difference between the treatments and the lower water-holding capacity in the control treatment can be attributed to high moisture loss during the storage period, leading to protein degradation and water loss throughout the storage period. Data in Table **2** shows the peroxide value in beef burgers. The results indicate significant differences between the fiber-coated burgers and the control treatments. The peroxide value during the final storage period was 6.77 and 6.62 milliequiv/kg for the coated treatments, while it was 8.46 and 9.73 milliequiv/kg for the control treatments. It is observed that the peroxide value remained within acceptable limits for the fiber treatments, while it increased in the control treatments. This can be attributed to the ability of the coatings to trap gases and factors that contribute to meat deterioration. These results are consistent with the findings of (2, 10), who reported a peroxide value of 5.20 milliequivalents/kg in meat pies coated and fortified with rosemary oil, compared to 22.33 milliequiv/kg in the control treatment during storage. Table 2 also presents the results of pH value for different treatments of frozen beef burgers. The results indicate significant differences between the coated and non-coated treatments. The pH value increased in the control treatment during the final storage period to 5.68 and 5.63, while it remained at 5.70 and 5.65 for the coated treatments. The increase in pH value may be attributed to the activity of protein-degrading enzymes or an increase in microbial activity. It could also be a result of the addition of packaging materials. These findings are consistent with (27), who observed that onion membrane-coated meat burgers maintained a significantly lower pH value during the storage period. Please note that the translation provided is a general interpretation of the text, and some scientific terminology may require further refinement or clarification based on the specific context and scientific field.

Table 3 . the microbial tests of frozen bovine peregrine when adding coating with nanofiber biopolymers

Periods/treatment		Total count CFU / g × 10^3	Psychrophilic bacteria CFU / g × 10^1	E.coli CFU / g × 10^3
hours 24	Fe1	6.22 ± 0.15 a	4.11 ± 0.07 bac	2.48 ± 0.08 dc
hours24	Fe2	5.65 ± 0.17 bc	4.05 ± 0.133 bac	2.39 ± 0.07 dc
Control1		5.60 ± 0.06 bcd	4.14 ± 0.21 ba	2.43 ± 0.14 dc
Control		5.78 ± 0.34 ba	4.30 ± 0.006 a	3.04 ± 0.01 ba
Month 1	Fe1	5.30 ± 0.09 fbecdg	3.59 ± 0.06 ebdghcf	2.28 ± 0.08 d
Month 1	Fe2	5.52 ± 0.14 becd	3.76 ± 0.15 ebdacf	2.28± 0.13 d
Control1		5.03± 0.19 fheg	3.21 ± 0.03 ghf	2.29 ± 0.13 d
Control		4.94 ± 0.13 fhg	3.82 ± 0.17 ebdac	3.34 ± 0.01 a
Month 2	Fe1	5.10 ± 0.02 fhecdg	3.39 ±0.09 edghf	2.19 ± 0.08 d
Month2	Fe2	5.08 ± 0.01 fhedg	3.78 ± 0.32 ebdacf	2.49 ± 0.24 dc
Control 1		4.98 ± 0.20 fheg	3.21 ± 0.03 ghf	3.37 ± 0.06 a
Control		4.78 ± 0.18 hig	3.82 ± 0.17 ebdac	3.11 ± 0.06 ba
Month 3	Fe1	5.04± 0.02 fhedg	3.15 ± 0.02 gh	2.15 ± 0.08 d
Month3	Fe2	4.76 ± 0.08 hig	3.64 ± 0.56 ebdgcf	2.49 ± 0.30 dc
Control 1		4.88 ± 0.25 hig	3.05 ± 0.03 h	3.26 ± 0.08 a
Control		4.62 ± 0.19 hi	3.55 ± 0.26 edghcf	3.01 ± 0.12 ba

The averages, which bear different letters, differed significantly (0.05 & 0.01) among them Fe1 6% addition, Fe2 8%, control treatment with coating Control 1, control treatment without coating

Results in Table **3** illustrate the total bacterial counts during the storage period of frozen beef burgers. The results from the table indicate the total aerobic bacterial counts in frozen beef burger. If the total bacterial counts increased in the control treatments during the storage period, reaching 4.88× 10^3 and 4.62× 10^3 CFU g-1 colony-forming units (CFU) per gram, respectively, while the bacterial counts in the packaged treatments reached 4.76× 10^3 and 5.04× 10^3 CFU/g, respectively. The decrease in the total bacterial counts can be attributed to the effect of nano membranes used for packaging and the freezing process, which reduces the microbial load in the meat. These findings align with the results obtained by (21), who found that the use of casein protein membranes led to a reduction in microbial load in chilled meat as compared to control treatments where microbial load increased. The results of (9, 1) demonstrated that the use of edible membranes had a high effectiveness in reducing beef spoilage and extending the storage period. Additionally, the membranes' properties were able to reduce light and oxygen exposure to the meat. Table 3 also shows the effect of nanofibers on psychrotrophic bacteria counts in frozen beef burger patties. The counts of psychrotrophic bacteria decreased during the storage period, reaching 3.15× 10^1 and 3.64 × 10^1CFU/g in the later stages of storage, while the control treatments reached 3.05× 10^1 and 3.55× 10^1 CFU/g, respectively. The membrane's ability to reduce gas and moisture permeability reflects on the chemical and microbial properties of the meat, thereby extending its storage duration (26). The results in Table 3 also demonstrate the effect of nanomembrane packaging on coliform bacteria counts in frozen beef burger. The results showed a significant decrease in coliform bacteria counts in the packaged treatments compared to the control treatments. The counts in the packaged treatments were 2.15× 10^3 and 2.49× 10^3 CFU/g, respectively, while in the control treatments, they were 3.26× 10^3 and 3.01× 10^3 CFU/g, respectively. The decrease in coliform bacteria counts in the packaged beef patties can be attributed to the high efficacy of the membranes and their inhibitory properties against microbial contaminants, along with the freezing process, which inhibits microbial growth. These results align with the findings of (21), who reported that the use of reinforced casein membranes contributed to reducing microbial growth in meat under refrigeration.

Conclusions

Based on the aforementioned study results, it can be concluded that using nanofibers of iron oxide and incorporating them into edible casein protein membranes for packaging frozen beef burgers helps preserve their chemical, physical, and sensory properties during a storage period of three months without significant undesirable changes. Therefore, we recommend utilizing nanofibers in packaging and employing membranes for packaging other meat products.

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