USING OF ELECTROSPUN CHROMIUM OXIDE NANPFIBER TO INCREASE THE SHELF LIFE OF FROZEN BEEF BURGER

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ABSTRACT

Electrospun Whey protein membranes reinforced with electrospun chromium oxide nanofibers were utilized for the packaging of beef burger, which were subsequently frozen and stored for 3 months. The chromium oxide nanofibers were incorporated into the membranes at two concentrations 6, 8 %. The study exhibited promising results for the electrospun nanofibers, as their addition contributed to reducing the total bacterial count, psychrophilic bacterial count, and coliform count during the storage period, at both concentrations: 4.89 × 10^3, 4.36 × 10^3, 3.28 × 10^1, 2.48 × 10^3, and 2.79 × 10^3 colony-forming units per gram (CFU/g), respectively. Meanwhile, the moisture, protein, fat, and ash percentage of the beef burger reached 60.19, 60.10, 18.41, and 18.49%, respectively, for both additive concentrations. The addition of nanofibers also improved water-holding capacity during storage, reaching 39.02 and 39.63% for the respective concentrations. Moreover, it helped maintain a stable pH level of 5.70 and 5.64 during the final storage period. Additionally, the inclusion of nanofibers ensured that the peroxide value remained within acceptable limits at 7.17 and 6.84 milliequivalents per kilogram, respectively.

Keywords: protein, meat processing ,WHC, food safety
Part on Ph.D.disseertaion of the 1st author

استعمال الالياف النانوية المغزولة كهربائيا لزيادة العمر الخزني للبيرغر البقري المجمد

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المستخلص

تم استعمال اغشية بروتينات الشرش المدعمة بألياف أوكسيد الكروم النانوية المغزولة كهربائيا في تغليف البيركر البقري وخزنت أقراص البيركر بالتجميد لمدة 3 أشهر. أضيفت الياف أوكسيد الكروم النانوية الى الاغشية بتركيزين 8,6 %. أظهرت الدراسة نتائج جيدة للألياف النانوية المغزولة كهربائيا اذ ساهمت إضافة الالياف النانوية الى خفض اعداد البكتيريا الكلية واعداد البكتيريا المحبة للبرودة كذلك اعداد بكتيريا القولون خلال فترة الخزن باستخدام كلا التركيزين 4.89 × 10^3 ,4.36× 10^3 , 3.28× 10^1, 2.48 × 10^3, 2.79 × 10^3 وحدة مكونة للمستعمرة لكل غم (CFU/g) على التوالي. في حين بلغت النسبة المئوية للرطوبة والبروتين والدهن والرماد لأقراص البيركر البقري 60.19, 60.10 , 18.41, 18.49 , 19.52 , 19.92 , 1.53, 1.35 % على التوالي لتركيزي الإضافة . ساهمت إضافة الالياف النانوية في تحسين قابلية حمل الماء خلال فترة الخزن 39.02 , 39.63 % على التوالي , كذلك ساهمت في الحفاظ على الاس الهيدروجيني pH 5.70 ,5.64 على التوالي خلال فترة الخزن الأخيرة, كذلك ساهم إضافة الالياف النانوية في الحفاظ على قيمة رقم البيروكسيد ضمن الحدود المقبولة 7.17 , 6.84 ملي مكافئ/كغم على التتابع.

الكلمات المفتاحية: بروتين، تصنيع اللحوم، قابلية حمل الماء ، سلامة الغذاء
جزء من أطروحة الدكتوراه للباحث الأول

Received:22/7/2023, Accepted:8/10/2023

INTRODUCTION

The primary objective of food packaging is to extend the shelf life of food during storage and transportation (7, 13). In this context, the concept of "shelf life" becomes crucial for a better understanding of food preservation. Shelf life refers to the period between the packaging after production and storing the food with approved specifications without exhibiting signs of spoilage under specific storage conditions. Consequently, the shelf life of food is closely related to the inherent characteristics of packaged foods, the environmental conditions during their transportation and storage, and most importantly, the quality of the packaging system used (17, 18, 22). The packaging and labeling sector has become an essential part of the global industry, accounting for 2% of the Gross National Product (GNP) in advanced countries (25,15). Various material systems have been developed and exploited to manufacture highly efficient food packaging materials. In recent years, particular attention has been given to the electrospinning technique for preparing nanoscale-structured or surface-functionalized food packaging materials using electrospun functional nanofibers (23). Progress in research and development of new packaging materials has been significant to meet the requirements of effective food protection against oxidation and microbial attacks (1, 11). Additionally, smart food packaging materials containing integrated or encapsulated sensory elements can indicate the freshness and characteristics of the food (25). Food packaging materials, besides the fundamental need for barrier function against moisture and oxygen, can be engineered to be active by incorporating functional components, such as antimicrobial nanoparticles, to deter microbes from the food (37). Over the past decade, electrospinning has also been exploited to prepare packaging materials to extend the shelf life of processed and raw foods, either using electrospun-produced packaging materials or blending them with other (biodegradable) polymers, such as cellulose and chitosan (6). The application of nanotechnology has emerged as an innovative alternative increasingly applied in the meat production chain to ensure extended storage life while enhancing food quality and safety (33, 40). The continuous increase in demand for meat products, intensified competition, and health concerns have led to the adoption of new and innovative methods in the meat industry (26, 39). Overall, the meat industry worldwide is focused on developing new productive and manufacturing methods to meet consumers' demands, making the use of technologies like nanotechnology potentially impactful in the meat industry by improving sensory acceptance, acting as antimicrobial agents, and accurately delivering active bioactive compounds to the target (30, 31).

MATERIALS AND METHODS

Chromium oxide nanoparticles:

All chemicals and reagents used were of synthetic grade and employed without further purification. In a typical procedure, 50 mL of molar chromium oxide ($Cr(NO3)3 \cdot 9H2O$) solution (0.2 M) was mixed with an appropriate amount of triethanolamine (C6H15NO3) as a template (20 and 30 mmol). After stirring for an hour, the mixture was microwave irradiated for 3 minutes. The resulting green solid product was centrifuged and air-dried at room temperature (8).

Preparation of whey proteins membrane and electrospun nanofibers:

Prepare the membrane solution according to the method described previously (18) Using processed whey proteins from a company Bypro (USA)

Electrospinning process:

Nanofibers are fibers with diameters in the nanometer range. Nanofibers can be produced from various polymers, giving them different physical properties and potential applications. There are several methods for preparing nanofibers, but the electrospinning method is considered more efficient and significant. 2 grams of PVP K60 were dissolved in distilled water, and 0.06 grams of Cr2O3 were added. The solvent was stirred at 100 degrees Celsius for two hours. To obtain a well-homogeneous solvent with good viscosity, the solvent was subjected to ultrasonic probing for 30 minutes at 70 dB. After achieving high homogeneity, the solvent was injected into a syringe, and the nanofibers were prepared by applying 15 kilovolts and a flow rate of approximately 50 micro-liters/second for 4 hours (21). The nanofibers were prepared with concentrations of 6% and 8% of chromium oxide nanoparticles. The pH was measured according to the method described by (14).Water Holding Capacity (WHC) was estimated by the method of (35). The (23) method was used for peroxide value determination.

RESULTS AND DISCUSSION

Table(1) illustrates the chemical analyses of beef burger when incorporating 6% and 8% concentrations of chromium oxide nanofibers. The results revealed significant ($p< 0.05$)differences between the burger treated with nanofiber coatings compared to the control treatment during the storage periods. The findings indicate a decrease in moisture percentage in frozen burger with the progression of the storage period, and significant differences were observed among the treatments. The control treatment without any coating showed the highest moisture loss, reaching 60.19% and 60.10%, respectively, during the final storage period. On the other hand, the coated and uncoated nanofiber-treated samples exhibited moisture losses of 59.38% and 57.43%, respectively. Food packaging, especially for meat products, aims to minimize moisture loss from the meat . Thus, the use of edible films for food packaging has shown essential benefits by controlling the transfer of water between the food material and the external environment. This improves food quality, shelf life, and reduces shrinkage and moisture loss, which can affect both the physical and chemical properties of the meat. The decrease in moisture percentage is attributed to the loss of free water from the uncoated burger . These results align with the findings of (21) who reported that using Whey protein coatings for fish meat packaging contributed to reducing moisture loss. The ability of nanofiber coatings to preserve moisture and minimize moisture loss in food products makes them a valuable option for enhancing food preservation during storage.

Table 1 Shows the chemical composition % of frozen beef burger when adding nanofibers

Period/ treatment		Moisture	protein	fat	Ash
hours 24	cr1	63.16 ± 0.06 b	16.58 ± 0.19 h	18.09 ± 0.44 khjgi	1.57± 0.23 bac
hours 24	cr2	63.74 ± 0.59 ba	16.52 ± 0.21 h	17.41 ± 0.33 kj	1.69 ± 0.21 ba
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	cr1	61.82 ± 0.13 dc	17.24 ± 0.03 egdfh	18.92 ± 0.23 fcegd	1.41 ± 0.14 bac
Month 1	cr2	62.17 ± 0.29 c	17.52 ± 0.52 egdfh	18.18 ± 0.41 hjgi	1.44± 0.21 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	cr1	60.24 ± 0.11 fheg	18.04 ± 0.19 egdfc	19.53 ± 0.16 cbd	1.56 ± 0.01 bac
Month2	cr2	60.61 ± 0.30 feg	18.15 ± 0.57 ebdfc	19.29± 0.18 cebd	1.37± 0.11 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
Month3	cr1	60.19 ± 0.04 fheg	18.41 ± 0.21 bdac	19.52 ± 0.08 cbd	1.53± 0.04 bac
Month3	cr2	60.10± 0.009 fheg	18.49 ± 0.72 bdac	19.92 ± 0.46 b	1.35 ± 0.13 bac
Control 1		59.38 ± 0.30 h	19.41 ± 0.23 a	19.76 ± 0.09 cb	1.13 ± 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,cr1 6% addition, cr 8%, control treatment with coating Control 1, control treatment without coating

Table (1), show observe the protein percentage in beef burger coated with nanofibers. The results indicate significant ($p< 0.05$) differences among the treatments. The protein percentage increased during the storage periods, attributed to the decrease in moisture percentage during storage. On the first day of storage, the protein percentage in the nanofiber-coated burger was 16.58% and 16.52%, respectively. However, during the final storage period, the protein percentage in the coated reached 18.41% and 18.49%, respectively. In contrast, the control treatment, both coated and uncoated, had protein percentages of 19.41% and 21.05%. These findings align with the results reported by Yaghoubi (36), where chicken meat coated with chitosan membranes exhibited higher protein percentage compared to the uncoated control. This increase in protein percentage was proportional to the decrease in moisture loss in different treatments. The results also correspond with the findings of (39), who reported a protein percentage of 19.0% when fish meat was coated with chitosan membranes, leading to increased protein levels. Furthermore, Table 1 illustrates the fat percentage in chromium oxide nanofiber-coated burger . The results indicate a significant increase in fat percentage during the storage period in the coated compared to the control treatments. During the final storage period, the fat percentage in the nanofiber-coated was 19.52% and 19.92%, respectively. In contrast, the fat percentage in the control-coated and uncoated was 19.76% and 21.05%, respectively. The increase in fat percentage during the storage period is attributed to the overall moisture reduction in the burger during storage.

Table 2 shows the chemical composition of frozen beef burger when nanofibers are added

period /	treatment	WHC%	PH
Hours 24	cr1	47.90 ± 0.31 b	5.83 ± 0.02 bac
Hours 24	cr2	48.84 ± 0.37 ba	5.80 ± 0.01 ebdac
Control 1		48.70± 0.28 ba	5.84 ± 0.02 ba
Control		49.11 ± 0.26 a	5.83 ± 0.01 a
Month 1	cr1	44.74 ± 0.44 d	5.76± 0.006 ehdgf
Month 1	cr2	44.32± 0.28 d	5.74 ± 0.006 ehgif
Control 1		44.90± 0.11 d	5.78 ± 0.01 ebdacf
Control		44.24 ± 0.10 d	5.77 ± 0.006 edgcf
Month 2	cr1	42.16 ± 0.31 fe	5.72 ± 0.01 hjgif
Month 2	cr2	41.80 ± 0.33 fe	5.70 ± 0.021 khji
Control 1		41.96 ± 0.27 fe	5.74 ± 0.01 ehgif
Control		40.99 ± 0.23 f	5.70 ± 0.03 khjgi
Month 3	cr1	39.02 ± 0.50 g	5.70 ± 0.03 khjgi
Month 3	cr2	39.63 ± 0.51 g	5.64 ± 0.003 kl
Control 1		38.76± 0.65 hg	5.68 ± 0.006 kjli
Control		36.36± 0.06 i	5.63 ± 0.008 l

The averages, which bear different letters, differed significantly (0.05 & 0.01) among them,cr1 6% addition, cr 8%, control treatment with coating Control 1, control treatment without coating

Table 2 presents the chemical composition of burger treated with nanofiber membranes. The results indicate water-holding capacity in frozen burgers, showing significant differences between that nanofiber-coated burger and the control treatments during storage periods. In the initial storage period, the water-holding capacity was 47.90% and 48.84%, respectively, for both nanofiber treatments, while it was 48.70% and 49.11%, respectively, for the coated and uncoated control. As the storage period progressed, that water-holding capacity decreased significantly, reaching 39.02% and 39.63% in the nanofiber-coated, and 38.76% and 36.36% in the coated and uncoated control, respectively. The nanofiber membranes likely played a role in protecting the cell membranes from damage, preserving proteins from degradation, and reducing water loss from the burger by maintaining water association with proteins. Alternatively, the increase in pH due to the addition of nanofiber-reinforced membranes may have enhanced the meat's ability to retain water (3). The table also displays the pH values of the nanofiber-coated burger, showing significant ($p< 0.05$) differences between coated and uncoated treatments. In the initial storage period, the pH values were 5.83 and 5.80 for the nanofiber treatments and 5.84 and 5.83 for the control treatments, respectively. As the storage period progressed, the nanofiber-coated burger maintained their pH values were reaching 5.70 and 5.64, while the pH values in the control treatments increased to 5.68 and 5.63, respectively. The increase in pH in the control treatments may be due to protein degradation by enzymes in the burger during the storage period or could be attributed to the addition of packaging materials. These findings align with (36,39), who observed increased pH values in chicken meat samples during refrigerated storage for 12 days, suggesting that the enzymatic self-degradation of proteins is the main reason for the pH changes during refrigerated storage.

Table 3. Shows the peroxide value (mequival / kg) of frozen beef burger when nanofibers are added

period / treatment	Hours 24	Month 1	Month 2	Month 3
Cr1	3.42 ± 0.16 i	4.97 ± 0.009 h	5.22 ± 0.03 hg	7.17 ± 0.04 dc
Cr2	3.32 ± 0.05 i	4.87± 0.01 h	5.10 ± 0.04 hg	6.84± 0.01 dc
Cont1	4.93 ± 0.35 h	6.02 ± 0.31 fe	7.27 ± 0.35 dc	8.46 ± 0.60 b
Cont	5.66 ± 0.21 fg	7.44 ± 0.33 c	8.39 ± 0.32 b	9.73 ± 0.32 a

The averages, which bear different letters, differed significantly (0.05 & 0.01) among them,cr1 6% addition, cr 8%, control treatment with coating Control 1, control treatment without coating

Table (3) shows the results of peroxide value in nanofiber-reinforced coated beef burger. The results indicate significant ($p< 0.05$)differences between treatments as the storage period progresses. In the initial storage period, the peroxide value was 3.42 and 3.32 milliequivalents per kilogram (meq/kg) for the nanofiber-coated burger , while it was 4.93 and 5.66 meq/kg for both control treatments, respectively. As the storage period advanced, the peroxide value increased, but it remained within the required specifications for the nanofiber-coated burger, reaching 7.17 and 6.84 meq/kg, respectively. In contrast, the peroxide value reached 8.46 and 9.73 meq/kg in both control treatments, respectively. The ability of proteinaceous membranes to trap gases may have contributed to maintaining peroxide levels in the coated treatments, controlling oxidative factors in the meat. (5,10) pointed out that the shelf life of non-coated meat samples decreased to less than 5 days, compared to the coated models with mustard seed gum membranes, which extended the shelf life based on peroxide value within acceptable limits.

Table 4. shows the microbial Test of frozen beef burger when nanofibers were added

period	treatment	Total count CFU / g × 10^3	psychrophilic bacteria CFU / g × 10^1	E.coli CFU / g × 10^3
hours 24	cr1	5.46 ± 0.18 fbecd	4.10 ± 0.10 bac	2.36 ± 0.09 d
Hours24	cr2	5.51 ± 0.23 becd	4.09 ± 0.02 bac	2.56 ± 0.13 dc
Control 1		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	cr1	5.246± 0.10 fbecdg	3.56 ± 0.13 ebdghcf	2.29 ± 0.07 d
Month 1	cr2	4.89 ± 0.12 hig	3.88 ± 0.13 bdac	2.36 ± 0.02 d
Control 1		5.03± 0.19 fheg	3.76 ± 0.02 ebdacf	2.29 ± 0.13 d
Control		4.94 ± 0.13 fhg	4.03 ± 0.08 bac	3.34 ± 0.01 a
Month 2	cr1	5.06 ± 0.06 fhedg	3.34 ± 0.08 edghf	2.18 ± 0.08 d
Month 2	cr2	4.82 ± 0.20 hig	3.44 ± 0.03 edghf	2.54 ± 0.13 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	cr1	4.89 ± 0.19 hig	3.28 ±0.10 eghf	2.48 ± 0.21 dc
Month 3	cr2	4.36± 0.10 i	3.28 ±0.10 eghf	2.79 ± 0.10 bc
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,cr1 6% addition, cr 8%, control treatment with coating Control 1, control treatment without coating

Table (4) illustrates the microbial test of nanofiber-reinforced coated beef burger . The results indicate significant ($p < 0.05$)differences between treatments during the storage period. In the initial storage period, the total aerobic bacterial count was 5.46 and 5.51 colony-forming units per gram (CFU/g) for nanofiber-coated burger , while it was 5.60 and 5.60 CFU/g for both control treatments, respectively. In the final storage period, the total aerobic bacterial count decreased to 4.98 and 4.36 CFU/g for nanofiber-treated burger , whereas it was 4.88 and 4.62 CFU/g for both control treatments, respectively. The decrease in the total bacterial count can be attributed to the effectiveness of chromic nanofibers in inhibiting bacteria (12,9). These results are in agreement with previous studY by (9). Table 4 also shows the psychrophilicbacterial count in frozen beef burger treated with nanofiber coatings. In the initial storage period, the psychrophilic bacterial count was 4.10 and 4.09 CFU/g for nanofiber-treated burger , while it was 4.14 and 4.30 CFU/g for both control treatments, respectively. In the final storage period, the Psychrophiles bacterial count was 3.28 and 3.28 CFU/g for the nanofiber-coated , whereas it was 3.05 and 3.55 CFU/g for both control treatments, respectively. Studies by (33) indicated that the membranes' ability to reduce gas and moisture permeability in meat contributes to the biochemical and microbial properties, leading to extended storage duration. The decrease in psychrotrophic bacterial count during the storage period can be attributed to the coatings' ability to reduce meat exposure to light and their physical and barrier properties. These factors collectively help in reducing psychrotrophic bacterial counts (27).

Furthermore, Table 4 demonstrates the coliform bacterial count in frozen beef burger treated with nanofiber coatings. The results show significant differences between treatments during the storage period, with the coliform bacterial count being 2.48 and 2.79 CFU/g in the nanofiber-treated , while it was 3.26 and 3.01 CFU/g in both control treatments, respectively. The decrease in coliform bacterial count during the storage period in nanofiber-treated is attributed to the nanomaterials' role in inhibiting microbial enzymes, leading to increased production of reactive oxygen species (ROS), which damage pathogenic microorganisms (2,19).

Based on the results of the investigated characteristics in this study, we can infer that the utilization of edible chitosan-based nanofiber coatings with chromium oxide nanoparticles, used to package frozen beef burger and stored for a period of 3 months, demonstrated favorable outcomes in preserving the chemical, physical, and microbial characteristics throughout the storage period without encountering unacceptable changes in these treatment. Therefore, we recommend exploring the use of chromium oxide nanoparticles, at the same concentrations, in preserving other products as well as considering the use of other metallic materials to enhance edible coatings.

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