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Research Article

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The effect of active packaging hydrogel based on polyethyleneimine/polyacrylamide on the safety and shelf life of chilled fillet

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ABSTRACT

Chicken breast fillet was packaged with polyacrylamide (PAm) hydrogel supplemented with polyethylenimine (PEI) to investigate its effect on the shelf life and inhibition of pathogenic bacteria. Chicken fillet was divided into two categories the first contains 4 control groups packaged without hydrogel (non-contaminated and contaminated with each of S. Typhimurium, S. aureus and E. coli) and 4 groups like them packaged with hydrogel. Packaging with hydrogel extended the shelf life (according to aerobic plate count) of chicken fillet by 2 days more than the control. Packaging chicken breast with hydrogel significantly improved the overall sensory score as it kept the sensory score accepted two more days than the control one. In the same way, hydrogel packages significantly ($P<0.05$) decreased the count of each of S. Typhimurium, S. aureus and E. coli than the control one.

Key words: Active packaging, polyacrylamide hydrogel, Chicken fillet, shelf life, antimicrobial.

INTRODUCTION

Poultry have higher pathogenic and spoilage bacteria counts especially chicken than almost any other food[1]. pathogenic and spoilage microorganisms presence in poultry meat and its by-products is still of significant concern to suppliers, consumers, and public health officials worldwide and to international trade. Unless appropriate actions are taken, e.g. packaging, storage temperature, and transportation, the product can spoil in a relatively short time[2]. Diverse in consumer choices have directed to innovations and developments in new packaging technologies. Active packaging is one of unique technology for extending the shelf life of fresh, cooked and other meat products[3]. Antimicrobial packaging is a hopeful form of active packaging especially for meat products to avoid partial inactivation of the active compounds by meat constituents in case of using the antibacterial as sprays or dips[4]. Antimicrobial food packaging materials have to reduce the growth phase and extend the lag phase of microorganisms in order to extend shelf life and to maintain product quality and safety[5].

Those active packaging systems, which reduce the risks associated with foods[6]. The main advances in food packaging over the last two decades have been the development of new materials, combinations of materials, and containers with specific technical and economic benefits[7]. Most of these new materials are inactive technologies in that they act primarily as passive barriers from environment. However, many researches are directed to the development of packaging which actively contributes to the safety and preservation of foods[8]. Packaging material interacts directly with the food and its environment to improve shelf-life. Active packaging improves food safety include bioactive polymers and films against microorganisms by inhibit the growth of pathogenic and spoilage[9].

Antibacterial compounds incorporated with food packages extend the shelf life even when it was in indirect contact with the food[10].

A main reason cause of food spoilage is excess humidity. Soaking up moisture by using various absorbers or desiccants is very effective at improving food quality and extending shelf life by inhibiting microbial growth and moisture related degradation of texture and flavor[3]. Moisture drip absorber pads are commonly placed under packaged fresh meats, fish and poultry to absorb unsightly tissue drip exudates. It maintain aesthetic appeal by absorbing all visible moisture released from the meat during storage[11], thereby preventing discoloration of either the meat or the white foam tray[12]. Common superabsorbent polymers like starch, acrylate salts and carboxymethyl cellulose copolymers, which have a very strong affinity for water[13].

Hydrogel materials can be defined as polymeric matrices which are capable of absorbing water and swelling in aqueous solutions, thus forming a three-dimensional network structure. In the state of swelling, these hydrogels acquire elastic rubbery shape with a soft touch. The hydrogel itself has no antimicrobial activity[14].

Polyethylenimine (PEI) is a weekly basic, aliphatic, nontoxic synthetic polymer[15]. It is used as a common ingredient involved in microbicidal compositions in different formulations ranging from washing agents to packaging materials[15, 16]. The minimal inhibitory concentrations values and minimal lethal concentrations obtained for PEI were similar and ranged between 50 and 380 mg/l, trying the fungicidal and microbicidal activity of this compound. Antibiofilm activity was also proved for all the microorganisms causing severe lesion of the membrane and cell depolarization[17].

The aim of this study was to evaluate the effect of polyacrylamide hydrogel supplemented with polyethylenimine as absorbance packaging pad on the shelf life of chilled chicken breast fillet and its effect on pathogenic bacteria.

EXPERIMENTAL SECTION

1.1. Materials

2-Phenyl-2-oxazoline (PhOx, Aldrich, 99.3%), chloroform, dioxane, hydrochloric acid and diethylether were used as received. p-Toluenesulfonic acid (TsOH, Aldrich) was used without futher purification.

1.2. Synthesis of poly2-Phenyl-2-oxazoline(PPhOx)

The monomer was distilled at reduced pressure and collected in an protected flask (22.5 g, 150.5 mmol) and an initiator PhOx. TsOH (48 mg, 0.142 mmol) was added. After 3 freeze-thaw cycles, the protected flask was closed under reduced pressure. PhOx was polymerised at 150˚C for 48 hours. After cooling down, the contents of reactor consisted of a yellow transparent tough solid. The solid was dissolved in chloroform and the resulting very viscous solution was poured in 1 L of diethyl ether. The polymer precipitated and was collected. This procedure was repeated twice. The yield was close to 100% conversion.

1.3. Preparation of Linear Polyethyleneimine (LPEI)

A procedure for removing a portion of the pendent amide groups from the PPhOX by acid hydrolysis is provided. A PPhOX polymer (0.83 g, 0.043 mmol) was dissolved in 10 mL of dioxane in a 100-mL flask containing a magnetic stir bar and enclosed with a septum. 2M HCl (0.73 mL, 1.46 mmol) was added and the reaction temperature was raised to 90°C and maintained for 24 h. The PEI was isolated by precipitation into diethyl ether.

1.4. Blending Copolymerization of LPEI/Gelatin

PAm was prepared according to our previous work[18].LPEI and PAm were dissolved with equal amount in hot water, 80 ^{o}C. The mixture was mechanically stirred at 600 rpm for 30 min. The efficiency of glyceraldehydes 0.3 mol related to LPEI mole fraction, a potential food-grade cross-linking agent, was used to form polymer matrix hydrogel.

1.5. Water Uptake:

Water uptake of the crosslinked LPEI/gelatin blending copolymers was evaluated for granules. The seeds of blending copolymer were dried at 110°C under vacuum for 24 h and weighed to obtain the dry weights. To obtain fully-hydrated weights, copolymer granules was immersed in DI water at ambient temperature for 24 h, patted dry,

then weighed. The swelling percentage was calculated from the ratio of the increase in weight divided by the dry weight and expressed as a weight percent

1.6. Gel Permeation Chromatography (GPC)
GPC was measured on a Waters 510 HPLC pump with a Waters 410 StyragelTM HT column; column A: 103 Å and μ Differential Refractometer with a Waters column B: 103 Å + 104 Å combined column. N-methylpyrrolidone (NMP) as a eluent at 70˚C. Column C: eluent 85:15 v/v% THF/MeOH mixture at 30˚C and a 103 Å column and r.i. detector. Calibration by polystyrene standards.

1.7. Preparation of chicken fillet samples
Chicken fillet without skin were prepared in the laboratory from freshly slaughtered chicken obtained from local manufacture. Chicken fillet was divided into two categories the first contains 4 control groups packaged without hydrogel (non-contaminated andcontaminated with each of*S*. Typhimurium, *S. aureus* and *E. coli*) and 4 groups packaged with hydrogel (non-contaminated and contaminated with each of *S*. Typhimurium, *S. aureus* and *E. coli*).
S. aureus (ATCC 29213), *Salmonella* Typhimurium (ATCC 14028) and *Escherichia coli* (ATCC 8739) strains (acquired from the Department of Food hygiene, Animal Health Research Institute, Dokki, Giza) from frozen cultures were activated with two successive passes in 9 ml of triptych soy broth (TSB) (Oxoid) and incubated at 37^{o}C for 18 h. For each individual strain, 1 ml of the stock inoculums was added to 100 ml of TSB and incubated with shaking at 37^{o}C for 18 - 24 h, then further diluted to reach a final concentration of approximately 5 log cfu/mL (determined by plating on specific media). Then, 2.5 ml of the stock inoculum was added to 250 ml of sterilized saline to give final concentration of approximately 4 log CFU/mL in the dipping solution. Chicken fillet (previously tested to be free of concerned microorganisms) were inoculated by being placed for 20 s in the dipping solution followed by drying under a hood at least 20 min to allow attachment of bacteria[19].

All samples were stored at 4^{o} C and examined for sensory and bacteriological characteristics at zero, 2nd, 4th, 6th, 7th, and 8thdays. Samples examination ended by reaching aerobic plate count higher than log 5 cfu/g and/or coliforms count 100 MPN/g according to Egyptian Standards (2005) for chilled poultry and rabbits No. 1651.

1.8. Sensory examination
A five trained test panel evaluation of the samples was done for the color, odor and texture characteristics then the average was recorded as overall sensory score ranging from 5 = very good, 4 = good, 3= accepted, 2= dislike to 1= very dislike .

1.9. Microbiological examination
According to (APHA)[20] samples were homogenized with peptone water (Oxoid) (1:10). Ten-fold serial dilutions were prepared using peptone water for further bacterial counts. Aerobic plate count was done using pour plate technique onto plate count agar (Oxoid) and incubating at 35^{o} C for 48 h. Meanwhile, coliforms count was done by most probable number technique on lauryl tryptose broth (Oxoid) at 35^{o} C for 48h and confirmed on brilliant green bile (2%) broth (Oxoid) at 35^{o} C for 48h. contaminated samples were counted on selective media for each strain (Baird Parker for *S. aureus*, XLD for *S*. Typhimuriumand EMB, for *E. coli*) in duplicate.

1.10. Statistical analysis
A completely randomized design was applied. The experiment was conducted in triplicate repetitions. Data were analyzed by using the mixed procedure from SPSS software (release 20, IBM CO) after logarithmic transformation. Means were separated by T-test, and significance was tested at $\alpha = 0.05$.

RESULTS AND DISCUSSION

LPEI with Mn 5400 g/mol and PDI 2.1 exhibit nice water uptake properties when blended with PAm with 1200 % swelling ratio related to dry sample.

The swelling data displayed in Figure 1 highlight the LPEI/PAm has high equilibrium swelling ratio, a common characteristic found as superabsorbent hydrogels (SH). The main property of SHs is their capacity of absorbs hundred times its own weight of water.

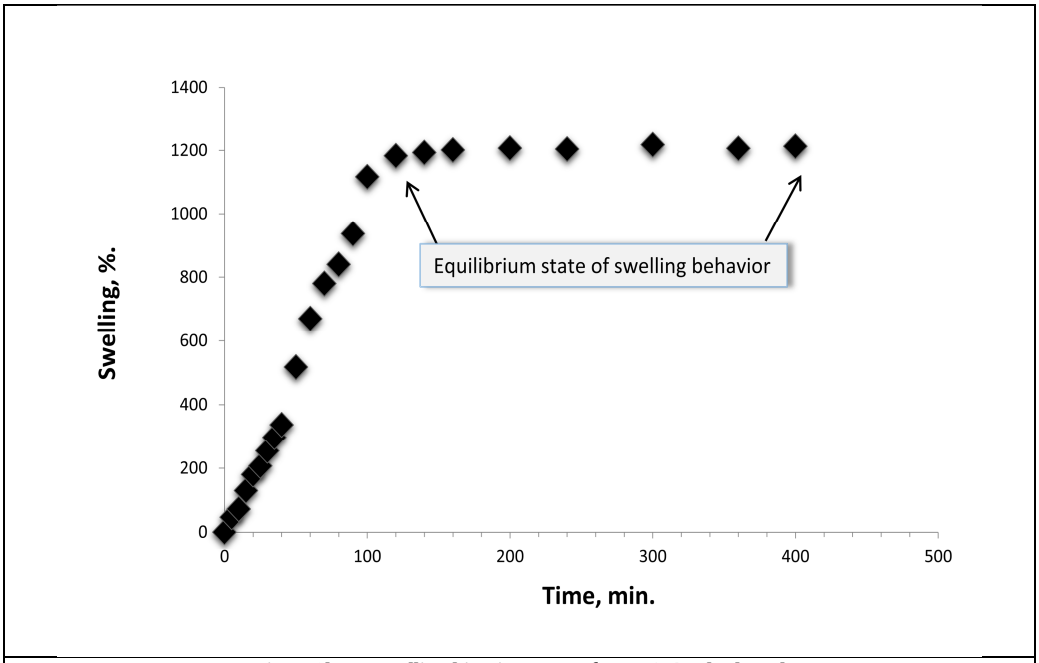

Fig. 1. Shows swelling kinetics curve of LPEI/PAm hydrogel

LPEI/PAm sample showed very similar swelling kinetics curves to superabsorbent hydrogel, where the equilibrium swelling is achieved fast and such equilibrium remains up to the assays end. The W values calculated for LPEI/PAm hydrogel at the equilibrium were 1218 g water to each g for hydrogel. The swelling kinetic curve of LPEI/PAm showed a quick increasing during the first 25 min of immersion, reaching about 90% of the equilibrium value followed by a slower process until the equilibrium being reached at about 30 min. The mechanism of swelling process of obeyed second-order kinetics model of swelling[21].

Table (1): Mean Overall sensory score of chicken breast during storage at 4° C

Days / Packaging	zero	2nd	4th	5th	6th	7th	8th
Control	5 ^{a}	4.5 ^{a}	4 ^{a}	3.5 ^{a}	3 ^{a}	-	-
Hydrogel	5 ^{a}	5 ^{b}	4.5 ^{b}	4.5 ^{b}	4 ^{b}	3.5	3

** Means having different letters in the same column is significantly differ (p<0.05)*

According to the aerobic plate count, the samples was deemed unfit by exceeding the log 5 cfu/g, nevertheless the overall sensory score still accepted to the end of the storage period in both groups (score 3).The overall sensory score of hydrogel packaging group was significantly higher (P<0.05) than control beginning from the 2nd day of storage. This difference in sensory score continued through the storage time giving the chicken breast packaged with hydrogel two more days than the control one. This effect can be attributed to the absorption of tissue drip exudate and visible moisture released from the meat during storage, thereby preventing meat discoloration[11, 12].

The initial aerobic plate count (APC) in chicken breast fillet was 3.3 log cfu/g (Fig.2), which was lower than that reported by de Melo *et al.*[22], but higher than that reported by Ibrahim and El-Khawas[23]. This count increased continuously in control samples until the 6th day of storage where it reached 5.3 (log cfu/g). On the other hand, APC significantly (P<0.05) decreased by the 2nd day of storage in hydrogel packaged fillet than control, then increased continuously during storage, but still significantly lower than control until the 8th day where it reached 5.4 (log cfu/g).

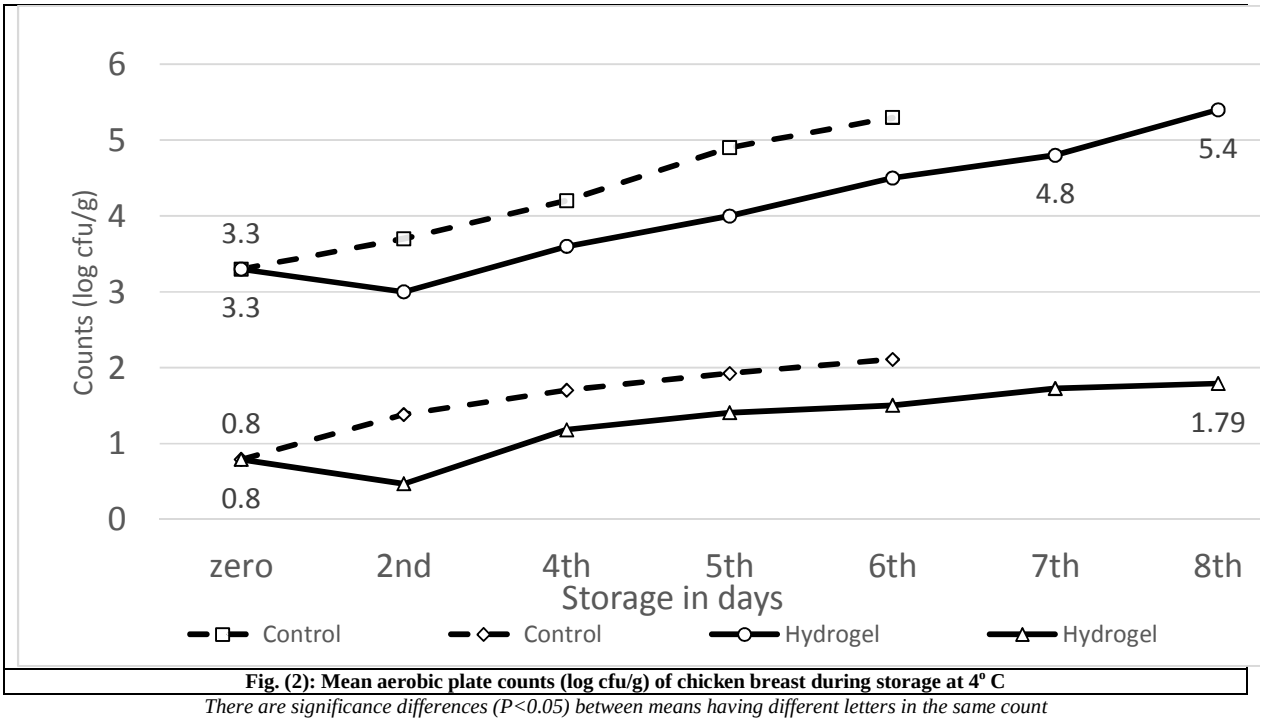

Fig. (2): Mean aerobic plate counts (log cfu/g) of chicken breast during storage at 4° C

There are significance differences (P<0.05) between means having different letters in the same count

The initial coliforms count was 0.79 log MPN/g, which was near that recorded by Ibrahim and El-Khawas[23], then continued to increase in control samples during storage to reach 2.11 log MPN/g by the 6th day. On the other hand, hydrogel packaged fillet significantly (P<0.05) decreased by the 2nd day of storage than control then continued to increase by storage to reach 1.79 log MPN/g by the 8th day, but still significantly lower than control.

The control of microorganism growth by antimicrobial agents can occur through a reduction in the growth rate, an increase in the lag phase or through direct inactivation by contact between the active agents and microorganisms[4]. Therefore, the treatment may have presented at least one of these roles to increase the storage period in treated samples than control. Regarding APC stated by the Egyptian standards[24] (ES No. 1651) packaging of chicken fillet with hydrogel extended the shelf life by two days more than the control. This effect of hydrogel supplemented with Polyethylenimineas antimicrobial may be attributed to inhibiting microbial growth and moisture related degradation of texture and flavor[3] due to absorbance of the drip into the hydrogel.

Fig. (3) illustrated the mean count of contaminated samples with tested pathogenic bacteria. Each of the inoculated bacteria slightly decreased throughout the storage period (about 0.1 - 0.2 log cfu/g) in the control groups. Similar results were recorded by previous studies[25-27]. This decrease can be attributed to the low storage temperature. Most food poisoning bacteria associated with meat are mesophiles, whose growth is prevented by refrigeration to below 5^{o} C[28].

For the hydrogel packaged groups, the count significantly (P<0.05) decreased than the control by the second day (about one log cfu/g), then continued significantly less than the control until the end of the storage time. This decrease could be attributed to the effect of drip absorbance by polyacrylamide hydrogel and neutralization by polyethylenimine as the moisture plays an important role in the attachment of bacteria[29].Besides that, moisture markedly increased the pathogen transference and contamination of the surrounding[26].

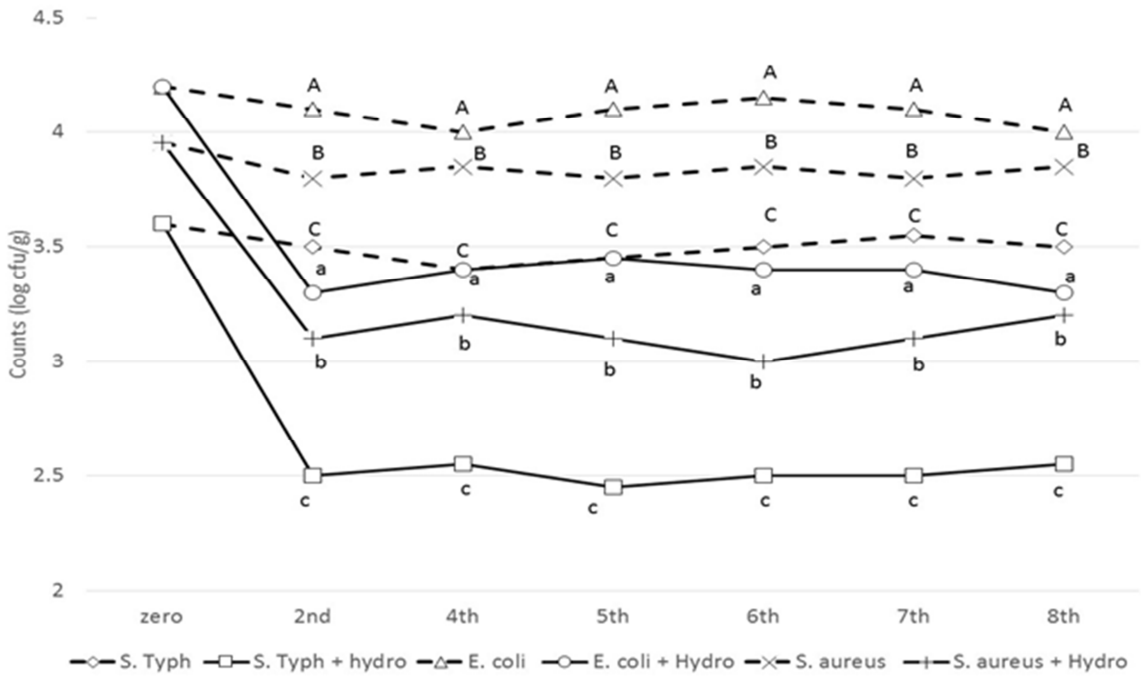

Fig. (3): Mean counts (log cfu/g) of food poisoning bacteria on chicken breast during storage at 4 °C

** There are significance differences (P<0.05) between means having the same capital and small letter*

CONCLUSION

Linear PEI was successfully synthesized as nontoxic antimicrobial polymer. In Addition, superabsorbent hydrogel based on PEI/PAm was prepared through crosslink matrices. Packaging chicken breast fillet with polyacrylamide hydrogel supplemented with Polyethylenimine, as absorbance pad, increased the shelf life by two days more than the control and significantly decreased the mean counts of pathogenic bacteria.

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