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Antimicrobial Effect for Both of Carboxy Methyl Cellulose and Chitosan Treated with Ferulic Acid or Nanosilver Particles as Edible Coatings used for Some Refrigerated Beef Samples

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ABSTRACT

Two edible coatings namely carboxy methyl cellulose (CMC) and chitosan were examined as edible coatings either alone or with addition of ferulic acid or silver nanoparticles for some refrigerated beef samples. During 12 days experiment, the microbiological quality was carried out by examining some spoilage and pathogenic bacteria. The examined bacteria were total viable count, *salmonella* and *shigella*, *Staphylococcus* spp. plus molds and yeasts. Obtained results showed that using of chitosan has better effect than CMC as an edible coating of meat. In addition, results showed that addition of ferulic acid to either CMC or to chitosan increased the reduction percent of all examined bacteria, and moreover, the use of silver nanoparticles with either CMC or chitosan increased the removing percent of examined bacteria. This results indicated that addition of silver nanoparticles was more efficient in removing of viable bacteria than ferulic acid with chitosan than that of CMC.

Keywords: CMC, chitosan, ferulic acid, silver nanoparticles, *salmonella* and *shigella, Staphylococcus* spp. molds and yeasts and meat.

INTRODUCTION

Edible Coatings act as barrier between food and the surrounding environment to improve the quality of food products protecting them from physical, chemical, and biological deterioration. Edible films and coatings offer some benefits such as edibility, biocompatibility, aesthetic appearance, barrier properties, Being non-toxic, non-polluting and having low cost (Han, 2000). Also, coatings and biofilms, by themselves or acting as carriers of foods additives (i.e.: antioxidants, antimicrobials), have been principally considered in food preservation because of their facility to extend the shelf life (Franssen and Krochta 2003)

Antimicrobial or antioxidant compounds integrated into the polymer matrix may inhibit growth of spoilage and pathogenic microbes, interval of meat fat rancidity, discoloration prevention, and even improvement of the nutritional quality of coated foods Soliva-Fortuny, R. *et al*. (2012). Meanwhile, Darmadji and Izumimoto, (1994) reported that higher concentrations (0.1%) were required to inhibit *E.coli* growth.

Among noble-metal nanomaterial's, silver nanoparticles (SNPs) have received considerable attentions owed to their attractive antimicrobial properties (Rai *et al*., 2009).

Silver compounds have been used to treat burns, wounds and infections. Various salts of silver and their derivatives are used as antimicrobial agents (Ip et al, 2006). Contemporary studies have informed that nanosized silver particles reveal antimicrobial properties (Petica *et al*. 2008, Rai *et al.* 2009). Nanoparticles of silver have been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating ingredients (Kim *et al.* 2007, Ruparelia *et al.* 2006). Accordingly, this study was conducted to investigate the ability of using two kinds of edible coating namely carboxy methyl cellulose and chitosan without additive or incorporated with ferulic acid or silver nanoparticles against some spoilage and pathogenic microbes.

MATERIALS AND METHODS

Materials:

Carboxy methyl cellulose with an average molecular weight of 41.000 g , medium viscosity 400-800 cp (practical grade), and glycerol were obtained from El-Gomhouria Company, Cairo, Egypt. Chitosan extracted from crab shells was obtained from *Roth*, Germany. Starch was purchased from *Pio Chem* Cairo, Egypt, acetic acid glacial (100%), from *Tedia Company*, USA. Ferulic acid (95%) was purchased from Sigma Company. Nanosilver particle was purchased from Bavaria Company, Germany.

Malt extract media was obtained from Qualikems Company, India, MacConkey agar No.3 and Plate Count Agar were purchased from LAB M Limited, United Kingdom. Agar S. S. was obtained from BioCen, Cuba. Baired parker agar base was purchased from Titan media Company, India.

Beef samples:

Beef sirloin retail cuts were obtained from a private shop in a local market in Mansoura city immediately after slaughtering. Then transferred in an ice box to the laboratory.

Methods:

Treatment was applied except the control sample (T1) as uncoated, samples were coated with CMC edible coating , CMC +Ferulic acid and CMC+ nanosilver particles ,respectively, as T2, T3 and T4.chitosan as edible coating used for the rest of samples, chitosan + ferulic acid and chitosan + nanosilver particles as T5,T6 and T7 respectively. All samples were refrigerated at 4±1°C, The microbial pollution on the treated samples was determined at zero time and every 96 hrs.

Preparation of CMC coatings solutions:

With an average molecular weight of 41.000 g/mol , medium viscosity of 400-800 cp and glycerol as plastizer ,CMC (1g) was dissolved in 100 ml distilled water , glycerol was added (1 ml) and solution was stirred for another 15 min.Ferulic acid was added at concentration of 100 p.p.m to coating solution. Nanosilver particles 40 nm

at concentration of 50 p.p.m (Bavaria Company Germany) was added to coating solution.

Preparation of chitosan coating solutions

Chitosan (1% w/w) was dispersed in 2 % (w/w) acetic acid glacial solution under gentle stirring. Starch 1% was added. Glycerol as plastizer was added at a concentration of 1 % (w/w) María B. Vásconez*et et al*, (2009). Ferulic acid was added at concentration of 100 p.p.m to coating solution. Silver nanoparticles (40 nm) at concentration of 50 p.p.m was added to coating solution.

Microbiological examination:

Sample preparation.

Ten grams of each sample were added to 90 ml of saline solution and mixed thoroughly to give 1/10 dilution. Serial dilutions were prepared to be used for microbiological examination using different cultivation media.

Total bacterial count:

The total bacterial counts were determined using the plate counts technique on a nutrient agar medium according to the procedures described in Merck, (2000). The plates were incubated at 37ºC for 48 hrs.

Spore- forming mesophilic bacterial count:

The previous dilutions were heated to 85˚C for 15 min before being plated on the same previous medium for aerobic bacterial counts (tryptone glucose yeast extract agar). Plates were incubated at 37˚C for 24 and 48 h according to the method described in Merck, (2000).

Psychrophilic bacterial count:

Psychrophilic bacterial count was carried out as described in typical procedure of the total plate bacterial count methods, except, incubation was achieved at 8ºC for 5 days according to Merck, (2000).

Coliform bacterial count:

The coliform bacteria were detected using McConkey agar medium according to the procedures described in Merck, (2000). The plates were incubated at 37ºC for 24 hrs.

Detection of Salmonella and Shigella:

The presence or absence of Salmonella and Shigella was detected according to the methods described by FAO, (2013).

Staphylococcus* spp. *count:

Staphylococcus spp. were detected according to the method described in Merck, (2000). Using 5 ml of egg yolk tellurite emulsion to each 100 ml of sterilized medium which mixed well before pouring in the plates. The plates were incubated at 37ºC for 24 or 48 hr.

Yeasts and molds:

The procedures of Merck, (2000) were followed for the examination of yeast and mold counts using potato-dextrose agar medium. The plates were incubated at 28ºC for 5 days. When excessive growth develops, colonies counted after 3 days and reported as yeasts and molds count per gram of examined sample.

RESULTS AND DISCUSSION

Results in Tab. 1 showing decrease in the total viable bacterial count in a percent of 38.56 , 2.69 and 23.31 in case of T1 (control) the examined sample without coating after 4 , 8 and 12 days of refrigeration periods , respectively.

In case of using CMC alone (T2) , the count of total viable bacterial count decreased from 223 cfu ×103 /g to 92 cfu ×103 /g of examined sample in a percent of 58.74 while this percent was 5.38 and 50.67 after 8 and 12 days , respectively. This decreasing percent became lower when using CMC (T3) with ferulic acid to be 67 cfu ×103 /g, 219 and 103 cfu ×103 /g of examined sample expressed in 69.95, 1.79 and 53.81 %, respectively.

Tab. 1. Effect of treated CMC and Chitosan edible coatings with or without ferulic acid or nanosilver particles on total viable bacterial count on some refrigerated beef sample:

	Total viable bacterial count , cfu×10^3/g examined sample			
Refrigeration period (day) Treatment (T)	**0**	**4**	**8**	**12**
T1	223	137	217	171
T2	223	92	211	110
T3	223	67	219	103
T4	223	58	169	70
T5	223	28	130	13
T6	223	27	122	N.D
T7	223	N.D	72	N.D

T1: uncoated **T2: CMC**
T3: CMC + Ferulic acid **T4: CMC + Nanosilver particles**
T5: Chitosan **T6: Chitosan + Ferulic acid**
T7: Chitosan + Nanosilver particles **N.D.: Not Detectable**

Results of T4 of CMC with silver nanoparticles, the decreasing percent became 73.99, 24.21 and 68.60 % after 4, 8 and 12 days, respectively.

These results indicated that using of the silver nanoparticles achieved superiority of bacterial decreasing than ferulic acid with CMC since the decreasing percent were 69.95 and 53.81 % in case of ferulic acid while these percent were 73.99 and 68.60 % in case of silver nanoparticles after 4 and 12 days.

Results of chitosan (T5), showing that after 4 days of refrigeration the total viable bacterial count decreased by 87.44% while 41.70 % was found after 8 days. After 12 days of refrigeration, the decreasing percent was 94.17% in case of using chitosan alone. These percent were 87.89%, 45.29 % after 4 and 8 days, respectively, when using silver nanoparticles the decrease percent was 67.71% after 8 days of refrigeration. Not detectable results were noticed in case of using ferulic (T6) acid after 12 days and when using silver nanoparticles (T7) after 4 and 12 days. Again, this means that silver nanoparticles was more efficient in removing viable bacteria than ferulic acid with chitosan.

Sharifi, *et al.* (2012) showed that Ag-Nps have antimicrobial activity and it also have a great effect on total bacterial count reduction as a new generation of antimicrobial. Shahverdi, *et al.* (2007) reported that silver nanoparticles has an antibacterial activity against Gram-positive and Gram-negative bacteria.

Concerning storage for 8 days, the microorganisms count was increased. This increase was not reasonable.

Detection of spore forming bacteria:

Examining spore forming bacteria , Tab. 2 showed that using of CMC (T1) as an edible coating decreased their count from 182 cfu $\times 10^3$ /g to 123 cfu $\times 10^3$ /g of examined sample (T2) in percent of 32.41% after 8 days of refrigeration. This decrease percent was 54.87% after 12 days.

Tab. 2. Effect of treated CMC and Chitosan edible coatings with or without ferulic acid or nanosilver particles on Spore forming bacteria on some refrigerated beef sample:

	Spore forming bacteria , cfu$\times 10^3$/g examined sample			
Refrigeration period(day) Treatment (T)	**0**	**4**	**8**	**12**
T1	97	30	182	109
T2	97	31	123	59
T3	97	9	141	35
T4	97	5	8	12
T5	97	2	7	N.D
T6	97	N.D	3	N.D
T7	97	N.D	N.D	N.D

T1- T7 see Tab. 1

The removing power of spore forming bacteria was increased by adding ferulic acid and silver nanoparticles to be 90.72, 94.84 % and 63.91, 87.62 % after 4 and 12 days, respectively.

The bacterial removing was also increased when using chitosan either alone (T5) or after adding ferulic acid (T6) or silver nanoparticles (T7) as shown as in Tab. 2. Results showed that chitosan decreased the count of spore formers from 97 cfu $\times 10^3$ /g to 2.0 cfu $\times 10^3$ /g of examined sample (97.93 %) and to 7.0 cfu $\times 10^3$ /g of examined sample (92.78 %) after 4 and 8 days of refrigeration, respectively.

Not detectable results was found after 12 days of refrigeration. In case of addition of ferulic to chitosan , the removing of spore formers is increased to be N.D. , 3.0 cfu $\times 10^3$ /g of examined sample and not detectable after 4 , 8 and 12 days of refrigeration , respectively.

The efficient of bacterial removal was also increased when using silver nanoparticles with chitosan being not detectable numbers in the three examined times 4, 8 and 12. This means that the silver nanoparticles was more efficient than ferulic acid in bacterial removal.

Dave and Ghaly (2011) reported that pathogenic microbes do not survive through meat cooking but several of their toxins and spores do.

Detection of coliform bacteria:

For coliform bacteria, results in Tab. 3 showed that both CMC and chitosan are good edible coating either alone or with addition of ferulic acid or silver nanoparticles. In case of using CMC alone (T2) the removing of coliform bacteria were 66.4, 84.8 and 30.4 % after 4, 8 and 12 days, respectively. These percent were 84, 87.2 and 83.2% when using CMC with silver nanoparticles (T4) after 4, 8 and 12 days, respectively.

Tab. 3. Effect of treated CMC and Chitosan edible coatings with or without Ferulic acid or Nanosilver particles on coliform bacteria on some refrigerated beef sample:

	Coliform bacteria cfu$\times 10^3$/g examined sample			
Refrigeration period (day) Treatment (T)	**0**	**4**	**8**	**12**
T1	125	105	27	154
T2	125	42	19	87
T3	125	23	17	28
T4	125	20	16	21
T5	125	11	15	24
T6	125	7	11	9
T7	125	6	8	2

T1- T7 see Tab.1

The same trend of obtained results was found in case of using chitosan. The reduction percent of coliform bacteria were 91.2, 94.4 and 95.2 % after the 4th day in case of using chitosan alone T5, T6 and T7, respectively, these reduction percent were found to be 88, 91.2 and 93.6 % after the 8th day and 80.8, 92.8 and 98.4 % after the 12th day, respectively. These results indicated that the chitosan was better than CMC as an edible coating either alone or after adding either ferulic acid or silver nanoparticles.

This result is in agreement with those obtained by Wen-Ru, et al. (2010) who found that using of silver nanoparticles delayed the growth of *E.coli*. They also found that the activity of respiratory chain dehydrogenase of *E.coli* could be whibited by silver nanoparticles.

Detection of *Staphylococcus* spp.:

Regarding *Staphylococcus* spp. Results at zero time for all treatments and content gave positive detection while all treatments gave not detectable results after 4, 8 and 12 days of refrigeration. This means that examined meat samples were free of *Staphylococcus* spp. During all examined periods as shown in Tab.4.

Tab. 4. Effect of treated CMC and Chitosan edible coatings with or without ferulic acid or nanosilver particles on *staphylococcus* spp. on some refrigerated beef sample:

	***Staphylococcus* spp. cfu $\times 10^3$ /g examined sample**			
Refrigeration period (day) Treatment (T)	**0**	**4**	**8**	**12**
T1	+	-	-	-
T2	+	-	-	-
T3	+	-	-	-
T4	+	-	-	-
T5	+	-	-	-
T6	+	-	-	-
T7	+	-	-	-

T1 – T7 see Tab. 1

Irais et al. (2014) reported that red meat involved in outbreak due to the presence of *staphylococcus* spp.

Detection of Mold and Yeast:

Regarding ,using of CMC as an edible coating showed removing of molds and yeasts count and decreased from 106 cfu $\times 10^3$ /g of uncoated sample to 102 cfu $\times 10^3$

/g after 4 days of refrigeration. These decreases were observed more with addition of either ferulic acid or silver nanoparticles. In case of ferulic acid (T3), the decreasing percent, were 46.89, 55.36 and 91.52 % after 4, 8 and 12 days, respectively. These values were found to be 61.01, 59.88% and N.D when using silver nanoparticles with CMC (T4) as shown in Tab. 5.

Tab. 5. Effect of treated CMC and Chitosan edible coatings with or without ferulic acid or nanosilver particles on mold and yeast on some refrigerated beef sample:

	Mold & Yeast cfu $\times 10^3$ /g examined sample			
Refrigeration period (day) Treatment (T)	**0**	**4**	**8**	**12**
T1	177	106	113	31
T2	177	102	98	17
T3	177	94	79	15
T4	177	69	71	N.D
T5	177	N.D	64	N.D
T6	177	N.D	25	N.D
T7	177	N.D	19	N.D

T1- T7 see Tab.1

Using chitosan either alone or with addition of ferulic acid or silver nanoparticles, were the superior of this edible coating than CMC. Not detectable results were found after 4 and 12 days of refrigeration. After 8 days of refrigeration , the count of mold and yeasts decreased from 177 cfu $\times 10^3$ /g to 25 cfu $\times 10^3$ /g and from 177 to 19 cfu $\times 10^3$ /g of examined sample T6 and T7 , respectively. While T5 decreased from 177 cfu $\times 10^3$ /g to 64 cfu $\times 10^3$ /g.

Again, this means that the chitosan was more efficient than CMC in molds and yeasts removing either alone or with addition of ferulic acid or silver nanoparticles.

Detection of psycrophilic bacteria:

Psycrophilic bacteria, were not detected in all treatments except T3 after 8 days of refrigeration giving 10 cfu $\times 10^3$ /g of examined samples. The treatment of T6 of using chitosan with ferulic acid recorded 18 cfu $\times 10^3$ /g of examined samples. When using silver nanoparticles with chitosan after 8 days gave 3 cfu $\times 10^3$ /g of examined samples. Silver nanoparticles was more sufficient in psycrophilic bacteria than ferulic acid with CMC and chitosan as shown in Tab. 6.

Tab. 6. Effect of treated CMC and Chitosan edible coatings with or without ferulic acid or nanosilver particles on psychrophilic bacteria on some refrigerated beef sample:

	Psychrophilic bacteria , cfu $\times 10^3$ /g examined sample			
Refrigeration period (day) Treatment (T)	**0**	**4**	**8**	**12**
T1	N.D	N.D	N.D	N.D
T2	N.D	N.D	N.D	N.D
T3	N.D	N.D	10	N.D
T4	N.D	N.D	N.D	N.D
T5	N.D	N.D	N.D	N.D
T6	N.D	N.D	18	N.D
T7	N.D	N.D	3	N.D

T1-T7 see Tab.1

Detection of *Salmonella* and *Shigella*:

Examining *Salmonella* spp. and *Shigella* spp. in examined meat samples showing that control sample (T1) showed positive detection after 8 days of refrigeration while not detectable results were found with 0.0, 4 and 12 day. In case of using CMC with silver nanoparticles, results showed positive detection after 4 days of refrigeration while the rest of all treatments gave not detectable results. This means that the examined meat samples were free of both *Salmonella* spp. and *Shigella* spp. as shown in Tab.7.

Tab.7. Effect of treated CMC and Chitosan edible coatings with or without ferulic acid or nanosilver particles on salmonella and shigella on some refrigerated beef sample:

	Salmonella and shigella , cfu $\times 10^3$ /g examined sample			
Refrigeration period (day) Treatment (T)	**0**	**4**	**8**	**12**
T1	-	-	+	-
T2	-	-	-	-
T3	-	-	-	-
T4	-	+	-	-
T5	-	-	-	-
T6	-	-	-	-
T7	-	-	-	-

T1-T7 see Tab.1

Irais et al. (2014) found that red meat in frequently involved in outbreaks mainly due to the presence of *salmonella* spp. and *listeria* spp.

Finally**,** from obtained results, it could be concluded that chitosan either alone or with incorporated with silver nanoparticles or ferulic acid were the superior against spoilage and pathogenic microbes more than CMC alone or with the addition of ferulic acid or silver nanoparticles.

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تأثير النشاط المضاد للميكروبات لكل من كربوكسي ميثيل سليلوز و الشيتوزان المعامل بحمض الفيروليك أو جزيئات الفضة المتناهية الصغر كمغلف غذائي لبعض عينات اللحوم المبردة

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تم اختبار نوعين من الاغلفة الغذائية و هما الكربوكسي ميثيل سليلوز (CMC) و الشيتوزان سواء بمفردهما أو باضافة حمض الفيروليك أو الجزيئات متناهية الصغر من الفضة و ذلك لتغليف عينات من اللحوم الحمراء تحت ظروف التبريد 4±1°م. تم دراسة الجودة الميكروبيولوجية خلال 12 يوم لعينات اللحم تحت الدراسة من خلال اجراء تعداد لبعض انواع البكتيريا الملوثة للأغذية و الممرضة للانسان مثل العد البكتيري الكلي , و البكتيريا المتجرثمة , البكتيريا المحبة للحرارة المنخفضة , بكتيريا القولون , السالمونيلا و الشيجيلا , استافيلوكوكس و كذلك الفطريات و الخمائر. أوضحت النتائج المتحصل عليها أن الشيتوزان كان أفضل من الـ CMC كمغلف غذائي للحوم. علاوة علي ذلك بينت النتائج أن اضافة حمض الفيروليك الي CMC أو الشيتوزان عمل علي زيادة النسبة المئوية لاختزال الميكروبات تحت الدراسة فى عينات اللحوم. بالاضافة الي ذلك كانت اضافة جزيئات الفضة متناهية الصغر سواء مع CMC او الشيتوزان عمل علي زيادة في النسبة المئوية لازالة لكل المجموعات البكتيرية تحت الدراسة. و قد دلت هذه الدراسة علي إضافة جزيئات الفضة متناهية الصغر كانت أكثر كفاءة فى ازالة البكتيريا الحية أكثر من حمض الفيروليك سواء مع الشيتوزان كمغلف غذائي للحوم تحت ظروف التبريد أكثر من CMC.