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Influence of $Zn2^{+}$, Sodium Bicarbonate, and Citric Acid on the Antibacterial Activity of Ovotransferrin against *E. coli* O157:H7 and *L. monocytogenes* in Model Systems and Ham

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Summary and Implications

The antibacterial activity of natural apo-ovotransferrin against *E. coli* O157:H7 and *L. monocytogenes* in model systems increased as the concentration of sodium bicarbonate increased. $NaHCO_3$ at 100 mM markedly increased antibacterial activity of ovotransferrin against *E. coli* O157:H7 and *L. monocytogenes*. Citric acid at 0.5% enhanced antibacterial activity of apo-ovotransferrin against *E. coli* O157:H7, but 0.5% citric acid alone also showed a strong bactericidal activity against *L. monocytogenes.* Addition of $NaHCO_3$ negated the strong antibacterial activity of ovotransferrin plus citric acid against the two pathogens. The antimicrobial activity of ovotransferrin was greatly enhanced by acidic pH conditions. Zn-bound ovotransferrin produced a bacteriostatic effect against *L. monocytogenes*, but Fe-bound ovotransferrin had little or no antibacterial activity against *E. coli* O157:H7 and *L. monocytogenes.* Considering these results, iron bind capacity of ovotransferrin is not the major cause of antibacterial action of ovotransferrin. Previous studies indicate that ovotransferrin directly interacts with bacterial membranes causing a variety of physiochemical changes which affect the survival of microorganisms. Ovotransferrin plus 100 mM $NaHCO_3$ did not exhibit any antibacterial activity against two pathogens in commercial hams, whereas ovotransferrin + 0.5% citric acid suppressed *L. monocytogenes* in irradiated hams but not in non-irradiated hams. There are some limitations of using ovotransferrin to control pathogens in meat or meat products. To overcome these problems, further studies are needed to determine the mechanisms of antibacterial activity of ovotransferrin and to identify various factors that can improve the antibacterial activity of ovotransferrin.

Introduction

Ovotransferrin is the second major avian egg white protein constituting 12% of total egg white proteins and contributes to the egg's defense against microbial infection and rotting. Ovotransferrin has demonstrated antibacterial activity against a wide spectrum of bacteria. The antimicrobial activity of ovotransferrin is influenced by several factors such as bicarbonate and citrate concentrations, pH conditions, composition of medium or metal irons like $Zn2^{+}$ as well as bacterial species or strains. Bicarbonate, known as a synergistic anion, is a prerequisite for the iron uptake by transferrin and lactoferrin because it inhibits the iron-chelating ability of citrate. The anion may serve as a bridging ligand between proteins and metal ions. An excess of citrate can negate the antimicrobial activity of ovotransferrin by chelating iron, and thus the iron binding capacity of ovotransferrin is affected by metal chelaters such as citrate. However, the effect of citrate on antibacterial activity of ovotransferrin depends on the strain of microorganism.

As ovotransferrin can bind metal ions such as Cu, Al, Zn, and Fe, its antimicrobial effect is logically expected to decrease by binding other metal ions. Generally, iron, copper, and zinc have been shown to bind to the same sites in the ovotransferrin molecules. However, ovotransferrin saturated with metals such as zinc and iron still displayed antimicrobial activities. $Zn2^{+}$-loaded ovotransferrin had more bactericidal activity than apo-ovotransferrin and other metal complexes, and the antimicrobial effect of Zn^{2+}-ovotransferrin was attributed to the direct interaction of Zn^{2+}-ovotransferrin with the surface of bacteria rather than iron depletion from the medium. However, other researchers reported that the antibacterial action of ovotransferrin is due to more complex mechanisms related to direct interactions between ovotransferrin and microorganisms in addition to iron depletion. Ovotransferrin has a great potential as a natural antimicrobial agent like lactoferrin. However, there is a scarcity of published reports on the use of ovotransferrin to control pathogenic bacteria in meat. To increase the potential of using ovotransferrin as an antibacterial agent in meat, methods to activate or improve antimicrobial capability of natural apo-ovotransferrin against foodborne bacteria is required. In the present study, the influence of bicarbonate and citric acid as synergistic anions on the antibacterial activity of apo-ovotransferrin was investigated. Also, viable ovotransferrin combined with bicarbonate and citric acid was applied to ham to determine the possibility of using ovotransferrin as a natural antimicrobial agent in meat processing.

Materials and Methods

Apo-ovotransferrin used in this study was produced by the method of Ko and Ahn. A five-strain mixture of *L. monocytogenes*, including strains H7962 serotype 4b, H7762 serotype 4b, H7969 serotype 4b, H7764 serotype ½ a, and Scott A NADC 2045 serotype 4b, or *E. coli* O157:H7 was used in this study. For each pathogen equal amounts of

each strain were combined to prepare a five-strain mixture of *L. monocytogenes* or *E. coli* O157:H7. Disc diffusion assays were performed using ovotransferrin (5, 10, 15, or 20 mg/ml) and $NaHCO_3$ (30, 40, or 50 mM) in combination to determine optimal inhibitory concentrations of ovotransferrin and bicarbonate against *E. coli* O157:H7 and *L. monocytogene*s. Ovotransferrin solution (20 mg/ml) of was used to determine the effect of sodium bicarbonate on antibacterial activity of ovotransferrin. Also, $10^3 \sim 10^4$ level of viable *E. coli* O157:H7 and *L. monocytogenes* were inoculated into the sterilized BHI broth media containing ovotransferrin and sodium bicarbonate. The broth culture was incubated at 35° C for 1, 2, 4, 6, and 8 d and then the turbidities of BHI broth culture were measured to estimate the change in population of *E. coli* O157:H7 and *L. monocytogenes* in each treatment.

Commercial hams were were sliced to 0.2 cm-thick pieces and vacuum packaged in low oxygen-permeable bags, irradiated at 5 kGy using an electron beam Linear Accelerator to destroy background microflora. Viable *E. coli* O157:H7 and *L. monocytogenes* cocktail stock suspension (0.1 ml) were inoculated aseptically on the surface of a sliced ham to yield a population of 10^6 CFU/cm^2. After inoculation, ham samples were manually mixed for 30 seconds to evenly distribute the inocula and then packaged samples were further separated into 3 groups. One ml of the ovotransferrin solutions (20 mg/ml) containing 100 mM $NaHCO_3$ or 0.5% of citric acid was distributed evenly on the same surface of sliced ham, manually mixed for 30 seconds, vacuum-packaged in nylon-polyethylene bags, and stored in a refrigerator. The number of surviving target pathogens was enumerated following refrigeration for 0, 5, 10, 15, 22, 29 and 34 d.

Results and Discussion

Natural apo-ovotransferrin showed a weak antibacterial property against *E. coli* O157:H7 after 6 to 8 d of incubation (Figure 1A), but its antimicrobial activities against *L. monocytogenes* were apparent after 4 d of refrigerated storage (Figure 1B). The antibacterial activity of apo-ovotransferrin was more effective in inhibiting the growth of *L. monocytogenes* than *E. coli* O157:H7. As the concentration of $NaHCO_3$ increased, the turbidity of BHI broth culture inoculated with *E. coli* O157:H7 decreased. However, sodium bicarbonate did not restrain the growth of *E. coli* O157:H7. In contrast to *E. coli* O157:H7, as the concentration of sodium bicarbonate increased the turbidity of BHI broth culture with *L. monocytogenes* increased, and the turbidity of sample with 100 mM sodium bicarbonate had the highest value (Figure 1B). *E. coli* O157:H7 showed some susceptibility to 100 mM sodium bicarbonate, while *L. monocytogenes* exhibited little resistant to the sodium bicarbonate.

The antibacterial activity of ovotransferrin plus $NaHCO_3$ depended on the concentration of sodium bicarbonate added (Figure 1). As the concentration of sodium bicarbonate increased, antimicrobial activity of ovotransferrin plus $NaHCO_3$ increased. Sodium bicarbonate increased the antibacterial effect of apo-ovotransferrin against *E. coli* and *L. monocytogenes*. Sodium bicarbonate alone had no effect on the antibacterial activity of apo-ovotransferrin against *E. coli* O157:H7. The combination of ovotransferrin with 100 mM sodium bicarbonate was the best condition for inhibiting the growth of *E. coli* O157:H7. Ovotransferrin + 25 mM sodium bicarbonate or ovotransferrin + 50 mM sodium bicarbonate did not inhibit the growth of *L. monocytogenes* during the first 4 d of incubation, whereas ovotransferrin containing 100 mM $NaHCO_3$ prevented the growth of *L. monocytogenes* from the beginning. There was an apparent synergistic effect of 100 mM-$NaHCO_3$ on the antimicrobial activity of apo-ovotransferrin against *L. monocytogenes* (Figure 1B). Considering ovotransferrin plus 100 mM $NaHCO_3$ has the lowest turbidity value in the two strains (Figure 1), 100 mM $NaHCO_3$ was the most appropriate concentration to improve the antibacterial activity of apo-ovotransferrin against *E. coli* O157:H7 and *L. monocytogenes*. This suggested that a certain concentration of sodium bicarbonate could increase the antibacterial effectiveness of ovotransferrin toward *E. coli* O157:H7 and *L. monocytogenes.*

L. monocytogenes was more sensitive to citric acid than *E. coli* O157:H7 (Figure 2). Citric acid at 0.5% level showed much greater antibacterial activity than 0.25% citric acid. Also, 0.5% citric acid alone did not completely inhibit the growth of *E. coli* O157:H7 until 8 days of incubation (Figure 2A and Table 1), but induced 2.5 ~ 3 log reduction of viable *L. monocytogenes* cells after 2 days of incubation (Figure 2B). Citric acid showed stronger antibacterial activities against *L. monocytogenes* than *E. coli* O157:H7. Considering that sodium citrate has antibacterial activity against gram positive microorganisms, the cause of antimicrobial action of citric acid against *L. monocytogenes* was not due to pH.

Even though ovotransferrin plus 0.25% citric acid did not show antibacterial activity, ovotransferrin added with 0.5% citric acid showed bactericidal effect against *E. coli* O157:H7. Also, 0.5% citric acid and ovotransferrin combination produced a larger reduction in the number of viable *E. coli* O157:H7 cells than that with 0.5% citric acid alone (Figure 2A). This indicated that 0.5% citric acid and ovotransferrin combination has some synergistic effect against *E. coli* O157:H7. Ovotransferrin containing 0.5% citrate showed bactericidal effect against *L. monocytogenes*, but its antimicrobial activity was lower than that of 0.5% citric acid alone after 4 days of incubation at 35° C (Figure 2B). Therefore, the bactericidal effect of ovotransferrin solution added with 0.5% citric acid against *L. monocytogenes* was attributed to antibacterial activity of 0.5% citric acid rather than their synergistic effect. In addition, the citric acid effects on antibacterial activity of apo-ovotransferrin against *E. coli* O157:H7 and *L. monocytogenes* were lost in the presence of 50 mM-

$NaHCO_3$. Also, the number of viable cells in ovotransferrin plus 0.5% citric acid in the presence of 50 mM-$NaHCO_3$ was similar to that of ovotransferrin with 50 mM-$NaHCO_3$ in both pathogens. Ovotransferrin plus 25 mM-sodium citrate solution did not inhibit the growth of the two pathogens in contrast to ovotransferrin plus 0.5% citric acid (Tables 1 and 2).

The antimicrobial action of ovotransferrin was enhanced by acidic pH conditions. The synergistic effect of citric acid on antibacterial activity of ovotransferrin against *E. coli* O157:H7 was due to proton formation since addition of $NaHCO_3$ or sodium citrate did not result in acidic environments. As ovotransferrin reacted directly to bacterial membrane, the permeability of outer membrane was assumed to be increased. Under such a circumstance, many protons provided by citric acid can enter inside the cell membrane. Thus, the proton motive force of *E. coli* O157:H7 could have been destroyed and resulted in cell death. This suggested that the synergistic effect of citric acid on antibacterial activity of ovotransferrin is not related to the iron binding capability of ovotransferrin.

Natural apo-ovotransferrin showed little antibacterial activity, but ovotransferrin added with 50 mM $NaHCO_3$ has antibacterial activity against *E. coli* O157:H7 and *L. monocytogenes* (Figure 3). Ovotransferrin plus 100 mM $NaHCO_3$ showed the strongest bacteriostatic activity against both pathogens. Ovotransferrin saturated with iron had little or no bactericidal activity against *E. coli* O157:H7 and *L. monocytogenes* (Figures 3A and 3B). Ovotransferrin bound with zinc also did not show antibacterial activity against *E. coli* O157:H7 (Figure 3A), but it had a similar degree of antibacterial activity as apo-ovotransferrin added with 50 mM sodium bicarbonate against *L. monocytogenes* (Figure 3B). Fe-bound ovotransferrin had little antimicrobial effect and the antibacterial activity of Zn-bound ovotransferrin varied with microorganisms. $Zn2^+$ affected antibacterial action of ovotransferrin against *L. monocytogenes*, but not *E. coli* O157:H7. Considering Zn-bound ovotransferrin possesses bacteristatic activity, it is not likely to come to such a conclusion that the cause of antibacterial activity of ovotransferrin is attributed to iron deprivation or metal binding capacity. Ovotransferrin may permeate bacterial outer membranes, reach the inner membrane and lead to permeation of other ions, which dissipate electrical potential so that it can exert antibacterial action against Gram-negative bacteria.

Based on the results obtained from *in vitro* tests, the ovotransferrin solutions added with 100 mM sodium bicarbonate or 0.5% citric acid were applied on commercial hams to determine their antibacterial activity. During storage at 4^o C for 4 weeks, the number of viable *E. coli* O157:H7 cells decreased slowly (Table 3), whereas that of *L. monocytogenes* resulted in 1 ~ 2 log increase (Table 4). This indicated that *E. coli* O157:H7 is more sensitive to low temperature than *L. monocytogenes.* Moreover, commercial hams contain a variety of antibacterial substances such as nitrite, salt, low water activity etc., but *L. monocytogenes* are resistant to low temperature and grew slowly. There was little significant difference in the numbers of viable *E. coli* O157:H7 cells between non-irradiated and irradiated hams during 4^o C storage (Table 3). The number of viable *L. monocytogenes* cells between non-irradiated and irradiated hams treated with OTF + 100 mM-sodium bicarbonate was not different. OTF + 0.5% citric acid did not exhibit any antibacterial activity in non-irradiated hams, but inhibited the growth of *L. monocytogenes* in irradiated hams (Table 4). This indicated that the *L. monocytogenes* existed originally in commercial hams affected the antibacterial activity of OTF + 0.5% citric acid in this study.

The ovotransferrin plus 100 mM $NaHCO_3$ treatment had higher number of viable *L. monocytogenes* cells than control at 10, 15, 22 and 29 d of storage. However, combination of ovotransferrin with 0.5% citric acid exhibited 1.1 log lower viable *L. monocytogenes* cells than control at Day 29 (Table 4). This indicated that ovotransferrin combination with 100 mM $NaHCO_3$ did not have any antibacterial activity against *E. coli* O157:H7 and *L. monocytogenes*, but the combination of ovotransferrin with 0.5% citric acid suppressed the survival of *L. monocytogenes* in hams (Tables 3 and 4). Figures 1 and 2 showed that the antibacterial activities of ovotransferrin + 100 mM $NaHCO_3$ and ovotransferrin + 0.5% citric acid against two pathogens in non-irradiated hams and model system are significantly different. Such differences might be attributed to media or matrix compositions, difficulties in treatment distribution, or binding of ovotransferrin to the matrix. As hams have a variety of factors that affect antibacterial activity, the antibacterial activity of ovotransferrin on hams was modulated by these factors.

Table 1. Influences of citric acid and citrate on antibacterial activity of ovtransferrin against 10^5 CFU/ml of *E. coli O157:H7.*

Treatments	Storage period (days)			
	1	2	4	8
	Number of viable cell ($\log_{10}$ CFU/ml)			
Citric acid	$7.90^{cw} \pm 0.65$	$6.88^{cwx} \pm 0.43$	$5.90^{bx} \pm 1.00$	$2.23^{cy} \pm 0.43$
OTF + Citric acid	$4.71^{dw} \pm 0.09$	$3.92^{dx} \pm 0.10$	$2.04^{cy} \pm 0.00$	$1.00^{dz} \pm 0.00$
OTF + Citric acid +$NaHCO_3$	$8.64^{abx} \pm 0.10$	$8.41^{bx} \pm 0.36$	$8.97^{aw} \pm 0.19$	$8.74^{ax} \pm 0.15$
OTF + Na-Citrate	$9.24^{aw} \pm 0.06$	$9.35^{aw} \pm 0.08$	$8.97^{ax} \pm 0.05$	$8.22^{by} \pm 0.07$
OTF + $NaHCO_3$	$8.40^{bcw} \pm 0.10$	$8.64^{abw} \pm 0.39$	$8.69^{aw} \pm 0.18$	$7.75^{bx} \pm 0.03$

[a-c] Values with different letters within a column with the same storage day are significantly
[w-z] Different letters within a row with the same treatment are different ($p < 0.05$). n=4.
This study used 0.5% citric acid, 25 mM sodium citrate, and 50 mM-$NaHCO_3$.
OTF: Apo-ovotransferrin (20 mg/ml).

Table 2. Effect of citric acid and citrate on antibacterial activity of ovotransferrin against a 104 population of *L. monocytogenes* in BHI broth.

Treatments	Storage period (days)			
	1	2	4	8
	Number of viable cell ($\log_{10}$ CFU/ml)			
Citric acid	$3.52^{bw} \pm 0.32$ **	$2.78^{bwx} \pm 1.14$	$1.52^{bx} \pm 0.96$	$1.00^{bx} \pm 0.0$
OTF * + Citric acid	$3.52^{bw} \pm 0.83$	$2.86^{bwx} \pm 0.99$	$2.05^{bw} \pm 1.67$	$1.91^{bw} \pm 1.78$
OTF + Citric acid +$NaHCO_3$	$8.86^{aw} \pm 0.14$	$8.60^{awx} \pm 0.41$	$8.14^{axy} \pm 0.29$	$7.67^{ay} \pm 0.60$
OTF + Na-Citrate	$9.23^{aw} \pm 0.11$	$8.62^{ax} \pm 0.17$	$8.57^{ax} \pm 0.23$	$8.43^{ax} \pm 0.31$
OTF + $NaHCO_3$	$8.60^{aw} \pm 0.19$	$7.74^{ay} \pm 0.01$	$8.14^{ax} \pm 0.07$	$7.13^{az} \pm 0.04$

[a-b] Values with different letters within a column with the same storage day are significantly different ($p < 0.05$, n=4)
[w-z] Different letters within a row with the same treatment are different ($p < 0.05$). n=4.
This study used 0.5% citric acid, 25 mM sodium citrate, and 50 mM-$NaHCO_3$.
OTF: Apo-ovotransferrin (15 mg/ml).

Table 3. Antibacterial activity of ovotransferrin solutions (20 mg/ml) plus either 100 mM-$NaHCO_3$ or 0.5% citric acid on non-irradiated and irradiated vacuum-packaged hams inoculated with *E. coli O157:H7* during storage at 4° C.

Sample	Treatments	Storage period (days)					
		0	5	10	15	22	29
		Number of viable cell ($\log_{10}$ CFU/ml)					
NR	Control *	$6.1^{ax} \pm 0.21$	$5.5^{ay} \pm 0.22$	$5.3^{abyz} \pm 0.12$	$5.2^{ayz} \pm 0.10$	$5.0^{bz} \pm 0.08$	$5.5^{ayz} \pm 0.01$
	OTF +$NaHCO_3$	$6.0^{ax} \pm 0.11$	$5.7^{ay} \pm 0.50$	$5.2^{bz} \pm 0.01$	$5.2^{az} \pm 0.17$	$5.0^{bz} \pm 0.10$	$5.0^{cz} \pm 0.01$
	OTF + Citric acid	$5.9^{ax} \pm 0.10$	$5.7^{axy} \pm 0.04$	$5.2^{byz} \pm 0.05$	$5.1^{az} \pm 0.42$	$5.0^{bz} \pm 0.07$	$5.1^{cyz} \pm 0.11$
IR	Control	$6.0^{ax} \pm 0.0$	$5.5^{ay} \pm 0.06$	$5.5^{ay} \pm 0.05$	$4.9^{ay} \pm 0.32$	$5.3^{ay} \pm 0.17$	$5.4^{aby} \pm 0.01$
	OTF + $NaHCO_3$	$6.1^{ax} \pm 0.19$	$5.6^{ay} \pm 0.08$	$5.5^{ayz} \pm 0.06$	$4.9^{az} \pm 0.19$	$5.0^{bz} \pm 0.07$	$5.3^{abyz} \pm 0.04$
	OTF + Citric acid	$6.0^{ax} \pm 0.0$	$5.7^{ax} \pm 0.05$	$5.5^{axy} \pm 0.08$	$5.0^{ay} \pm 0.40$	$5.0^{by} \pm 0.02$	$5.3^{abxy} \pm 0.10$

[a-b] Values with different letters within a column with the same storage day are significantly different ($p < 0.05$, n=4)
[w-z] Different letters within a row with the same treatment are different ($p < 0.05$).
[*] Only *E. coli O157:H7* inoculation
OTF: Ovotransferrin (20 mg/ml); NR: non-irradiated ham; IR^2: irradiated ham
This study used 0.5% citric acid and 100 mM sodium bicarbonate.

Table 4. Antibacterial activity of ovotransferrin solution (20 mg/ml) plus either 100mM-sodium carbonate or 0.5% citric acid on non-irradiated and irradiated vacuum-packaged hams inoculated with *L. monocytogenes* during storage at 4° C.

Sample	Treatments	Storage period (days)					
		0	5	10	15	22	29
		Number of viable cell (log_{10} CFU/ml)					
NR	Control*	$6.5^{ax} \pm 0.15^{**}$	$6.3^{abx} \pm 0.01$	$6.5^{bx} \pm 0.03$	$6.9^{bxy} \pm 0.10$	$7.5^{aby} \pm 0.10$	$8.2^{az} \pm 0.09$
	OTF *** +$NaHCO_3$	$6.4^{abx} \pm 0.11$	$6.4^{ax} \pm 0.04$	$7.7^{ay} \pm 0.09$	$7.6^{ay} \pm 0.09$	$8.3^{az} \pm 0.21$	$8.4^{az} \pm 0.05$
	OTF + Citrate	$6.4^{abx} \pm 0.14$	$6.5^{ax} \pm 0.04$	$6.6^{bx} \pm 0.14$	$7.1^{aby} \pm 0.03$	$7.3^{aby} \pm 0.12$	$8.2^{az} \pm 0.10$
IR	Control	$6.4^{abx} \pm 0.04$	$6.2^{bx} \pm 0.08$	$6.4^{bx} \pm 0.13$	$6.6^{byz} \pm 0.25$	$7.0^{by} \pm 0.08$	$8.0^{az} \pm 0.30$
	OTF + $NaHC0_3$	$6.2^{bx} \pm 0.14$	$6.5^{ayz} \pm 0.01$	$7.5^{axy} \pm 0.23$	$7.7^{axy} \pm 0.45$	$7.7^{abxy} \pm 0.63$	$8.3^{az} \pm 0.47$
	OTF + Citrate	$6.2^{abx} \pm 0.18$	$6.2^{bx} \pm 0.03$	$6.4^{bx} \pm 0.07$	$6.5^{bx} \pm 0.22$	$6.9^{bx} \pm 0.53$	$6.9^{bx} \pm 0.57$

[a-b]Values with different letters within a column with the same storage day are significantly different ($p < 0.05$). n=4.
*Only *L. monocytogenes* inoculation.
OTF: Ovotransferrin (20 mg/ml); NR: non-irradiated ham; IR: irradiated ham
This study used 0.5% citric acid and 100 mM sodium bicarbonate.

Figure 1. The effect of sodium bicarbonate on antibacterial activity of ovotransferrin (20mg/ml) against *E. coli O157:H7* (A : 10^3 CFU/ml inoculation) and *L. monocytogenes* (B: 10^4 CFU/ml inoculation) during 35° C incubation. Control was inoculated with only *E. coli O157:H7*. OTF: 20 mg/ml apo-ovotransferrin.

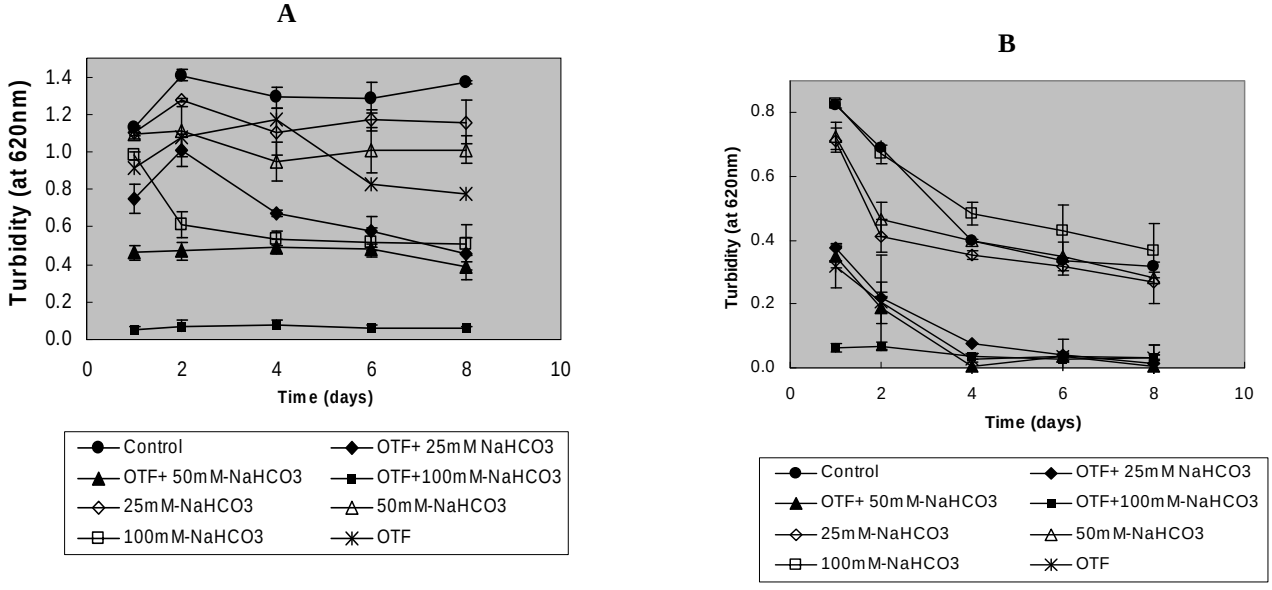

Figure 2. The influence of citric acid on antibacterial activity of ovotransferrin against 10^5 CFU/ml of *E. coli O157: H7* (A) and 10^4 CFU/ml of *L. monocytogenes* (B) after 2 days of incubation at 35°C. The control was inoculated with only *E. coli O157:H7* or *L. monocytogenes*. OTF in A: 20 mg/ml apo-ovotransferrin, OTF in B: 15 mg/ml apo-ovotransferrin. Different letters on bars represent significantly different groups ($p < 0.05$, $n = 4$).

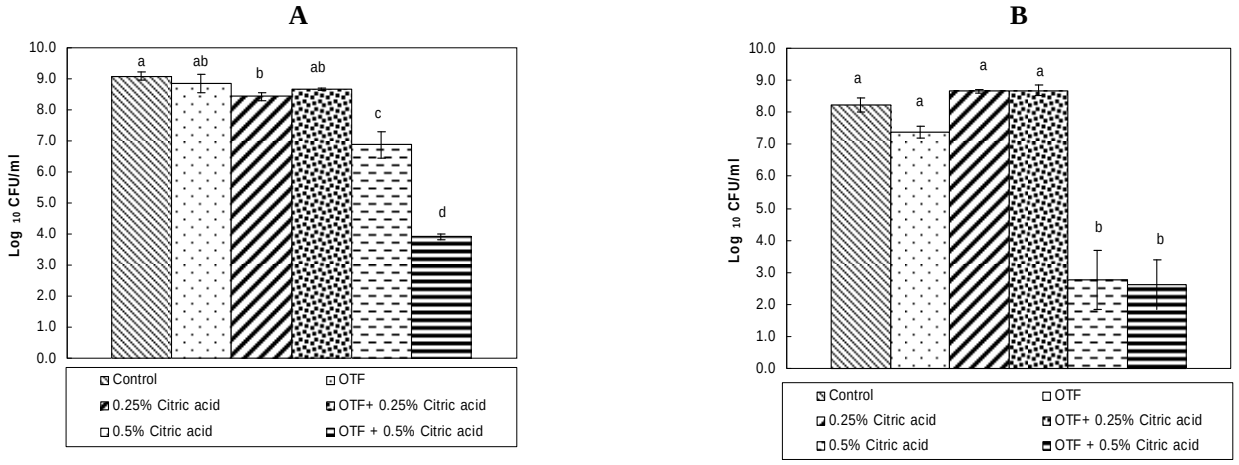

Figure 3. Antibacterial activity of OTF, Fe-OTF, and Zn-OTF against *E. coli O157:H7* (10^4 CFU/ml, A) after 1day of incubation at 35°C and *L. monocytogenes* (10^5 CFU/ml, B) after 2 days of incubation at 35°C. Control: only *E. coli O157:H7* or *L. monocytogenes.* OTF: 20 mg/ml of apo-ovotransferrin, OTF 50: OTF + 50 mM-$NaHCO_3$, OTF 100: OTF + 100 mM-$NaHCO_3$, Fe-OTF: Fe-bound ovotransferrin (20 mg/ml), Zn-OTF: Zn-bound ovotransferrin (20 mg/ml). Different letters on bars represent significantly different groups ($p < 0.05$, $n = 4$).