POSSIBLE APPLICATIONS OF OVOTRANSFERRIN-INCORPORATED K-CARRAGEENAN-BASED FILM AS PACKAGING FOR FRESH CHICKEN FILLET DURING COLD STORAGE

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Introduction

Ovotransferrin (OTf) is the major component of the avian egg's antimicrobial defense system (Tranter and Board, 1982) as it renders iron unavailable to microbial growth within the albumin; this is the so called nutritional immunity of albumin (Ibrahim, 2000). Antimicrobial food packaging is one of the most promising concepts of active packaging. Cha *et al.* (2002) investigated antimicrobial films based on Na-alginate and κ-carrageenan with some antimicrobials such as lysozyme, nisin, grapefruit seed extract and chelating agent (EDTA) against food spoilage bacteria and pathogens.However, the possibility of using OTf as an antimicrobial agent and the effect of antimicrobial films on shelf-life of fresh meat has not been thoroughly researched. Thus, we have investigated the effects of κ-carrageenan-based film incorporating OTf on the shelf-life of fresh chicken fillet.

Materials and Methods

κ-carrageenan-based films were prepared by modification of the methods used by Pavlath *et al.* (1999) and Cha *et al.* (2002). The compositions of κ-carrageenan-based films tested are listed in Table 1. Typical meat product bacterial contaminants used were *S. aureus* (ATCC 12692), *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 14028), and the mould *C. albicans* (ATCC 10231). Chicken fillets were purchased from market to investigate the effect of films on shelf-life of fresh chicken fillet during cold storage (4~5℃). Antimicrobial activity of films was measured by a modified agar diffusion method according to Chen *et al.*(1996). Effects of the prepared films on chicken fillet were evaluated by modification of the method used by Ouattara *et al.* (2002);. experiments were replicated five times. One-way ANOVA with Duncan's multiple range test was carried out to analyse the significant differences between treatments ($P<0.05$) using the SAS program for Windows (V9.1; SAS Institute, Cary, NC, USA).

Results and Discussion

The effect of films on inhibition zone area against indicator microorganisms is given in Table 2 and Figure 1. In all indicator microorganisms, control (C), OTf (T1), and potassium sorbate-incorporated film (T3) revealed little inhibitory effect (indicated by minimal growth underneath film pieces) while EDTA-incorporated films (T2, T4 and T5) did reveal an inhibitory effect. The film incorporating OTf and EDTA (T2) revealed a strong inhibitory effect against *E. coli* and *C. albicans*. The EDTA may induce antimicrobial agents to kill microorganisms by destabilizing cell membranes and limiting the availability of cations (Shelef and Seiter, 1993). Total microbial counts on chicken fillets increased during storage in all treatments and after 7 days of storage those of C were >7.0 log CFU/g (Figure 2). Total microbial counts on chicken fillets covered by T1 and T3 showed no significant difference with C during the entire storage period ($p>0.05$). However, the fillets covered with T2 had significantly lower total microbial counts throughout the storage period ($p<0.05$) and those of fillets covered with T4 and T5 were significantly lower than those of the control after 7 days storage ($p<0.05$). The number of *E. coli* on chicken covered with T2 did not increase significantly during storage ($p>0.05$) and it was significantly lower than any other treatments ($p<0.05$). Those of fillets covered with all treated films (from T1 to T5) were significantly lower than C during the storage period ($p<0.05$). These results agree with the result that T2, T4 and T5 had inhibitory effects on microbial growth and show the possibility of using OTf and chelators in combination as an additive for antimicrobial films.

Table 1: Compositions of κ-carrageenan-based films.

Code	Film Treatments
Control (C)	κ-carrageenan (2%) + plasticiser[a] (1.5%)
T1	Control + Ovotransferrin (OTf, 25mg/g κ-carrageenan)
T2	Control + Ovotransferrin (OTf, 25mg/g κ-carrageenan) + EDTA (5mmol)
T3	Control + Potassium sorbate (PS, 10mg/g κ-carrageenan)
T4	Control + Potassium sorbate (PS, 10mg/g κ-carrageenan) + EDTA (5mmol)
T5	Control + EDTA (5mmol)

[a]Plasticiser : 50% PEG (polyethylene glycol) + 50% glycerol.

Table 2: Effect of κ-carrageenan-based films on inhibition zone area against indicator microorganisms.

Film type	*S. aureus*	*E. coli*	*S. typhimurium*	*C. albicans*
C	nd	nd	nd	nd
T1	nd	nd	nd	nd
T2	+	++	+	++
T3	nd	nd	nd	nd
T4	+	+	+	++
T5	+	+	+	+

nd : not detected, + : Inhibition is weak (diameter of inhibition zone is < 2mm), ++ : Sensitive (diameter of inhibition zone is 2~5mm)
C: κ-carrageenan + plasticiser; **T1**: C + OTf; **T2**: C + OTf + EDTA; **T3**: C + PS; **T4**: C + PS + EDTA; **T5**: C + EDTA

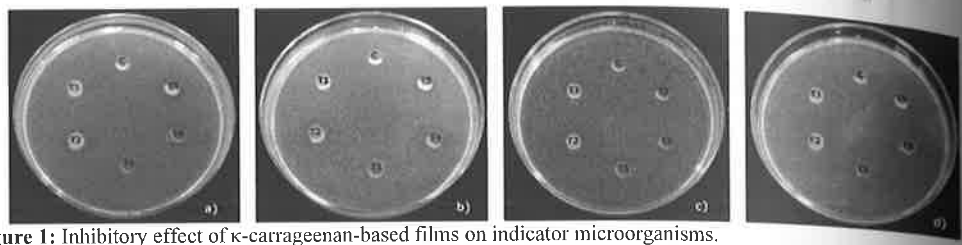
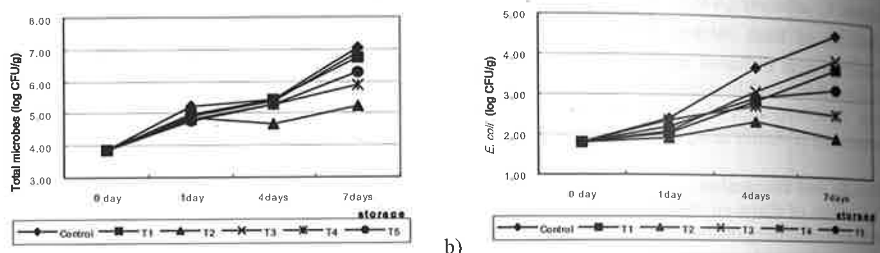


Figure 1: Inhibitory effect of κ-carrageenan-based films on indicator microorganisms.
a) *S. aureus*, **b)** *E. coli*, **c)** *S. typhimurium*, **d)** *C. albicans*
C: κ-carrageenan + plasticiser; **T1**: C + OTf; **T2**: C + OTf + EDTA; **T3**: C + PS; **T4**: C + PS + EDTA; **T5**: C + EDTA



a) b)

Figure 2: Changes in the number of total microbes and *E. coli* on chicken fillet covered with κ-carrageenan-based films during storage. **a)** total microbes, **b)** *E. coli* **Control**: κ-carrageenan + plasticiser; **T1**: C + OTf; **T2**: C + OTf + EDTA; **T3**: C + PS; **T4**: C + PS + EDTA; **T5**: C + EDTA

Conclusions

OTf combined with EDTA showed a strong inhibitory effect against various microorganisms and has possibilities for application in active food packaging. Further studies concerning the applicability of OTf antimicrobial peptide are required.

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