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Effectiveness of Antimicrobial Starch Coating Containing Thyme Oil against *Salmonella*, *Listeria*, *Campylobacter*, and *Pseudomonas* on Chicken Breast Meat

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Abstract: Antimicrobial coating on chicken carcasses may reduce the effects of cross-contamination and improve product shelf-life and safety. Thyme oil was mixed at 0.5%(v/v) with a pre-gelatinized pea starch coating solution. The coating solution was spread on chicken breast meat after inoculation with selected spoilage and pathogenic bacteria. After inoculation, the chicken meats were packaged in plastic bags and stored at 4°C. During 12 day storage, total aerobic bacteria, lactic acid bacteria, and inoculated organisms were counted at 4 day intervals. Thyme oil treatments reduced the viability of *Salmonella* as well as the growth of *Listeria* and *Pseudomonas* by 2 log CFU/g, and appeared to eliminate inoculated *Campylobacter* during storage. The addition of thyme oil increased the viscosity of the pre-gelatinized pea starch solution. The results suggested that thyme oil inclusion in an edible starch coating may be a satisfactory delivery system to enhance the safety of processed fresh meat.

Keywords: chicken meat, food-borne pathogen, natural antimicrobial coating, edible coating, thyme oil

Introduction

Most research has been concerned with the contamination of chicken carcasses and fresh poultry products by *Salmonella* or *Campylobacter* which are predominant pathogens, and *Pseudomonas* which are the major psychotropic spoilage bacteria of refrigerated poultry products (1-3). Poultry processing lines operate at high-speed, often processing over 150 bird/min. At this high-speed poultry meat is very vulnerable to cross-contamination. Various processing methods are used to reduce levels of undesired microorganisms on broiler carcasses in poultry processing lines. Among them one of the important unit processes is washing using an inside-outside bird washer before immersion or air chilling (1,4). It appears that after washing followed by chilling, there is no unit process in use which can satisfactorily remove pathogens or spoilage microorganisms from poultry carcasses.

Edible coatings are produced from edible biopolymers and food-grade additives. Film-forming biopolymers can be selected from proteins, polysaccharides (carbohydrates and gums), or lipids (5). Various antimicrobial agents may be incorporated into edible coating materials to produce antimicrobial coating systems, as they allow a slow migration of the antimicrobial agents from the coating materials and extend the shelf-life of coated foods.

Starch and calcium alginate gels incorporating trisodium phosphate and acidified sodium chlorite, respectively, effectively inhibited an inoculated *Salmonella* cocktail on chicken wings (6). Nisin was mixed with protein and carbohydrate coating materials and reduced the number of *Salmonella* and *Listeria* on chicken meats (7-9). Among available antimicrobial agents, oils of plant or spice extracts are attractive since they are natural ingredients (which require no or a reduced label declaration), are accepted by consumers (10-13) and they can be extracted easily from herbs, spices, and aromatic plants by solvents or steam distillation. Many of these essential oils contain antimicrobial as well as antioxidant activity. Examples include rosemary, clove, thyme, oregano, and basil oils, plus horseradish and mustard extracts. They are mostly phenolics or terpenes while the latter two contain isothiocyanates (14,15).

Thyme oil mainly contains thymol, *p*-cymene, and carvacrol, which demonstrate antimicrobial and antioxidant activities (16-18). Thyme oil has been reported to inhibit the growth of *Escherichia coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Penicillium* spp., and many other bacteria (17,19-22).

In the present study thyme oil was incorporated into a high amylose pea starch gel and applied on chicken breast meats pre-inoculated with spoilage or pathogenic microoranisms. The objective was to characterize: (i) the rheological characteristics of the starch-based coating material with and without thyme oil; and (ii) the antimicrobial effectiveness of thyme oil in a starch-based coating material against food borne pathogens and spoilage bacteria on chicken meat. The goal of this project was to determine whether the formation of an antimicrobial coating containing thyme oil applied to chicken carcasses would be suitable to reduce the effects of contamination by a high-speed poultry line, enhance the safety of poultry products and extend their shelf-life.

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Materials and Methods

Materials Air-chilled fresh chicken breast meats were obtained from a local poultry processing plant (Dunn-Rite,

Winnipeg, MB, Canada) about 4 hr before the experiment. The meats were cut into 2×2 cm cubes (10±1 g) with a knife disinfected in 70% ethanol. Starch extracted from Canadian yellow field peas (*Pisum sativum* L. Century) by a conventional wet milling process was supplied by Nutri-Pea Ltd. (Portage-La-Prairie, MB, Canada). Pea starch is a C-type starch containing 37-40% amylose. One g of phosphatidyl choline (Fisher Scientific, Nepean, ON, Canada) was dissolved in 15 mL of thyme oil (Sigma-Aldrich, St. Louis, MO, USA) to increase surface tension of the mixture and stored at 4°C until used.

Ampicillin resistant *Salmonella entericia* serovars (i.e., Typhimurium and Heidelberg) and *Campylobacter jejuni* were obtained from R. Ahmed, Canadian Centre for Human and Animal Health (Winnipeg, MB, Canada). *L. monocytogenes* and *Pseudomonas aeruginosa* were obtained from the culture collections of the Department of Food Science and the Department of Microbiology, respectively, at the University of Manitoba (Winnipeg, MB, Canada).

Antimicrobial pea starch coating solution Pea starch suspension was prepared by mixing 25 g pea starch and 12.5 g glycerol (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) in 1 L sterile cold distilled water. This suspension was boiled for 20 min with agitation to gelatinize pea starch, and cooled in a water bath at 50°C. The thyme oil and phosphatidyl choline mixture was blended into the pea starch coating solution to give a 5%(v/v) concentration and stirred for 5 min.

Consistency profile of starch coating solution Fully gelatinized 2.5%(w/v) aqueous starch solution (prepared by boiling 20 min) containing 1.25%(w/v) glycerol was mixed with 5%(v/v) thyme oil and phosphatidyl choline mixture at room temperature, and its consistency was determined using a rheometer (AR1000; TA Instruments, New Castle, DE, USA). The volume of samples was 0.99 mL. Operating conditions of the rheometer were 25°C using a 60 mm diameter 1° angle steel cone. Initial shear rate was 1.275/sec and was ramped to 1,000/sec. Shear rate was increased by steady state flow mode with a logarithmic ramp pattern. Consistency index and fluid behavior index were calculated using the power law equation by parameter estimate of regression analysis. Each treatment was tested in triplicate.

Bacterial inoculum preparation All bacterial cultures were maintained in brain heart infusion (BHI) broth and enumerated on BHI agar (Difco Division, Becton Dickinson Co., Sparks, MD, USA) after incubation at 35°C for 24 to 48 hr. For *Campylobacter* culture BHI agar and broth media were used with 0.5%(w/v) yeast extract and 10%(w/v) laked horse blood (Oxoid Ltd., Nepean, ON, Canada), and were incubated at 35°C under microaerophilic conditions created by the CampyPak Plus system (Becton Dickinson Co., Cockeysville, MD, USA) for 48 hr. Bacterial culture broth was centrifuged at 3,000×g for 15 min at 10°C (Sorvall RC2-B; Refrigerated Centrifuge, Du Pont, Newtown, CT, USA). The sedimented culture pellet was suspended in 0.85% sterile saline solution to wash and was recentrifuged. The pellet was diluted to yield an optical density of 0.80 at 600 nm and the live bacterial population was determined using a spiral plating unit (Autoplate 4000; Spiral Biotech, Bethesda, MD, USA). The equivalent number of bacteria for 0.8 optical density units was 10^9 CFU/mL. The 2 *Salmonella* cultures were mixed at equal numbers of cells to obtain a cocktail of *S.* Typhimurium and *S.* Heidelberg.

Inoculation of chicken meat Chicken meat cubes (approximately 2 kg) were placed in a sterile aluminum tray and 2 L of inoculum containing 10^6 CFU/mL of each of the test organisms and the *Salmonella* cocktail were separately poured on the chicken cubes. The tray was shaken 2 to 3 times during 15 min exposure to allow the meat to adsorb bacteria, then the excess liquid was drained. The inoculated meats were dried for 5 min in the tray. One quarter of the inoculated cubes (approximately 0.5 kg) were enclosed in a high-barrier plastic bag (Deli*1; WinPak, Winnipeg, MB, Canada) composed of nylon/ethylene vinyl alcohol/polyethylene, and heat-sealed. The film was 75-μm thick with an oxygen transmission rate of 2.3 $cm^3/m^2 \cdot day$ at 23°C, and water vapor transmission rate of 7.8 $g/m^2 \cdot day$ at 37.8°C and 98% relative humidity. The second quarter of the inoculated cubes was transferred onto a sterile tray and 1 L of pea starch coating solution was poured onto the cubes. After shaking for 1 to 2 min, the excess starch solution was drained. The coated cubes were dried for 1 hr in the tray, and each cube was packaged in the high-barrier plastic bag. The third quarter of inoculated cubes were placed in a sterile tray, and 1 L of pea starch coating solution containing 5% thyme oil was poured on the chicken cubes. The last quarter of inoculated chicken cubes were mixed with 1 L sterile water containing 5% thyme oil. Both thyme oil treatments were mixed, dried, and packaged as described earlier. Chicken meats without inoculation and coating were packaged as control samples (i.e., no treatment). All samples were stored at 4°C.

Viable numbers of bacteria At 0, 4, 8, and 12 day of storage after inoculation, 3 bags/treatment were opened aseptically and 90 mL of 0.1% peptone water was added. This bag was placed in a stomacher and pummeled for 1 min. After appropriate serial dilutions, the samples were plated on agar media using the spiral plating unit, and incubated. All plates were counted in duplicate from each sample (total 6 analyses/treatment). Types of agar media used and incubation conditions used for inoculated bacteria were:

- Total aerobes: BHI agar at 35°C for 24 hr
- Lactic acid bacteria: MRS agar (Difco) at 32°C for 48 hr
- *Salmonella*: XLD agar (Difco) containing 100 ppm ampicillin (Sigma-Aldrich) at 35°C for 24 hr
- *Campylobacter*: Karmali agar (Oxoid Ltd.) containing a growth supplement (Oxoid SR 139) at 35°C for 48 hr under microaerophilic conditions
- *Listeria*: *Listeria* selective agar (Oxford Selective, Oxoid Ltd.) at 35°C for 24 hr
- *Pseudomonas*: *Pseudomonas* agar (Oxoid Ltd.) with a supplement (Oxoid SR 103) at 35°C for 24 hr

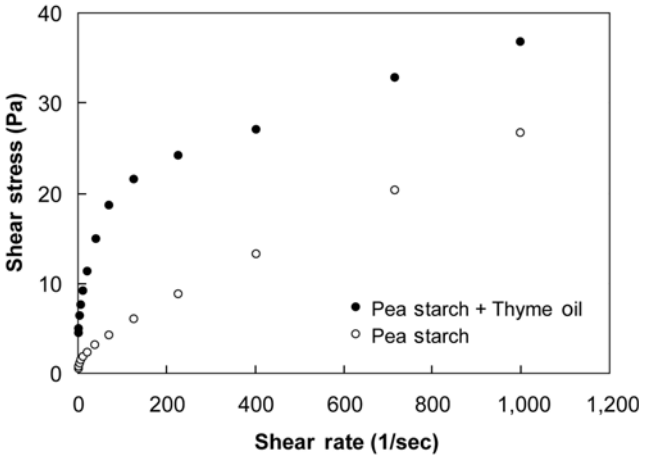

Fig. 1. Consistency profile of pea starch gels with and without thyme oil at 25°C.

Results and Discussion

Flow properties of starch-based coating solution Figure 1 shows the shear stress-strain curve of the pea starch coating solution with and without thyme oil. From this figure the consistency index and power law flow behavior index were calculated, and these results are summarized in Table 1. The consistency of the gelatinized pea starch coating solution was affected significantly by the presence of thyme oil, which caused increased viscosity at low shear rate range. The addition of thyme oil decreased the power law flow behavior index and made the starch gel more viscous and pseudoplastic.

Figure 1 shows that both starch coating solutions, regardless of thyme oil addition, exhibited shear-thinning pseudoplastic behavior below 100/sec of shear rate. However, above 100/sec, the pseudoplastic characteristics were converted to Newtonian behavior, specifically Bingham flow. Starch solutions possess intermolecular interactions and form elastic starch gels when the deformation is not significant, such as occurred below 100/sec of shear. However, above this critical shear, the intermolecular interaction of starch gels could not be maintained and were converted from an elastic gel to a viscous solution. The corresponding critical shear stresses of 100/sec shear rate were approximately 20 and 5 Pa for pea starch with and without thyme oil, respectively. Yield stresses (the Y-intercept of Bingham) were 22.4903 and 5.3486 Pa for pea starch solutions with and without thyme oil, respectively, which reflects the dramatic increase in the yield stress of the starch solutions caused by thyme oil addition. This result implies that thyme oil enhanced the intermolecular interaction of starch, perhaps by the formation of starch (amylose)-lipid complexes, which may be a different phenomenon from the case of oil and beeswax additions in the starch solution. It is found that the addition of beeswax to gelatinized pea starch did not change the starch structure and related characteristics until 30%(w/w) of beeswax had been added to the starch gel (23). Therefore, the changes in viscoelastic properties of pea starch gels by 5% thyme oil are remarkable. Thyme oil contains mostly phenolic compounds that have very small molecular weight compared to those of beeswax. It is hypothesized that the small hydrophobic molecules can be incorporated within the amylose helix much easier than macromolecular lipids, and consequently exhibit higher viscosity than that of starch solutions with macromolecular lipids. For the practical application of a thyme oil-starch coating for poultry processing, it is suggested that an inside-outside bird washer be used. The washer would spray the starch coating solution at both high pressure and high speed feeding rate. Therefore, within the practical operating range of feeding, which will be definitely over 100/sec shear rate, the thyme oil-starch solution will behave as a Bingham fluid. A minimum 22.49 Pa of pressure is required for the bird washer to initiate the flow of the starch coating containing thyme oil. The higher yield stress produces a thicker coating weight. Since the yield stress of the coating solution increased 5 times after thyme oil addition, theoretically on a smooth surface hanging vertically (e.g., chicken carcass on an overhead conveyor), the thickness of the coating containing thyme oil will be 5 times greater than that of a starch coating without thyme oil. Therefore, understanding the effects of yield stress upon coating viscosity is critical to optimize coating application and uniformity. After washing, chicken carcasses are warm and the antimicrobial coating solution can be sprayed at ambient processing room temperature. Before commercial adoption of this technology, temperature/viscosity relationships should be established to allow description of optimal application conditions.

Microbial viability on *Salmonella*-inoculated chicken Application of the starch coating to chicken cubes had little effect on the numbers of total organisms, the lactic acid bacteria present, and the viability of inoculated (ampicillin resistant) *Salmonella* during 12 day storage at 4°C (Table 2). Numbers of total organisms (psychrotrophs) and lactic acid bacteria increased similarly in the presence or absence of the starch coating. MRS agar is a non-selective enriched medium and *Salmonella* were able to form colonies on this agar. *Salmonella* numbers decreased by about 1 log CFU/g during refrigerated storage in treatments with and without the starch coating. Inclusion of thyme oil in the coating delayed the growth of psychrotrophs until day 4 and the lactic acid bacteria until after day 8. Thyme oil

Table 1. Flow characteristics of gelatinized pea starch coating material with and without thyme oil at 25°C[1)]

	Consistency index ($Pa \cdot sec^n$)	Power law flow behavior index, n	Viscosity of Bingham ($Pa \cdot sec$)	Yield stress of Bingham (Pa)
Pea starch	0.738±0.3686 (42.9%)	0.5405±0.0370 (6.8%)	0.029±0.0072 (25.1%)	5.349±1.7621 (32.9%)
Pea starch+Thyme oil	3.425*±0.8748 (25.5%)	0.3933*±0.0454 (11.5%)	0.016±0.0008 (4.8%)	22.490*±1.7236 (7.7%)

[1)]Values in parentheses are coefficients of variance (CV); Viscosity and yield stress of Bingham were obtained from data over 100/sec of shear rate; *Indicates significant difference of mean value of (pea starch+thyme oil) from mean value of (pea starch) at 90% confidence interval.

Table 2. Effects of thyme oil treatments on the numbers (log CFU/g) of *Salmonella* Typhimurium and *S.* Heidelberg on chicken breast meat at 4°C[1)]

Treatments	Day 0	Day 4	Day 8	Day 12
Total aerobes				
No treatment	3.1±0.2	4.6±0.1	6.1±0.2	6.6±0.3
Salmonella inoculation	4.7±0.0	5.0±0.1	6.6±0.5	7.1±0.1
Salmonella+Pea starch coating	4.8±0.05	5.2±0.3	7.0±0.4	7.5±0.1
Salmonella+Pea starch coating+Thyme oil	4.0±0.05[a]	3.4±0.5[b]	5.8±0.3	7.2±0.4
Lactic acid bacteria				
No treatment	2.6±0.3	3.6±0.1	5.2±0.6	5.4±0.7
Salmonella inoculation	4.7±0.0	4.7±0.2	5.3±0.3	6.1±0.6
Salmonella+Pea starch coating	4.9±0.2	4.8±0.1	5.4±0.2	6.9±0.1
Salmonella+Pea starch coating+Thyme oil	3.9±0.1[b]	3.0±0.0[c]	2.8±0.7[c]	5.8±0.4[a]
Salmonella				
No treatment	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Salmonella inoculation	5.2±0.0[a]	4.5±0.1[b]	4.3±0.2[b]	4.2±0.1[b]
Salmonella+Pea starch coating	5.1±0.1[a]	4.2±0.2[b]	4.4±0.2[b]	3.9±0.1[c]
Salmonella+Pea starch coating+Thyme oil	4.3±0.4[a]	2.9±0.2[b]	2.0±1.7[b]	2.2±0.4[b]

[1)]Experiments with *Salmonella* + H_2O + thyme oil treatment were not conducted. Different superscripts indicate a significant difference of mean values in rows (*t*-test, $n=6$, $p<0.05$).

Table 3 Effects of thyme oil treatments on the survival (log CFU/g) of *Campylobacter jejuni* on chicken breast meat at 4°C

Treatments	Day 0	Day 4	Day 8	Day 12
Total aerobes				
No treatment	3.2±0.0	5.0±0.1	7.7±0.1	7.5±0.1
Campylobacter inoculation	3.4±0.2	4.9±0.1	6.9±0.1	7.2±0.3
Campylobacter+Pea starch coating	3.5±0.3	4.9±0.1	7.7±0.0	7.8±0.1
Campylobacter+H_2O+Thyme oil	ND	3.4±0.4	5.3±0.5	5.3±0.6
Campylobacter+Pea starch coating+Thyme oil	2.0±0.0	2.4±0.1	5.0±0.05	5.7±1.0
Lactic acid bacteria				
No treatment	2.4±0.1	4.3±0.0	6.3±0.2	6.9±0.0
Campylobacter inoculation	3.2±0.0	4.4±0.0	6.1±0.2	7.0±0.1
Campylobacter+Pea starch coating	3.4±0.2	4.2±0.1	6.3±0.1	7.5±0.2
Campylobacter+H_2O+Thyme oil	ND	2.0±0.0	2.3±0.0	4.1±0.5
Campylobacter+Pea starch coating+Thyme oil	ND	ND	2.3±0.0	4.7±0.2
Campylobacter jejuni				
No treatment	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Campylobacter inoculation	4.6±0.1[a1)]	4.2±0.1[b]	3.8±0.2[c]	3.7±0.1[c]
Campylobacter+Pea starch coating	4.2±0.5[a]	3.4±0.1[b]	4.6±0.3[a]	2.5±0.1[c]
Campylobacter+H_2O+Thyme oil	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Campylobacter+Pea starch coating+Thyme oil	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

[1)]Different superscripts indicate a significant difference of mean values in rows (*t*-test, $n=6$, $p<0.05$). ND, not detectable (<100 CFU/g).

inclusion in the coating had a significant negative effect on *Salmonella* viability with recoveries being 2 log CFU/g lower at day 4 and this reduction was increased to 3 log CFU/g at day 8 and 12.

Microbial viability on *Campylobacter*-inoculated chicken

As with the previously reported trial (Table 2), the starch coating had essentially no effect on the growth of psychrotrophs and lactic acid bacteria during storage of the chicken meat at 4°C for 12 day (Table 3). However, addition of starch coating containing thyme oil significantly reduced the extent of both psychrotrophic and lactic acid bacterial growth by 2 and 3 log CFU/g at day 8 and 12, respectively. Direct addition of thyme oil as a water emulsion without the coating caused a similar delay in psychrotrophic bacterial growth, but had a greater initial inhibitory effect on the lactic acid bacteria. These latter recovered by day 8 to reach about the same numbers as were present on chicken coated with starch containing thyme oil. These latter levels were 2 to 3 log CFU/g less than in treatments where thyme oil was not used. *Campylobacter* were absent from the chicken meat used in this study, and following inoculation their numbers were relatively stable during storage at 4°C. A very slight reduction in *Campylobacter*

Table 4. Effects of thyme oil treatments on the growth (log CFU/g) of *Listeria monocytogenes* on chicken breast meat at 4°C

Treatments	Day 0	Day 4	Day 8	Day 12
Total aerobes				
No treatment	3.0±0.6	4.6±0.1	6.8±0.1	7.7±0.0
Listeria inoculation	5.6±0.0	5.2±0.4	6.9±0.2	8.1±0.9
Listeria+Pea starch coating	4.7±0.1	6.1±0.1	7.2±0.1	8.3±0.0
Listeria+H_2O+Thyme oil	4.0±0.4[a1)]	3.5±0.1[b]	5.3±0.6	5.1±0.6
Listeria+Pea starch coating+Thyme oil	4.5±0.3	5.1±0.1	5.9±0.8	6.8±0.5
Lactic acid bacteria				
No treatment	2.5±0.5	4.6±0.1	6.7±0.2	7.6±0.1
Listeria inoculation	5.5±0.1	5.6±0.0	6.9±0.2	7.7±0.2
Listeria+Pea starch coating	4.8±0.1	5.8±0.2	7.0±0.4	7.8±0.1
Listeria+H_2O+Thyme oil	3.9±0.4[a]	3.3±0.1[b]	3.9±0.6[a]	5.0±0.1[b]
Listeria+Pea starch coating+Thyme oil	4.5±0.3	4.5±0.5	5.3±0.3	5.5±1.0
Listeria monocytogenes				
No treatment	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Listeria inoculation	5.5±0.1	5.5±0.0	5.9±0.1	6.4±0.2
Listeria+Pea starch coating	4.7±0.0	5.9±0.3	6.6±0.3	7.2±0.2
Listeria+H_2O+Thyme oil	6.0±0.4[a]	3.1±0.0[d]	3.6±0.3[c]	5.1±0.0[b]
Listeria+Pea starch coating+Thyme oil	4.3±0.3	4.6±0.5	4.8±0.1	5.1±0.2

[1)]Different superscripts indicate a significant difference of mean values in rows (*t*-test, $n=6$, $p<0.05$).

viability was noted in response to starch coating at day 12, but use of thyme oil alone (H_2O+thyme oil) or use of thyme oil following its incorporation into the starch coating (pea starch coating+thyme oil) caused an immediate reduction in *Campylobacter* viability to below detectable levels, and this inhibitory or lethal effect was maintained for the remainder of the study (Table 3).

Microbial viability on *Listeria*-inoculated chicken As noted in Table 2 and 3, psychrotrophic and lactic acid bacteria naturally present on uninoculated chicken grew rapidly and reached 7 to 8 log CFU/g by 12 day of storage at 4°C (Table 4). There was little difference in bacterial recoveries (psychrotrophs, lactic acid bacteria, or inoculated *L. monocytogenes*) among the media used when starch-coated chicken (with or without *L. monocytogenes* inoculation) was stored at 4°C for 12 day. *L. monocytogenes* was able to grow on the MRS medium used for lactic acid bacteria recovery, and contributed to the number of colonies recovered as lactic acid bacteria.

The extent of bacterial growth on BHI and MRS agars was reduced in treatments containing thyme oil, and inhibition caused by direct addition of thyme oil was only slightly greater than that caused by the thyme oil-starch coating (Table 4). The inhibitory effects were not as great as noted with *Campylobacter* (Table 3).

L. monocytogenes was not recovered on *Listeria* selective agar from uninoculated chicken during storage, but following its inoculation the organism increased one log CFU/g during storage. In addition, growth of *L. monocytogenes* was unaffected by the presence of the starch coating as noted with *Salmonella* and *Campylobacter*. Thyme oil alone or when incorporated into the starch coating was inhibitory to *L. monocytogenes* (on *Listeria* agar) to about the same extent (>1 log CFU/g reduction) by 12 day storage.

Microbial viability on *Pseudomonas*-inoculated chicken The microbial growth profile on chicken inoculated with *P. aeroginosa* as monitored on BHI and MRS agars (Table 5) did not differ from results obtained with the other inoculated organisms when thyme oil was not used (Table 2-4). In addition, the pea starch coating did not further alter bacterial recoveries on these media or *Pseudomonas* agar during storage at 4°C. Thyme oil alone or when incorporated in the pea starch coating significantly delayed the growth of bacteria on chicken monitored with all 3 media. These differences from control (no treatment) were from 1 to 2 log CFU/g and were noted at 12 day of storage (Table 5), however, there was no significant difference in effectiveness of thyme oil action alone (H_2O+thyme oil) and after incorporation in the starch coating (pea starch +thyme oil).

Antimicrobial effectiveness of thyme oil Thyme oil has been shown to one of several potently antimicrobial essential oils during tests against a range of spoilage and pathogenic bacteria. Its major component, thymol, was as effective as eugenol and carvacrol against most of the pathogens tested in the present study (14). Generally, essential oils are more effective against Gram-positive bacteria, but Gram-negative bacteria can be vulnerable (14,15). In the present work, delayed growth of aerobic psychrotrophs and lactic acid bacteria was exhibited. Inhibition of L. *monocytogenes* growth and reduction in *Salmonella* viability in the presence of thyme oil reported here are consistent with the results from other studies where different substrates and temperatures of incubation were used (14). The delayed growth of *P. aeroginosa* reported here is a positive finding since *Pseudomonas* frequently show resistance to essential oil treatment (15), however, it is likely that during longer storage *P. aeroginosa* would recover from the inhibitory effects of thyme oil

Table 5. Effects of thyme oil treatments on the growth (log CFU/g) of *Pseudomonas aeruginosa* on chicken breast meat at 4°C

Treatments	Day 0	Day 4	Day 8	Day 12
Total aerobes				
No treatment	3.2±0.1	5.5±0.0	6.9±0.2	7.9±0.2
Pseudomonas inoculation	5.1±0.1	5.6±0.6	7.0±0.3	7.5±0.6
Pseudomonas+Pea starch coating	4.8±0.1	5.5±0.6	6.9±0.1	8.2±0.1
Pseudomonas+H_2O+Thyme oil	4.2±0.0	4.6±0.5	4.9±0.6	6.8±0.4
Pseudomonas+Pea starch coating+Thyme oil	4.0±0.3$^{b1)}$	3.1±0.7^{a}	5.1±0.8^{c}	5.6±1.1^{c}
Lactic acid bacteria				
No treatment	2.4±0.4	4.9±0.3	6.0±0.2	7.3±0.3
Pseudomonas inoculation	5.0±0.1	4.9±0.1	6.3±0.2	6.9±0.3
Pseudomonas+Pea starch coating	4.2±1.2	4.8±0.5	5.9±0.5	7.4±0.0
Pseudomonas+H_2O+Thyme oil	4.1±0.1	4.3±0.5	4.6±0.5	5.9±0.5
Pseudomonas+Pea starch coating+Thyme oil	3.9±0.3^{b}	2.7±0.8^{a}	4.5±0.7^{b}	5.1±0.9^{b,c}
Pseudomonas aeruginosa				
No treatment	3.2±0.1	5.0±0.2	7.6±0.1	7.9±0.5
Pseudomonas inoculation	5.1±0.2	5.3±0.3	7.8±0.0	7.7±0.2
Pseudomonas+Pea starch coating	4.8±0.1	5.2±0.3	7.6±0.1	8.4±0.1
Pseudomonas+H_2O+Thyme oil	4.1±0.0	4.5±0.5	6.0±0.6	6.8±0.1
Pseudomonas+Pea starch coating+Thyme oil	4.0±0.2^{b}	2.8±0.9^{a}	5.6±1.9^{b,c}	6.0±1.0^{c}

[1)]Different superscripts indicate a significant difference of mean values in rows (*t*-test, $n=6$, $p<0.05$).

exposure. One of the more important observations made here was the drastic reductions in numbers of *C. jejuni* which occurred immediately upon exposure to thyme oil alone or to the starch-thyme oil coating, which was sustained during 12 day storage. Surprisingly little work is reported in the literature concerning *C. jejuni* inhibition by thyme oil. In a study by Friedman *et al.* (24) thyme oil was found to be as effective as cinnamaldehyde, eugenol, carvacrol, citral, geranol, and benzaldehyde against *C. jejuni* in a microplate assay.

In the *C. jejuni* and *L. monocytogenes* tests reported here where thyme oil was directly added to the chicken meat surface, a more immediate inhibitory effect was found against the lactic acid bacteria, however, this difference was not evident at 12 day storage. In *P. aeruginosa* tests the starch-thyme oil coating initially showed a greater inhibitory effect but this difference was resolved by day 8 of storage. In separate test it was found that *Salmonella*, *L. monocytogenes*, and *P. aeruginosa* were able to form small colonies on MRS agar. Thus, lactic acid bacterial recoveries may have been over-estimated to some extent. However, this observation does not affect the overall conclusions from the study.

It is concluded that thyme oil reduced *C. jejuni* viability below detectable levels, significantly inhibited the growth of *S. enterica* serovars as well as *L. monocytogenes*, and delayed the growth of *P. aeruginosa* on chicken breast meats. Pea starch coating was used as a delivery vehicle for thyme oil and also served as a viscosity enhancer to extend the contact of thyme oil with the chicken meat surface. High yield stress is an essential factor for controlling coating thickness and maintaining stable coating layer on food surfaces. The increase yield stress of pea starch coating solution will extend the covering time of the antimicrobial coating layer on chicken carcasses, and enhance the antimicrobial effectiveness. The benefit of this yield stress effect would be greater under the situation of high-speed inside-outside bird washer spraying operation at the poultry processing plant than the individual dipping situation conducted in this study. This study has shown that thyme oil either alone or in a gelatinized pea starch coating was effective in delaying growth of spoilage and pathogenic bacteria on chicken meat surfaces during refrigerated storage. These treatments were effective in essentially eliminating large numbers of *C. jejuni* from the chicken meat and significantly reduced the viability of *S.* Typhimurium. The pea starch coating may be a useful vehicle for application of natural antimicrobials to control undesirable organisms on chicken carcasses.

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