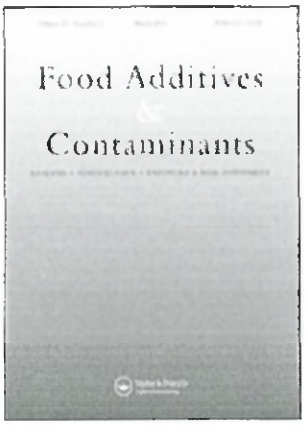


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# Effectiveness of some recent antimicrobial packaging concepts

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# Effectiveness of some recent antimicrobial packaging concepts

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*A new type of active packaging is the combination of food-packaging materials with antimicrobial substances to control microbial surface contamination of foods. For both migrating and non-migrating antimicrobial materials, intensive contact between the food product and packaging material is required and therefore potential food applications include especially vacuum or skin-packaged products, e.g. vacuum-packaged meat, fish, poultry or cheese. Several antimicrobial compounds have been combined with different types of carriers (plastic and rubber articles, paper-based materials, textile fibrils and food-packaging materials). Until now, however, few antimicrobial concepts have found applications as a food-packaging material. Antimicrobial packaging materials cannot legally be used in the EU at the moment. The potential use would require amendments of several different legal texts involving areas such as food additives, food packaging, hygiene, etc. The main objective of this paper is to provide a state of the art about the different types of antimicrobial concepts, their experimental development and commercialization, and to present a case study summarizing the results of investigations on the feasibility of a low-density polyethylene (LDPE)-film containing triclosan to inhibit microbial growth on food surfaces and consequently prolong shelf-life or improve microbial food safety. In contrast with the strong antimicrobial effect in* in-vitro *simulated vacuum-packaged conditions against the psychrotrophic food pathogen* L. monocytogenes, *the 1000 mg kg$^{-1}$ containing triclosan film did not effectively reduce spoilage bacteria and growth of* L. monocytogenes *on refrigerated vacuum-packaged chicken breasts stored at 7°C.*

## Introduction

Active food-packaging concepts provide some additional functions in comparison with traditional passive packaging materials that are limited to protection of the packaged food product against external influences. Active packaging materials change the condition of the packaged food product to extend shelf-life and/or improve microbial food safety and/or improve sensorial properties. One promising type of active packaging is the incorporation of antimicrobial substances in food-packaging materials to control undesirable growth of microorganisms on the surface of foods (Vermeiren *et al.* 1999). The main cause of spoilage of many refrigerated foods is microbial growth on the product surface. The application of antimicrobial agents to packaging materials could be useful to prevent the growth of microorganisms on the product surface and hence may lead to an extension of the shelf-life and/or improved microbial safety of the product (Collins-Thompson and Hwang 2000).

The market for biocidal additives for plastics is growing as consumers demand products with increased hygiene benefits and as new polymers incorporated with these biocides are being developed (Lee 1998). Antimicrobial packaging attracts more and more attention from the food and packaging industry, because of an increasing consumer demand for minimally processed, preservative-free products (Collins-Thompson and Hwang 2000). The use of preservative food-packaging films can offer advantages compared with the direct addition of preservatives to the food product as the preservative agents are applied to the packaging material in a way that only low levels of preservative come into contact with the food. Furthermore, the production process can be

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simplified by combining the packaging step with the addition of preservatives.

The antimicrobial action can be obtained by two types of mechanisms. Preservative-releasing or migrating concepts contain a preservative within their mass or on their surface of which the purpose is to migrate partly or completely into the food or in the space surrounding the food and exercise there its preservative action (Lück and Jager 1997). Non-migrating concepts contain within their mass or on their surface a compound or produce, in certain conditions, a compound that acts antimicrobial when the target microorganism comes into contact with the antimicrobial surface (Kourai *et al.* 1994). The antimicrobial materials can be prepared by incorporating the antimicrobial agent either into the packaging material or by coating the active compound on the surface of the packaging film.

For both concepts, migrating and non-migrating, intensive contact between the active material and the food product is required. Therefore, potential food applications include especially vacuum-packaged products such as vacuum-packaged meat, fish, poultry or cheese, although fruits and vegetables can benefit from the coating technique.

Another possibility, mainly used for fruits, is to incorporate the antimicrobial compound into an edible film or coating, applied by dipping or spraying onto the food (Collins-Thompson and Hwang 2000). However, these coatings, which are supposed to be eaten, are not considered or legally defined as packaging materials.



## Antimicrobial technologies

In recent years, a whole range of antimicrobial technologies has been developed. Applications are mainly situated in the medical sector (medical devices, healthcare products, personal hygiene products, dental care products) and in the food-contact and household area (cutting boards, knife handles, protective clothing, etc.). Other applications are biofilm-sensitive systems such as pipe lines, laboratory, and other scientific equipment and materials of construction for food equipment (Kane 1999). Until now, however, very few of these concepts have applications as food-packaging materials.

The earliest commercial developments were J-Mac (Johnson Matthey Co.), an antibacterial silver-releasing coating for steels and masterbatches containing OBPA (oxybisphenoxarsine) (Morton plastic additives). The latter, however, has some toxicological limitations, due to the presence of arsenic and cannot be used in food-type applications (Lee 1998, Kane 1999). In Japan, Ag-containing components are the most common antimicrobial agents incorporated into plastics (Ishitani 1995). The biological activity of silver is related to the active silver ion released from silver salts, complexes and halides. The silver cation binds strongly to electron donor groups containing sulphur, oxygen or nitrogen. Biological molecules generally contain these components in the form of thio-, amino-, imidazole, carboxylate and phosphate groups. Silver ions act by displacing other essential metal ions such as $Ca^{2+}$ or $Zn^{+}$. The binding of silver to bacterial DNA may inhibit a number of important transport processes such as phosphate and succinate uptake and can interact with cellular oxidation processes as well as the respiratory chain. In comparison to other heavy metal ions, silver is probably the most useful as it combines a high antimicrobial activity with a remarkable low human toxicity (Schierholz *et al.* 1998). Information from producing companies and literature about all these silver-containing materials is scarce and sometimes confusing and contradictory. A first clear distinction has to be made between Ag-zeolites and Ag-substituted zirconium phosphate ceramics.

Silver zeolites are crystalline aluminosilicate materials continuously releasing a small amount (~10 ppb) of silver ions resulting in long-term antimicrobial activity that is not harmful to tissue cells (Matsuura 1997). Examples of commercialized Ag-zeolites are Zeomic® (Shinagawa Fuel Co., Japan) and Bactekiller (Kanebo Co., Japan). The structural formula of Zeomic® is $MX_{2/n}O.Al_2O_3.YSiO_2.ZH_2O$ (M: a cation, e.g. $Ag^{+}$, $Zn^{2+}$, etc.; X, Y and Z: mole fraction of each component). The unique feature of Ag-zeolites is their broad antimicrobial spectrum. As Ag-zeolite is expensive, it is laminated as a thin co-extruded layer of 3–6 μm containing Ag-zeolite particles. The normal incorporation level varies from 1 to 3% (w/w). The effectiveness of this Ag-zeolite incorporated materials towards control of contamination of food products depends on the nutrient level, presence of salts and pH of the product. As amino acids can react with the silver substituted in the zeolite molecular matrix, effects cannot be expected when nutrient-rich foods are packaged at the state of small

relative contact areas, whereas very high effects can be expected with nutrient-poor drinks like mineral water or tea (Ishitani 1995).

Recently, master batches containing Ag-substituted zirconium phosphate ceramics ($Ag_xH_{1-x}Zr_2(PO_4)_3$), called Novaron® (Toagosei Co. Ltd, Japan), are brought onto the European market. However, according tot the European food additive regulation, silver is permitted only to very few foodstuffs, as a colouring. Kourai *et al.* (1994) found that the bactericidal activity of zirconium phosphate ceramics containing silver ion was extremely enhanced by white light irradiation and oxygen. When $HZr_2(PO_4)_3$ was irradiated, Ag-surface$^+$ would work as an electron pool and $O_2$ molecules adsorbed on this site would be reduced to $O_2^-$ radical (Miyoshi 1998):

$$HZr_2(PO_4)_3 + h\nu \rightarrow HZr_2(PO_4)_3 \quad (e^- \text{ and } h^+)$$

$$e^- + (Ag^+_{surface})\text{–}O_2 \rightarrow (Ag^0_{surface})\text{–}O_2$$
$$\rightarrow O_2^- + Ag^+_{surface} \quad \text{(electron pool)}$$

$$h^+ + OH^- \rightarrow OH^\cdot$$

This mechanism in which oxygen is changed into active oxygen through the catalytic action of silver is the so-called oligodynamic action (Yoshida *et al.* 1999). However, the mechanism of photogeneration for superoxide radicals and the role of the silver ion is not clear (Miyoshi 1998) as it is still not clear if these Novaron-containing materials do release silver ions. It is quite possible that both a release of silver ions and the production of super oxide and hydrogen peroxide, formed by certain photochemical reactions on the surface of the Novaron particles, are responsible for the inhibitory effect of Novaron-materials.

Another interesting commercial development was the marketing of triclosan-based master batches such as Microban® by Microban Products Co., UK. The non-ionic and broad-spectrum antimicrobial compound triclosan ($C_{12}H_7Cl_3O_2$) is a difenyletherderivate (2,4,4′-trichloro-2′-hydroxy-diphenyl ether), produced by Ciba Geigy under the trade name Irgasan® DP-300. Microban Products has found a technology to encapsulate triclosan in almost any type of plastic in a way that it is still free to migrate to the surface to start it's work against developing bacteria. The main applications are situated in the food-contact and household areas for instance conveyor belts and cutting boards, as well as medical applications such as surgical drapes (Ciba Technical Information 1998). Triclosan was widely thought to be a non-specific biocide that acts by attacking and disrupting bacterial cell membranes in a way that bacteria cannot assimilate nutrients anymore. It was thought that through this discrete mechanism of action, the acquisition of cellular resistance was unlikely. However, recent work revealed that triclosan blocks bacterial fatty acid synthesis by specifically inhibiting the enzyme enoyl-acyl carrier protein (enoyl-ACP) reductase (FabI). Triclosan acts as a site-specific inhibitor by mimicking the natural substrate of enoyl-ACP (Heath *et al.* 1998, 1999, Levy *et al.* 1999). According to Heath *et al.* (1999), the ability of *E. coli* to acquire genetic resistance to triclosan through mutations in the *fabI* gene suggests that the widespread use of triclosan will lead to the appearance of resistant organisms. However, more research is necessary to confirm this thesis.

In Europe, the legislative status of triclosan is unclear. According to the European regulation, triclosan is not on the list of the approved food additives and is not mentioned in the list of additives notified to the EC as substances that may be used in the manufacture of plastics intended for contact with foodstuffs. Whether triclosan is a food additive or not should be decided through an evaluation of the intention of the use of the substance and of whether it is covered by the definition of food additives. If the technological background for the use is covered by this definition, then triclosan would be a regarded as a food additive. However, as it is not on the list of approved food additives, it cannot be legally used. As to the plastic additive's positive list, this is not a complete list, meaning that substances outside the list can be used, provided their safety in the meaning of the framework Directive is ascertained, and provided the substance does not have a food preservation effect (Fabech *et al.* 2000). Triclosan was submitted to the EC by Ciba in April 1998 with a complete dossier of toxicological and efficacy data (Kane 1999). The Scientific Committee on Food has published their preliminary opinion about triclosan. A quantitative restriction of 5 mg $kg^{-1}$ of food is mentioned but the Commission remarks that the migration could exceed 5 mg $kg^{-1}$ of food and that the use of this compound should not lead to lowering of the hygienic standards in food handling (Scientific Committee on Food 2000).

The list of companies producing biocidal materials gets longer every day. Table 1 lists some important commercialized antimicrobial materials.

*Table 1. Commercialized antimicrobial materials.*

| Company | Trade name | Active compound | Applications |
|---|---|---|---|
| Sanitized AG, Switzerland/Clariant | Sanitized® | triclosan and others | textiles, plastics, leather and paper |
| | Actigard® | | home textiles and PU-foams |
| | Saniprot® | | films and in-can preservation |
| DuPont, USA | MicroFree™ | Ag, copper oxide and zinc silicate | textile and carpet fibres, paints, packaging films, etc. |
| Milliken Co., USA | Novaron® | Ag-substituted zirconium phosphate | many |
| Microban Products, UK | Microban® | triclosan | many |
| Thomson Research Associates, Canada | Ultra-Fresh® | triclosan and others | polymers, adhesives, latexes, plastics, foams, etc. |
| Surfacine Development Company, USA | Surfacine® | Ag-halide/polymer complex | many |
| Ishizuka Glass Co., Japan | Ionpure | Ag/glass | many |



## Antimicrobial food-packaging materials

A whole range of additives have been proposed and tested for use in antimicrobial food-packaging materials including Ag-based compounds (Ishitani 1995), organic acids (Han and Floros 1997, Devlieghere *et al.* 2000a) and the related acid anhydrides (Weng and Chen 1997), bacteriocins, e.g. nisin and pediocin (Ming *et al.* 1997, Dobias *et al.* 1999, Scanell *et al.* 1999, Siragusa *et al.* 1999), hexamethylenetetramine (Devlieghere *et al.* 2000b), enzymes such as lysozyme (Appendini and Hotchkiss 1997, Padgett *et al.* 1998), fungicides such as benomyl (Halek and Garg 1989) and imazalil (Weng and Hotchkiss 1992) and organic compounds such as triclosan (Cutter 1999). However, few of the experimental studies have resulted in effective concepts ready for commercial applications. Often, the inhibitory activity is lost by combining the compound with the polymeric materials due to incompatibility of the component with the packaging material or during extrusion due to the heat lability of the component (Devlieghere *et al.* 2000a). Other systems are effective in *in-vitro* conditions but are not working in combination with real food products because of interactions between food constituents and the active compound (Ishitani 1995, Cutter 1999). Furthermore, the legislative status of the incorporated antimicrobials, as mentioned above, is limiting the application of antimicrobial systems.

A lot of research (Weng and Hotchkiss 1993, Han and Floros 1997, Weng and Chen 1997, Weng *et al.* 1999, Devlieghere *et al.* 2000a) has focussed on the incorporation of sorbic acid and benzoic acid or their corresponding acid anhydrides into food-packaging materials. Han and Floros (1997) showed that 1% potassium sorbate in a low-density polyethylene (LDPE) film inhibited the growth of *Saccharomyces* spp. on agar plates. However, Weng and Hotchkiss (1993), who also tested LDPE films with up to 1% (w/w) incorporated sorbic acid, failed to inhibit mould growth on inoculated media. According to Weng and Hotchkiss, the sorbic acid was insufficiently released from the film to be antimycotic because of the incompatibility of the polar sorbate with the apolar LDPE. These findings were confirmed by Devlieghere *et al.* (2000a). Acid anhydrides were thought to be more compatible than free acids and their salts because of their lower polarity. However, Weng *et al.* (1999) developed the technique of combining poly(ethylene-co-methacrylic acid) (PEMA) with benzoic acid and sorbic acid. Films modified with NaOH (1 mol $l^{-1}$) before incorporation with sorbic acid exhibited dominantly antimicrobial properties in fungal growth inhibition tests, presumably due to the higher amount of preservatives released from the films than other types of films.

Related to food applications, two commercial biocidal films are currently marketed in the USA. One is composed of a chlorinated phenoxy compound and the other of chlorine dioxide (Microgarde, Bernard Technologies, Chicago). Both films have the biocidal agent residing in the polymer spaces and the agents are released upon food contact. A commercial antifungal coating produced from chitosan is also sold as a shelf-life extender for fresh fruit (Padgett *et al.* 1998). In Europe, such a chemical would fall under the definition of food additives and could not legally be used. Rengo Co. (Japan) offers antibacterial labels and sheets, e.g. Wasouro is a combined antibacterial and antimould agent using the volatile constituents extracted from Japanese horseradish and mustard (allyl-mustard oil) (http://www.lintec.co.jp). CSIRO

(Australia) is developing systems gradually releasing $SO_2$ to control mould growth in some fruits. This application is not allowed in the EU. Excessive release of $SO_2$ from pads of sodium bisulphite incorporated microporous material has been shown to cause partial bleaching problems in grapes. Furthermore, the accumulation or absorption of the excess of $SO_2$ by foods could cause toxicological problems (http://www.dfst.csiro.au).



## Case study on a triclosan film as antimicrobial food packaging

### *Introduction*

As mentioned above, the organic compound triclosan has recently gained a lot of interest. Cutter *et al.* (1999) evaluated the effectiveness of plastic containing 1500 mg $kg^{-1}$ triclosan in plate overlay assays and meat experiments. The study demonstrated that the incorporation of triclosan into plastic resulted in activity in plate overlay assays, but that when the plastic is combined with vacuum-packaging and refrigerated storage, bacteria are not sufficiently reduced on meat surfaces. The possible interaction of triclosan with adipose components of the meat product may be responsible for this inactivity. In this study, the feasibility of a low-density polyethylene (LDPE) film containing triclosan to inhibit microbial growth on food surfaces and consequently prolong shelf-life or improve safety of foods was investigated. The antimicrobial activity of the triclosan-containing LDPE was determined qualitatively in an agar diffusion assay and quantitatively in an *in-vitro* storage experiment on agar plates. The experiments were finalized by performing a storage experiment with poultry inoculated with *L. monocytogenes*.

### *Materials and methods*

*Agar diffusion assay.* The inhibitory effect of LDPE films containing 0, 500 and 1000 mg $kg^{-1}$ triclosan was determined qualitatively in an agar diffusion test towards a range of food pathogens and spoilage organisms: *L. monocytogenes*, *St. aureus*, *S. enteritidis*, *E. coli* 0157:H7, *Br. thermosphacta*, *B. cereus*, *L. sake*, *L. brevis*, *P. roqueforti*, *A. niger* and *C. albicans*. TSA-plates were inoculated by spreadplating with $10^5$ cfu/plate of the test organisms and covered with a circular piece ($\phi = 3$ cm) of the triclosan-containing films. Any antimicrobial effect could be observed as a growth inhibition zone around the plastic. Experiments were performed in duplicate.

In vitro *storage experiment.* The inhibitory activity of the film against *L. monocytogenes* was quantified in an *in vitro* storage experiment on agar plates. During 20 days, the growth was followed at 7°C on TSA-plates (pH 5.7), which were uncovered, covered with LDPE or covered with the 1000 mg $kg^{-1}$ triclosan-containing LDPE film.

TSA-plates were inoculated by spreadplating with $10^4$ cfu/plate of *L. monocytogenes* (LMG 13305) and covered with test films, which had the same diameter as a Petri dish. To determine the effect of covering on the growth of *Listeria*, a non-covered series of Petri dishes was also prepared. All Petri dishes were packaged in an atmosphere of 100% $N_2$ to simulate vacuum-packaged conditions and stored at 7°C. Every day the counts of *L. monocytogenes* were determined. The agar of the Petri dishes was weighed and based on the weight of the agar a first decimal dilution of both agar and covering film in pepton physiologic salt solution was prepared. After mixing in a stomacher, further decimal dilutions were prepared and spreadplated onto *Listeria*-selective agar base (Oxford formulation Oxoid CM856). After incubation of the Oxford plates at 37°C for 48 h, countings were performed.

*Storage experiment with chicken breasts.* A storage experiment with chicken breasts, vacuum-packaged in the LDPE film containing 1000 mg $kg^{-1}$ triclosan, was conducted. The chicken breasts were inoculated with *L. monocytogenes*. During storage at 7°C, the evolution of total aerobic count, total anaerobic count, lactic acid bacteria, *Enterobacteriaceae* and yeasts, and the growth of *L. monocytogenes* (LMG 13305) was followed.

Twenty-eight chicken breasts (pH 5.7) with a mean weight of 200 g were inoculated with $10^4$ cfu $g^{-1}$ of *L. monocytogenes* by spreading 0.5 ml of an inoculum of $2 \times 10^6$ cfu $ml^{-1}$ on the top and bottom side of the chicken's surface (0.25 ml on each side). Half of the chicken pieces were packaged in the 1000 mg $kg^{-1}$ triclosan-containing film, the other half was packaged in the reference LDPE film free of triclosan. All

packages were packaged once more in a barrier film (PA/PE 15-60 Südpack, verpackungen Ochsenhauwsen, Germany) to ensure the vacuum and stored at 7°C. At day 0, 4, 8 and 12 microbial analyses were performed on two samples (in duplicate) of the chicken breasts. For the determination of the total aerobic count, respectively total anaerobic count, lactic acid bacteria and *Enterobacteriaceae* the pour-plating method was performed onto PCA (plate count agar, Oxoïd CM325) respectively RCA (reinforced clostridial agar, Oxoïd CM151), MRS (Man Rogosa Sharpe, Oxoïd CM361) and VRBG (Violet Red Bile Glucose agar, Oxoïd CM485). Yeasts and *L. monocytogenes* were determined by spreadplating on YGC (Yeast Glucose Chloramphenicol, Sanofi 64104) and *Listeria* selective agar base (Oxford formulation, Oxoïd CM856) combined with Selective supplement (Oxford formulation, Oxoïd SR 140) respectively. PCA, RCA, MRS and YGC were incubated for 3–5 days at 30°C. VRBG was incubated for 1 day at 37°C and *Listeria* selective agar base for 2 days at 37°C. After incubation, countings were performed.



## Results

### *Agar diffusion assay*

The observed inhibition zones (zones of no visible growth) are shown in table 2. The zones of reduced growth were zones around the inhibition zones showing smaller colonies. The width of an inhibition zone (1) is the difference between the radius of the inhibition zone and the radius of the plastic test circle (1.5 cm). The value indicating the zone of reduced growth (2) is the width of the zone around the inhibition zone. Around the control films—LDPE free of triclosan — no inhibition zones were observed, as expected.

The growth inhibition zones indicate that the triclosan film has a clear antimicrobial effect against the food pathogens *L. monocytogenes*, *St. aureus*, *S. enteritidis* and *E. coli* O157:H7. For the spoilage organism *Br. thermosphacta*, only a zone of reduced was obtained. No effect was found towards *B. cereus*, *C. albicans*, *L. sake*, *L. brevis*, *P. roqueforti* and *A. niger*.

A doubling of the triclosan concentration did not result in a doubling of the radius of the inhibition zone. Only a slightly larger inhibition zone was observed when the concentration of triclosan in the LDPE films was increased from 500 to 1000 mg triclosan $kg^{-1}$ LDPE. The results partly correspond to the plate overlay assay with the triclosan-incorporated plastic of Cutter *et al.* (1999). They also detected antimicrobial activity against *E. coli* O157:H7, *St. aureus* and *Br. thermosphacta*, but not against *L. monocytogenes*. However, in this work, the activity of the film against *L. monocytogenes* was unarguable.

*Table 2. (1) Width of the inhibition zones (cm) and (2) zones of reduced growth* (cm) produced by a 500 and 1000 mg $kg^{-1}$ triclosan-containing film in an agar diffusion assay at the optimal growth temperature of the test organisms.*

| | 500 mg $kg^{-1}$ triclosan film | | 1000 mg $kg^{-1}$ triclosan film | |
|---|---|---|---|---|
| Test strains | 1 | 2 | 1 | 2 |
| *L. monocytogenes* | 0.61 (±0.35) | 0.30 (±0.47) | 0.85 (±0.44) | 0.26 (±0.65) |
| *St. aureus* | 1.12 (±0.08) | 0.55 (±0.02) | 1.34 (±0.04) | 0.80 (±0.01) |
| *S. enteritidis* | 0.41 (±0.04) | 0.20 (±0.05) | 0.67 (±0.02) | 0.15 (±0.01) |
| *E. coli O157:H7* | 0.24 (±0.60) | 0.39 (±0.02) | 0.55 (±0.02) | 0.25 (±0.02) |
| *Br. thermosphacta* | 0.00 | 0.55 (±0.00) | 0.00 | 0.85 (±0.00) |
| *B. cereus* | 0.00 | 0.00 | 0.00 | 0.00 |
| *L. sake* | 0.00 | 0.00 | 0.00 | 0.00 |
| *L. brevis* | 0.00 | 0.00 | 0.00 | 0.00 |
| *P. roqueforti* | 0.00 | 0.00 | 0.00 | 0.00 |
| *A. niger* | 0.00 | 0.00 | 0.00 | 0.00 |
| *C. albicans* | 0.00 | 0.00 | 0.00 | 0.00 |

* Standard deviation (SD) is in parentheses.

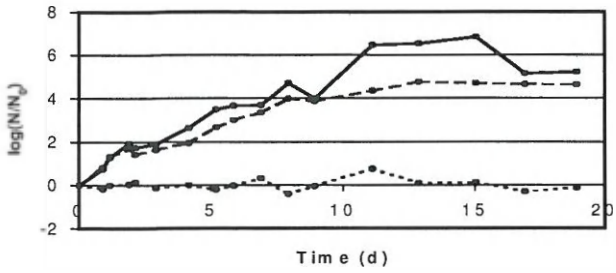


*Figure 1. Growth of* L. monocytogenes *at 7°C in 100% $N_2$ on TSA plates, which were uncovered (—), covered with LDPE (– –) and covered with LDPE containing 1000 mg kg$^{-1}$ triclosan (- - -).*



In vitro *storage experiment*

The growth of *L. monocytogenes* on the uncovered and covered TSA-plates is shown in figure 1 as log($N/N_0$) as a function of time. On the plates covered with the 1000 mg kg$^{-1}$ triclosan film, no growth of *L. monocytogenes* could be observed after 20 days. On uncovered plates and plates covered with LDPE free of triclosan, a normal growth pattern was observed: on the plates covered with LDPE, a lower maximum cell density was reached compared with uncovered plates because of a lack of oxygen created by covering with the film. These results show that the 1000 mg kg$^{-1}$ containing triclosan film has a strong antimicrobial effect in *in vitro* simulated vacuum-packaged conditions against the psychrotrophic food pathogen *L. monocytogenes*.

*Storage experiment with chicken breasts*

Results of the microbial analyses are presented in figures 2 and 3. Packaging of chicken breasts in

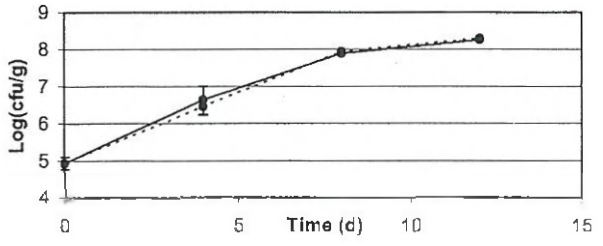


*Figure 2. Evolution of total aerobic count at 7°C on chicken breasts vacuum-packaged in LDPE (—) and LDPE containing 1000 mg kg$^{-1}$ triclosan (- - -).*

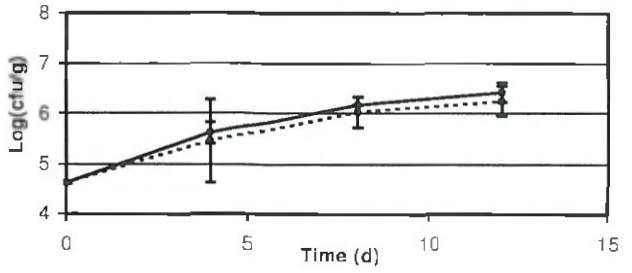


*Figure 3. Growth of* L. monocytogenes *at 7°C on chicken breasts vacuum-packaged in LDPE (—) and LDPE containing 1000 mg kg$^{-1}$ triclosan (- - -).*

LDPE containing 1000 mg kg$^{-1}$ triclosan had no influence on the evolution of the spoilage bacteria. Total aerobic count, total anaerobic count, lactic acid bacteria, *Enterobacteriaceae* and yeasts were not reduced by the presence of the triclosan-containing film.

In addition, the growth of *L. monocytogenes* was not inhibited by the triclosan-incorporated film in contrast with the inhibitory effect observed in the *in vitro* storage experiment.

These results correspond to those of Cutter *et al.* (1999), who also demonstrated that while antimicrobial activity is detected against bacterial cultures in antimicrobial plate assays, the triclosan-incorporated plastic does not effectively reduce bacterial populations on refrigerated vacuum-packaged meat surfaces. Additional experiments by Cutter *et al.* suggest that the presence of fatty acids or adipose may diminish the antimicrobial activity of the triclosan-incorporated plastic. The possible interaction of triclosan with adipose components of the meat product may be responsible for this inactivity.

*Conclusions*

The LDPE films containing 500 and 1000 mg kg$^{-1}$ (= 0.5 and 1.0% Sanitized MB E 97-65) of triclosan exhibited antimicrobial activity against *L. monocytogenes*, *St. aureus*, *S. enteritidis*, *E. coli* O157:H7 and *Br. thermosphacta* in an agar diffusion assay. The inhibitory activity of the film against *L. monocytogenes* was quantified in an *in vitro* storage experiment on agar plates. The 1000 mg kg$^{-1}$ containing triclosan film had a strong antimicrobial effect in *in vitro* simulated vacuum-packaged conditions against the

psychrotrophic food pathogen *L. monocytogenes*. However, the triclosan-incorporated plastic did not effectively reduce spoilage bacteria and growth of *L. monocytogenes* on refrigerated vacuum-packaged chicken breasts stored at 7°C. The results show that the benefit of packaging chicken breast for refrigerated storage into a triclosan-incorporated LDPE film is reduced because of the ineffectiveness towards microbial growth.



## General conclusion

Nowadays, the incorporation of antimicrobial compounds in all types of articles is gaining a lot of interest, both by the industry and the consumer. However, few antimicrobial concepts have found applications as food-packaging material. Further research on antimicrobial food-packaging materials is necessary not only to reveal the mechanisms of action of existing systems, but also to develop new materials. One of the problems in getting an antimicrobial-packaging concept commercialized for food applications is the legal status of the tested additives. In the European FAIR-project (CT 98-4170) Actipak, a workgroup on legislation is working on the need for change in regulations in the EU countries. The use of antimicrobial packaging and active packaging in general involves the consideration and possible amendments of several different parts of the legislation such as the food-packaging legislation, legislation on food additives, EU directives on hygiene, etc. Amendments to regulation might require extensive toxicological and other testing of compounds before approval to secure safe food and environment.

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