

## Introduction

Polymer films containing active substances were produced and tested for their antimicrobial potential against certain bacteria in vitro and with different types of raw meat. The different results could be linked to the individual migrational behaviour of the substances.

## Material preparation

The active substances were mixed with an ethylene-octene copolymer by extrusion on a laboratory-sized twin-screw extruder at 140 °C, and subsequently pressed to films with ~150-200 µm thickness on a hydraulic press. The active substances were: **citric acid, mono-sodium citrate, sorbic acid, and potassium sorbate**.

## Experimental methods, results and discussion

### Migration of active substances

- Migration into distilled water
- Two-sided extraction for 7 days at different temperatures
- Citric acid, citrate: enzymatic test (photometric): citrate lyase, lactate dehydrogenase
- Sorbic acid, sorbate: quantitative thin-layer chromatography after extraction with petrol ether/diethylether 1:1 (v/v), eluent: hexane/acetic acid 96:4 (v/v)

Table 1: Dissolved substance in water after 7 days (% of the original amount), average of at least 2 samples

	40 °C	20 °C	4 °C
<b>Citric acid (25 %)</b>	99.4	94.3	74.1
<b>Mono-sodium citrate (50 %)</b>	98.8	95.9	85.6
<b>Sorbic acid (5 %)</b>	10.5	8.8	**
<b>Potassium sorbate (25 %)</b>	76.0	60.3	43.5

\*\* below detection limit

Citric acid and citrate show very high migration rates even at lower temperatures.

The migration of potassium sorbate is lower and obviously more dependent on temperature.

Sorbic acid does not migrate very well into water.

### Microbiological tests with meat

- Storage of beef and turkey meat samples in active films and reference LDPE films for 7 days at 5 °C
- Determination of total plate count according to EN ISO 6887-2:2003 (Plate count agar, 30 °C, 72h)

Tables 6-9: cfu/g aerobic mesophilic bacteria in meat samples after storage of 7 days in active films and reference

#### 25 % Citric acid

##### Beef

Fresh	7 days / reference	7 days / active film
1.4 * 10 <sup>3</sup>	5.4 * 10 <sup>7</sup>	4.2 * 10 <sup>4</sup>

##### Turkey

Fresh	7 days / reference	7 days / active film
1.5 * 10 <sup>3</sup>	2.0 * 10 <sup>9</sup>	6.4 * 10 <sup>5</sup>

#### 50 % Mono-sodium citrate

##### Beef

Fresh	7 days / reference	7 days / active film
2.0 * 10 <sup>2</sup>	1.5 * 10 <sup>8</sup>	2.2 * 10 <sup>4</sup>

##### Turkey

Fresh	7 days / reference	7 days / active film
1.5 * 10 <sup>3</sup>	2.0 * 10 <sup>9</sup>	8.7 * 10 <sup>4</sup>

With 5 or 10 % sorbic acid and 30 % potassium sorbate there was no significant difference between the active films and the references. This might be linked to the differences in the migration of the substances to the aqueous surface of the meat.

### Simple microbiological tests

- Spraying of active films and reference LDPE films with bacterial suspension (pieces of 6 x 5 cm, 1 ml of suspension (~10<sup>4</sup> cells/ml))
- Overlaying with nutritional medium, incubation
- Counting of colonies after 2, 5 and 7 days
- Bacterial strains: E. coli K-12, Carnobacterium divergens
- Nutritional media: plate count agar, trypticase soy broth

Tables 2-5: cfu/30 cm<sup>2</sup> film after 7 days (average of 3 samples)

#### Citric acid

	LDPE	Film with 15 % citric acid	Film with 25 % citric acid
<b>E. coli</b>	> 200	31	0
<b>C. divergens</b>	98	37	6

#### Mono-sodium citrate

	LDPE	Film with 26 % citrate	Film with 51 % citrate
<b>E. coli</b>	> 200	81	0
<b>C. divergens</b>	102	49	2

#### Sorbic acid

	LDPE	Film with 2 % sorbic acid	Film with 5 % sorbic acid
<b>E. coli</b>	> 200	195	2
<b>C. divergens</b>	> 200	200	8

#### Potassium sorbate

	LDPE	Film with 20 % sorbate	Film with 30 % sorbate
<b>E. coli</b>	> 200	13	3
<b>C. divergens</b>	> 200	125	13



LDPE film (left) and copolymer film containing 25 % citric acid (right) sprayed with E. coli after 5 days.

E. coli is generally more sensitive than C. divergens. Effective inhibition of microorganisms is possible, but (except for sorbic acid) only with very high concentrations of the active substances.

## Conclusions

Polymer films containing citric acid or citrate might be an interesting option for antimicrobial packaging in the future as they are able to slow down the growth of aerobic bacteria on meat surfaces. Further research will however be necessary before the product is ready for the market.