# Effect of some spices on the development of the spoilage flora in minced meat preparations*

Matthias Upmann[a], Susanne Stiegler[a] and Johannes Novak[b]

[a]Institute for Meat Hygiene, Meat Technology and Food Science and [b]Institute for Applied Botany, University of Veterinary Medicine Vienna, Austria

**Key words**
Spice, essential oil, antibacterial activity, fresh meat, spoilage flora.

**Background**
Nowadays, a convenient and time efficient manner of food preparation is one of the main detterminants of consumers' buying behaviour. In the fresh meat sector, convenience foods are often prepared in advance and stored under refrigeration. Various herbs and spices possess antimicrobial activity. In this study their food preserving potential was determined.

**Objective**
Oregano, thyme, rosemary and marjoram possessing a distinct antimicrobial activity are widely used spices for flavouring meat and meat products. The aim of this study was to determine (i) which spice concentrations are necessary in order to produce an antimicrobial effect, (ii) if the intensity of antimicrobial activity changes when ground spices or essential oils are used instead of spice leaves, and (iii) if there are differences between the effect of spices on the main bacterial species responsible for meat spoilage.

**Material and Methods**
Oregano (*Origanum* sp.), rosemary (*Rosmarinus officinalis*) and marjoram (*Origanum majorana*) were purchased from local wholesale operations as whole leaves and as ground spice. Thyme leaves (*Thymus vulgaris*) were obtained directly from a german trader. Ground thyme was produced by milling the thyme leaves to a granule size of 0.25 mm with an ultra centrifugation mill. Essential oils were extracted from whole and ground leaves by steam destillation.
Minced meat was purchased at various local supermarkets. Whole leaves and ground spices were added to meat portions of 200 g in concentrations of 0.5%, 1.0%, 2.0% and 5.0%. Essential oils extracted from whole leaves were added in quantities corresponding to those introduced by the addition of whole spice leaves. For this purpose, oil quantities between 9.2 mg (0.5% marjoram) and 249.0 mg (5.0% thyme) were suspended in 5.0 g of lard and this mixture was subsequently introduced homogeneously into 195.0 g minced meat by use of a commercial electrical hand mixer. All meat samples were stored for 4 days at 3 ± 1 °C.

The oil concentrations in the spices under study were determined by using a modified Clavenger apparatus. The chemical compositions of the essential oils were determined by GC/MS on a HP 6890 in connection with a HP 5972 MSD. For this purpose, 5 µl of the essential oil was diluted with 495µl $CH_2Cl_2$ and the oil compounds were analysed with a Rtx-5MS 30 x 0.25 mm capillary column (carrier gas: Helium; injection temperature: 250 °C, split ratio: 50:1, temperature program: 60 °C to 240 °C with an increase of 3 °C /min).

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The microbiological condition of the spiceless meat was determined at the day of purchase (day 0). Subsequently, 15 g to 20 g samples from spice or essential oil containing- as well as spiceless meat portions were taken daily up to the fourth day of storage. After homogenization in a Stomacher 400, total mesophilic counts (30 °C, plate count agar), *Enterobacteriaceae* counts (VRBG-agar), *Pseudomonaceae* counts (GSP-agar) and *Brochothrix thermosphacta* counts (STAA-agar) were determined in duplicat by the drop plating technique. Each storage trial was repeated 6 times.

**Results and Discussion**
Generally, none of the spices or essential oils had a marked shelf life prolonging effect when added to minced meat, neither with regard to total aerobic counts nor to *Enterobacteriaceae*-, *Pseudomonaceae*- and *Brochothrix thermospacta* counts. Even with spice concentrations of 5 % the median of the differences did generally not exceed 0.5 $\log_{10}$ cfu/g. Contrarily, some authors have described a reduced specific growth rate and a 2 to 3 $\log_{10}$ lower microbial count for meat spoilage organisms by addition of spices or essential oils, respectively. However, a direct comparison to these results is difficult, since in one case a non-specified spice mixture was used as underlayer for 2 $cm^3$ pork cubes (5), in the other 0.8 % oregano oil was added to beef muscle pieces of about 25 g (12).

Different intensities of the antimicrobial activity due to the spice's grinding grade (whole leaves, ground, essential oil) were seen only exceptionally. Expectedly, spice leaves and essential oils produced similar effects because the same oil was added to meat in comparable concentrations. However, ground thyme seemed to be less inhibitory to pseudomonads than were thyme oil and thyme leaves (see fig. 1). This can be explained by the lesser oil content, which was nearly halved during grinding (leaf: 2,49 %; ground: 1,27 %). The quantity of thymol - the most inhibitory compound - introduced into meat was three times lower for ground thyme than for thyme leaves or thyme oil (see tab. 1).

Differences in the intensity of antimicrobial activity due to the spice or essential oil concentration were small. In two cases only, a marked growth reduction was observed. 5.0% of ground rosemary resulted in 0.34 to 0.52 $\log_{10}$ cfu/g lower total viable counts in comparison to samples without spices. The reason for this is not clear. Although β-pinene and δ-3-carene, which are known to act inhibitory on a variety of microorganisms, were only detected in ground rosemary, some other oil compounds with similar inhibitory effects (limonene, linalool, borneol, terpinen-4-ol, α-terpineole) have been detected exclusively or in larger amounts in whole leaves (see tab. 1). In contrast to this, the inhibitory effect of 5.0% ground oregano on *Brochothrix thermosphacta* (0.24 to 0.73 $\log_{10}$ cfu/g: see fig. 2) seems to be due to the inhibitory substances carvacrol and thymol, which have been added to meat in larger quantities by the ground spice (see tab. 1). However, spice concentrations of 5.0 % have not been organoleptically acceptable for any of the spices used.
The spices showed diverging effects on the different groups of microorganisms under study. As described above, ground rosemary was able to reduce the total viable counts without having a visible effect on the other microbial groups, while ground oregano showed growth

reducing potential only against *Brochothrix thermosphacta*. Furthermore, marjoram leaves appear to have a growth increasing rather than reducing influence on *Brochothrix thermosphacta* (3/4 of the differences have been in the 'growth increasing' sector), while the same seems to hold for ground thyme and pseudomonads (see fig. 1). Similar observations have been made in *in vitro* tests. While pseudomonads seem to be rather resistant against the essential oils under study (13), *Escherichia coli* is rather sensitive to oregano and thyme oil and *Brochothrix thermosphacta* in particular to thyme oil (6, 8, 10, 13). The inhibitory effect of marjoram oil and rosemary oil on *Escherichia coli* and *Brochothrix thermosphacta* is less pronounced as compared to thyme oil (4).

## Conclusions

- The possibility to delay bacterial spoilage of minced meat preparations by the addition of spice leaves, ground spices or essential oils from thyme, oregano, rosemary or marjoram is limited.
- In order to produce an inhibitory effect with pure thyme, oregano, rosemary or marjoram, concentrations beyond their organoleptic acceptability have to be added.
- Different intensities of antimicrobial activity of whole leaves, ground leaves and essential oil are due to differences in oil content and oil composition of the spice rather than to differences in oil liberation and homogenisation in the food matrix.
- A spices effect on a complex microflora is difficult to predict since inhibitory effects on one bacterial species may be compensated by increased growth of another bacterial species, resulting in similar overall bacterial counts.

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**Table 1:** Amount of chemical essential oil compounds with known antibacterial actitvity added to minced meat*

| Compound | strength antibact. activity[ref] | *thyme* | | *oregano* | | *rosemary* | | *marjoram* | |
|---|---|---|---|---|---|---|---|---|---|
| | | l | g | l | g | l | g | l | g |
| α-pinene | -[2], ±[3,4,9] | | | 0.5 | | 11.7 | 11.9 | | 0.1 |
| camphene | -[3] | | | 0.5 | | 4.5 | 4.0 | | |
| β-pinene | ±[3], +[4,9] | | | | | | 0.5 | | 0.1 |
| α-phellandrene | +[3] | | | | | | | | 0.2 |
| δ-3-carene | -[3], +[4] | | | | | | 0.4 | | 0.1 |
| α-terpinene | ±[3,7] | | 0.8 | 1.2 | 1.1 | | | 1.5 | 2.1 |
| para-cymene | -[2], ±[11], +[3] | 10.2 | 16.6 | 8.0 | 2.4 | 2.5 | 2.7 | 0.4 | 1.4 |
| limonene | ±[4,7], +[3] | | 0.6 | | | 3.2 | | 0.8 | |
| 1,8-cineole | -[7,9] | 1.3 | 1.4 | 0.6 | 0.3 | 36.1 | 78.3 | | |
| γ-terpinene | -[2] | 4.6 | 6.2 | 4.2 | 1.9 | | | 3.3 | 3.2 |
| cis-sabinene hydrate | | | | | | | | 17.2 | 1.0 |
| linalool | ±[2,7], +[3,4] | 3.0 | 5.9 | 2.8 | 10.2 | 2.7 | 0.6 | | 0.9 |
| camphor | -[3,9] | | 0.8 | | | 52.0 | 6.5 | | |
| borneol | -[9], ±[4] | 2.5 | 3.3 | 1.9 | 1.1 | 14.6 | 6.9 | | |
| terpinen-4-ol | +[4] | 1.0 | 1.7 | 1.1 | 5.4 | 6.0 | 1.5 | 22.2 | 9.7 |
| α-terpineole | ±[2], +[4] | | 0.5 | 0.3 | 1.0 | 13.4 | 4.8 | 7.6 | 2.0 |
| verbenone | | | | | | 8.0 | | | |
| linalyl acetate | ±[7] | | | | | | | 11.5 | 0.3 |
| thymol | ++[1,2,4] | 191.6 | 67.2 | 5.3 | 9.0 | | | | |
| carvacrol | ++[1,2,4,11] | 20.6 | 6.0 | 80.4 | 119.3 | | | 0.5 | |
| geranyl acetate | ±[4] | | | | | | | 0.3 | |
| β-caryophyllene | | 5.0 | 5.5 | 1.3 | 1.9 | | 2.9 | 7.1 | 1.5 |

[ref]: reference  l: cutted spice leaves;  g: ground spice

* calculated by multiplication of the contents of chemical compound in the essential oil after destillation [in %(w/v)] and the oil content in spice [in %(v/w)])

**Figure 1:** Differences between *Pseudomonaceae* counts of minced meat samples with thyme (oil, leaves, ground) and samples without spice during 4 days at 3±1 °C.

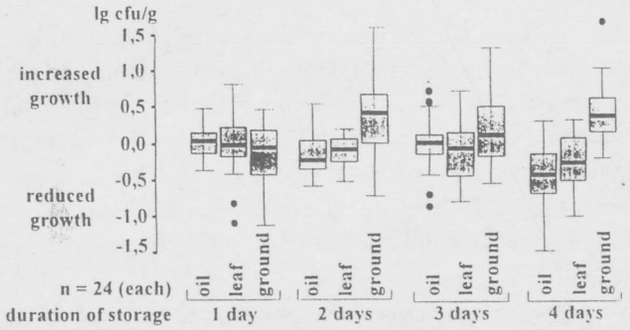


**Figure 2:** Differences in *Brochothrix thermosphacta* counts between minced meat samples with different concentrations of ground oregano and without spice during 4 days at 3±1 °C.