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USE OF ESSENTIAL OILS AND OLEORESINS FROM SPICES AND HERBS FOR CONTROL OF L. MONOCYTOGENES IN COOKED, CURED MEAT PRODUCTS

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Background

Essential oils and oleoresins from spices and herbs could be an alternative to using chemical preservatives in cooked, cured meat products. Many essential oils and oleoresins are shown to exert antimicrobial effects against *Listeria monocytogenes* in laboratory assays (Aureli *et al*, 1992; Lis-Balchin *et al*, 1996b). However, when tested in real food matrices, the effects are reduced, and higher concentrations are needed (Aureli *et al*, 1992; Mendoza-Yepes *et al*, 1997). At the same time, different preparations of essential oils and oleoresins from different producers will exert different antimicrobial activity (Lis-Balchin *et al*, 1996a); and different strains of *L. monocytogenes* are not equally sensitive to essential oils (Lis-Balchin & Deans, 1997). These factors, combined with a very sparse knowledge about the influence of essential oils on organoleptic properties in "real life" meat products, provided the background for the initiation of the present study.

Objectives

The primary aim of the study was to identify essential oils and oleoresins capable of preventing growth of *L. monocytogenes* in cooked, cured meat products during storage in abusive temperatures without having a negative effect on the organoleptic properties. A secondary aim was to investigate whether results from *in vitro* screening were valid also for commercial meat products.

Methods

Preliminary screening A 1:1:1 cocktail of three strains of *L. monocytogenes*, two isolated from meat products and one isolated from a meat production facility, was mixed with ISO-Sensitest agar at a level of 4 log cfu/ml. 20-25 ml agar were poured into a petri dish, and four holes of 4 mm diameter were punched in the agar. 20 µl essential oil/oleoresin were pipetted into each hole. 46 different essential oil/oleoresin preparations from 9 different producers were tested. They represented 12 spices/herbs: clove, thyme, bay, nutmeg, pimento, rosemary, cinnamon, origanum, cumin, lemongrass, tea tree and sage. One positive control (20 µl nisin 5000 IE) and one negative control (20 µl sterile water) were included. After incubation (10°C/6 days) the diameters of the inhibition zones were measured.

Meat slurry model One part of a cooked, cured, emulsion type meat product (2.3% sodium chloride in the aqueous phase, 15% fat, 30 ppm nitrite) was dispersed in two parts 1.5% w/v sterile saline making a 33% meat slurry model. Essential oils and oleoresins, selected from the preliminary screening, were diluted in glycerin 1:1 and added to the meat slurry model in concentrations of 2% and 0.5% v/v. Two controls, one with 2% glycerin and one with no addition, were included. The same cocktail of *L. monocytogenes* strains as mentioned above was added to the meat slurry in an amount of 3 log cfu/ml. After incubation (10°C/7 days) *L. monocytogenes* was enumerated on Oxford agar (37°C/2 days).

Testing in emulsified sausage system A cooked, cured, emulsion type sausage was made (2.3% sodium chloride in the aqueous phase, 15% fat, 30 ppm nitrite). Thyme essential oil (preparation 44) in concentrations of 2% and 1% and cinnamon bark essential oil (preparation 42) in concentrations of 2%, 1%, 0.25% and 0.1% w/w were added to the batter during chopping. After pasteurisation, the sausages were sliced and inoculated with 3 log cfu/g of a 1:1:1:1:1 cocktail of five strains of *L. monocytogenes*, three isolated from meat products and two isolated from a meat production facility. The slices were packed in a modified atmosphere (20% CO_2 /80% N_2; product:head space volume 1:2.5) in a 40 µm PA/EVOH/PA, 75 µm LDPE/oriented LDPE plastic laminate. The samples were stored at 10°C for 4 weeks with sampling after 1 day, 1 week, 2 weeks and 4 weeks. *L. monocytogenes* was enumerated on Oxford agar (37°C/2 days). Sensoric properties were assessed in an open test on uninoculated samples kept at 5°C for two weeks.

Results and discussion

Preliminary screening The 46 preparations of essential oils and oleoresins were selected to represent different spices and herbs, but also to ensure that the same spice/herb was represented from different manufacturers (e.g. thyme 1, 10, 25, 33 and 44). The preparations showed major differences in their effect against *L. monocytogenes* (*figure 1*). Some preparations of cinnamon bark (18, 32 and 42), lemongrass (21 and 43) and thyme (25 and 33) gave maximum inhibition zones (50 mm), whereas e.g. thyme 1, 10 and 43 produced a smaller inhibition effect. A number of preparations had no effect at all. Essential oils from different spices/herbs contain different components (Tainter & Grenis, 1993) and the different components exert different antimicrobial effects (Bullerman *et al*, 1977; Kim *et al*, 1995). A great variation in effectiveness was therefore expected.

Meat slurry model To ensure correlation between the preliminary screening in ISO-Sensitest agar and a meat system, 12 of the 46 preparations were selected for further studies in the meat slurry model (*table 1*). They represented preparations that gave no, small and large inhibition zones in ISO-Sensitest agar. Seven of these preparations showed listericidal effect in the meat slurry (thyme 1, clove 3, thyme 25, cinnamon bark 42, lemongrass 43, thyme 44 and origanum 46) in 2% concentrations; two (thyme 25 and cinnamon bark 42) also in 0.5% concentration. Rosemary 5 had a listeriostatic effect, whereas cumin 9, nutmeg 15, cumin 20 and tea tree 27 had no antilisterial effect at all. The controls with and without glycerin had no effect (data not shown). Qualitatively, this correlated well with results obtained in the ISO-Sensitest agar. Quantitatively the correlation was less obvious. Some preparations with small diameter inhibition zones in ISO-Sensitest agar exerted a strong effect in the meat slurry model (e.g. thyme 1 and clove 3). Poor quantitative correlation was also shown by Manou *et al* (1998) and could be due to differences in viscosity and solubility of the many active components. This would influence the effectiveness in the two different test matrices.

Testing in emulsified sausage system Cinnamon bark 42 and thyme 44 were selected for testing in the emulsified sausage system as representatives of preparations with strong antilisterial effect (cinnamon bark 42) and medium antilisterial effect (thyme 44). Thyme 44 could not prevent growth in low concentrations in the meat slurry model while cinnamon bark could. The latter was therefore tested in a broader range of concentrations (*table 2*). Thyme 44 had very limited effect on the growth of *L. monocytogenes*. It only reduced growth approx. one log-unit the first two weeks when used in a 2% concentration. This was not expected after the meat slurry model result. On the other hand, as expected from the other assays, cinnamon bark 42 not only prevented growth, but seemed to kill *L. monocytogenes* when used in concentrations of 1% or more. Unfortunately even the lowest concentration (0.1%) of cinnamon bark had a very strong negative influence on the organoleptic properties of the product. Both taste, flavour and colour were affected. In a 1% concentration the sensory panel found the sausage inedible. Thyme in 1% and 2% concentrations was also unacceptable. A possible next step could be to identify antimicrobial components without taste and flavour in the herb and spice preparations.

Conclusions

This study has shown that many preparations of essential oils and oleoresins from herbs and spices exert an antilisterial effect, although variations are found in different test assays. The effect of specific preparations could be reproduced in different assays, but with different levels of inhibition. When using essential oil preparations in meat products, complete inhibition even for the worst case storage situations (10°C/28 days) could be achieved with cinnamon bark (1%), but the product was organoleptically unacceptable.

Literature

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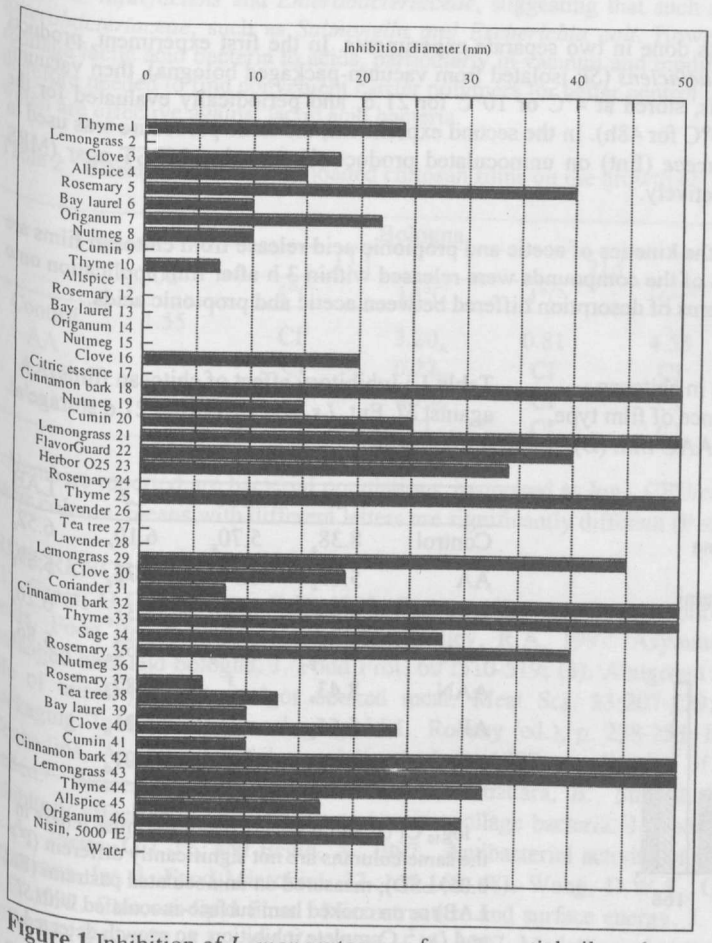

Figure 1 Inhibition of *L. monocytogenes* from essential oils and oleoresins in ISO sensitest agar (10°C/6 days).

Table 1 Growth of *L. monocytogenes* (log cfu/g) in a meat slurry model incubated at 10°C

Preparation (2%)	Day 0	Day 3	Day 5	Day 7
Thyme 1	<1	<1	<1	<1
Clove 3	<1	<1	<1	<1
Rosemary 5	2.8	3.3	3.1	3.2
Cumin 9	3.2	8.3	8.7	8.7
Nutmeg 15	3.2	8.7	8.7	8.7
Cumin 20	3.2	4.1	5.7	7.2
Thyme 25	<1	<1	<1	<1
Tea tree 27	3.3	7.2	8.7	8.7
Cinnamon bark 42	1.6	<1	<1	<1
Lemongrass 43	2.1	<1	<1	<1
Thyme 44	<1	<1	<1	<1
Origanum 46	<1	<1	<1	<1

Table 2 Growth of *L. monocytogenes* (log cfu/g) in a cooked, cured emulsion type sausage incubated at 10°C, packed in 20% CO_2 / 80% N_2

Batch	Day 1	Day 7	Day 14	Day 28
Control	3.2	6.5	8.3	8.1
0.1% cinnamon bark 42	3.0	7.5	7.3	7.7
0.25% cinnamon bark 42	3.2	7.3	7,2	7.0
1% cinnamon bark 42	2.7	2.6	2.3	1.9
2% cinnamon bark 42	2.8	2.6	2.4	2.3
1% thyme 44	3.0	5.9	7.8	8.0
2% thyme 44	3.1	5.2	7.1	7.7