



Antibacterial activity and acceptability of plant extracts in minced meat

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Introduction

Food spoilage is a serious problem in terms of food safety, nutritional value and economical losses. In general, foods have been preserved from ancient times by use of heat, cold, drying, salting and fermentation. In recent years synthetic food preservatives are extensively used and some advanced technologies for increasing shelf-life have been developed. Although being effective, chemical preservatives have been associated with possible adverse effects on human health. Therefore, is an increased interest in search for other, preferably, natural antimicrobials, such as organic acids, essential oils, extracts, bacteriocins, and dried fermentation- based products. Taking into account variability in the chemical composition of such materials depending on plant cultivar, growing conditions and some other factors, as well as differences in the sensitivity of various microbial strains testing of antimicrobial properties of natural products remains of some interest. The main objective of this study was to determine chemical composition and evaluate antimicrobial activity and acceptability of commercial oils of thyme and marjoram in minced meat against selected bacteria and yeasts strains.

Materials ir methods

The yeast strains were obtained from Food Institute of Kaunas University of Technology. They are typically associated with food spoilage and were originally isolated from dairy products, equipment washing liquid and the air of industrial premises. After collecting typical colonies, their belonging to the yeast genera was confirmed by microscopy. The antimicrobial properties were evaluated by the agar well diffusion method. Bacteria and yeasts were grown on a slant plate count agar (CM 325, OXOID) and potato – dextrose agar (LAB 98, LAB M), respectively. Bacteria and yeasts were cultivated for 18 hours at 37 °C and 24 h at 25 °C, respectively. After cultivation, test culture cells were washed with saline and mixed using an MS 1 minishaker (Wilmington, USA). The bacteria and yeasts cell suspensions were diluted according to the procedure of McFarland No 0.5 and No 1, respectively. A suspension of cells was introduced into a dissolved and cooled to 47 °C medium. 20 ml of the suspension was pipetted into a 90 mm diameter Petri plate. After cooling wells nine-millimeter in diameter were pushed in the agar and filled with 50 µl of 5.0% ethanolic solutions of extracts. The plates with bacteria and yeasts were incubated overnight at a temperature of 37 °C and 25 °C, respectively.

Pure ethanol was used as a control.

Bacillus subtilis suspensions of vegetative cells and spores were used to measure the dependence of the physiological phase of bacteria cells. *B. subtilis* were grown on a slant plate count agar (CM 325, OXOID). For vegetative cell and spore suspensions bacteria were cultivated for 18 hours and 5 days at 37 °C, respectively. After 5 days cultivation the test cultures were washed with saline and pasteurized for 10 min at 80 °C. Vegetative cells and spore solutions were prepared as described above.

Minced meat was kept with plant extracts in 4 – 6 °C temperature for evaluating antimicrobial activity of extracts and acceptability of meat.

Ranking test was used for evaluating acceptability of minced meat. The evaluation was carried out with a trained panel of 7 pannelists, consisting of KTU Food Institute staff. The panelists had experience in sensory analysis of meat products. All sessions were conducted in a climate-controlled sensory analysis laboratory, equipped with individual booths.

Data analysis. Statistical analysis was performed using SPSS software (v. 11.0, SPSS Inc. Chicago, IL). Two-way analysis of variance was used for testing possible interactions in sensory data. Treatment means were compared using Duncan's comparison. Differences were considered significant at $p < 0.05$.

Results and discussion

The results showed that grampositive bacteria were more sensitive to extracts of celery, parsley, coriander, tarragon and lemon balm than gramnegative bacteria. The possibility to stabilize a number of coliforms in meat mince was also evaluated. The extracts of plants did not have significant influence on the number of coliforms during cold storage. The test with ginger showed that it was contaminated with microorganisms which increased total number of bacteria in mince, and it did not have any preservative effect during storage. Caraway extract inhibited growth of all test cultures, but its influence was not strong, zones of inhibition were not wide. Differences between sensitivity of salmonella were determined. *S. typhimurium* and *S. agona* had smallest sensitivity to caraway extract. Count of bacteria and coliform bacteria had growth in all samples with coriander or caraway extracts. In case with higher amount of extracts growth rate was slower.

Term keeping of minced meat, h	Total bacteria count, CFU/g				
	Amount of caraway extract in minced meat, %.				
	0,0	0,2	0,4	0,6	1,0
0	$(2,8 \pm 1,5) \cdot 10^7$				
24	$(9,0 \pm 6,5) \cdot 10^6$	$(8,1 \pm 3,5) \cdot 10^6$	$(1,6 \pm 1,3) \cdot 10^7$	$(1,4 \pm 1,1) \cdot 10^7$	$(9,6 \pm 3,7) \cdot 10^7$
48	$(1,1 \pm 0,5) \cdot 10^7$	$(1,1 \pm 0,8) \cdot 10^7$	$(1,1 \pm 0,9) \cdot 10^7$	$(1,2 \pm 1,2) \cdot 10^7$	$(1,1 \pm 0,7) \cdot 10^7$
72	$(7,7 \pm 3,5) \cdot 10^7$	$(3,2 \pm 2,1) \cdot 10^7$	$(8,8 \pm 3,5) \cdot 10^7$	$(9,5 \pm 7,3) \cdot 10^7$	$(4,7 \pm 2,9) \cdot 10^7$
168	$(7,8 \pm 2,9) \cdot 10^7$	$(6,6 \pm 3,0) \cdot 10^7$	$(8,8 \pm 7,2) \cdot 10^7$	$(7,7 \pm 5,5) \cdot 10^7$	$(6,0 \pm 1,5) \cdot 10^7$

Influence of addition of caraway extract on all test cultures in minced meat

Antimicrobial properties of caraway extract on salmonella and *B. subtilis* group were evaluated by method of diffusion to agar. Caraway extract inhibited growth of all test cultures, but its influence was not strong, zones of inhibition were not wide. Differences between sensitivity of salmonella were determined. *S. typhimurium* and *S. agona* had smallest sensitivity to caraway extract. Even salmonella depend to gram-negative group and *B. subtilis* to gram-positive one, differences between sensitivity of these groups were not determined. There were not significant differences in sensitivity between vegetative cells and spores.

Influence of addition of coriander extract on variation of coliform bacteria in minced meat

Used amount of coriander extract had no significant effect on growth of coliform bacteria in minced meat. Number of coliform bacteria in control meat sample after keeping 5 days increased from $(4,0 \pm 2,3) \cdot 10^1$ to $(2,9 \pm 1,9) \cdot 10^5$. Growth of coliform bacteria in meat samples with more concentrations of extract was slower both after 3 and 5 days

Term keeping of minced meat, h	Number of coliform bacteria, CFU/g				
	Amount of coriander extract in minced meat, %.				
	0,0	0,2	0,4	0,6	1,0
0	$(4,0 \pm 2,3) \cdot 10^1$				
72	$(2,6 \pm 1,6) \cdot 10^4$	$(2,3 \pm 2,0) \cdot 10^4$	$(2,0 \pm 1,5) \cdot 10^4$	$(2,1 \pm 0,9) \cdot 10^4$	$(1,9 \pm 1,3) \cdot 10^4$
120	$(2,9 \pm 1,9) \cdot 10^5$	$(6,2 \pm 3,5) \cdot 10^4$	$(1,4 \pm 1,1) \cdot 10^5$	$(8,3 \pm 3,5) \cdot 10^4$	$(8,1 \pm 4,7) \cdot 10^4$

Results	Amount of caraway extract in meat samples, %.				
	0,0	0,2	0,4	0,6	1,0
Mean	4,4 c	4,33 c	3,53 b	3,2 b	1,73 a
Mode	5	5	3	4	1
Median	5	5	3	3	1
a, b – different superscripts within column denote significant differences ($p < 0,05$). Treatment means were compared using Duncan's comparison					

Acceptability of cooked minced meat samples with addition of caraway extraxt

Sensory panel evaluated acceptability of cooked minced meat samples with plant extracts. No influence of 0,2 % caraway extract on sensory attributes were determined. Control and sample with addition of 0,2 % caraway extract were evaluated as most acceptable and it was no significant difference among samples. Increasing amount of caraway extract had negative influence on sensory quality of cooked minced meat and sample with 1,0 % of plan extraact was not acceptable for panelists.

Acceptability of cooked minced meat samples with addition of coriander extraxt

The same situation for evaluation influence of coriander extracts was established. Addition of extracts had negative influence on sensory quality of meat samples. 0,2 % coriander extract had no significant influence on sensory quality of meat samples in compare with control and these samples were evaluated as most acceptable. Increasing amount of extract had negligible effect on sensory quality of cooked minced meat and significant differences among samples with 0,2 0,4, 0,6 and 1,0 % of plant extracts were determined. Sample with 1,0 % coriander extract had strong off-flavour and it was not acceptable for panelists.

Results	Amount of coriander extract in meat samples, %.				
	0,0	0,2	0,4	0,6	1,0
Mean	4,6 d	4,2 d	2,6 c	1,9 b	1 a
Mode	5	4	3	2	1
Median	5	4	3	2	1
a, b, c, d – different superscripts within column denote significant differences ($p < 0,05$). Treatment means were compared using Duncan's comparison					

Conclusions

Caraway extract inhibited growth of all test cultures, but its influence was not strong and zones of inhibition were not wide. Differences between sensitivity of salmonella were determined. *S. typhimurium* and *S. agona* had smallest sensitivity to caraway extract. Count of bacteria and coliform bacteria had growth in all samples with coriander or caraway extracts. In case with higher amount of extracts growth rate was slower.

No influence of 0,2 % plant extracts on sensory quality were determined. Increasing amount of extracts had negligible effect on the acceptability of cooked minced meat and sample with 1,0 % of plant extracts was not acceptable for panelists.