Study on antibacterial activity of some spice extracts used in the meat industry

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Abstract. *The antibacterial activity of freeze-dried extracts from 5 spices against Staphylococcus aureus, Esherichia coli and common microflora of ground pork meat in nutrient broth was investigated. The samples were inoculated with tested microorganisms and stored at 4°C and 20°C.The results showed inhibitory effect of all spices against all used microorganisms at two applied concentrations (1 and 2%). S. aureus and E.coli were more sensitive to spice extracts in comparison with natural meat microflora. The marjoram, savory and thyme extracts demonstrated a strong antibacterial effect against all studied microorganism strains. Savory extract inhibits completely the growth of S. aureus and E.coli and non-pathogenic microorganisms of meat at 4°C. Such extracts may have potential as preservatives in raw meat products.*

Key words: plant extract, pork meat, toxins, antibacterial effect

Introduction

Herbs and spices have been used for centuries in culinary art. They don't only impart flavour to food but also improve the healthiness and quality of life. It has been established that a number of them have bactericide and bacteriostatic effect. The main factors, determining the intensity and the type of antibacterial activity are plant chemical composition, quantity, microorganism type, product composition (proteins, lipids, salts), pH, storage temperature.

The leaves and the over-ground parts of plants, belonging to Lamiaceae family, such as thyme, basil, sage, marjoram, oregano, savory are traditionally added to meat, fish and their products to improve the taste and flavour. Due to their antioxidant effect, some of them prolong the storage term and prevent lipid oxidation of foods with high lipid content (Shahidi et al., 1995).

During the last decade the attention of a lot of consumers has been directed towards healthy lifestyle and healthy nutrition. The results from the sociological surveys show that consumers wish to consume foods that have preserved their natural qualities, without artificial preservatives. Spices are appropriate ingredients meeting these requirements. They not only improve the food taste but also have an useful effect on human health due to their antibacterial and antioxidant effect.

It is widely known that some bacteria, yeast and fungi, which don't belong to the pathogenic microorganisms, can cause a change in the qualitative characteristics of foodstuff by making it smell unpleasantly, change in colour and consistence and reduce the product shelf life. On the other side, the diseases caused by contamination of foods with pathogenic microorganisms and/or their toxins are of great importance for the public health. The foods of animal origin can be contaminated with different microorganisms, including also potential pathogens. *E. coli* is the most often met microorganism isolated from raw meats. *Staphylococcus aureus* is a second microorganism, after *Salmonella* sp. on the list of causative agents reported for foodborne outbreaks in Europe (Anon, 2002).

It is considered that the antibacterial effect of spices is due mainly to the essential oils contained in them. The essential oils extracted from various spices have shown a significant effect on 25 different bacteria species, including food pathogens and spoilage bacteria (Afifi, 2001; Dorman and Deans, 2000).

Hydrophobic phenolic compounds derived from the terpenoid pathway - thymol and carvacrol present in essential oil of thyme, oregano, savory and sage are efficient against yeast, gram-positive and gram-negative microorganisms (Farag et al., 1989; Kim et al., 1995)**.** Rosemary contains essential oil, ursolic acid, phenolic acids, flavons and other. According to the studies of Prabuseenivasan et al., rosemary essential oil inhibits the growth of *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus* and to a lower extent of *Klebsiella pneumoniae* **(**Prabuseenivasan et al., 2006). In other studies, growth of *E. coli*, *Salmonella spp*. *L. monocytogenes* and *S. aureus* were inhibited by oregano essential oil in broth cultures and its effect in emulsion systems depended on pH, temperature and other environmental factors (Shetty et al, 2006).

Notwithstanding the proven strong bactericide effect of essential oils, they are not appropriate to be added to meat products in concentrated form. Besides, other phytochemicals are also obtained by the extraction of the plant material that can modify and increase the antibacterial effect of the respective spice.

It is traditionally accepted to input dry spices in meat products which, however, are naturally contaminated with big quantities of microorganisms depending on the ecosystems they originate from

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(Enikova et al., 2004**).** From microbiological point of view their application in a dry form carries a lot of risks and their antioxidant effect is decreased by the drying process.

That is why our investigations were directed to a comparative study of the effect of lyophilized extracts of 5 spice types against standard strains *Staphylococcus aureus* and *Escherichia coli* and natural microflora of raw pork meat.

Materials and methods

As initial plant material were used: oregano (Origanum vulgarae), rosemary (Rosmarinus officinalis), savory (Satureja hortensis), majorana (Origanum majorana) and wild thyme (Thymus serpyllum), preliminarily dried and cut fine up to 0,5-1 mm. The plant extracts were obtained by extraction with water-alcoholic mixtures and then concentrated and freeze- dried.

Two experiments were conducted to evaluate the effect of selected spice extracts.

In the first study freeze dried extracts were added to the nutrient broth at concentrations of 0, 1.0, and 2.0%. Samples of nutrient broth were inoculated with *Staphylococcus aureus* ATCC 43300 or *Esherichia coli* ATCC 25922. Test microorganisms used in experiments were obtained from the National Center of Infectious and Parasitic Diseases (NCIPD), Bulgaria.

Cultures of *S. aureus* were incubated in nutrient broth for 24 h at 37ºC before inoculation. Initial suspensions were prepared by dilution of overnight cultures by adding sterile meat-peptone

Table 1. Effect of freeze-dried plant extracts on *S. aureus* in broth stored at 4°C (Log10 CFU/ml)

Extract	Extract concentration	Storage time (h)			
		0	24	48	72
Control	0,00%	3,15	3,36	3,51	3,52
Oregano	1,00%	2,50	0,90	1,50	2,15
Oregano	2,00%	2,24	ND	1,48	1,85
Rosemary	1,00%	1,23	1,00	1,30	1,80
Rosemary	2,00%	ND	0,78	ND	1,78
Savory	1,00%	1,64	1,70	ND	ND
Savory	2,00%	0,30	0,52	ND	ND
Marjoram	1,00%	0,50	ND	2,30	1,30
Marjoram	2,00%	2,57	ND	1,48	1,70
Thyme	1,00%	2,85	1,13	1,60	0,30
Thyme	2,00%	2,69	1,08	1,60	ND

ND= not detected by the method used

Table 2. Effect of freeze-dried plant extracts on *S. aureus* in broth stored at 20°C (Log10 CFU/ml)

Extract	Extract concentration	Storage time (h)			
		0	24	48	72
Control	0,00%	3,07	4,20	5,15	6,31
Oregano	1,00%	0.60	0,15	ND	ND
Oregano	2,00%	ND	ND	ND	ND
Rosemary	1,00%	1,81	1,54	0,60	1,45
Rosemary	2,00%	0,91	1,11	0,60	0,73
Savory	1,00%	0,60	0,90	ND	0,30
Savory	2,00%	0,30	0,52	ND	ND
Marjoram	1,00%	0,50	0,65	0,61	0.30
Marjoram	2,00%	0,30	ND	ND	ND
Thyme	1,00%	0,85	1,00	ND	0,30
Thyme	2,00%	0,58	0,83	ND	ND

ND= not detected by the method used

broth (MPB) to achieve absorbance corresponding to 0,5 McFarland turbidity standard. The suspensions were diluted to obtain inoculum ca.: 3.8 x 10^3 CFU/ml (for *S. aureus*) and 4 x 10^2 CFU/ml (for E. coli). Inoculated broth samples, containing plant extracts, were stored at 4ºC and at 20ºC for 3 days. Numbers of microbial cells in samples were determined after 0, 24, 48 and 72 h of storage. Non-selective nutrient agar prepared according EN ISO 4833:2004 was used for *S. aureus* enumeration. The plates were incubated for 24 h at 37ºC. *E. coli* cells in samples were enumerated on selective agar Endo. The experiment was repeated three times.

In the second study an isolate of common microflora from pork meat was used. For that purpose 10g of minced meat were added to 90 ml MPB and incubated for 24 h at 37°C. The achieved suspension was diluted to a certain number microorganisms by applying the McFarland optical standard.

Every freeze-dried extract was rehydrated with distilled water (proportion 1:10). To this solution was added MPB and 1 ml of bacterial suspension so that in the final dilution the content of the respective extract was 2.5%. For comparison, a control sample was prepared in a similar way but instead of extract, 1 ml of distilled water was added. The samples were stored at temperature 4±1°C for a period of 48 hours. Bacterial counts were transformed to logarithms and calculations were conducted on the transformed data.

From the data in Table 1 it is seen that in the control samples stored at 4°C the cells number is approximately one and the same during the whole storage period – from 3,15 log units at 0 h to 3,52 at 72 h. *Staphylococcus aureus* is a mesophylic microorganism and temperature is one of the most important growth controlling factors. Temperature growth range is 7-47.8^{0}C with an optimum of 35-37^{0}C, and decreasing the storage temperature significantly inhibited the growth rate of *S.aureus* in different foods **(**Halpin-Dohnalek and Marth, 1989**).** Temperature under 7^{0}C deactivates the cells without destroying them and under the suitable conditions they restore their growth capacity. The adding of extracts decreases the number of staphylococci in the samples stored at 4 ^{0}C, and in savory (1% and 2%) and thyme (2%), viable cells were not established at the end of the period.

For the control samples stored at 20°C an exponential increase of the total counts of staphylococci was established from 3,07 at the beginning of the experiment up to 6,31 log units at 72 hour. For all samples with added extracts a very strongly expressed growth inhibition was observed. The intensity of the antibacterial effect depends on the type of the extract used and increases with the increase of its concentration. It is typical, however, that the growth inhibition in the samples with oregano extract starts at 0 h and remains unchanged till the end of the investigated period. In the samples with savory, marjoram and thyme extracts colonies of *S. aureus* were not established. Most probably, at the higher temperature the diffusion of the terpenoid components and their interaction with the bacterial cells is increased.

In Tables 3 and 4 the data from the testing of the extracts effect on *E.coli* are presented.

In the control sample, stored at 4°C, the cells number was not changed during the whole period, as after 48 h even a certain decrease – from 2,23 to 1,95 log units - was observed. The refrigerator temperature inhibits growth of this bacteria type but does not destroy it. As *E. coli* is part of the common microflora in the large intestine, it is accustomed to a body temperature of 37^{0}C.

After adding the extracts, still at 0 h - a reduction of the order of 1,00 lg units was observed, as at the end of the period *E. coli* colonies were not established in almost all of the samples. It should be necessarily mentioned that this effect is a result not only of the influence of spice extracts but of low temperature as well. Van Derlinden et al., determined, that the optimal temperature for *E. coli* is within [15 °C - 45 °C] (Van Derlinden et al., 2008)**.** The strongest inhibiting effect was established in both concentrations of savory, marjoram and thyme extracts.

For the samples, stored at 20°C, a strong inhibiting effect of the extracts was also observed. For the samples with oregano and rosemary extracts a slight increase in the number of *E. coli* colonies was observed, but to a lower degree compared to the control sample

Table 3. Effect of freeze-dried plant extracts on *E. coli* in broth stored at 4 °C (Log10 CFU/ml)

Extract	Extract concentration	Storage time (h)			
		0	24	48	72
Control	0,00%	2,23	2,93	1,95	1,95
Oregano	1,00%	1,48	1,00	0,70	0,30
Oregano	2,00%	1,18	1,00	0,70	0,30
Rosemary	1,00%	1,20	1 08	0,85	0 30
Rosemary	2,00%	1,11	0,90	0,48	0,30
Savory	1,00%	1,34	0,60	0,48	ND
Savory	2,00%	1,00	0,85	ND	ND
Marjoram	1,00%	1,30	0,70	ND	ND
Marjoram	2,00%	1,00	0,60	ND	ND
Thyme	1,00%	1,28	0,76	ND	ND
Thyme	2,00%	1,08	0,48	ND	ND

ND= not detected by the method used

Table 4. Effect of freeze-dried plant extracts on *E. coli* in broth stored at 20 °C (Log10 CFU/ml)

Extract	Extract concentration	Storage time (h)			
		0	24	48	72
Control	0,00%	2 23	2,36	3,17	4,80
Oregano	1,00%	1,48	1,10	1,50	1,50
Oregano	2,00%	1,18	1,00	1,32	1,20
Rosemary	1,00%	1,20	1,48	1,78	1,70
Rosemary	2,00%	1,11	1,26	1,60	1,60
Savory	1,00%	1,34	1 00	0,70	0,30
Savory	2,00%	1,00	0,90	0,70	0.30
Marjoram	1,00%	1,30	1,11	0,30	ND
Marjoram	2,00%	1,00	0,78	0,30	ND
Thyme	1,00%	1,28	0.60	0,30	ND
Thyme	2,00%	1,08	0.60	ND	ND

ND= not detected by the method used

(Table 4). The antibacterial effect of savory, marjoram and thyme extracts (2%) was expressed still at 24 h, and at the end of the storage period, *E.coli* were not discovered in the samples. The same trend was also observed for the lower extracts concentration but with a slightly weaker effect. Besides with *E. coli* and *S. aureus* strains, the testing for antimicrobial activity of the extracts was also carried out with microorganisms isolated from minced pork meat.

The preliminary analysis of 4 meat lots showed the following results: total count of aerobic mesophylic microorganisms (LgCFU/g) from 4,46 to 7,00. Microbial population *Proteus (P.mirabilis, P.morganii, P.vulgaris*), *Enterobacter,* non-pathogenic staphylococci and a few colonies of *B.cereus* and fungi. Most generally, it can be said that in the samples studied by us gram-negative flora dominated.

Samples of the different freeze-dried extracts were added to liquid nutrient medium (MPB) inoculated with bacterial suspension of natural microflora isolated from pork meat. (Table 5).

Table 5. Effect of freeze-dried plant extracts on the common microflora from minced pork meat (log10 CFU/ml)

Extract	Inoculum	0 h	24 h	48 h
Control	3.75	4,00	5.10	6.45
Oregano	3.75	1.00	2.00	2.00
Rosemary	3.75	2,20	2,39	2,17
Savory	3.75	0,78	ND	ND
Marjoram	3.75	1,30	1,00	ND
Thyme	3.75	1,80	1,70	1.00

ND= not detected by the method used

For all samples with extracts we observed a strong reduction of the total counts of microorganisms compared to the control sample, which at 48 hour of storage varies from 4.00 to 6.00 lg units. From the studied spice types, the savory, marjoram and thyme extracts showed the strongest inhibiting effect on the common meat microflora. In the sample with savory extract at 24 and 48 hour microorganisms were not found. Our results for the marjoram extract confirm studies of Vagi et al. that ethanol extracts of this spice type demonstrate antimicrobial activity on food borne strains of bacteria and fungi (Vagi et al., 2005).

There are many studies dealing with the effect of natural antimicrobial substances of plant origin on microorganisms isolated from several sources but they pertain mainly to essential oils (Burt, 2004; Dorman and Deans, 2000). Sage, thyme and rosemary extracts were found to possess antimicrobial properties against a large variety of pathogenic microorganisms (Miguel et al., 1997).

The addition of 0,05% alcoholic extracts of thyme can inhibit the growth of *S aureus*. Rosemary extract of 0,1% substantially inhibited the growth of *S.aureus* and *Salmonella typhimurium* (Shetty et al, 2006).

In our experiments we established a strong antibacterial effect of oregano extract on *S. aureus*, but a weaker one on the remaining studied microorganisms. The marjoram, savory and thyme extracts showed a strong antibacterial effect on all studied microorganism types.

Conclusion

The freeze-dried water-ethanolic extracts from oregano, rosemary, savory, majorana and wild thyme show a strong antibacterial effect in an "in vitro" investigation in liquid nutrient medium on S.aureus, E.coli and common microflora of minced pork meat. This action is due not only to the essential oils contained therein but also to other components which have an additional impact on bacterial cells. Contrary to the concentrated essential oils which have a rather strong flavour and can be added to food products only in very low concentrations, the extracts are more appropriate as meat additives and in addition to imparting flavour, they can be also used for improving the microbial status of various meat products.

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