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ARTICLE

Antimicrobial effects of commercial herbs, spices and essential oils in minced pork

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ABSTRACT Effect of fresh and dried garlic, onion, thyme and oregano on the bacterial germ count of minced pork stored at 5°C was evaluated. All spices were added in 1% concentration. Furthermore, MIC (Minimal Inhibitory Concentration) values of marjoram and thyme essential oils on *Escherichia coli* were determined *in vitro*, and their antibacterial effect was tested in minced pork inoculated with *E. coli.* In general, fresh spices showed weak or no inhibition on the total cell count of minced pork, or even caused increased microbe count. On the contrary, dried garlic and thyme decreased total cell count with 1 or two orders of magnitude. MIC values for marjoram and thyme essential oils were 0.5 µl/ml and 2 µl/ml, respectively. The essential oils decreased *E. coli* cell number in minced pork with 1 log cfu after 24 h storage at 5°C.
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Herbs and spices are used for flavoring foods but also, since ancient times, as medicines against a variety of diseases. Garlic (*Allium sativum*) and onion (*Allium cepa*) possess many biological activities including antimicrobial, antioxidant, anticarcinogenic, immunomodulatory and prebiotic action. The biological and medical effects of garlic and onion are mainly due to their high content of organo-sulphur compounds, such as alliin and allicin and their breakdown products. Flavonoids, abundant in onion but practically absent in garlic, are also responsible for a great part of the health benefits of both vegetables (Corzo-Martinez et al. 2007). *In vitro* and *in vivo* studies have shown a great effectiveness of garlic and onion against a broad spectrum of fungi and yeasts, including certain *Candida, Torulopsis, Cryptococcus* and *Aspergillus* species. Garlic has been proven to inhibit the growth of gram positive and gram-negative bacteria including strains of, e.g. *Pseudomonas, Proteus, Escherichia,, Staphylococcus, Salmonella* (Iwalokun et al. 2004; Uhart et al. 2006). Onion, however, unlike garlic, was not effective against gram-negative bacteria (Corzo-Martinez et al. 2007).

Essential oils in aromatic plants are among the most important active constituents of herbs and spices. Their efficiency against a wide range of microorganisms is well documented. *Thymus* species are used as medicinal and aromatic plants, as well as in cosmetics and perfumery. The essential oil of *Thymus vulgaris* contains various levels of thymol and/or carvacrol, phenolic derivatives with strong and wide-spectrum antimicrobial activity (Nevas et al. 2004).

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Origanum vulgare has been applied in traditional medicine, as well as in agriculture, pharmaceutical and cosmetic industry. Antimicrobial activity of *Origanum vulgare* essential oil (with carvacrol as the main compound) against bacteria and fungi has been reported (Nevas et al. 2004; Carvo et al. 2008). *Origanum majorana* also possesses substantial antimicrobial effects (Tserennadmid et al. 2010).

There is a growing interest in using natural compounds in food protection instead of chemical preservatives (Burt 2004; Abdel-Hammiad et al. 2009). Minced meat is one of the most perishable foods. Several strategies have been investigated to extend the shelf life of fresh meat products; one is the use of natural antioxidants and antibacterials of plant origin, alone or in combination with other preservation techniques (Busatta et al. 2008; Del Nobile et al. 2009).

We studied the germ count reducing effect of fresh and dried garlic, onion, thyme and oregano in 1% concentration in minced pork stored at 5°C. We supposed that fresh spices contain bioactive ingredients in higher concentrations than dried ones, which probably lose part of the drug during drying. Antibacterial effect of thyme and marjoram essential oils against *E. coli* in inoculated minced pork was also evaluated.

Materials and Methods

Bacterium and growth conditions

Escherichia coli SZMC 0582 (Szeged Microbiological Collection) was grown on Luria-Bertani medium (LB; 1% tryptone, 0.5% yeast extract, 1% NaCl) and was incubated at 37°C.

Table 1. Effect of onion and garlic on the MPN of minced pork stored at 5°C. (Onion was added in 2% and garlic in 1%.).

Time (h)	Onion			Garlic		
	Control	Dried	Fresh	Control	Dried	Fresh
0	2.3×10^2	2.3×10^2	2.3×10^2	2.3×10^2	2.3×10^2	2.3×10^2
24	2.4×10^3	2.4×10^3	6.6×10^4	6.6×10^3	6.6×10^3	6.6×10^4
48	2.4×10^4	6.6×10^4	2.4×10^5	2.4×10^5	6.6×10^4	2.4×10^5
72	1×10^6	2.4×10^4	2.4×10^5	7×10^4	7×10^4	2.4×10^5

Table 2. Effect of thyme and oregano on the MPN of minced pork stored at 5°C. (The herbs were added in 1%.).

Time (h)	Thyme			Oregano		
	Control	Dried	Fresh	Control	Dried	Fresh
0	6.2×10^1	6.2×10^1	6.2×10^1	2.4×10^2	2.4×10^2	2.4×10^2
24	2.4×10^4	6.6×10^3	2.4×10^5	2.4×10^2	2.4×10^2	2.4×10^2
48	2.4×10^3	2.4×10^3	2.4×10^4	2.4×10^3	6.2×10^2	2.4×10^4
72	2.3×10^4	1.3×10^5	6.2×10^4	2.4×10^3	2.4×10^4	2.4×10^4

Table 3. Combined effect of onion or garlic and 1% salt on the MPN of minced pork stored at 5°C. (Onion was added in 2% and garlic in 1%.)

Time (h)	Onion + salt			Garlic + salt		
	Control	Dried	Fresh	Control	Dried	Fresh
0	2.4×10^2	2.4×10^2	2.4×10^2	6.2×10^1	6.2×10^1	6.2×10^1
24	2.4×10^4	6.6×10^4	6.6×10^3	2.4×10^2	6.1×10^1	2.4×10^2
48	5×10^3	2.4×10^3	6.1×10^3	2.4×10^3	2.4×10^2	6.6×10^3
72	2.3×10^4	6.1×10^3	6.1×10^4	2.4×10^5	2.4×10^3	2.4×10^5

Herbs, spices and essential oils

All investigated materials were purchased in local shops in Szeged (Hungary). The essential oils were purchased from Aromax Natural Products (Budapest, Hungary).

The bulbs of *Allium sativum* (garlic) and *Allium cepa* (onion) were peeled and crushed before use. The aerial parts of *Thymus vulgaris* (thyme) and *Origanum vulgare* (oregano) were washed, rinsed with sterile distilled water, and chopped just before addition to the meat. All spices and essential oils were stored at room temperature.

In vitro tests of antibacterial activity

Agar hole diffusion test was used to investigate the antibacterial activity of the fresh and dried spices on *E. coli*. Solid culture media were overlaid with a suspension of 10^5 cells in 1 ml of distilled water and were dried. One gram of the crushed or chopped fresh herbs was mixed with 5 ml sterile distilled water. After 30 min, samples were centrifuged and the supernatant was used in the tests. Dried spices were extracted with hot water (5 ml for 1 g), centrifuged and the supernatants were used. Aliquots (100 µl) of the supernatants were filled into wells of 8 mm diameter; and sterile water was used as negative control. The diameter of the inhibition zones was measured after incubation at 37°C for 24 h. The experiments were repeated three times.

Evaluation of MIC values for essential oils

The MIC values of the essential oils were determined by macrodilution assays. The thyme and marjoram oils were added directly to LB medium resulting in final concentrations of from 0.0625 µl/ml to 2 µl/ml in twofold increments. The tubes were inoculated with 10^5 CFU/ml *E. coli* and then incubated at 37°C for 24 h. MICs were taken as the lowest concentration at which no visible growth occurred.

Antibacterial activity in minced pork: Fresh and dried herbs/spices alone or in combination, with and without 1% salt, were mixed in 1% concentration to minced pork (100 g). The meat was stored at 5°C. Every day, total and coliform MPN (Most Probably Number) was determined (Roberts and Greenwood 2003) for 72 h. Essential oils of thyme and marjoram were added to the minced pork, previously inoculated with 2×10^5 *E. coli*, in a concentration range from 0,125 to 1%

Table 4. Combined effect of thyme or oregano with 1% salt on the MPN of minced pork stored at 5°C. (The herbs were added in 1%.)

Time (h)	Thyme + salt			Oregano + salt		
	Control	Dried	Fresh	Control	Dried	Fresh
0	<10	<10	<10	6.2×10^1	6.2×10^1	6.2×10^1
24	2.4×10^2	2.4×10^2	2.4×10^3	2.4×10^3	2.4×10^3	6.6×10^3
48	2.4×10^4	2.4×10^3	6.1×10^4	6.6×10^3	6.2×10^4	2.4×10^4
72	2.4×10^5	2.4×10^5	7×10^5	2.4×10^5	2.3×10^4	6.2×10^4

Table 5. Effect of mixed spices and salt on the germ count of minced pork after 72 h incubation at 5°C.

Onion + thyme + salt			Onion + garlic + salt			Onion + oregano + salt		
Control	Dried	Fresh	Control	Dried	Fresh	Control	Dried	Fresh
$2.4x10^3$	$2.4x10^4$	$1.3x10^4$	$2.3x10^4$	$7x10^5$	$6.6x10^5$	$6.6x10^2$	$2.4x10^3$	$2.4x10^2$

Table 6. Effect of spices and herbs on the MPN of coliforms in minced pork after 72 h storage at 5°C.

Spice/herb	Control	Dried	Fresh
Onion	6.6×10^2	6.6×10^2	2.4×10^3
Onion + salt	6.2×10^2	<10	<10
Garlic	2.4×10^3	2.4×10^2	6.2×10^2
Garlic +salt	6.2×10^1	2.2×10^1	6.2×10^2
Thyme	6.0×10^1	2.3×10^2	1.3×10^3
Thyme + salt	2.3×10^1	2.3×10^1	<10
Oregano	2.3×10^1	2.4×10^2	2.3×10^1
Oregano + salt	2.3×10^1	<10	<10

Table 7. *E. coli* CFU count of minced pork treated with different concentrations of essential oils. (Size of inoculum: 4×10^5 CFU/ml).

Concentration (% w/w)	0	0.125	0.25	0.5	1.0
Thyme	7.5×10^5	7.5×10^5	4.1×10^5	2.2×10^5	1.3×10^5
Marjoram	6.5×10^5	6.5×10^5	4.0×10^5	3.0×10^5	7.5×10^4

(w/w). After 24 h incubation *E. coli* CFU (Colony Forming Unit) was determined on VRBG agar (Merck).

Results and Discussion

Inhibition zones for water extracts of dried and fresh garlic were 16.5 ± 2.32 mm and 18.82 ± 1.84 mm, respectively. No inhibition zone was detected with the other herbs and spices. Iwalokun et al. (2004) found an inhibition zone of 23.7 ± 2.4 mm of aqueous garlic extract for *E. coli.* In their study, garlic also exerted an excellent antibacterial and antifungal effect against various multidrug-resistant bacterial isolates and certain *Candida* species. In general, fresh and dried spices showed weak or no inhibition on the total cell count of minced pork (Table 1 and 2). Furthermore, in some cases, the microbe number increased. Supplementation with 1% salt resulted in germ count reduction by dried onion and garlic, and dried and fresh oregano (Table 3 and 4). The combination of onion with the other spices plus salt had no reducing effect on the germ count, MPN was the same or even greater than that of the control (Table 5). These spices exerted more considerable antimicrobial effect on coliform bacteria when salt was added (Table 6). In general, it can be said, that the spices mostly failed to exert considerable growth reducing effect in fresh or in dried form. The number of germs was influenced by the initial number of germs in minced meat. Our results agreed well with the results of Uhart and coworkers (2004) who found that spices (garlic, ginger and turmaric) inhibited *Salmonella* Typhimurium DT 104 when they were in direct contact. However, when the spices were added to a complex food system such as ground beef, the inhibitory activity was considerably decreased.

The MIC values of thyme and marjoram essential oils were 0.5 and 2 µl/ml in LB medium. We were not able to determine MIC values in minced pork, only the highest concentration (1%) of marjoram oil reduced the *E. coli* CFU/ml with 1 log (Table 7). In the study by Busatta et al (2008), MIC of marjoram essential oil against *E. coli* was 0.92 mg/ml. They applied marjoram oil in fresh sausage inoculated with *E. coli*, and after 25 days of incubation the MPN was reduced by approximately 1 log. The main component in marjoram EO having antibacterial activity is terpinene-4-ol, causing changes in membrane permeability. The main component of thyme essential oil, thymol was effective on coliforms and Enterobacteriaceae in minced beef patties (Del Nobile et al. 2009). Thyme, oregano and savory essential oils showed the broadest antibacterial activity in the study of Nevas et

al. (2004). In our experiments, thyme EO had only a slight CFU reduction effect which might be due to differences in its composition.

Herbs, spices and essential oils in minced pork had variable and moderate antibacterial effect in our study. Best candidates for prolonging minced pork shelf life are garlic and marjoram essential oil. Further experiments are needed to evaluate the antibacterial effect of these substances combined with other hurdle techniques.

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