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IN VITRO INHIBITION ACTIVITY OF THE SPICE MIX USED IN THE „PAPRIKÁŠ“ SAUSAGES

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Abstract

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The aim of this work was to test *in vitro* the ability of the components in the spice mix usually used for the production of the „paprikáš“ sausage (P) to inhibit the growth of tyramine and histamine forming microorganisms. The ability of the P spice mix components to inhibit the growth of the *Pediococcus pentosaceus* and *Enterococcus faecalis* CNRZ 238 species was tested by the agar diffusion method. The tested cultures were chosen as positive to a gene sequence for tyrosindecarboxylase (tyrDC). None of the tested P spice components or the mix as a whole inhibited growth of *Pediococcus pentosaceus* and *Enterococcus faecalis* CNRZ 238.

antimicrobial activity, spice, biogenic amines, *Enterococcus faecalis*, *Pediococcus pentosaceus*

Flavour of fermented meat products is significantly influenced by the addition of a spice mix which forms an important part of the final product. Moreover, some kinds of spices was also antioxidant effect (Firuzi *et al.*, 2010; Vaštag *et al.*, 2010). Another reason for using spices and their derivatives is their ability to increase shelf life of foods by inhibition of growth of undesirable microorganisms, including pathogens such as *Clostridium botulinum*, which represent a great health risk (Cui *et al.*, 2010), and even reduce the development of the pathogen microorganisms resistance to antibiotics (Goñi *et al.*, 2009). Antimicrobial effect of spices was to bacteria, fungi and viruses, and resistance of organisms is different. Bacteria are compared with mold resistant (Pasqua *et al.*, 2005). Spices used in the production of dry fermented meat products are added as such or in the form of extracts. Complex spice mixtures containing about ten components are often used with objectives to influence the fermentation process. The total quantity of the spice mix is usually between 5–10 g.kg^{-1} of batter (Kameník, 2005). Therefore, the presumption of the authors of the previous experiment articulated on the basis of an operational experiment that the spice mix „paprikáš“ sausages (P) inhibits the growth of microorganisms capable of creating BA (biogenic amines) was not, therefore, proved (Komprda *et al.*, 2004). It has been proved that garlic is very important in the inhibition of microorganisms, including the pathogenic ones.

Qualitative determination of the spice mix components

The objectives of the present experiment were to test the ability of the components of the spice mix used in production of the typical Czech dry sausage „paprikáš“ to inhibit growth of microorganisms with proved potential to decarboxylate amino-acids and to form one of toxicologically most important biogenic amines (BA), tyramine.

MATERIALS AND METHODS

The spice mix used in the present experiment has been kindly provided by the TRUMF International (Czech Republic) and was designed in such a way in order to resemble the spice mix used in our previous experiment (Komprda *et al.*, 2004). The spice mix has the following composition (recalculated to mg.kg^{-1} of the final product): red pepper (6 000), hot pepper (1 000), black pepper (500), sodium iso-ascorbate (500), red pepper oleoresin (100), garlic granules (50), cumin essential oil (10), coriander oil (10),

coriander oleoresin (1), rosemary extract (10) garlic essential oil (2), hot pepper oleoresin (1) and antioxidant BHT (0.3). The producer has provided the information on the spice mix composition from the view point of the possible antimicrobial activity of its individual components. The qualitative composition of the mixture (the quantitative representation of individual components has not been provided) determined using GC/MS (chromatograph Agilent 6890, column DB5) was as follows: red pepper and hot pepper: δ-karene, octamethyl-cyctotetrasiloxane, DL-limonene, 2-methyl-5-cyclohexen-1-on, trans-caryophyllene, benzthiazol-2-spiro-4-oxane, 2-hydroxy-9-octadecan acid, capsaicine, 14-methyl-pentadecan acid, N-acetyl-N-butyl-acetamide; black pepper: piperidine, trans-caryophyllene, DL-limonene, sabinene, δ-karene, α-pinene, α-kubenene; cumin essential oil: 2-methyl-5-cyklohexen-1-on, DL-limonene; coriander oleoresin: linalool, camphor, γ-terpinen, α-pinene, DL-limonene; garlic essential oil: di-2-propenyl-disulfide, 3,4-dimethyl-isothiasole, 2-ethyl-tetrahydro-thiophene.

In vitro testing of the inhibition effect of the spice mix components

Individual chemical substances with a potential bioactive effect were extracted by a supercritical fluid extraction CO_2 (these extractions were not carried out in our laboratory but the respective extracts were provided by the TRUMF company). The ability of the spice mix components to inhibit the growth of lactic acid bacteria (LAB), including genus Enterococcus was tested by the agar diffusion method according to López–Malo Vigil *et al.* (2005). *Enterococcus faecalis* CNRZ 238 (Czech collection of microorganisms, Masaryk University, Brno, Czech Republic) and *Pediococcus pentosaceus* (Almi Ges.m.b.H. & Co KG, Austria) were used as the testing microorganisms. Both testing cultures were chosen based on their positivity on a gene sequence for tyrosinedecarboxylase (tyrDC).

300 µl of the 24-hour suspension (6 log $CFU.ml^{-1}$) of *Enterococcus faecalis* CNRZ 238 (substrate Slanetz-Bartley with added TTC supplement, Noack, Austria), or *Pediococcus pentosaceus* (substrate M17, Noack, Austria) was separately pipetted into Petri dishes and poured with a prepared fresh liquid medium with temperature of 45 °C. Supercritical CO_2 extracts of the spice mix components were then individually put (amount 200 µl) into holes (diameter 9 mm, depth 10 mm) formed by an aseptic cork drill. Each hole was then covered with a cover glass and poured with a pure aseptic agar (Agar, type E, Noack, Austria). Petri dishes were cultivated for 48 hours at the temperature of 37 °C and the diameter of the formed inhibition zones was measured. Positivity was assessed based on comparison with the inhibition zone diameter of the standard antimicrobial agent butyl hydroxytoluen (BHT). All measurements were performed three times.

RESULTS AND DISCUSSION

The present experiment was in a sense a continuation of the previous experiment carried out by the same laboratory (Komprda *et al.*, 2004). One of the results of the quoted paper was a finding that the dry fermented sausage produced with a spice mix usually used in the production of the „paprikáš“ sausage had in comparison with a control sausage significantly lower biogenic amine (BA) including tyramine, which is usually the most significant BA in these product (BA) (Eerola *et al.*, 1998). However, the authors of the quoted experiment were not able to carry out a more detailed analysis of the mentioned spice mix and to find out, which of its components would possibly inhibit the growth of the microorganisms capable of forming BA in fermented meat products.

Table I. shows that in comparison with *Pediococcus pentosaceuc, Enterococcus faecalis* CNRZ 238 was inhibited more ($P < 0.05$) by the extracts of the following components of the spice mix: hot pepper, garlic granules, garlic essential oil, coriander oil and rosemary extract. Klancnik *et al.* (2009) mentioned a high sensitivity of G+ bacteria to lipotropic rosemary extracts, which they explain by the presence of carnosic acid, which represents the most bioactive fenolic compound in this type of extract. The present experiment has proved this assumption only in the case of *Enterococcus faecalis* CNRZ 238, where rosemary extract showed the same inhibition effect as antioxidant butylhydroxytoluene (BHT); BHT is usually used as a control substance for testing of inhibition activity in the above mentioned context (Klancik *et al.*, 2009).

As follows from Table I, the inhibition zones of the tested spice mix components were mostly smaller in comparison with the BHT (butylhydroxytoluene) inhibition zones. One possible reason is the use of the supercritical CO_2 extracts. Lipophilic properties of the extracts could cause a lower diffusion of the inhibitory substances from the sample to the test agar as pointed out López-Malo Vigil *et al.* (2005). The reason why this type of extract was used in the present experiment was that it showed a higher antimicrobial activity as compared to the water extracts in an experiment of Klancnik *et al.* (2009).

Moreover, the results of the present experiment did not confirmed (Romano *et al.*, 2009) a possibility of a synergic inhibitory effect of the tested spice mix components on neither *Enterococcus faecalis* CNRZ 238 nor *Pediococcus pentosaceuc* (compare last line in Table I with effects of particular components).

If can be concluded that no significant ability of either particular components of the tested spice mix or the mix as a whole to inhibit growth of the tyrosine decarboxylase-positive microorganisms was found in the present experiment. Therefore, the results of the previous experiment of the same laboratory (Komprda *et al.*, 2004) could not be explained *in vitro* by the agar diffusion method using biogenic amine-forming strains of microorganisms.

I: *In vitro inhibition effect of the components of the tested spice mix (agar diffusion test according to López-Malo Vigil et al. (2005) using holes formed by aseptic cork drill; spice components were tested in a form of supercritical CO_2 extracts (n = 2). Tested microorganisms: Enterococcus faecalis CNRZ 238 (Czech collection of microorganisms, Masaryk University, Brno); Pediococcus pentosaceus; both cultures containing gene sequences for tyrosindecarboxylase; BHT – butylhydroxytoluen.*

Component	Tested microorganisms			
	Enterococcus faecalis **CNRZ 238**		**Pediococcus pentosaceus**	
	Inhibition zone		**Inhibition zone**	
	mm	**% of inhibition zone BHT**	**mm**	**% of inhibition zone BHT**
Pepper	6	46	7	58
Hot pepper	12	92	7	58
Black pepper	7	54	9	75
Sodium iso-ascorbate	6	46	6	50
Pepper oleoresin	6	46	9	75
Garlic granules	7	54	3	25
Cumin essential oil	7	54	6	50
Coriander oil	11	85	5	42
Coriander oleoresin	10	77	12	100
Rosemary extract	13	100	6	50
Garlic essential oil	12	92	4	33
Chilli oleoresin	4	31	2	17
Butylhydroxytoluen	13	100	12	100
Total compound	11	85	7	58

SUMMARY

The objectives of the experiment was to test *in vitro* the ability of individual components of the spice mix usually used for the production of the typical Czech dry fermented sausage „paprikáš“ growth of biogenic amine-forming microorganisms and thus clarify the results of the previous experiment of the same laboratory: significant decrease of biogenic amines content in the „paprikáš“ sausage compared to a control sausage.

In the present experiment, none of the components of the tested spice mix or the mix as a whole inhibited microorganisms with proved tyrosine-decarboxylase activity. Therefore, the hypothesis that the components of the „paprikáš“-type spice mix inhibit the growth of biogenic amine-forming microorganisms was not confirmed *in vitro*.

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