YEASTS RESISTANCE TO PLANT AND BERRY EXTRACTS

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Abstract

Undesirable in food products eight yeasts species *Debaryomyces hansenii, Trichosporon cutaneum, Kluyveromyces marxianus var. lactis, Sacharomyces cerevisiae, Candida parapsilosis, Torulaspora delbrueckii, Pichia kluyveri, Rhodotorula rubra* were used in the test cultures. Antimicrobial effect of various extracts and essential oils against yeasts was determined by diffusion in agar method.

Tarragon, savory and parsley essential oils solutions at concentration of 0.5% inhibited the growth of yeasts. *T. cutaneum* and *R. rubra* were more sensitive to these essential oils than other yeasts cultures. The essential oil of long curcuma inhibited only before mentioned yeasts cultures. The extract of parsley also possessed inhibitory effect against all yeasts. The extracts of black cumin, cayenne pepper and red bell pepper did not influence the growth of yeasts. The inhibitory effect of green paprika and chilly pepper extracts was weaker comparing to the extracts of parsley. The extracts of leaves and seeds of coriander, leek and basil possessed inhibitory effect and inhibited growth of yeasts.

Yeast shows minimal sensitivity to the ethanol extracts from cranberry, black currant and bilberry berries and berry press cakes. Only *T. cutaneum* and *S. cerevisiae* makes bigger transparence zone. Yeast shows resistance to berry juice and water extracts from berry press cakes.

The main objective of this study was to evaluate antimicrobial activity of spices extracts obtained by carbon dioxide and ethanol extracts of cranberry, bilberry and black currant berry and their press cakes against selected yeasts strains.

Key words: yeasts species, essential oils, extracts

Introduction

Spices have been widely consumed throughout history in the human diet not only as flavoring substances but also as antimicrobial agents. Numerous reports demonstrate an inhibitory effect of essential oils and extracts, as well as purified compounds isolated from various plants on the growth of microorganisms; such substances have been used for preserving foods and drinks (Liu *et. al*., 1996; Azzouz *et. al*., 1982; Ozean *et al*., 2001).

Since the very first scientific experiments on the antimicrobial properties of spices, herbs and their components, which were performed carried out in the end of 19th century, the interest in this topic has not diminished. Hoffman and Evans (1911) were the first who performed a laboratory study on the effect of spices in food preservation. They found that cinnamon, mustard and clove were useful in preserving apple juice (Zaika, 1988).

Several studies have reported that garlic bulb extract can inhibit the growth of bacteria, fungi and viruses in culture media and food systems. It has also been shown that the antimicrobial activity of garlic bulbs is due to allicin (diallyl thiolsulfinate), ajoene and other sulfur compounds (Yin *et al.,* 1998; Conner *et al.,* 1984). It is well established that such compounds as thymol, anethole, menthol as well as essential oils and extracts from Jamaican pepper, cinnamon, clove, garlic, oregano, sage and thyme can inhibit the growth of pathogens and yeast found in foods. These substances were strong agents in terms of their capability to reduce the number of various microorganisms (Marino *et al.,* 1999).

Materials and Methods

The yeast strains were isolated from dairy products, equipment washing liquid and the air of industrial premises: *Debaryomyces hansenii, Trichosporon cutaneum, Kluyveromyces marxianus var. lactis, Sacharomyces cerevisiae, Candida parapsilosis, Torulaspora delbrueckii, Pichia kluyveri, Rhodotorula rubra.*

Fruit juices of cranberry, bilberry and black currant were pressed out in a conventional juicer and the press cake was stored in a freezer until extraction. The ethanol extractspigments were

Plants essential oil	Concentration, %	Inhibition zone, cm							
		D. hansenii	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbrueckii*	*S. cerevisiae*	*C. parapsilosis*	*P. kluyveri*	*R. rubra*
Essential oil of savory from plant extract	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	1	2.9±0.1	4.0±0.0	3.9±0.1	3.2±0.0	3.1±0.1	2.1±0.1	2.6±0.0	4.0±0.0
	0.5	1.8±0.0	4.0±0.0	2.0±0.0	2.4±0.0	1.8±0.0	1.8±0.1	1.5±0.0	4.0±0.0
Essential oil of parsley from plant extract	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	1	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	3.9±0.1	4.0±0.0
	0.5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	2.8±0.0	4.0±0.0

The essential oil of long curcuma inhibited only before mentioned yeasts cultures. The combined effect of different pH of media and extracts was also investigated and it was found that pH did not have significant effect on antimicrobial properties of extracts.

After investigation yeast sensitivity on various plant extracts, it was found that yeasts are resistant on most of them. Only parsley leaves extract inhibit test cultures growing – *D. hansenii*, *T. cutaneum*, *C. parapsilosis* and *R. rubra* made inhibition zone in 4.0 cm diameter. Better resistance showed *K. marxianus* var. *lactis*, *T. delbrueckii*, extracts of concentration 10 and 5 % made lesser inhibition zone, their diameter was from 1.6 to 3.5 cm. The extract of parsley also possessed inhibitory effect against all yeasts. The extracts of black cumin, cayenne pepper and red bell pepper did not influence the growth of yeasts. The inhibitory effect of green paprika and chilly extracts was weaker comparing to the extracts of parsley.

In literature it is mentioned, that composition of several essential oils some times shows stronger inhibition effect compared to single component. After mixing extracts of savory, coriander and tarragon in proportion 1:1:1, obtained composition showed antimicrobial effect, but it was not very strong (Table 2).

Table 2

Sensitivity of yeasts on extracts compositions

Plants extract and their composites	Concentration, %	Inhibition zone, cm							
		D. hansenii	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbrueckii*	*Sac. cerevisiae*	*C. parapsilosis*	*P. kluyveri*	*R. rubra*
Horseradish, garlic, rosemary	50	2.9±0.1	2.0±0.1	1.8±0.1	2.3±0.1	1.8±0.0	2.0±0.0	1.6±0.1	4.0±0.0
	10	1.9±0.1	1.6±0.1	1.6±0.0	1.7±0.0	1.6±0.0	1.8±0.0	1.4±0.1	2.8±0.0
	1	1.2±0.0	0.0	0.0	1.0±0.0	1.2±0.0	0.0	0.0	1.1±0.0
Extract of horseradish	50	2.3±0.3	1.6±0.2	1.5±0.0	1.4±0.1	2.3±0.1	1.5±0.1	1.5±0.0	2.7±0.2
	10	2.0±0.1	1.5±0.1	1.4±0.1	1.3±0.0	2.0±0.0	1.6±0.1	1.9±0.0	2.3±0.4
	1	1.3±0.0	0.0	0.0	1.1±0.0	1.2±0.0	0.0	1.2±0.0	1.2±0.0
Extract of garlic	50	2.6±0.1	2.4±0.1	3.2±0.3	1.8±0.3	2.6±0.1	2.8±0.4	1.7±0.0	3.2±0.3
	10	2.0±0.1	2.0±0.0	2.6±0.1	1.7±0.1	2.0±0.1	2.1±0.9	1.3±0.1	2.4±0.0
	1	1.4±0.1	1.3±0.0	1.3±0.0	1.0±0.0	1.1±0.0	0.0	1.1±0.0	1.2±0.1
Extract of rosemary	50	1.5±0.5	1.8±0.0	1.7±0.3	1.4±0.1	2.0±0.0	1.6±0.1	1.8±0.1	4.0±0.0
	10	1.6±0.0	1.8±0.0	1.6±0.1	1.3±0.1	1.3±0.0	0.0	1.6±0.0	3.0±0.3
	1	1.2±0.0	1.2±0.0	1.1±0.0	1.2±0.0	1.1±0.0	0.0	1.1±0.0	1.3±0.1
Peel of	50	1.9±0.1	1.8±0.0	1.2±0.0	1.3±0.0	1.4±0.0	1.6±0.1	0.0	0.0
	10	1.3±0.1	0.0	0.0	1.1±0.0	0.0	0.0	0.0	0.0

Plants extract and their composites	Concen-tration, %	Inhibition zone, cm							
		D. hansenii	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbrueckii*	*Sac. cerevisiae*	*C. parapsilosis*	*P. kluyveri*	*R. rubra*
lemon. caraway, fenugreek	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Extract of lemon peel	50	0.0	1.2±0.0	1.1±0.0	1.1±0.0	1.5±0.0	1.2±0.0	0.0	2.0±0.0
	10	0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.3±0.1	0.0	0.0	1.5±0.0
	1	0.0	0.0	1.0±0.0	1.0±0.0	1.1±0.0	0.0	0.0	1.1±0.1
Extract of fenugreek *Trigonella foenum-graecum*	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Savory, coriander, tarragon	50	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0 ±0.0	4.0±00
	10	4.0±0.0	3.8±0.0	4.0±0.0	3.8±0.0	4.0±0.0	3.8±0.1	3.5±0.1	4.0±0.0
	1	4.0±0.0	1.8±0.0	4.0±0.0	1.8±0.0	4.0±0.0	3.6±0.0	0.0	4.0±0.0
Extract of starragona-vory	50	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	1	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
Extract of coriander	50	1.3±0.07	4.0±0.0	1.9±0.0	1.7±0.0	1.9±0.1	2.9±0.1	1.2±0.3	4.0±0.0
	10	1.3±0.0	4.0±0.0	1.6±0.1	1.3±0.0	1.6±0.0	2.1±0.1	1.3±0.0	4.0±0.0
	1	1.1±0.0	4.0±0.0	0.0	1.2±0.0	1.1±0.1	1.4±0.1	0.0	4.0±0.0
Extract of tarragon	50	1.50±0.0	3.3±0.1	2.9±0.1	2.7±0.4	3.2±0.0	2.4±0.1	2.2±0.1	4.0±0.0
	10	1.20±0.0	3.3±0.1	2.4±0.0	2.4±0.0	2.8±0.0	2.2±0.0	2.0±0.0	4.0±0.0
	1	0.0	2.8±0.0	1.5±0.0	1.2±0.0	2.0±0.0	1.8±0.0	1.2±0.0	4.0±0.0
Chilli pepper, marjoram, curcuma	50	0.0	1.2±0.0	1.3±0.0	1.2±0.0	1.6±0.0	1.1±0.0	0.0	2.0±0.0
	10	0.0	0.0	0.0	0.0	1.1±0.0	0.0	0.0	1.4±0.1
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9±0.0
Extract of chilli pepper	50	1.3±0.0	0.0	1.0±0.0	0.0	1.3±0.1	1.3±0.1	0.0	1.8±0.1
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2±0.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0±0.0
Extract of marjoram	50	2.0±0.0	4.0±0.0	3.4±0.3	4.0±0.0	4.0±0.0	3.0±0.0	1.5±0.1	4.0±0.0
	10	1.3±0.0	1.7±0.0	2.0±0.0	1.6±0.0	2.8±0.0	2.3±0.3	1.3±0.0	4.0±0.0
	1	1.0±0.1	1.0±0.0	1.3±0.0	1.0±0.0	1.2±0.1	1.0±0.0	0.0	4.0±0.0
Extract of curcuma	50	1.3±0.0	1.9±0.0	1.3±0.2	1.3±0.1	1.3±0.1	1.1±0.0	1.5±0.0	1.7±0.2
	10	0.0	1.4±0.1	1.6±0.4	1.2±0.0	1.3±0.0	1.1±0.0	1.2±0.0	1.4±0.0
	1	0.0	1.3±0.0	1.3±0.1	1.1±0.0	1.1±0.1	1.0±0.0	1.1±0.0	1.2±0.0

Extract of 50, 10 and 1% concentration inhibit only *D. hansenii*, *K. marxianus*, *S. cerevisiae* test cultures. Other yeast made smaller inhibition zones, especially with extracts of concentration in 1%. After mixing extracts of horseradish, garlic and rosemary, increase of affectivity was not observed too. The extracts of leaves and seeds of coriander, leek and basil possessed inhibitory effect and inhibited growth of yeasts.

Yeast shows minimal sensitivity to the ethanol extracts from cranberry, black currant and bilberry berries and berry press cakes (Table 3). Only *T. cutaneum* and *S. cerevisiae* makes bigger transparence zone. *K. marxianus* var. *lactis* was the most sensitive to ethanol extracts of bilberry and black currant berry press cakes than other yeasts cultures.

Yeast shows resistance to water extracts from berry press cakes. (the results are not shown). Berry juice has weak inhibitory effect, in this case only cranberry juice and black currant juice inhibit growing of *T. cutaneum* and makes transparence zone in 0.8 and 1.0 cm respectively (the results are not shown).

Table 3

Inhibitory effect of berry and berry cakes ethanol extracts on yeasts

Ethanol extract	Inhibition zone, cm							
	S. cerevisiae	*C. parapsilosis*	*P. kluyveri*	*P. kluyveri*	*D. hansenii*	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbruecki*
Cranberry berries	1.1±0.0	0.9±0.0	0.8±0.0	0.9±0.0	1.0±0.0	1.5±0.1	1.2±0.0	0.9±0.0
Bilberry berries	1.2±0.2	0.9±0.0	0.1±0.0	1.0±0.0	0.8±0.0	1.3±0.1	1.0±0.0	0.9±0.2
Black currant berries	1.2±0.2	0.9±0.0	0.9±0.0	0.9±0.0	0.8±0.0	0.9±0.0	0.9±0.4	0.9±0.0
Cranberry press cakes	1.3±0.2	0.9±0.0	0.9±0.0	0.9±0.0	0.8±0.2	1.2±0.0	1.0±0.0	1.0±0.0
Bilberry press cakes	1.2±0.4	0.9±0.0	0.9±0.1	0.9±0.1	0.8±0.2	1.2±0.2	1.9±0.1	1.0±0.1
Black currant press cakes	1.2±0.0	0.9±0.0	0.8±0.0	0.8±0.0	0.9±0.2	1.3±0.2	1.9±0.1	1.0±0.0

Conclusions

1. Essential oil of savory, tarragon and parsley showed strong inhibitory effect on yeast cultures. Effective concentration was from 0.5 to 50 %.
2. After investigation of various plants extracts mixtures, synergistic effect was not observed.
3. Yeast shows minimal sensitivity to the ethanol extracts from cranberry, black currant and bilberry berries and berry cakes. Only *T. cutaneum* and *S. cerevisiae* makes bigger transparence zone. Yeast shows resistance to berry juice and water extracts from berry cakes.

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