

ANTIBACTERIAL ACTIVITY OF ISOTHIOCYANATES, ACTIVE PRINCIPLES IN ARMORACIA RUSTICANA ROOTS (II)

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Abstract

The aim of this paper is to study the inhibitor effect of ITCs, compounds present in homogenized horseradish extracts by comparing the effects of this compounds on cutting horseradish extracts on some microorganisms.

Keywords: *horseradish, isothiocyanates, antibacterial activity*

Introduction

Isothiocyanates (ITCs) are a group of naturally occurring compounds that occur as thioglucoside conjugates, termed glucosinolates, in plants and cruciferous vegetables such as watercress, Brussels sprouts, broccoli, cabbage, kai choi, kale, horseradish, mustard, radish and turnip (Holst, 2004). Isothiocyanates are sulphur-containing phytochemicals with the general formula R-NCS. Isothiocyanates are also responsible for the typical flavour of these vegetables. ITCs inhibit the development of tumors in many of the experimental models investigated, and are being investigated as possible chemo-preventive agents for specific human cancers (Manici, 1997; Shapiro, 1998; Smolinsko, 2003; Rouzand, 2004).

Amounts of isothiocyanates formed from glucosinolates in foods are variable and depend partly on the processing and preparation of those foods. Glucosinolates are water-soluble compounds that may be leached into cooking water (Fenwick, 1986; Ono, 1998).

Experimental

The experimental procedeeing, the obtaining extracts and microbiological tests are presented in the first part of the paper (Mucete, 2006).

Results and Discussions

The experimental results are given in the tables 1 - 6.

Table 1. Effect of ITCs from homogenized horseradish extract on *Bacillus subtilis* after 24 respectively 48 hours of incubation, and enzymatic activation temperature of 55°C

Microbial species	Samples/ Reaction time (120-330 min)	Time (hours)	ITCS (mg/100g product)	Sensibility/ resistance of microbial species	
<i>Bacillus subtilis</i>	P ₁ /120 min.	24	117.32	0.1	
		48		0.1	
	P ₂ /150 min.	24	119.27	0.1	
		48		0.1	
	P ₃ /180 min.	24	123.89	0.2	
		48		0.2	
	P ₄ /210 min.	24	124.38	0.3	
		48		0.3	
	P ₅ /240 min.	24	130.25	0.5	
		48		0.5	
	P ₆ /270 min.	24	128.5	0.2	
		48		0.2	
	P ₇ /300 min.	24	125.72	0.2	
		48		0.2	
	P ₈ /330 min.	24	122.21	0.1	
		48		0.1	
	Witness sample				++
					++

Note: 0.1 ÷ 0.5 cm (negative reaction) represents the free zones of the microorganisms diameter values indicating the sensibility degree of the microorganism to ITCs; ++ the microorganism developed on the entire surface of culture medium.

From table 1, we can see that after 24 and 48 hours of incubation, microorganism presents sensitiveness at ITCs action from

homogenized horseradish extracts (free zone's diameter being of maximum 0.5cm).

Table 2. Effect of ITCs from homogenized horseradish extract on *Staphylococcus aureus* after 24 respectively 48 hours of incubation, and enzymatic activation temperature of 55°C

Microbial species	Samples/ Reaction time (120-330 min)	Time (hours)	ITCS (mg/100g product)	Sensibility/ resistance of microbial species	
<i>Staphylococcus aureus</i>	P ₁ /120 min.	24	117.32	0.2	
		48		0.1	
	P ₂ /150 min.	24	119.27	0.4	
		48		0.2	
	P ₃ /180 min.	24	123.89	0.5	
		48		0.3	
	P ₄ /210 min.	24	124.38	0.6	
		48		0.4	
	P ₅ /240 min.	24	130.25	0.8	
		48		0.4	
	P ₆ /270 min.	24	128.5	0.5	
		48		0.2	
	P ₇ /300 min.	24	125.72	0.2	
		48		0.2	
	P ₈ /330 min.	24	122.21	0.1	
		48		0.1	
	Witness sample				++
					++

0.1 ÷ 0.8 cm (negative reaction) represents the free zones of the microorganisms' diameter values indicating the sensibility degree of the microorganism to ITCs;

++ the microorganism developed on the entire surface of culture medium.

From table 2, it can be observed that the microorganism reaction, in presence of homogenized horseradish extracts, is similar with that of the cutting horseradish, after 24 and also after 48 hours so, the

sensitiveness of *Staphylococcus aureus* species reduces once with the growth of action period of these compounds of natural origin.

Table 3. Effect of ITCs from homogenized horseradish extract on *Candida albicans* after 24 respectively 48 hours of incubation, and enzymatic activation temperature of 55°C

Microbial species	Samples/ Reaction time (120-330 min)	Time (hours)	ITCS (mg/100g product)	Sensibility/ resistance of microbial species	
<i>Candida albicans</i>	P ₁ /120 min.	24	117.32	0.1	
		48		0.1	
	P ₂ /150 min.	24	119.27	0.1	
		48		0.1	
	P ₃ /180 min.	24	123.89	0.2	
		48		0.2	
	P ₄ /210 min.	24	124.38	0.2	
		48		0.2	
	P ₅ /240 min.	24	130.25	0.3	
		48		0.3	
	P ₆ /270 min.	24	128.5	0.2	
		48		0.2	
	P ₇ /300 min.	24	125.72	0.1	
		48		0.1	
	P ₈ /330 min.	24	122.21	0.1	
		48		0.1	
	Witness sample				++
					++

0.1 ÷ 0.3 cm (negative reaction) represents the free zones of the microorganisms' diameter values indicating the sensibility degree of the microorganism to ITCs;

++ the microorganism developed on the entire surface of culture medium.

From table 3, we can see that after 24 and 48 hours, *Candida albicans* presents a lower sensitiveness to ITCs action from homogenized

horseradish extracts (free zone's diameter is of 0.1 ÷ 0.3 cm), in comparison with her reaction to cutting horseradish.

Table 4. Effect of ITCs from homogenized horseradish extract on *Escherichia coli* after 24 respectively 48 hours of incubation, and enzymatic activation temperature of 55°C

Microbial species	Samples/ Reaction time (120-330 min)	Time (hours)	ITCS (mg/100g product)	Sensibility/ resistance of microbial species	
<i>Escherichia coli</i>	P ₁ /120 min.	24	117.32	+	
		48		+	
	P ₂ /150 min.	24	119.27	0.1	
		48		0.1	
	P ₃ /180 min.	24	123.89	0.1	
		48		0.1	
	P ₄ /210 min.	24	124.38	0.2	
		48		0.2	
	P ₅ /240 min.	24	130.25	0.3	
		48		0.3	
	P ₆ /270 min.	24	128.5	0.2	
		48		0.2	
	P ₇ /300 min.	24	125.72	0.1	
		48		0.1	
	P ₈ /330 min.	24	122.21	0.1	
		48		0.1	
	Witness sample				++
					++

0.1 ÷ 0.3 cm (negative reaction) represents the free zones of the microorganism's diameter values indicating the sensibility degree of the microorganism to ITCs;

+ has the significance of a positive reaction, the microorganisms resisting to ITCs action from homogenized extracts;

++ the microorganism developed on the entire surface of culture medium.

From table 4, we can see that after 24 respectively 48 hours, *Escherichia coli* presents a lower sensitiveness (free zone's diameter

is only of 0.1 ÷ 0.3 cm), to 7 from tested concentrations, excepting P₁/120min. sample (conc. of ITCs 117.32 mg/100 g product) where presents a positive reaction.

Table 5. Effect of ITCs from homogenized horseradish extract on *Agrobacterium tumefaciens* after 24 respectively 48 hours of incubation, and enzymatic activation temperature of 55°C

Microbial species	Samples/ Reaction time (120-330 min)	Time (hours)	ITCS (mg/100g product)	Sensibility/ resistance of microbial species	
<i>Agrobacterium tumefaciens</i>	P ₁ /120 min.	24	117.32	0.2	
		48		0.2	
	P ₂ /150 min.	24	119.27	0.2	
		48		0.2	
	P ₃ /180 min.	24	123.89	0.3	
		48		0.3	
	P ₄ /210 min.	24	124.38	0.3	
		48		0.3	
	P ₅ /240 min.	24	130.25	0.5	
		48		0.5	
	P ₆ /270 min.	24	128.50	0.2	
		48		0.2	
	P ₇ /300 min.	24	125.72	0.2	
		48		0.2	
	P ₈ /330 min.	24	122.21	0.2	
		48		0.2	
	Witness sample				++
					++

0.1 ÷ 0.3 cm (negative reaction) represents the free zones of the microorganisms' diameter values indicating the sensibility degree of the microorganism to ITCs;

++ the microorganism developed on the entire surface of culture medium.

From table 5, we can see that after 24 respectively 48 hours, ITCs presents an inhibiting action, relatively reduced, free zone's diameter is only of 0.2 ÷ 0.5 cm.

Table 6. Effect of ITCs from homogenized horseradish extract on *Rhizopus nigricans* after 24 respectively 48 hours of incubation, and enzymatic activation temperature of 55°C

Microbial species	Samples/ Reaction time (120-330 min)	Time (hours)	ITCS (mg/100g product)	Sensibility/ resistance of microbial species	
<i>Rhizopus nigricans</i>	P ₁ /120 min.	24	117.32	+	
		48		+	
	P ₂ /150 min.	24	119.27	+	
		48		+	
	P ₃ /180 min.	24	123.89	+	
		48		+	
	P ₄ /210 min.	24	124.38	+	
		48		+	
	P ₅ /240 min.	24	130.25	+	
		48		+	
	P ₆ /270 min.	24	128.5	+	
		48		+	
	P ₇ /300 min.	24	125.72	+	
		48		+	
	P ₈ /330 min.	24	122.21	+	
		48		+	
	Witness sample				++
					++

+ has the significance of a positive reaction, the microorganisms resisting to ITCs action from homogenized extracts;

++ the microorganism developed on the entire surface of culture medium.

From table 6, we can see that after 24 and 48 hours *Rhizopus nigricans* presents a positive reaction, I mean a growing resistance to ITCs action from homogenized horseradish extracts.

Conclusions

On the bases of obtained results we can asses that the ITCs compounds present in homogenized horseradish extracts have a lower inhibitory effect on the microorganisms than the ITCs present in cutting horseradish extracts.

The antimicrobial activity of isothiocyanates has been studied in order to be able observing their potential as alimentary preservatives in comparison with other preservatives, sodium benzoate for example.

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