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

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Abstract

In this study we want to emphasis the bactericidal, bacteriostatical and antifungal effect of isotiocyanates from horseradish roots on some microbial culture: Escherichia coli, Candida albicans, Bacillus subtilis, Staphylococcus aureus, Agrobacterium tumefaciens and Rhizopus nigricans. For this, at first were established the best conditions of working, namely: phosphate buffer pH was 7, reaction time was of 120 ÷ 330 minutes, temperature of 55°C, with a view to extracts obtained from cutting horseradish. Then, through inoculate dissemination technique on culture medium surface, were done microbiological tests. The obtained results, distinguished the bactericidal, bacteriostatical and antifungal effect of isothiocyanates on studied microorganisms.
Keywords: *horseradish, isothiocyanates, antibacterial activity*

Introduction

Horseradish (*Armoracia rusticana Lam - Fam. Cruciferae*) is an annual edible plant from south-east Europe. A class of important compounds from *Armoracia rusticana* composition is glucosinolates (GLS). Glucosinolates are a class of secondary plant metabolites found in dicots, particularly in the order Capparales, comprising the Capparaceae, Brassicaceae (Cruciferae), Koeberliniaceae, Moringaceae, Resedaceae and Tovariaceae (Rosa, 2001).

Because of their high bioactivity and because of the variety of compounds that can be obtained from them, GLS exhibit a great potential for their use in chemistry, food processing and food applications. In spite of being considered antinutritional compounds at

the beginning, after wards their efficiency in preventing sickness and in preparing and storage at some foods, was proved (Palmieri, 1999).

Upon plant tissue disruption during food processing (e.g. by cutting), GLS presumably stored in the cell vacuole are released and hydrolysed by the enzyme myrosinase (thioglucoside glucohydrolase EC 3.2.3.1.), which is located in the cytoplasm. Myrosinase hydrolytically cleaves off the glucose, resulting in an unstable intermediate (aglycone).

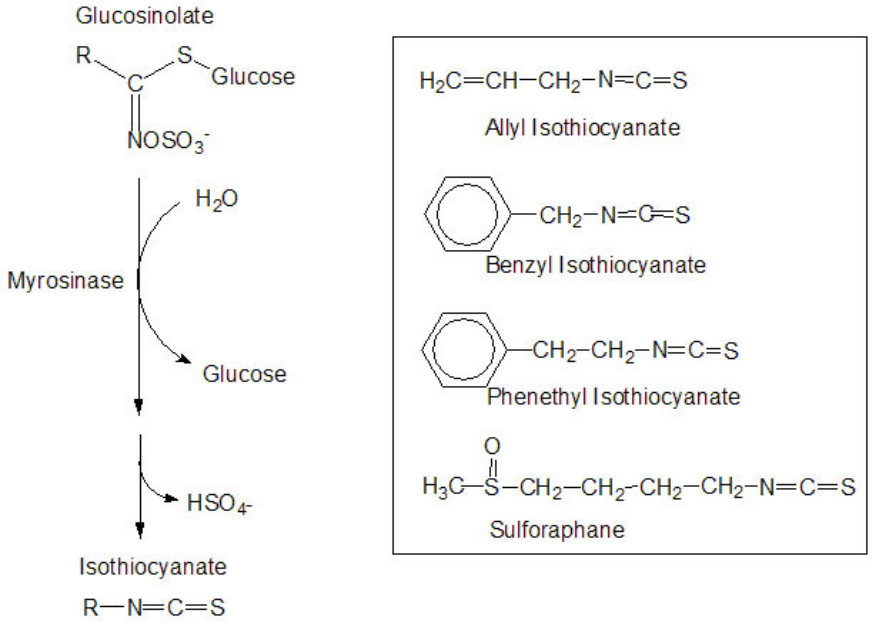

Fig. 1. Hydrolysis of glucosinolates in *Armoracia rusticana*

This aglycone spontaneously rearranges into the potential cancer-protective isothiocyanates (ITCs), nitriles or other products, such as thiocyanates. Which breakdown products will be formed, depends on the GLS substrate as well as the reaction conditions, such as: substrate, pH, temperature and availability of ferrous ions (Fenwick, 1983). The chemical structure of a GLS and the breakdown products formed on myrosinase activity are shown in *Figure 1*. But, from the hydrolysis products of GLS, only ITCs have the biggest bactericidal, bacteriostatical and antifungal effects (Shofran, 1998; Conaway, 2002).

Glucosinolates and/or their breakdown products have long been known for their fungicidal, bactericidal and bacteriostatical properties, and have recently attracted intense research interest because of their cancer chemo-protective attributes. The activity of ITCs against numerous human pathogens (e.g. *Escherichia coli, Candida albicans, Bacillus subtilis*) could even contribute to the medicinal properties ascribed to cruciferous vegetables (Drobnica, 1967; Fahey, 2001).

Taking in account the presented reasons, we can say that ITCs may be used as preservatives in food industry (Delaquis, 1995; Shofran, 1998).

Experimental

Obtaining extracts: The extracts for analysis were obtained from horseradish root (cutting, 1g each one) dissolved in 10mL phosphate buffer solution (pH=7). Then, the extracts were heated and maintained at best temperature of forming ITCs (55°C) in the interval of 120 ÷ 330 minutes in a shaker. After every 30 minutes was taken a sample, which was cooled, treated with 1mL $AgNO_3$ 0.1M for the enzymatic reaction inhibition, and then filtered. The condition of working for obtaining extracts were established after there were done some kinetically, thermodynamically and pH studies, researches which showed the best conditions (pH=7, temperature of 55°C, and reaction time of 120 ÷ 330minutes), and the ITCs concentration was maximum. The concentrations of ITCs from cutting horseradish extracts were determined by GC-MS.

Microbiological tests: It was followed the behavior of the following microbial cultures: *Escherichia coli, Candida albicans, Bacillus subtilis, Staphylococcus aureus, Agrobacterium tumefaciens and Rhizopus nigricans,* in the presence of ITCs from cutting horseradish extracts.

The nutritive mediums used were prepared in accordance with Zarnea (1996). Then, the mediums were distributed in Petri sterile plates (10mL in every plate) and after cooling and solidification of mediums, it was effected the insemination procedure with four microbial culture. For the insemination of microbial cultures it was used *"the inoculate dissemination technique"*. In incubation, on the surface of inoculate medium from Petri plates, were deposited 5 micro

tablets for every adequate reaction time. The Petri plates were then incubated to thermostat for 24 respectively 48 hours, at different temperatures depending on the microbial cultures requirements. It was followed the sensibility/resistance of microbial species to cutting horseradish extracts.

Results and Discussions

The experimental results are given in the tables 1 – 6. We must mention that the witness samples mean the microbial species developed on the two culture mediums, in absence of ITCs developed very well, they occupied to entire surface of Petri plates, so they had a positive reaction.

Table 1. Effect of ITCs from cutting 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
Bacillus subtilis	P_1/120 min.	24	142.25	0.3
		48		0.2
	P_2/150 min.	24	145.83	0.4
		48		0.3
	P_3/180 min.	24	148.25	0.5
		48		0.4
	P_4/210 min.	24	155.88	1
		48		0.5
	P_5/240 min.	24	147.38	0.8
		48		0.4
	P_6/270 min.	24	144.75	0.5
		48		0.3
	P_7/300 min.	24	141.82	0.2
		48		0.2
	P_8/330 min.	24	139.98	0.2
		48		0.2
	Witness sample			++
				++

0.2. ÷ 1 cm it means a negative reaction, the microorganism is sensitive at ITCs action from tested extract;
++ the microorganism developed on the entire surface of culture medium.

From table 1, it can be observed that after 24 hours of incubation, *Bacillus subtilis* presents a bigger sensitiveness at cutting horseradish extracts (free zone's diameters presents constant values between 0.2 ÷ 1 cm), and after 48 hours inhibition areas reducing having values between 0.2. ÷ 0.5 cm.

Table 2. Effect of ITCs from cutting 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
Staphylococcus aureus	P_1/120 min.	24	142.25	0.2
		48		0.2
	P_2/150 min.	24	145.83	0.5
		48		0.2
	P_3/180 min.	24	148.25	0.8
		48		0.4
	P_4/210 min.	24	155.88	1
		48		0.5
	P_5/240 min.	24	147.38	0.7
		48		0.4
	P_6/270 min.	24	144.75	0.6
		48		0.3
	P_7/300 min.	24	141.82	0.3
		48		0.2
	P_8/330 min.	24	139.98	0.2
		48		0.2
	Witness sample			++
				++

0.2. ÷ 1 cm it means a negative reaction, the microorganism is sensitive at ITCs action from tested extract;
++ the microorganism developed on the entire surface of culture medium.

From table 2, we can see that *Staphylococcus aureus* after 24 hours presents sensitiveness enough pronounced to ITCs action from cutting horseradish extracts, (free zone's diameter has values between 0.2 ÷ 1cm). After 48 hours free zone's diameter reduces, has values between 0.2 ÷ 0.5cm, so we can say that the microorganism sensitiveness to ITCs from cutting horseradish is reducing.

Table 3. Effect of ITCs from cutting 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
Candida albicans	P_1/120 min.	24	142.25	0.2
		48		0.2
	P_2/150 min.	24	145.83	0.3
		48		0.3
	P_3/180 min.	24	148.25	0.4
		48		0.4
	P_4/210 min.	24	155.88	0.5
		48		0.5
	P_5/240 min.	24	147.38	0.3
		48		0.3
	P_6/270 min.	24	144.75	0.3
		48		0.3
	P_7/300 min.	24	141.82	0.2
		48		0.2
	P_8/330 min.	24	139.98	0.2
		48		0.2
	Witness sample			++
				++

0.2 ÷ 0.5 cm it means a negative reaction, the microorganism is sensitive at ITCs action from tested extract;
++ the microorganism developed on the entire surface of culture medium.

From table 3, we can see that *Candida albicans* after 24 hours, respectively 48 hours of incubation presents a lower sensitiveness at ITCs action (free zone's diameter has constant values between 0.2 ÷ 0.5 cm).

Table 4. Effect of ITCs from cutting 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
Escherichia coli	P_1/120 min.	24	142.25	0.2
		48		0.2
	P_2/150 min.	24	145.83	0.5
		48		0.5
	P_3/180 min.	24	148.25	0.8
		48		0.8
	P_4/210 min.	24	155.88	1
		48		1
	P_5/240 min.	24	147.38	0.8
		48		0.8
	P_6/270 min.	24	144.75	0.6
		48		0.6
	P_7/300 min.	24	141.82	0.5
		48		0.5
	P_8/330 min.	24	139.98	0.1
		48		0.1
	Witness sample			++
				++

0.1 ÷ 1 cm it means a negative reaction, the microorganism is sensitive at ITCs action from tested extract;
++ the microorganism developed on the entire surface of culture medium.

From table 4, we can see that after 24 and 48 hours, *Escherichia coli* presents as a rule a bigger sensitiveness at tested samples, (free zone's diameter has values between 0.1 ÷ 1cm).

Table 5. Effect of ITCs from cutting 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
Agrobacterium tumefaciens	P_1/120 min.	24	142.25	0.2
		48		0.2
	P_2/150 min.	24	145.83	0.2
		48		0.2
	P_3/180 min.	24	148.25	0.4
		48		0.4
	P_4/210 min.	24	155.88	0.5
		48		0.5
	P_5/240 min.	24	147.38	0.3
		48		0.3
	P_6/270 min.	24	144.75	0.2
		48		0.2
	P_7/300 min.	24	141.82	0.2
		48		0.2
	P_8/330 min.	24	139.98	0.2
		48		0.2
	Witness sample			++
				++

0.2 ÷ 0.5 cm it means a negative reaction, the microorganism is sensitive at ITCs action from tested extract;
++ 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, because free zone's around the micro tablets which contain these compounds with sulphur, have values between 0.2 ÷ 0.5 cm.

Table 6. Effect of ITCs from cutting 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
Rhizopus nigricans	P_1/120 min.	24	142.25	+
		48		+
	P_2/150 min.	24	145.83	+
		48		+
	P_3/180 min.	24	148.25	+
		48		+
	P_4/210 min.	24	155.88	+
		48		+
	P_5/240 min.	24	147.38	+
		48		+
	P_6/270 min.	24	144.75	+
		48		+
	P_7/300 min.	24	141.82	+
		48		+
	P_8/330 min.	24	139.98	+
		48		+
	Witness sample			++
				++

+ has the significance of a positive reaction, the microorganism is resisting to ITCs action from extract;
++ the microorganism developed on the entire surface of culture medium.

From table number 6, it can be observed that the reaction is positive even after 48 hours; the mould grows, occupying the entire surface of culture medium.

Conclusions

On the base of obtained results, we can infer, that, as a rule, the majority of tested microbial species, present a sensitiveness more or less increased (with some exceptions), which determine us to recommend the utilization of these compounds obtained from horseradish, in food and medicine domain. Also, we recommend, the utilization as a primary source of ITCs, cutting horseradish, knowing that in cutting horseradish extracts, their content is bigger and the inhibiting action to tested prokaryotes and eukaryotes is enough pronounced.

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