

RESEARCHES ABOUT THE ANTIMICROBIAL ACTION OF SOME ACTIVE PRINCIPLES IN ARMORACIA RUSTICANA

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Abstract

*In this paper the antimicrobial action of isothiocyanate, active principles from horseradish (*Armoracia rusticana*), on some bacteria: *Escherichia coli*, *Candida albicans* and *Aspergillus niger* were studied. In order to do that the extracts were obtained and then microbiological tests have been performed, using the technique of inocul dissemination on the media surface. It was remarked that the extracts from horseradish have antimicrobial effect on the studied bacteria.*

Keywords: *antimicrobial action, isothiocyanates, horseradish*

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, horseradish, mustard, cabbage, radish and turnip (Bones, 1996).

When tissues of plants containing glucosinolates are damaged or chewed, isothiocyanates are released by the hydrolytic action of the enzyme myrosinase (EC 3.2.3.1); the reaction (figure 1) involve initial cleavage of the thioglucoside linkages, yielding D-glucose and unstable thiohydroximate-O-sulphonate that spontaneously forms sulphate and isothiocyanates by a Lossen rearrangement (Bjorkman, 1972; Conaway, 2002; Fenwick, 1983). Myrosinase isoenzymes from plant sources show substantial diversity in physico-chemical characteristics. All myrosinases isolated and purified so far have been reported to be glycoproteins (Fahey, 2001).

Although isothiocyanates are formed during hydrolysis at neutral pH, at low pH and in the presence of Fe²⁺ ions or other compounds that modify the action of myrosinase, nitriles, epithionitriles, thiocyanates or oxazolidine-2-thiones may also be produced. This intermediate rapidly reverts resulting in the production of sulphate

and thiocyanate, isothiocyanate or nitrite depending on different factors such as: substrate, pH, temperature and availability of ferrous ions) Fenwick, 1983).

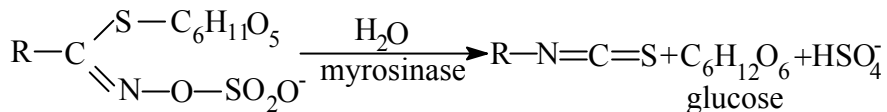


Fig. 1. Hydrolysis of glucosinolates in *Armoracia rusticana*

After glucosinolates hydrolysis catalyzed by myrosinase enzyme, more hydrolysis products result, but among these, isothiocyanates have the most powerful antimicrobial properties [Conaway, 2002, Shofran, 1998]. Also, the isothiocyanates inhibit the development of tumors in many of the experimental models investigated, and are being investigated as possible chemo preventive agents for specific human cancer. Factors influencing the generation of isothiocyanates may be important in regulating the fermentation of vegetables containing its precursor compound sinigrin. Starting up from these grounds, it may be said that the isothiocyanates can be used as preservative (Conaway, 2002; Fenwick, 1983; Rosa, 2001; Shofran, 1998).

Experimental

Obtaining extracts from whole/cutting horseradish: The sample extracts were prepared from cutting horseradish roots (approximately 1g each) dissolved in 10ml buffer phosphate (pH = 7). The samples have been warmed up in a shaker at 55°C (the best reaction temperature) for 30-300 minutes. After each 30 minutes, in this interval, a sample has been taken one by one, each being cooled, treated with AgNO₃ 0,1M, and then filtered.

Microbiological determination: It has been aimed the behavior of the next (following) microbial culture: *Escherichia coli*, *Candida albicans* and *Aspergillus niger* under isothiocyanates action-active principles from horseradish (*Armoracia rusticana*) with antimicrobial effect. The nourishing media that have been used, were:

- Sabouraud for the fungus species: *Candida albicans*, *Aspergillus niger*;
- Gelose for the bacterial species: *Escherichia coli*.

After preparation, the culture media have been distributed in Petri plates; have been layer down, for each extract, 5 micro tablets one by one (micro

tablets inhibited in the extract corresponding to reaction time). After that, the Petri plates have been incubated in thermostat for 24 and 48 hours at different temperatures depending on the microbial cultures needs namely: *Escherichia coli* at 37°C temperature and *Aspergillus niger*, *Candida albicans* at 27°C temperature. It has been aimed the microorganisms reaction in the presence of these extracts, after an incubation time of 24 hours, 48 hours respectively (Zarnea, 1970, 1996).

Results and Discussions

The experimental determination results are presented in the tables 1 – 3.

Table 1. Sensibility/multiple resistance of the *Escherichia coli* species, toward to ITCs from horseradish extracts.

Samples/Reaction time [30-300 min.]	Time [hours]	Samples concentration in ITCs [mmol]	Microorganisms reaction to the ITCs concentration
P ₁ /30 min.	24	1.862	0.2
	48		0.2
P ₂ /60 min.	24	2.019	0.2
	48		0.2
P ₃ /90 min.	24	2.125	0.2
	48		0.2
P ₄ /120 min.	24	3.183	0.5
	48		0.5
P ₅ /150 min.	24	3.368	0.5
	48		0.5
P ₆ /180 min.	24	3.781	0.5
	48		0.5
P ₇ /210 min.	24	3.965	0.5
	48		0.5
P ₈ /240 min.	24	4.429	0.5
	48		1
P ₉ /270 min.	24	4.589	1
	48		1
P ₁₀ /300 min.	24	4.653	1
	48		1

Note: 0.2-1 cm (negative reaction) represents the free zones of the microorganisms diameter values indicating the sensibility degree of the microorganism to ITCs.

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Table 2. Sensibility/multiple resistance of the *Candida albicans* species, toward to ITCs from horseradish extracts.

Samples/Reaction time [30-300 min.]	Time [hours]	Samples concentration in ITCs [mmol]	Microorganisms reaction to the ITCs concentration
P ₁ /30 min.	24	1.862	0.2
	48		0.2
P ₂ /60 min.	24	2.019	0.2
	48		0.2
P ₃ /90 min.	24	2.125	0.2
	48		0.2
P ₄ /120 min.	24	3.183	0.2
	48		0.2
P ₅ /150 min.	24	3.368	0.2
	48		0.2
P ₆ /180 min.	24	3.781	0.4
	48		0.4
P ₇ /210 min.	24	3.965	0.4
	48		0.4
P ₈ /240 min.	24	4.429	0.5
	48		0.5
P ₉ /270 min.	24	4.589	1
	48		1
P ₁₀ /300 min.	24	4.653	1
	48		1

Note: 0.2-1 cm (negative reaction) represents the free zones of the microorganisms' diameter values indicating the sensibility degree of the microorganism to ITCs.

From table number 1, it was ascertained that:

- *Escherichia coli* shows high sensibility towards samples P₄-P₁₀, at concentrations of 3.183mM till 4.653mM. The microorganisms' free zone's diameters are 0.5 cm till 1 cm, maintaining unchanged after 48 hours, too.
- For samples P₁, P₂ and P₃ it may be that this microbial species sensibility is low, at 1.862mM till 2.125mM concentrations. The situation remains unchanged after 48 hours, too.

From table number 2, it was ascertained that:

- After 24 hours, *Candida albicans* shows a negative reaction, with microorganism's free zone's diameters between 0.4 and 1

cm, towards to samples P₆-P₁₀. The diameters maintain after 24 hours.

- In the presence of samples P₁-P₅, at concentrations of 1.862 mM till 3.368mM, respectively, the microorganisms' presents a negative reaction with microorganisms' free zone's diameters of 0.2cm. After 48 hours, the diameters remain unchanged.

Table 3. Sensibility/multiple resistance of the *Aspergillus niger* species, toward to ITCs from horseradish extracts.

Samples/Reaction time [30-300 min.]	Time [hours]	Concentration samples in ITCs [mmol]	Microorganisms reaction of the ITCs concentration
P ₁ /30 min.	24	1.862	0.2
	48		0.2
P ₂ /60 min.	24	2.019	0.2
	48		0.2
P ₃ /90 min.	24	2.125	0.2
	48		0.2
P ₄ /120 min.	24	3.183	0.2
	48		0.2
P ₅ /150 min.	24	3.368	0.2
	48		0.2
P ₆ /180 min.	24	3.781	0.2
	48		0.2
P ₇ /210 min.	24	3.965	0.2
	48		0.2
P ₈ /240 min.	24	4.429	0.4
	48		0.4
P ₉ /270 min.	24	4.589	0.5
	48		0.5
P ₁₀ /300 min.	24	4.653	0.5
	48		0.5

Note: 0.2-1 cm (negative reaction) represents the free zones of the microorganisms' diameter values indicating the sensibility degree of the microorganism to ITCs.

From table number 3, it was ascertained that:

- After 24 hours, *Aspergillus niger* shows sensibility towards samples P₈-P₁₀, at concentrations of 4.429mM till 4.653mM, with microorganisms free zone's diameters of 0.4cm till 0.5cm, remaining unchanged after 48 hours, too.

- Towards samples P₁-P₇, the microorganism shows a low sensibility, with microorganism free zone's diameters of 0.2 cm, diameters that remain unchanged after 48 hours, too.

Conclusions

Following the performed experimental determinations it can be said that isothiocyanates, compounds present in horseradish have inhibitory effect, higher on *Escherichia coli* and *Candida albicans* microorganisms, and lower towards *Aspergillus niger* microorganism. 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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