ANTIBACTERIAL ACTIVITY OF ISOTHIOCYANATES, ACTIVE PRINCIPLES IN *BRASSICA NIGRA* SEEDS (IV)

Daniela STOIN, Florina RADU,
Mariana-Atena POIANĂ, Diana DOGARU

Faculty of Food Processing Technology,
Banat′s University of Agricultural Sciences
and Veterinary Medicine Timisoara
e-mail: mucetedaniela@yahoo.com

This paper belongs to a more complex study, study that has as aim the emphasize of antibacterial activity of "isothiocyanates" bioactive compounds, compounds present in more vegetable sources (Armoracia rusticana and Brassica nigra). In this case, the biological material studied, were the Brassica nigra seeds. On the basis of kinetics, thermodynamics and pH studied, the optimum conditions corresponding to obtaining the extracts were the following: phosphate buffer pH was 7, reaction time was of 120 ÷ 330 minutes, temperature of 55°C.The emphasize of antibacterial activity of there compounds was done by taking microbiological tests on the following microbiological cultures: Escherichia coli, Candida albicans, Bacillus subtilis, Staphylococcus aureus, Agrobacterium tumefaciens and Rhizopus nigricans, using the inoculate microbiological technique on culture medium surface. After 24 hours of incubation, the ITCs exhibit a average inhibitory action towards to Bacillus subtilis, because the free zones around the filter paper with extract (that contain sulphur compounds), have the values in the range 0.2-0.6 cm. Candida albicans exhibit a significant sensitivity to words ITCs action, from mustard seeds extracts, the free zone diameters to maximum concentration is 0.7 cm and decreases to the other concentrations, maintaining themselves unchanged after 48 hours, too respectively. In the presence of ITCs from mustard seeds extracts, Escherichia coli exhibits a average negative reaction, with free zone diameters in the range of 0.2-0.5 cm. The obtained results have shown a relative antibacterial activity, induced by these compounds, comparing to results obtained in the case of crushed down mustard extracts (results shown in a previous paper). 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 seeds mustard, in food and medicine domain.

***Keywords**: isothiocyanates, glucosinolates, enzymatic activity, mustard seeds, antibacterial activity*

Glucosinolates (GSL), found in *Brassica* species, are of interest due to the potential for using their degradation products as fumigants. When hydrolyzed by

the enzyme myrosinase, GLS produce D-glucose, sulfate, isothiocyanates (volatile mustard oils), thiocyanates and nitriles [1, 3, 10, 12].

Glucosinolates are a class of secondary plant metabolites found in dicots, particularly in the order Capparales, comprising the Capparaceae, Brassicaceae (Cruciferae), Koeberliniaceae, Moring-aceae, Resedaceae and Tovariaceae. 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 [3,4].

Isothiocyanates (ITCs) are sulphur-containing phytochemicals with the general formula R-NCS. Different molecules belong to this group, such as [4]. But, from the hydrolysis products of GLS, only isothiocyanates have the biggest bactericidal, bacteriostatical and antifungal effects [1,10].

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. These compounds are also responsible for the typical flavour of these vegetables [11]

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). 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 [1,8].

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 [5, 8, 9,12].

Taking in account the presented reasons, we can say that ITCs may be used as preservatives in food industry [2,10].

MATERIAL AND METHOD

Obtaining extracts: The extracts for analysis were obtained from seeds mustard grinding (1 g each one) dissolved in 10 mL 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 seeds mustard 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 seeds mustard extracts after Delaquis and Mazza [2].procesure.

The nutritive mediums used were prepared in accordance with Zarnea [11]. 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 seeds mustard extracts.

RESULTS AND DISCUSSIONS

The obtained results emphasizes the antibacterial activity of ITCs from biological studied materials, being in accordance with those presented by Delaquis [2], Manici [5], Ono [7] and Shofran [8]. 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.

The results of microbiological tests are given in tables 1 to 4. It is important to know that beside the four species presented in the tables, also were studied the *Agrobacterium tumefaciens* and *Rhizopus nigricans* species, but the obtained results were not conclusive.

From table 1-4 it can be said the following:

After 24 hours of incubation, the ITCs exhibit a average inhibitory action towards to *Bacillus subtilis*, because the free zones around the filter paper with extract (that contain sulphur compounds), have the values in the range 0.2-0.6 cm;

The same sensitivity degree maintains and after 48 hours of incubation.

Candida albicans exhibit a significant sensitivity to words ITCs action, from mustard seeds extracts, the free zone diameters to maximum concentration is 0.7cm and decreases to the other concentrations, maintaining themselves unchanged after 48 hours, too respectively. In the presence of ITCs from mustard seeds extracts, *Escherichia coli* exhibits a average negative reaction, with free zone diameters in the range of 0.2-0.5 cm.After 48 hours of incubation, the free zones diameter stays the same. *Staphylococcus aurous* after 24 and 48 hours of incubation, respectively exhibits a average sensitivity to inhibitory action of ITCs from the mustard seeds extracts, the free zones diameters being in the range of 0.3-0.6 cm.

Table 1

Effect of ITCs from seeds mustard 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 concentration from samples (mg/100g product)	Sensibility/ resistance of microbial species
Bacillus subtilis	P_1/120 min.	24	127.25	0.2
		48		0.2
	P_2/150 min.	24	137.42	0.3
		48		0.3
	P_3/180 min.	24	148.62	0.4
		48		0.4
	P_4/210 min.	24	154.19	0.5
		48		0.4
	P_5/240 min.	24	162.31	0.6
		48		0.6
	P_6/270 min.	24	157.09	0.5
		48		0.5
	P_7/300 min.	24	145.90	0.2
		48		0.2
	P_8/330 min.	24	136.29	0.2
		48		0.2
	Witness sample			++
				++

***Note**: 0.2 ÷ 0.6 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.*

Table 2

Effect of ITCs from seeds mustard 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 concentration from samples (mg/100g product)	Sensibility/ resistance of microbial species
Candida albicans	P_1/120 min.	24	127.25	0.3
		48		0.3
	P_2/150 min.	24	137.42	0.3
		48		0.3
	P_3/180 min.	24	148.62	0.5
		48		0.5
	P_4/210 min.	24	154.19	0.5
		48		0.5
	P_5/240 min.	24	162.31	0.7
		48		0.7
	P_6/270 min.	24	157.09	0.6
		48		0.6
	P_7/300 min.	24	145.90	0.4
		48		0.3
	P_8/330 min.	24	136.29	0.3
		48		0.3
	Witness sample			++
				++

***Note**: 0.3 ÷ 0.7 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.*

Table 3

Effect of ITCs from seeds mustard 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 concentration from samples (mg/100g product)	Sensibility/ resistance of microbial species
Escherichia coli	P_1/120 min.	24	127.25	0.2
		48		0.2
	P_2/150 min.	24	137.42	0.2
		48		0.2
	P_3/180 min.	24	148.62	0.4
		48		0.4
	P_4/210 min.	24	154.19	0.4
		48		0.4
	P_5/240 min.	24	162.31	0.5
		48		0.4
	P_6/270 min.	24	157.09	0.4
		48		0.4
	P_7/300 min.	24	145.90	0.3
		48		0.3
	P_8/330 min.	24	136.29	0.2
		48		0.2
	Witness sample			++
				++

***Note**: 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.*

Table 4

Effect of ITCs from seeds mustard 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 concentration from samples (mg/100g product)	Sensibility/ resistance of microbial species
Staphylococcus aurous	P_1/120 min.	24	127.25	0.3
		48		0.3
	P_2/150 min.	24	137.42	0.3
		48		0.3
	P_3/180 min.	24	148.62	0.4
		48		0.4
	P_4/210 min.	24	154.19	0.5
		48		0.5
	P_5/240 min.	24	162.31	0.6
		48		0.6
	P_6/270 min.	24	157.09	0.6
		48		0.5
	P_7/300 min.	24	145.90	0.4
		48		0.3
	P_8/330 min.	24	136.29	0.3
		48		0.3
	Witness sample			++
				++

***Note**: 0.3 ÷ 0.6 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.*

CONCLUSIONS

Comparing the obtained results, after studying the antibacterial activity of ITCs from rubbed out and unrubbed horseradish, crushed down and seeds mustard, it may be said that these biological active compounds have an inhibitory significant effect on the studied microorganisms.

Also, comparing the sensitivity degree of the studied microorganisms to *Armoracia rusticana* and *Brassica nigra* extracts, it could be seen the fact that the obtained extracts from processed biological material (crushed down, rubbed out) have a more microbiological activity significant than those obtained from unrubbed horseradish or mustard seeds.

Starting from those presented we can recommend the utilization of ITCs, in food and medical and medicine domain.

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