COBISS Code 1.01

DOI: 10.2478/v10014-009-0007-z

Agrovoc descriptors: rosemary, rosmarinus officinalis, antimicrobial properties, listeria monocytogenes, resistance to injurious factors, biological differences, plant extracts, concentrating, essential oils, foods, food additives, contamination, food processing, preservation

Agris category code: Q03, Q05

Antimicrobial activity of rosemary extracts (*Rosmarinus officinalis* L.) against different species of *Listeria*

Tanja ROŽMAN[1], Barbara JERŠEK[2]

Received September 11, 2008; accepted April 2, 2009.
Delo je prispelo 11. septembra 2008; sprejeto 2. aprila 2009.

ABSTRACT

Reduction or elimination of chemically synthesized additives from foods is a current demand in food industry. A new approach to prevent the proliferation of microorganisms or protect food from oxidation is the use of essential oils or plant extracts as natural additives in foods. We have studied antimicrobial activity of rosemary extracts (*Rosmarinus officinalis* L.) against different species of *Listeria* and against different strains of *L. monocytogenes*. We used two extracts of rosemary, VivOX 20 and VivOX 40 (Vitiva d.d., Slovenia) containing different levels of carnosic acid. We wanted to proof an antimicrobial activity of selected rosemary extracts with two most commonly used methods: disc diffusion method and broth dilution method. With the disc diffusion method we have obtained the inhibition zone and at the lowest concentrations, where no visible bacterial growth was recorded, were assumed as minimal inhibitory concentration values (MIC). We determined MIC values in the ranges from 625 µg extract/ml EtOH to 5000 µg extract/ml EtOH for VivOX 20 and from 312.5 µg extract/ml EtOH do 2500 µg extract/ml EtOH for VivOX 40 in the medium. We have established that the resistance of *Listeria* species against rosemary extracts depends on: selected extract, selected concentration, various species and strain of *Listeria*. With broth dilution method we have determined minimal bactericidal concentration (MBC), as the concentration giving 0.1 % bacterial survival. With this method we have tested two strains of *L. monocytogenes* and in determinate MBC values in the range from 15.63 µg/ml TSB to 98.5 µg/ml TSB for both tested extracts. Results have confirmed our assumption that resistance of *Listeria* against rosemary extracts depended on the selected strain.

Keywords: pathogens, *Listeria*, *Listeria monocytogenes*, plant extracts, rosemary, antimicrobial activity, carnosic acid, minimal inhibitory concentration, minimal bactericidal concentration

IZVLEČEK

Zahteve potrošnikov po celem svetu so zmanjšati oz. izločiti kemično sintetizirane konzervanse iz živil. Novejše metode preprečevanja mikrobne kontaminacije in oksidacije uporabljajo eterična olja ali rastlinske ekstrakte kot naravne konzervanse. Proučevali smo protimikrobno delovanje ekstraktov rožmarina (*Rosmarinus officinalis* L.) na različne vrste bakterij rodu *Listeria* in seve bakterij *L. monocytogenes*. Uporabili smo dva različna komercialno pripravljena ekstrakta rožmarina, VivOX 20 in VivOX 40 (Vitiva d.d., Slovenija), ki sta vsebovala različno koncentracijo karnozolne kisline. Protimikrobni učinek izbranih ekstraktov smo želeli dokazati z dvema najpogosteje uporabljenima metodama: metoda difuzije v trdnem gojišču in metoda razredčevanja v tekočem gojišču. Pri metodi difuzije v trdnem gojišču smo po inkubaciji odčitali nastale inhibicijske cone, s katerimi smo določili minimalne inhibitorne koncentracije (MIC), kot tiste koncentracije, pri katerih ni bilo vidne rasti bakterij na gojišču. Vrednosti MIC smo določili v območju med 625 µg ekstrakta/ml EtOH do 5000 µg ekstrakta/ml EtOH za ekstrakt VivOX 20 in med 312,5 µg ekstrakta/ml EtOH do 2500 µg ekstrakta/ml EtOH za ekstrakt VivOX 40. Ugotovili smo, da je odpornost listerij proti ekstraktoma rožmarina odvisna od izbranega ekstrakta, izbrane koncentracije ter vrste in seva listerij. Z metodo razredčevanja v tekočem gojišču smo določali minimalne baktericidne koncentracije (MBC), kot tiste koncentracije, pri katerih preživi 0,1 % testnih bakterij. Uporabili smo dva različna seva bakterij vrste *L. monocytogenes* in vrednosti MBC v večini poskusov določili med 15,63 µg/ml gojišča TSB in 98,5 µg/ml gojišča za oba uporabljena ekstrakta. Rezultati so ponovno potrdili našo domnevo, da je odpornost listerij proti ekstraktoma rožmarina odvisna od seva.

Ključne besede: patogeni mikroorganizmi, *Listeria*, *Listeria monocytogenes*, rastlinski ekstrakti, rožmarin, protimikrobno delovanje, karnozolna kislina, minimalna inhibitorna koncentracija, minimalna baktericidna koncentracija

[1] Univ. v Ljubljani, Biotehniška fakult., Odd. za živilstvo, Jamnikarjeva 101, 1111 Ljubljana, Slovenija

[2] Univ. v Ljubljani, Biotehniška fakult., Odd. za živilstvo, Jamnikarjeva 101, 1111 Ljubljana, Slovenija

1 INTRODUCTION

Food market trends are changing. Consumers demand more high-quality foods with fresh like attributes; consequently less extreme treatments and/or additives are being required. Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf-life reduction. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors. Oxidative processes and bacterial contamination, in turn, contribute to the deterioration in flavour, texture and color of food products (Fernandez-Lopez *et al*., 2004).

Growth of microorganisms in food may cause spoilage or foodborne disease (Del Campo *et al*., 2000). Synthetic additives have been widely used. The trend is to decrease their use because of the growing concern among consumers about such chemical additives. Consequently, search for natural additives, especially of plant origin, has notably increased in recent years. Therefore, the development and application of natural products with both antioxidants and antibacterial activities especially in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases (Fernandez-Lopez *et al*., 2004).

Rosemary (*Rosmarinus officinalis* L.) originally grows in southern Europe. Its herb and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and lately as antimicrobial agent (Lo *et al*., 2002; Ouattara *et al*., 1997). Moreno *et al*. (2006) reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity they attributed to carnosic acid and carnosol. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food (Del Campo *et al*., 2000).

Although the antimicrobial properties of essential oils and their components have been reviewed in the past (Shelef, 1983; Nychas, 1995), the mechanism of action has not been studied in great detail. Considering the large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell (Burt, 2004).

Listeria is aerobic, microaerophilic, facultatively anaerobic, catalase positive and oxidase negative, small, regular Gram-positive rod with rounded ends (Rocourt and Buchrieser, 2007) and is frequently present in human environment (Fenlon, 1999). Only two of the six species in this genus are currently recognized to be pathogenic: *L. monocytogenes* and *L. ivanovii*. They cause listeriosis, an opportunistic infection of humans and animals involving severe clinical manifestations such as meningoencephalitis, abortion and septicemia (Vazquez – Boland *et al*., 2001). Human cases of *L. ivanovii* infection are rare (Gandhi and Chikindas, 2007; Zhang *et al*., 2007), being pathogenic mostly for ruminants (Vazquez – Boland *et al*., 2001), whereas *L. monocytogenes* has been recognized as a human foodborne pathogen since 1929 (Painter and Slutsker, 2007; Zhang *et al*., 2007).

Listeriosis is foodborne illness and therefore the rapid and accurate detection of *L. monocytogenes* is important for food safety assurance. *L. monocytogenes* can be found in a wide variety of raw and processed foods. Milk and dairy products, various meats and meat products such as beef, pork, fermented sausages, fresh produce such as radishes, cabbage, seafood and fish products have all been associated with *Listeria* contamination (Gandhi and Chikindas, 2007). The temperature range that permits growth of *L. monocytogenes* is of particular interest to food processors because this pathogen is a psychotropic bacterium. *L. monocytogenes* was reported to grow at temperatures between –1.5 and 45°C (Lado and Yousef, 2007), between pH 4.5 and pH 9.2, optimally at pH 7. It can grow in 10 % (w/v) NaCl and survive at higher concentrations. Survival at low pH and high salt concentration depends strongly on temperature. *Listeria* is one of the few foodborne pathogens that can grow at a_w below 0.93 (Rocourt and Buchrieser, 2007).

The aim of our work was to investigate antilisterial activity of rosemary extracts VivOX 20 and VivOX 40 in concentrations that can be used as natural additives in foods.

2 MATERIALS AND METHODS

2.1 Bacterial cultures and preparation of rosemary extracts

We tested antibacterial activity of rosemary extracts against 4 different species of *Listeria*. As *L. monocytogenes* is well known foodborne pathogen, we decided to use 8 different strains. All used cultures are listed in table 1.

Cultures were grown in BHI broth (Brain Heart Infusion broth, Merck, 1.10493, Germany) at 37 °C for 20-24 h with shaking. Suspension from BHI was then diluted in sterile BP (Butterfield's phosphate buffered dilution water (pH 7.2 ± 0.1)) till final concentration 10^7 CFU/ml. Viable counts were obtained by plating 10-fold dilutions made in sterile BP and plated onto TSA (Tryptone Soy Agar, Oxoid, CM0131, England) in duplicates. After incubating plates for 24-48 h at 37 °C the number of bacteria was calculated as colony forming units.

Table 1: *Listeria* strains used for testing antibacterial activity of rosemary extracts

***Listeria* strain designation**	**Origin, serotype**
Listeria grayi ŽM66	IHG reference strain, /
Listeria innocua ŽM68	Isolated from sausage, /
Listeria ivanovii ŽM65	IHG reference strain, 5
Listeria monocytogenes ŽM51	IHG reference strain, 1/2a
Listeria monocytogenes ŽM52	IHG reference strain, 1/2b
Listeria monocytogenes ŽM53	IHG reference strain, 1/2c
Listeria monocytogenes ŽM58	IHG reference strain, 4b
Listeria monocytogenes ŽM80	Human isolate, 4b
Listeria monocytogenes ŽM92	Isolated from chicken salad, 1/2 c
Listeria monocytogenes ŽM108	Isolated from meat pasty, 4b
Listeria monocytogenes ŽM115	Isolated from Tatarian beefsteak, 1/2 b

ŽM and ŽMJ: Culture collections of laboratory of food microbiology, Department of Food Technology, Biotechnical Faculty, Ljubljana, Slovenia; IHG: Institute for Hygiene and Microbiology, Wuerzburg, Germany

2.2 Rosemary extracts

We used two extracts of rosemary, VivOX 20 and VivOX 40 (Vitiva d.d., Slovenia), containing different levels of carnosic acid (VivOX 20: 22.04 % and VivOX 40: 40.49 % of carnosic acid)

Both extracts, VivOX 20 and VivOX 40, were prepared in absolute ethanol (0.160 g/ml) and these 16 % stock solutions were than diluted ten times till final concentration 156.25 µg/ml. Antibacterial effect of prepared extracts was tested on TSA (Tryptone Soy Agar, Oxoid, CM0131, England) in case of agar diffusion method and in TSB (Tryptone Soy Broth, Oxoid, CM0129, England) in case of broth dilution method.

2.3 Determination of antimicrobial effect of rosemary extracts

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for each extract against eleven *Listeria* strains were determined using a disc diffusion method and/or broth dilution method as a well-standardized and reliable reference methods that are useful for research purpose (Woods and Washington, 1999).

2.3.1 Disc diffusion method

One milliliter of overnight culture of *Listeria* strain was added to each plate containing TSA agar. When the agar was solidified, 4 commercially prepared filter paper discs (6 mm diameter) were added on each plate. Ten microlitres of each dilution of extract was applied on each disc. The control samples were (1) 10 µl of sterile distilled water as negative control and (2) 10 µl of 0.01 % solution of OTC (oxytetracycline, Krka, 743054, Slovenia) as positive control to control the sensitivity of the strains. After a diffusion time of 15 min at room temperature, the plates were incubated at 37 ºC for 24 h. After incubation, the inhibition zones (IZ) was measured in 2 directions and the average values were used to define MIC. The MIC was determined as lowest concentration of the rosemary extract that prevented the growth of listeria during the incubation period. Two replications of this experiment were made.

2.3.2 Broth dilution method

In this part of experiment we used two strains *L. monocytogenes* ŽM58 and *L. monocytogenes* ŽM115. Overnight culture was inoculated in fresh TSB medium with suitable concentration of rosemary extract. Growth of bacteria was followed at 37 ºC with viable cell counts on TSA at regular time intervals for 24 and 48 h. All data are expressed as the average of the experimental results. The MBC was determined as concentration giving 0.1 % bacterial survival (Canillac and Mourey, 2003). Controls containing absolute ethanol or sterile distilled water and no extracts of rosemary were included to verify the effect of the diluent on growth of

Listeria. Using broth dilution method four different conditions were tested:

- incubation of suspensions with 10 % of supplements (0.5 ml culture and 0.5 ml extract in 9 ml sterile TSB medium) for 24 h,
- incubation of suspensions with 20 % of supplements (1 ml culture and 1 ml extract in 8 ml sterile TSB medium) for 24 h,
- incubation of suspensions with 10 % of supplements (0.5 ml culture and 0.5 ml extract in 9 ml sterile TSB medium) for 48 h
- and incubation of suspensions with 20 % of supplements (1 ml culture and 1 ml extract in 8 ml sterile TSB medium) for 48 h.

2.4 Statistical analysis

For statistical analysis SAS (SAS Software. Version 8.01, 1999) was used. Data were tested for normal distribution and analyzed by the GLM (General Linear Model). For data analyses four statistical models were used:

$Y_{ij} = \mu + E_i + e_{ij}$ (Model 1)
$Y_{ij} = \mu + K_i + e_{ij}$ (Model 2)
$Y_{ij} = \mu + B_i + e_{ij}$ (Model 3)
$Y_{ij} = \mu + S_i + e_{ij}$ (Model 4)

where y: the observation parameter, μ = general mean, E_i = effect of extract, K_i = effect of concentration of extract, B_i = effect of different species of *Listeria*, S_i = effect of different strain of *L. monocytogenes*, e = residual random term with variance σ_e^2.

The criterion for significance in the procedure was $p<0.05$ and this indicated that data sets were significantly different between examined places. A significant difference was assigned with a different capital letter.

3 RESULTS AND DISCUSSION

The aim of our research was to investigate antimicrobial activity of rosemary extracts against *Listeria* strains and to find out MIC and MBC values with two most commonly used methods.

3.1 Antimicrobial activity of rosemary extracts determined by disc diffusion method

The antibacterial activity of rosemary extracts against *Listeria* strains which are considered in this study was assessed by evaluating the presence of IZ and MIC values. Results (Table 2), showed that the rosemary extracts have great potential of antilisterial activity against all of the eleven strains tested.

Minimal inhibitory concentration (MIC) values are expressed as µg of rosemary extract per ml of absolute ethanol.

The MIC values for were in the range of 625–5000 µg/ml for extract VivOX 20 and in the range of 312.5-2500 µg/ml for extract VivOX 40. The results of our study showed that VivOX 40 rosemary extract, which contained 40.49 % of carnosic acid, had higher or the same antibacterial effect as VivOX 20, which contained 22.04 % of carnosic acid. These was in accordance with our proposal that carnosic acid was the major bioactive compound of the rosemary extract but also its derivative and other compounds like carnosol, rosmarinic acid, etc. have important antimicrobial activity. Rosemary plants are rich sources of phenolic compounds with high antioxidative and antimicrobial properties, but their antimicrobial activities have not been deeply characterized (Moreno *et al*., 2006). There is also some evidence that minor components have a critical part in antibacterial activity, possibly by producing a synergistic effect between other components (Burt, 2004). The absence of inhibition zone does not necessarily mean that compounds are inactive. For example, non-polar compounds may not diffuse into the culture medium (Moreno *et al*., 2006).

We have established with applied statistical analysis that the resistance of *Listeria* species against rosemary extracts depends on: selected extract, selected concentration of extract, species of *Listeria* and strain of *L. monocytogenes* (Table 3).

Table 2: *MIC values of rosemary extracts determined with agar diffusion method*

***Listeria* strain**	**MICs (µg/ml)**	
	VivOX 20	***VivOX 40***
L. monocytogenes ŽM51	2500	625
L. monocytogenes ŽM52	2500	1250
L .monocytogenes ŽM53	1250	625
L. monocytogenes ŽM58	2500	1250
L. monocytogenes ŽM80	2500	1250
L. monocytogenes ŽM92	625	625
L. monocytogenes ŽM108	2500	2500
L. monocytogenes ŽM115	1250	312,5
L. ivanovii ŽM65	1250	1250
L. grayi ŽM66	5000	1250
L. innocua ŽM68	2500	1250

***Table 3**: Statistical evaluation or antilisterial results obtained with disk diffusion method*

Selected parameter	*Tested concentration range (μg/ml)*	Inhibition zone (mm)	No. of observation	Statistical group[a]
Extract	VivOX 20	6.69	280	A
	VivOX 40	4.68	280	B
Concentration of rosemary extracts in absolute ethanol (μg /ml)	80000	10.48	56	A
	40000	10.25	56	AB
	20000	9.40	56	B
	10000	8.37	56	C
	5000	7.31	56	D
	2500	5.72	56	E
	1250	3.56	56	F
	625	1.51	56	G
	312.5	0.26	56	H
	156.25	0.00	56	H
Species of *Listeria*	*L. ivanovii*	6.94	40	B
	L. monocytogenes	6.01	320	BC
	L. grayi	5.97	40	BC
	L. innocua	5.02	40	C
Strain of *L. monocytogenes*	*L. monocytogenes* ŽM115	8.01	40	A
	L. monocytogenes ŽM92	7.43	40	A
		7.10	40	A
	L. monocytogenes ŽM51	6.25	40	A
		5.83	40	AB
	L. monocytogenes ŽM52	5.77	40	AB
		3.93	40	B
	L. monocytogenes ŽM108	3.77	40	B
	L. monocytogenes ŽM80			
	L. monocytogenes ŽM58			
	L. monocytogenes ŽM53			

[a] Values followed by the different letters are significantly ($p<0.05$) different from each other at selected parameter

On the basis of results obtained with agar diffusion method, for the further experiments two strains of *L. monocytogenes* were selected. *L. monocytogenes* ŽM115 on which rosemary extracts had the highest antimicrobial effect and *L. monocytogenes* ŽM58 as a strain with the biggest statistical difference comparing with the first selected one (Table 3 and Figure 2).

4.2 Antimicrobial activity of rosemary extracts determined by broth dilution method

MBC values of rosemary extracts for *L. monocytogenes* ŽM58 and *L. monocytogenes* ŽM115 were determined by a broth dilution method. All experiments were repeated at least two times on different days and all data are expressed as the average of the experimental results.

The MBC was read from the graph obtained by plotting the percentage of survival cells ($\log_{10}$) versus the percentage of the corresponding concentration of rosemary extracts (Figure 2). The MBC is the concentration of rosemary extract giving 0.1 % *L. monocytogenes* ŽM58 survival ($\log_{10}$ 0.1 = -1) after 24 h incubation as proposed by Canillac and Mourey, 2003.

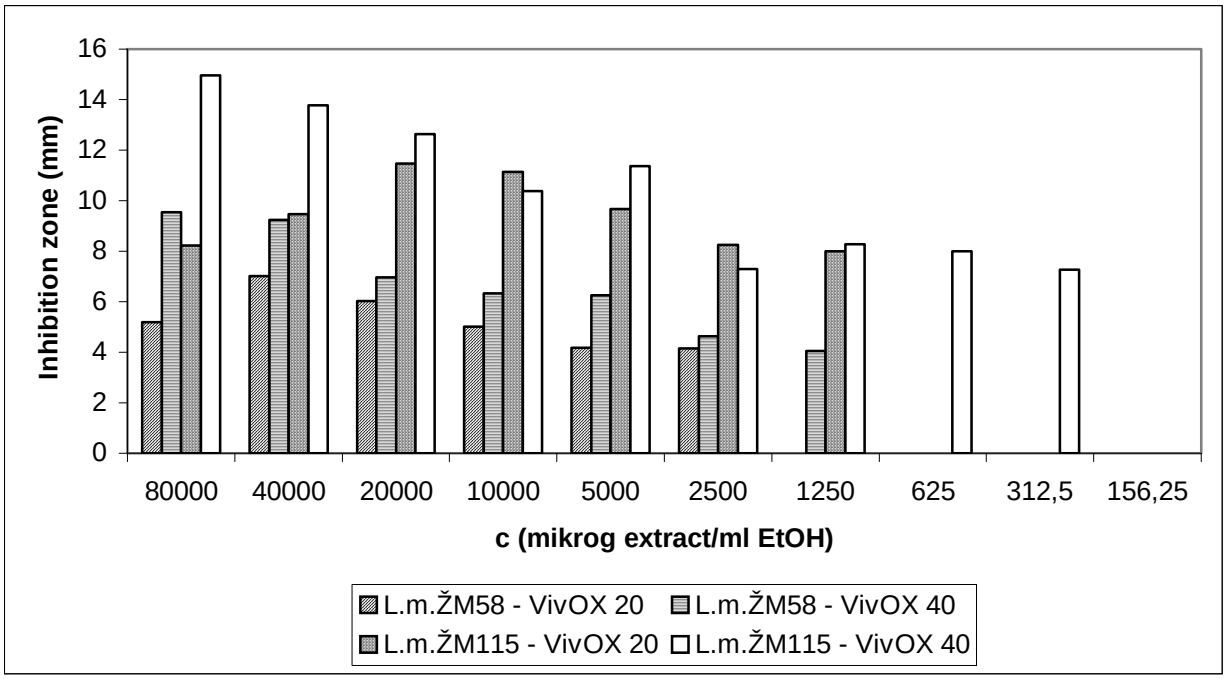

***Figure 1**: The average inhibition zones for L. monocytogenes ŽM58 and L. monocytogenes ŽM115 under the different concentrations of extracts VivOX 20 and VivOX 40*

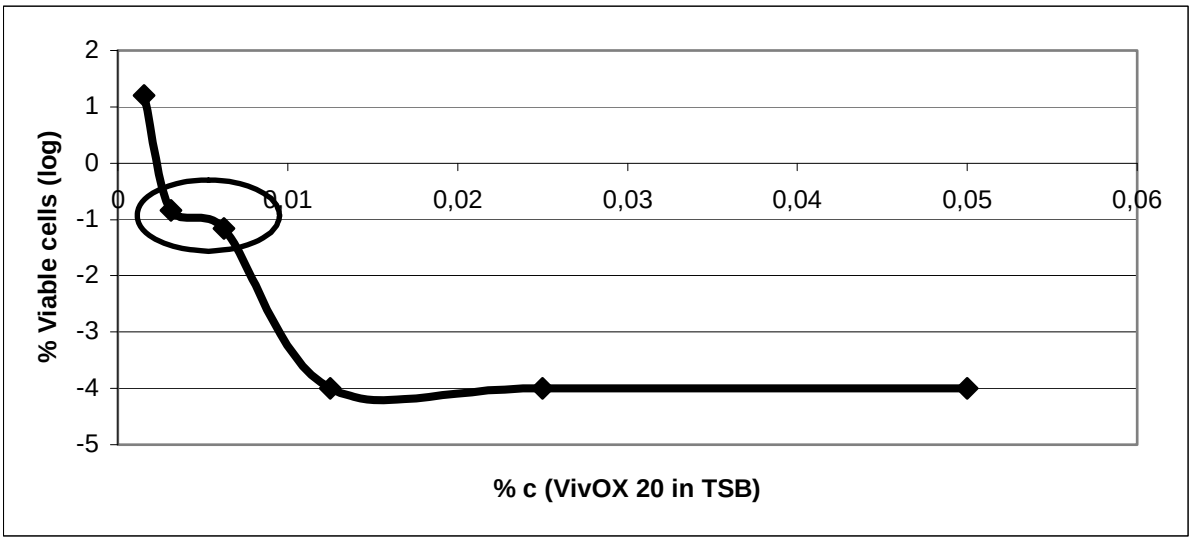

***Figure 2**: The effect of different concentrations of VivOX 20 on the percentage of viable cells of L. monocytogenes ŽM58 after 24 h incubation in TSB broth at 10 % volume of supplement*

Figure 2 shows that MBC is between 0 and 0.01 % of rosemary extract VivOX 20 in TSB broth. For accurate results, we have calculated straight line equation and MBC 0.00539 % extract in TSB broth.

In this example, the calculated MBC value for *L. monocytogenes* ŽM58 was 0.00539 % of VivOX 20 in TSB broth. On the same mode all MBC values were calculated from experimental data for extract VivOX 20 and VivOX 40 under different conditions and results are summarized in table 4.

***Table 4**: MBC values of VivOX 20 and VivOX 40 for L. monocytogenes ŽM115 and L. monocytogenes ŽM58*

Parameter	**MBC (µg/ml)**							
Type of extract	VivOX 20				VivOX 40			
Volume of supplement	10 vol. %		20 vol. %		10 vol. %		20 vol. %	
Incubation time	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
L. monocytogenes ŽM115	401.0	252.5	15.63	40.7	/	/	< 31.25	/
L. monocytogenes ŽM58	53.9	98.5	/	/	42.4	56.0	/	/

MBC: Minimal bactericidal concentration value is expressed as µg of rosemary extract per ml of TSB broth; /: not done

MBC values recorded for both rosemary extracts were often greater after 48 than 24 h incubation (Table 4). VivOX 20 extract was effective against *L. monocytogenes* ŽM115 with an MBC 401 µg/ml after 24 h incubation and 252.5 µg/ml after 48 h incubation, with 10 % of supplements. In contrast, with 20 % of supplements and the same strain of *Listeria*, MBC was 15.63 µg/ml after 24 h and 40.7 µg/ml after 48 h incubation. When 31.25 µg/ml of VivOX 40 extract was used, a 99.9 % inhibition was observed after 24 h. A higher bactericidal effect was found testing extract VivOX 20 with *L. monocytogenes* ŽM58, because 53.9 µg/ml were necessary to obtain MBC after 24 h and 98.5 µg/ml after 48 h incubation with 10 % addition of supplements. VivOX 40 showed similar MBC values against *L. monocytogenes* ŽM58 with also 10 % of supplements: 42.4 µg/ml after 24 h and 56.0 µg/ml after 48 h incubation. The strain of *L. monocytogenes* ŽM115 was slightly less sensitive. We assumed that this strain, isolated from Tatarian beefsteak was exposed to different stress conditions (change in T, pH, etc.) and this consecutively lead to increase strain′s resistance. The results also shows that bigger quantity of supplements in TSB broth decrease MBC, that is why we assumed that this lead to changes the composition of broth.

4 CONCLUSIONS

Here, we describe the antimicrobial activity of rosemary extracts against *Listeria* strains. Our results show that both extracts VivOX 20 and VivOX 40 had a good antimicrobial activity against several strains of *Listeria*. The definition of the MIC and MBC differs between publications and this is obstacle to comparison results between studies. More effective was extract VivOX 40, which contained a higher percent of carnosic acid, but the differences in MIC and MBC between the extracts were not so high, so we assumed that also other compounds in extracts had important antimicrobial activity. We confirmed that antimicrobial activity of rosemary extracts was dependent on selected rosemary extract, concentration of extracts, different species of *Listeria* and different strains of *L. monocytogenes*.

5 ACKNOWLEDGEMNT

The authors gratefully acknowledge Vitiva d.d., Slovenia for providing rosemary extracts VivOX 20 and VivOX 40.

6 REFERENCES

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in food – a review. International Journal of Food Microbiology, 94: 223-253

Carrasco E., Valero A., Perez-Rodriguez F., Garcia-Gimeno R.M., Zurera G. 2006. Management of microbiological modelling: *Listeria monocytogenes* as an example. International Journal of Food Microbiology, 114: 221-226

Cannilac N., Mourey A. 2001. Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. Food Microbiology, 18: 261-268

Del Campo J., Amiot M.J., Nguyen-The C. 2000. Antimicrobial effect of rosemary extracts. Journal of Food Protection, 10: 1359-1368

Duxbury D. 2004. Keeping tabs on *Listeria*. Food Technology, 58: 74-80

Fenlon D.R. 1999. *Listeria* in the natural environment. V: *Listeria*, listeriosis and food safety. 2nd ed. Ryser E.T., Marth E.H. (ur.). New York, Marcel Dekker, Inc.: 21-38

Fernandez-Lopez J., Zhi N., Aleson-Carbonell L., Perez-Alvarez J.A., Kuri V. 2004. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Science, 69: 371-380

Gandhi M., Chikindas M.L. 2007. Listeria: A foodborne pathogen that knows how to survive. International Journal of Food Microbiology, 113: 1-15

Lado B.H., Yousef A.E. 2007. Characteristic of *Listeria monocytogenes* important to food procesors. In: *Listeria*, listeriosis and food safety. 3rd ed. Ryser E.T., Marth E.H. (eds.). New York, CRC Press, Taylor & Francis Group, Inc.: 157-214

Lo A.H., Liang Y.C., Lin-Shiau S.Y., Ho C.T., Lin J.K. 2002. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down–regulating nuclear factor–κB in mouse macrophages. Carcinogenesis, 23: 983-991

Lopez-Malo Vigil A., Palou E., Alzamora S.M. 2005. Naturally occurring compounds-plant sources. In: Antimicrobials in food. 3rd ed. Davidson P.M., Sofos J.N., Branen A.L. (eds.). Boca Raton, Taylor & Francis Group: 429-451

Manzano M., Cocolin L., Ferroni P., Cantoni C., Comi G. 1997. A simple and fast PCR protocol to detect *Listeria nonocytogenes* from meat. Journal of the Science of Food and Agriculture, 74: 25-30

Moreno S., Scheyer T., Romano C.S., Vojnov A.A. 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research, 40: 223-231

Nychas G.J.E. 1995. Natural antimicrobials from plants. In: New methods of food preservation. Gould G.W. (ed.). London, Blackie Academic and Professional: 58-89

Ouattara B., Simard R.E., Holley R.A., Piette G.J.-P., Begin A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. International Journal of Food Microbiology, 37: 155-162

Painter J., Slutsker L. 2007. Listeriosis in humans. In: *Listeria*, listeriosis and food safety. 3rd ed. Ryser E.T., Marth E.H. (eds.). New York, CRC Press, Taylor & Francis Group, Inc.: 85-110

Rocourt J., Buchrieser C. 2007. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position, taxonomy, and identification. In: *Listeria*, listeriosis and food safety. 3rd ed. Ryser E.T., Marth E.H. (eds.). New York, CRC Press, Taylor & Francis Group, Inc.: 1-20

Shelef L.A. 1983. Antimicrobial effects of species. Journal of Food Safety, 6: 29-44

Vazquez–Boland J., Dominguez–Bernal G., Gonzalez–Zorn B., Kreft J., Goebel W. 2001. Pathogenical islands and virulence evolution in *Listeria*. Microbes and Infection, 3: 571-584

Woods G.L., Washington J.A. 1999. Antimicrobial susceptibility tests: dilution and disk diffusion methods. In: Manual of clinical microbiology. Murrey P.R., Baron E.J., Pfaler M.A., Tenover F.C., Yolken R.H. (eds.). Washington, American Society for Microbiology: 1327-1341

Zhang Y., Yeh E., Hall G., Cripe J., Bhagwat A.A., Meng J. 2007. Characterization of *Listeria monocytogenes* isolated from retail foods. International Journal of Food Microbiology, 113: 47-53