AN EFFECT OF THE ROSEMARY (*ROSMARINUS OFFICINALIS*) EXTRACT ON THE PRODUCTION OF AFLATOXIN B_1 BY *ASPERGILLUS FLAVUS*

Ljubiša Č. ŠariČ[1*], Marija M.Škrinjar[2], Marijana B. SakaČ[1], Bojana M. Beljkaš[1], Ivana S. Čabarkapa[1], Dragana V. PlavšiČ[1]

[1]Institute for Food Technology, Novi Sad, Serbia
[2]Faculty of Technology, University of Novi Sad, Serbia

UDC 633.812 .663.18

Abstract: Aflatoxins, metabolites of the fungus *Aspergillus flavus*, are potent liver toxins and carcinogens in animals, and may also be human carcinogens. There are more than 13 different subtypes of aflatoxin with B1 being the most toxic. The aim of this work was to investigate influence of the rosemary extract at three different concentrations on the production of the AFB1 by three isolates of *Aspergillus flavus*, that showed ability to produce AFB1 at high concentrations. Samples were analyzed by VICAM's AflaTest. The rosemary extract at two investigated concentrations (5.00 and 6.50 ml.l^{-1}) decresed content of AFB1 in YES broth. In the case of isolate 2, the decrease of aflatoxin production was not observed, what means that levels of AFB1 also depending on the isolate nature. The concentration of 6.50 ml.l^{-1} showed stronger effect than 5.00 ml.l^{-1} (isolate 3). The lowest concentration of rosemary extract (3.00 ml.l^{-1}) stimulated production of AFB1.

Key words: *rosemary extract, aflatoxin B1, Aspergillus flavus*

INTRODUCTION

The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial effects of natural compounds. Numerous investigations have confirmed the antimicrobial action of essential oils (EOs) in model food systems and in real food (Koutsoumanis et al., 1998; Tsigarida et al., 2000; Rasooli et al., 2008).

Since EOs are considered as generally regarded as safe (GRAS) (Kabara, 1991), the possibility of reinforcing their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be a way to attain a balance between sensory acceptability and antimicrobial efficacy. Mycotoxins are secondary metabolites produced by specific filamentous fungi that contaminate agricultural commo-

*Corresponding author:
e-mail: ljubisa.saric@fins.uns.ac.rs:
Tel: +381 21 485 3822: Fax: +381 21 450725

dities. They are toxic to humans and animals, cause significant reductions in crop yield and causeeconomic losses (Gourama & Bullerman, 1995). Aflatoxins are highly toxic secondary metabolites produced by the species of *Aspergillusi*, especially *A. flavus* and *A. parasiticus*. These fungi can grow on a wide variety of foods and feeds under favourable temperature and humidity. Aflatoxins have been found to contaminate a wide variety of important agricultural products world-wide, e.g., corn, wheat, rice, spices, dried fruits, and nuts. Contamination by aflatoxins can take place at any point along the food chain from the field, harvest, handling, shipment and storage (Giray et al., 2007).

They have been clearly identified as highly toxic, mutagenic, teratogenic and carcinogenic compounds and have been implycated as causative agents in human hepatic and extra hepatic carcinogenesis (Castells et al., 2008). Consumption of mycotoxin-contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, sometimes resulting in death (Bathnagar & Garcia, 2001).

Aflatoxin B_1 is the most powerfull hepatocancer agent known in mammals and is classified by the International Agency of Research on Cancer as Group 1 carcinogen (IARC, 1993). Aflatoxin B_1 is the substance without colour, smell or taste. It is stable and resistant to degradation during normal food cooking process. It could be completely destroyed by chromsulfat acid, Na hypochlorite, concentrate NaOH and by being exposed to solar light for an extended period of time (ŠutiČ & StojanoviČ, 1973).

Presence of aflatoxins is relatively rare in our country (MašiČ, 2000). But, we import many foodstuff, including spices, from regions with tropical and subtropical climate that are often contaminated by aflatoxins. Therefore, it is clear that all the examinations of aflatoxins and their producers have great significance, especially if we know their nature.

In addition, many natural compounds found in dietary plants, such as extracts of herbs and fruit extracts, possess antimicrobial activities against *Aspergillus species* (Soliman & Badeaa, 2002; Rasooli & Owlia, 2005). Many spices and herbs, such as cloves, anise and star anise seeds (Hitokoto et al., 1980), basil, cinnamon, marigold and spearmint (Soliman & Badeaa, 2002), garlic and onion (Benkeblia, 2004), thyme (Rasooli & Owlia, 2005), cassia and sweet basil (Atanda et al., 2007) have been reported to inhibit toxigenic and foodborne moulds.

Rosmarinus officinalis L. (family *Lamiaceae*), is also known as rosemary. This herb is an evergreen shrub, with aromatic linear leaves. The evergreen shrub originated in the Mediterranean area, but it is today cultivated almost everywhere in the world, primarily for its aromatic leaves. Rosemary is often used widely as a common household spice, and also as a fragrant aromatic flavoring agent in several commercially available products, such as vegetables, prepared meats, baked goods, etc.

Rosemary (*Rosmarinus officinalis* L.) extracts have a potent antioxidant activity and are widely used in the food industry. The antioxidant activity of rosemary extracts has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, which break free radical chain reactions by hydrogen donation (Aruoma et al., 1992; Basaga et al., 1997; Georgantelis et al., 2007). In addition to inhibition of lipid oxidation, several authors have reported that some of the compounds present in rosemary extracts possess antibacterial properties (Del Campo et al., 2000; Djenane et al., 2002).

The aim of this study was to investigate influence of rosemary extracts in different concentrations on the production of the aflatoxin B_1 by *Aspergillus flavus.*

MATERIALS AND METHODS

Mould

Three isolates of *Aspergillus flavus* that showed ability to produce aflatoxin B_1 (220, 18 and 320 $\mu g.kg^{-1}$) were used. Aflatoxigenic nature of these isolates was confirmed by thin layer chromatography (AOAC, 1995) and VICAM's AflaTest during the earlier examinations. Isolates were maintained on Sabouraud Maltose Agar

(Merck, Germany) at 4 °C. YES broth served as aflatoxin production medium.

Rosemary extract

Rosemary extract prepared in 70 % ethanole (1:2) was obtained from the Institute of Medicinal Plant Research „Dr Josif PanČČ" from Belgrade. Working concentrations of rosemary extract were 3.00, 5.00 and 6.50 ml per one litre of YES medium. The influence of all of three concentrations of rosemary extract on three isolates of *Aspergillus flavus* was examinated. Three controls without rosemary extract were also prepared.

Measurement of aflatoxin B_1

Erlenmeyer flasks containing 100 ml of YES broth and *R. officinalis* extract at appropriate concentrations were inoculated with 5 ml of fungal spores suspension (10^8 spores/ml). Spore population was counted using a standard Koch method. The flasks were then incubated at 25 ± 2 °C for 12 days in an incubator shaker. Aflatoxin B_1 measurement was determined at the end of the incubation period by VICAM's AflaTest.

AflaTest from VICAM is the trusted aflatoxin test that produces precise numerical results. Using monoclonal affinity chromategraphy, AflaTest can isolate aflatoxins B1, B2, G1, and G2 from feeds, foods and grains at levels as low as 0.1 ppb. Results may be recorded using a digital fluorometer readout with automatic printing devices. AOAC and FGIS established reliability and assurance of the Afla test.

RESULTS AND DISCUSSION

As can be seen from Table 1, addition of rosemary extract at the concentration of 6.50 $ml.l^{-1}$ decreased the production of AFB_1 by isolate 1 for 31.82% and by isolate 3 for 79.04%, in comparison with the control. In the case of isolate 2, the decrease of aflatoxin production was not observed.

Table 1.
The production of the aflatoxin B_1 (AFB_1) by *Aspergillus flavus* in YES broth with rosemary extract at the concentration of 6.50 $ml.l^{-1}$

Sample	Concentration of rosemary extract ($ml.l^{-1}$)	Concentration of AFB_1 ($\mu g.kg^{-1}$)	
		YES	YES + rosemary extract
1	6.50	220.00	150.00
2		18.00	19.00
3		320.00	67.00

Table 2.
The production of the aflatoxin B_1 (AFB_1) by *Aspergillus flavus* in YES broth with rosemary extract at the concentration of 5.00 $ml.l^{-1}$

Sample	Concentration of rosemary extract ($ml.l^{-1}$)	Concentration of AFB_1 ($\mu g.kg^{-1}$)	
		YES	YES + rosemary extract
1	5.00	220.00	150.00
2		18.00	17.00
3		320.00	300.00

Results in tab. 2. show that the addition of rosemary extracts at the concentration of 5.00 $ml.l^{-1}$ decrease production of AFB_1 by isolate 1 for 31.82% and by isolate 3 for 7.25%, in comparison with the control. In the case of isolate 2, significant decrease of aflatoxin production was not observed.

Table 3.
The production of the aflatoxin B_1 (AFB_1) by *Aspergillus flavus* in YES broth with rosemary extract at the concentration of 3.00 $ml.l^{-1}$

Sample	Concentration of rosemary extract ($ml.l^{-1}$)	Concentration of AFB_1 ($\mu g.kg^{-1}$)	
		YES	YES + rosemary extract
1	3.00	220.00	1100.00
2		18.00	60.00
3		320.00	2100.00

Results presented in Tab. 3. show that rosemary extract at the concentration of 3.00 $ml.l^{-1}$ possed stimulative effect on the production of AFB_1 in all three cases, especially for isolates 3 and 1. The production of AFB_1 by isolate 3 was increased 6.56 times in comparison with the control. In the case of isolate 1 the increase was 5 times.

Investigation performed by Rasooli et al. (2008) showed that aflatoxin biosynthesis was suppressed by *R. officinalis* essential oils. The inhibitory effect of the oils was proportional to their concentrations. The concentration of 450 ppm of *R. officinalis* essential oil showed the total inhibition of aflatoxin production (100%), whereas the lower concentration of 250 ppm decreased aflatoxin production only for 1.87%.

The antifungal and aflatoxin inhibition efficacy of *R. officinalis* essential oils may be attributable to the oil compositions (Cardenas-Ortega et al., 2005; Pinto et al., 2006). Antibacterial and antifungal activities of some substances have been reported (Cardenas-Ortega et al., 2005; Pinto et al., 2006).

It has been shown that the activity of rosemary could be attributed to borneol and other phenolics in the terpene fraction (Davidson & Naidu, 2000). The volatile terpenes carvacrol, and *p*-cymene have been suspected for being responsible for the antimicrobial activity of some essential oils. Also, a group of terpenes (borneol, camphore, 1,8 cineole, Č-pinene, camphone, verbenonone and bornyl acetate) was reported to possess an antimicrobial activity in rosemary (Davidson & Naidu, 2000).

Antimicrobial activities are mostly attributable to the presence of phenolic compounds such as thymol, and to hydrocarbons like Č-terpinene and *p*-cymene with limonene being more active than *p*-cymene (Vardar-Unlu et al., 2003).

CONCLUSION

Regarding the obtained results of preliminary examinations of the antimycotoxic activities of rosemary extracts, further investigations should be undertaken in order to established a relationship between antimycotoxigenic activity and chemical composition of rosemary extracts. In consideration of different seansitivity of three examined isolates of *A. flavus* on activitiy of rosemary extract on AFB1 synthesys, their genetic diversity should be investigated.

ACKNOWLEDGMENTS

This work is a part of the Project (TR – 20068) supported by the Ministry of Science and Technological Development, Republic of Serbia.

REFERENCES

1. Aruoma, O.I., Halliwell, B., Aeschbach, R., Löliger, J. (1992). Antioxidant and prooxidant properties of active rosemary constituents: carnosol and carnosic acid, *Xenobiotica*, *22*, (2), 257–268.
2. Atanda, O.O., Akpan, I., Oluwafemi, F. (2007). The potential of some spice essential oils in the control of *A. parasiticus* CFR 223 and aflatoxin production. *Food Control*, *18*, 601–607.
3. Basaga, H., Tekkaya, C., Acitel, F. (1997). Antioxidative and free radical scavenging properties of rosemary extract. *Lebensmittel – Wissenschaft und Technologie*, *30*, (1), 105–108.
4. Bathnagar D. & Garcia, S. (2001). *Aspergillus*. In: Labbe RG, Garcia, S. (eds), *Guide to foodborne pathogens*, pp 35–49, New York: John Wiley and Sons.
5. Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensmittel-Wissenschaft und Technologie*, *37*, (2), 263–268.
6. Cardenas-Ortega, N.C., Zavala-Sanchez, M.A., Aguirre-Rivera, J.R., Perez-Gonzalez, C., Perez-Gutierrez, S. (2005). Chemical composition and antifungal activity of essential oil of *Chrysactinia mexicana* gray. *Journal of Agricultural and Food Chemistry, 53*, 4347–4349.
7. Castells., M., Marin, S., Scanchis, V., Ramos, A. J. (2008). Distribution of fumonisins and aflatoxins in corn fractions during industrial cornflake processing. *International Journal of Food Microbiology*, *123*, 81-87.
8. Davidson, P.M. & Naidu, A.S. (2000). *Phytophenols*. In: A.S. Naidu, Editor, *Natural Food Antimicrobial Systems*, pp. 265–294. CRC Press, Boca Raton, FL.
9. Del Campo, J., Amiot , M.J., Nguyen, C. (2000). Antimicrobial effect of rosemary extracts. *Journal of Food Protection*, 63**,** (10), 1359–1368.
10. Djenane, D., Sánchez-Escalante, A., Beltrán, J.A., Roncalés, P. (2002). Ability of Č-tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. *Food Chemistry*, *76*, (4), 407–415.
11. Georgantelis, D., Ambrosiadis, I., Katikou, P., Blekas G., Georgakis, S. A. (2007). Effect of rosemary extract, chitosan and Č-tocopherol on microbiological parameters

and lipid oxidation of fresh pork sausages stored at 4 °C. *Meat Science*, *76*, (1), 172-181.

12. Giray, B., Girgin, G., Engin, A.B., Aydin S., Sahin, G. (2007). Aflatoxin levels in wheat samples consumed in some regions of Turkey. *Food Control*, *18*, 23–29.
13. Gourama, H. & Bullerman., L.B. (1995). Aspergillus flavus: Aflatoxigenic Fungi of Concern in Foods and Feed. F. *Food Protection*, *58*, 1395-1404.
14. Hitokoto, H., Morozumi, S., Wauke, T., Sakai, S., Kurata, H. (1980). Inhibitory effects of spices on growth and toxin production of toxigenic fungi. *Applied and Environmental Microbiology*, *39*, (4), 818–822
15. International Agency for Research on Cancer (1993). Some Naturally Occuring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. *Monographs on the Evaluation of Carcinogenic Risks to Humans*, *56*, 489.
16. Kabara, J.J. (1991). Phenols and chelators. *Food Preservatives*. 200–214.
17. Koutsoumanis, K., Tassou, C.C., Taoukis, P.S., Nychas, G.J.E. (1998). Modelling the effecttiveness of a natural antimicrobial on Salmonella enteritidis as a function of concentration, temperature and pH, using conductance measurements. *Journal of Applied Microbiology*, *84*, 981–987.
18. MašiČ, M. (2000). Mikotoksini u stoČnoj hrani kao faktor poremeČaja zdravlja životinja. *Zbornik radova i kratkih sadržaja*, *64*-74, 12. savetovanje veterinara Srbije, VrnjaČka Banja.
19. Pinto, E., Pina-Vaz, C., Salgueiro, L., Goncalves, M.J., Costa-de-Oliverira, S., Cavaleiro, C., Palmeria, A., Rodrigues, A., Martinez-de-Oliveria, J. (2006). Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. *Journal of Medical Microbiology*, *55,* (10), 1367–1373.
20. Rasooli, I., Fakoor, M. H., Yadegarinia, D., Gachkar, L., Allameh, A., Rezaei, M. B. (2008). Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. *International Journal of Food Microbiology*, *122*, 1-2, 135-139.
21. Rasooli, I. & Owlia, P. (2005). Chemoprevention by thyme oils of *Aspergillus parasiticus* growth and aflatoxin production. *Phytochemistry*, *66***,** 2851–2856.
22. Soliman, K.M. & Badeaa, R.I. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology*, 40, 1669–1675.
23. Tsigarida, E., Skandamis, P., Nychas, G.J.E. (2000). Behaviour of *L. monocytogenes* and autochthonous fora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C, *Journal of Applied Microbiology, 89*, 901–909.
24. Vardar-Unlu, G., Candan, F., Sokmen, A., Daferera, D., Polissiou, M., Sokmen, M., Donmez, E., Tepe, B. (2003). Antimicrobial and antitoxic activity of the essential oil and methanol extracts of *Thymus pectinatus Fisch. et Mey. var. pectinatus* (Lamiaceae). *Journal of Agriculture and Food Chemistry*, *51*, 63–67.
25. ŠutiČ, M. & StojanoviČ, M. (1973). Aflatoksini u životnim namirnicama. *Hrana i ishrana*, *1-2*, 84-92.