Arch. Biol. Sci., Belgrade, 66 (4), 1367-1370, 2014 DOI:10.2298/ABS1404367G

ANTIMICROBIAL EFFECT OF DIETARY OREGANO ESSENTIAL OIL AGAINST *VIBRIO* BACTERIA IN SHRIMPS

M. H. GRACIA-VALENZUELA[1*], M.J. VERGARA-JIMÉNEZ[2], M.E. BAEZ-FLORES[3] and F. CABRERA-CHAVEZ[2].

[1] *Instituto Tecnológico del Valle del Yaqui, Block 611, Municipio Bácum,* CP. 82276, Valle del Yaqui, Sonora, México
[2] *Unidad Académica de Ciencias de la Nutrición y Gastronomía, Universidad Autónoma de Sinaloa,* Culiacán, CP. 80019, México
[3] *Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa,* Culiacán, CP. 80019, México

*Corresponding author: hgracia@itvalledelyaqui.edu.mx

Abstract - The effect of dietary oregano essential oils on the growth of *Vibrio* bacteria in shrimps was evaluated. Shrimps were fed: (i) food with oregano oil with a high level of thymol; (ii) food with oregano oil with a high level of carvacrol, and (iii) food without oregano oil (the control). The animals were infected by three species of *Vibrio* (*vulnificus, parahaemolyticus* and *cholerae*). The microbial counts of *Vibrio* species were significantly lower ($p < 0.05$) in tissues from animals whose food was supplemented with oregano oil. We concluded that dietary supplementation of shrimps with oregano oil provides antimicrobial activity into the body of the penaeids.

Key words: Antimicrobial effect; *Vibrio* bacteria; shrimp culture; oregano essential oil.

INTRODUCTION

Although some chemicals are able to reduce the microbial count in foods, consumers desire more natural products. Several authors have indicated that some products containing essential oils from spices and herbs are an alternative to control the microbial growth in foods for human consumption (Govaris et al., 2010; Hulankova et al., 2013). However, their direct application on food products could affect the acceptability of the final product due to changes in the sensorial profile (e.g. strong flavor of essential oils) (Olmedo et al., 2013). Thus, dietary supplementation could be an approach to control food safety.

The essential oil of Mexican oregano (Lippia berlandieri Schauer) is a promising dietary supplement since it exhibits substantial in vitro antimicrobial activity (Skandamis and Nychas, 2001). Some studies have shown that the incorporation of oregano essential oil in the diet of farm animals exerts an inhibitory effect on microbial growth in meat products (Soultos et al., 2009). Nevertheless, there are no studies addressing the effectiveness of this kind of supplementation in shrimp farming. The aim of this study was to investigate the effect of dietary essential oils from oregano (Lippia berlandieri Schauer) on the microbial growth of Vibrio bacteria in the hepatopancreas and muscle of shrimps during refrigerated storage.

MATERIALS AND METHODS

Two types of oregano essential oils (OEOs) were extracted from the leaves and stems of oregano by hydro-distillation as described by Paredes-Aguilar et al. (2007). The two types of oils contained mainly thymol and carvacrol, one of them rich in thymol and the other one with high content of carvacrol (48% thymol/23% carvacrol and 25% thymol/40% carvacrol, respectively). Then, commercial food for shrimp (Purina 35™, México) was mixed with the OEOs by sprinkling 5μL of diluted essential oil (1:1 v/v, in soy lecithin) per gram of shrimp food.

Two hundred and seventy juvenile (15-18 g) shrimps (*Litopenaeus vannamei*) were divided by restricted randomization into three equal groups with three subgroups (ensuring the groups were balanced for body weight) and then each group was placed into an aquarium (50 l) for the treatments under controlled temperature (23-25 °C), pH (7.5-8.0), salinity (34-36 UPS) and dissolved oxygen concentration (4.5-5.5 mg/L). The shrimps groups were feed *ad libitum* (three treatments) food with oregano oil with a high level of thymol; food with oregano oil with a high level of carvacrol and food without essential oil (the control). At the same time, the shrimp infection (in subgroups of shrimps) was carried out by the inoculation of culture water with three species of *Vibrio* bacteria (*vulnificus, parahaemolyticus* and *cholerae;* 10^6 CFU/mL). The conventional identification system API 20E: BioMércurx (Basingstoke, UK) was used to identify the bacteria species (biochemical identification). The samples of hepatopancreas and muscle were taken at the time of sacrifice, after 4 days of refrigerated storing as well as at the beginning of the experiment.

Fifty mg of tissue (hepatopancreas or muscle) were manually separated, homogenized and diluted with 45 ml of sterile 0.1% peptone water. Decimal dilutions were prepared and plated in triplicate on agar TCBS specific for the *Vibrio* genus. Plates were incubated anaerobically at 32°C for 24 h. After incubation, microorganisms were enumerated; the average result of triplicated colony counts were presented as $\log_{10}$ CFU/g of tissue and their means were compared by Tukey test (Statistical software NCSS, 2001).

RESULTS AND DISCUSSION

During the experiment, no deaths were recorded for the groups of shrimps supplemented with oregano essential oils. However, after 3 days of treatment, 6, 10 and 16 shrimps died in the control subgroups inoculated with *V. vulnificus*, *V. cholerae* and *V. parahaemolyticus* bacteria, respectively. According to Panicker et al. (2004), these *Vibrio* species are most often associated with diseases in marine animals used for human consumption. However, the pathogenicity varies even with the strain. Thus, our results are in agreement with the previous report by Manilal et al. (2010), who reported that *V. parahaemolyticus* is highly pathogenic to shrimps than other *Vibrio* bacteria (e.g. *V. vulnificus*).

The effect of OEO dietary supplementation on microbial colonization of the hepatopancreas and muscle of shrimps is shown in Table 1. All microbial counts of *Vibrio* species on shrimp tissues of the control groups significantly increased ($p < 0.05$) during the treatment in aquarium (data not shown) as well as during refrigerated storage. Particularly, *V. parahaemolyticus* in the muscle of shrimps in the control group increased from 3.68±0.09 log CFU/g at the time of sacrifice to 9.81±0.21 log CFU/g at the fourth day of refrigerated storing. On the other hand, the dietary supplementation of shrimps with oregano essential oil resulted in significantly lower bacterial counts on the tissues (independently of whether they were from the hepatopancreas or muscle). Thus, although there were no differences due to the type of supplemented essential oil, the growth of *Vibrio* species in shrimp tissues was inhibited when the animals consumed OEOs.

The main antimicrobial mechanism of essential oils from herbs and spices is via structural and functional damage to the membranes of microorganisms by phenolic compounds (Govaris et al., 2010; Edris, 2007). There are some variations on their activity depending on Gram staining response. In fact, in

Table 1. Effect of oregano essential oil dietary supplementation on bacterial colonization of hepatopancreas and muscle of shrimps (log cfu/g of tissue).

Vibrio Genus	Ratio thymol (%): carvacrol (%) in oregano essential oil.			
	48:23	25:40	Control*	
	Bacterial count in hepatopancreas (log cfu/g of tissue ± sd)			At the time of sacrifice
V. vulnificus	2.81 ± 0.16a	2.83 ± 0.27a	4.23 ± 0.14b	
V. cholerae	2.82 ± 0.42a	2.81 ± 0.24a	3.45 ± 0.08b	
V. parahaemolyticus	2.45 ± 0.31a	2.44 ±0.52a	3.95 ± 0.71b	
	Bacterial count in muscle (log cfu/g of tissues)			
V. vulnificus	2.20 ± 0.15a	2.20 ± 0.31a	3.96 ± 0.43b	
V. cholerae	2.12 ± 0.15a	2.14 ± 0.26a	3.47 ± 0.51b	
V. parahaemolyticus	2.31 ± 0.09a	2.24 ± 0.25a	3.68 ± 0.09b	
	Bacterial count in hepatopancreas (log cfu/g of tissue± sd)			4 days after sacrifice and storage.
V. vulnificus	3.20± 0.11a	3.23 ± 0.25a	9.15 ± 0.21b	
V. cholerae	4.17 ± 0.22a	4.11 ± 0.15a	8.91 ± 0.19b	
V. parahaemolyticus	3.96 ± 0.16a	3.89 ± 0.08a	9.65 ± 0.34b	
	Bacterial count in muscle (log cfu/g of tissue ± sd)			
V. vulnificus	3.72 ± 0.17a	3.78 ± 0.19a	8.60 ± 0.42b	
V. cholerae	3.95 ± 0.37a	3.93 ± 0.40a	8.21 ± 0.18b	
V. parahaemolyticus	3.64 ± 0.24a	3.61 ± 0.17a	9.81 ± 0.21b	

*Control without oregano essential oil (food as commercially available)

a, b, different letter at the same row means statistically different ($p<0.05$) results.

previous studies (Gracia-Valenzuela et al., 2012) we reported that the cytoplasmic membrane could be avoided, depending on the lipopolysaccharide characteristics of the cell wall of Gram-negative bacteria. Although the antibacterial activity of OEOs is more pronounced against Gram-positive than Gram-negative bacteria (Marino et al., 2001), in this study we showed an antimicrobial effect on Gram-negative bacteria as well after nutrient supplementation. Since hydrophobic molecules can gain access to the periplasm of Gram-negative bacteria through the porin proteins of the outer membrane (Helander et al., 1998), carvacrol and thymol (the main antimicrobial phenolic compounds in OEOs) also have antimicrobial activity against the microorganisms in this study. In conclusion, this study shows that supplementation of shrimp diet with OEO can provide shrimps with antimicrobial activity.

REFERENCES

Edris, A.E. (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. *Phytother. Res.* **21**, 308-23.

Govaris, A., Solomakos, N., Pexara, A. and *P.S. Chatzopoulou* (2010). The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella enteritidis* in minced sheep meat during refrigerated storage. *Int. J. Food Microbiol.* **137**, 175-80.

Gracia-Valenzuela, M.H., Orozco-Medina, C. and *C. Molina-Maldonado* (2012). Antibacterial effect of essential oregano oil (*Lippia berlandieri*) on pathogenic bacteria of shrimp *Litopenaeus vannamei*. *Hidrobiológica*. **22**, 201-6.

Helander, I.M., Alakomi, H.L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E.J., Gorris, L.G.M. and *A. Von Wright* (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. *J. Agr. Food Chem.* **46,** 3590-5.

Hulankova, R., Borilova, G. and *I. Steinhauserova* (2013). Combined antimicrobial effect of oregano essential oil and caprylic acid in minced beef. *Meat Sci.* **95**, 190-4.

Manilal, A., Sujith, S., Selvin, J., Shakir, C., Gandhimathi, R. and *R.S. Kiran* (2010). Virulence of *Vibrios* Isolated from Diseased Black Tiger Shrimp, *Penaeus monodon*, Fabricius. *J. World Aquacul. Soc.* **41**, 332-43.

Marino, M., Bersani, C. and *G. Comi* (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *Int. J. Food Microbiol.* **67,** 187-95.

Olmedo, R.H., Nepote, V. and *N.R. Grosso* (2013). Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils. *LWT- Food Sci. Technol.* **53**, 409-417.

Panicker, G., Call, D.R., Krug, M.J. and *A.K. Bej* (2004). Detection of pathogenic *Vibrio spp.* in shellfish by using multiplex PCR and DNA microarrays. *Appl. Environ. Microbiol.* **70**, 7436-44.

Paredes-Aguilar, M.C., Gastelum-Franco, M.G., Silva-Váquez, R. and *G.V. Nevarez-Moorillon* (2007). Antimicrobial effect of Mexican oregano (*Lippia Berlandieri Schauer*) and its essential oil against five vibrio species. *Rev. Fit. Mex.* **30**, 261-7.

Skandamis, P.N. and *G.J. Nychas* (2001). Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. *J. Appl. Microbiol.* **91**, 1011-22.

Soultos, N., Tzikas, Z., Christaki, E., Papageorgiou, K. and *V. Steris* (2009). The effect of dietary oregano essential oil on microbial growth of rabbit carcasses during refrigerated storage. *Meat Sci*. **81**, 474-8.