THE ANTIMICROBIAL EFFECT OF ESSENTIALS OILS AGAINST SALMONELLA SPP. IN PORK BURGERS DURING REFRIGERATED STORAGE

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Abstract— **The aim of the present work was to study the anti-Salmonella activity of Oregano (*O*) and Rosemary (*R*) essentials oils (*EOs*) on inoculated pork burgers during refrigerated storage. For that 3 batches of pork burgers (minced to 5 mm and 2% salt) were prepared: the control group *C*, $Level_1$ (0.05% *EOs* of *O* or *R*), and $Level_2$ (0.4% *EOs*). The burgers were packed with modified atmosphere (70%O_2: 20%CO_2: 10% N_2) and stored for a maximum of 9 days at 4ºC in natural lighting conditions. The total Salmonella counts was determined using Brilliant green agar medium (BGA), 37ºC, 48h (ISO 6579, 2002). A validated PCR identification protocol from the EU ''Food PCR'' for Salmonella, was used both for the confirmation of presumptive colonies and for determining presence or absence of the pathogen. In general, all patties inoculated and stored in modified atmosphere, the results showed that *Salmonella* spp. survived after 9 days of storage. However, in treated patties with 0.4% of *EOs* of *O* or *R* the growth of *Salmonella* was significantly lower (*P* <0.05%) than control meat from day 3 of storage. This effect was dose dependent, ie the higher level of essential oils showed a greater effect (*P* <0.05) anti-pathogen. Among the examinated treatments, *R EO* at 0.4% proved the most efficient treatments to control Salmonella in pork burgers, since it showed a bacteriostatic effect against the pathogen. The results pointed to the possibility of using essentials oil of rosemary and oregano at 0.4% to preserve raw meat products.**

Keywords— **Rosemary, Oregano, *Salmonella*.**

I. INTRODUCTION

Salmonellas are one of the major sources of foodborne infections in humans. The consumption of undercooked ground meat has been recognized as a major risk factor for infection by foodborne pathogens of Salmonella [1]. Taking into accounts that *Salmonella* resistant to antimicrobial drugs appears to pose a particular health risk, there has been an increasing interest worldwide on therapeutic values of natural products. Nature has presented to humanity the gift of vast therapeutic antimicrobial agents of plant origins.

Certain essential oils (*EOs*) stand out as better antibacterial agents than the commonly used preservatives for meat applications [2], there has been a considerable interest in essential oils from common culinary herbs, spices and aromatic plants characterized by a notable antimicrobial activity. Such substances could be used to delay or inhibit the growth of pathogenic and/or toxin producing microorganisms in foods. In addition, the use of essential oils in consumer goods is expected to increase in the future due to the rise of 'green consumerism', which stimulates the use and development of products derived from plants.

Hence, to reduce health hazards and economic losses due to foodborne microorganisms, the use of essential oils as natural antibacterial compounds [3] seems to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of processed food. These compounds could be added during the food process. And this is the purpose of this study.

II. MATERIAL AND METHODS

3 batches of pork burgers (minced to 5 mm and 2% salt) were prepared: the control group *C*, Level 1: R_1, O_1 (0.05% *EOs*), and Level 2: R_2, O_2 (0. 4% *EOs*). The patties were packed with modified atmosphere

($70\%O_2$: $20\%CO_2$: 10% N_2) and stored for a maximum of 9 days at 4ºC. The meat and fat were picked (grain 5 mm) with a blender and mixed for 5 min in an air mixer RM-60 (Mainca Granollers, Spain). The temperature of the meat during the mixing did not exceed 12 ºC. The average size of the burgers was 10 cm in diameter and 1.5 cm thick. Subsequently, the burgers were packed in modified atmosphere (*MA*P) (70% O_2-20% CO_2-10% N_2) and stored for up to 9 days at 4 °C in a glass case display illuminated by white fluorescent light simulating the conditions of marketing.

Oregano (*Oringanum vulgare* L.; ref. F70900L) essential oil was obtained by steam extraction from flowers; its density at 20°C is 0.938 g/ml. The essential oil of rosemary (*Rosmarinus officinalis* L.; ref. F71371R) was obtained by steam distillation of the entire plant; its density at 20°C is 0.909 g/ml. Both essential oils were purchased from Ravetllat Aromatics (Barcelona, Spain).

The bacterial cells of *Salmonella* spp. were pelleted by centrifugation at 5000 g for 15 min at 5 °C, washed twice in 10 ml of 0.1 M phosphate buffered saline (PBS), pH 7.0, and diluted to 1.0×10^8 cfu/ml in PBS for the inoculation of burgers samples. Cell counts were determined by serial dilution and subsequent enumeration on tryptone soy agar (Oxoid).

Contamination with Salmonella spp. was performed at a level close to 100 ufc/g/cm^2. Analyses were performed on days 0, 3, 6 and 9 of storage. 3 hamburgers were analyzed for each day of control. The samples used in the microbiological analysis were homogenized with a Masticator (IUL Instruments GmbH, Königswinter, Germany) and diluted in peptone water (Merck. 64271, Darmstadt, Germany). The total Salmonella counts was determined using Brilliant green agar medium (BGA), 37ºC, 48h (ISO 6579, 2002). The plates were incubated in a culture oven ST 6120 (Heraeus SA, Boadilla, Madrid, Spain).

Specific PCR-based Salmonella confirmation was performed, as proposed by Malorny et al. [4]. Colonies of *Salmonella* spp. were picked from BGA (Brilliant Green Agar) plates and suspended in 30 µl of sterile distilled water. The conditions used for PCR are shown in Table nº 1.

Table 1 PCR conditions.

	Sequences	***PCR conditions***
Salmonella	139 (5’-GTGAAATTATCGCCACGTTCGGGCAA-3’) 141 (5’-TCATCGCACCGTCAAAGGAACC -3’)	94°C 1min, 30 cycles 94 C 30s, 64 C 30s, 72 C 1min, final extension 5min at 72 C.

III. RESULTS AND DISCUSSION

The antimicrobial effects of oregano and rosemary *EOs* at 0.05 and 0.4%, on *Salmonella* spp. in pork burgers during storage at 4 °C, are shown in Table 2. The initial populations of *Salmonella* spp. (2.55 log cfu/g) in control samples (*C*) were increased ($P<0.05$) and reached a value of 2.70 log cfu/g by the end of storage. In contrast, the populations of *Salmonella* spp. in *EO*s samples at the two levels (R_1, O_1 and R_2, O_2) were decreased during the storage.

Table 2 Average total viable count of *Salmonella* spp. in burgers made with two levels of rosemary and oregano essential oils (Level 1: R_1, O_1= 0.05% *EOs*), and (Level 2: R_2, O_2= 0.4% *EOs*) at 0, 3, 6 and 9 days under retail conditions.

		Day 0	***Day 3***	***Day 6***	***Day 9***
	C	2,57 ± 0,05	2,48 ± 0,21^a	2,57 ± 0,14^a	2,60 ± 0,21^a
R	$\boldsymbol{R_1}$	2,55 ± 0,09	2,51 ± 0,11^a	2,40 ± 0,15^a	2,42 ± 0,05^a
	$\boldsymbol{R_2}$	2,51 ± 0,15	1,97 ± 0,13^b	1,46 ± 0,11^b	1,29 ± 0,17^b
	C	2,57 ± 0,05	2,48 ± 0,21^a	2,57 ± 0,14^a	2,60 ± 0,21^a
O	$\boldsymbol{O_1}$	2,50 ± 0,09	2,51 ± 0,24^a	2,44 ± 0,05^a	2,40 ± 0,12^a
	$\boldsymbol{O_2}$	2,55 ± 0,15	2,37 ± 0,15^b	1,98 ± 0,01^b	1,79 ± 0,19^b

M ± SD: mean ± standard deviation. *C*: control. a, b, c: different letters within a same columns (different *EOs* treatment) differ significantly (P < 0.05).

The addition of rosemary and oregano essential oils at 0.05% in minced pork meat did not show any antibacterial activity against Salmonella. Treatment of pork burger meat with oregano *EO* at 0.4% showed lower antimicrobial (P<0.05) activity against *Salmonella* than those of the treatments rosemary *EO* at 0.4% from day 3 of storage at 4°. Among the examined treatments, the addition of rosemary *EO* at 0.4% proved the most efficient treatments to control *Salmonella* in minced pork meat. The level 0.4% of the essential oils studied showed a bactericidal effect against the pathogen.

In the same line that our results, the antibacterial activity of oregano *EO* against *Salmonella* was previously found in vitro experiments or in other food tests. In vitro experiments, the inhibitory activity of the oregano *EO* against *Salmonella* was proved by using the disc diffusion method [5] or on agar plates [6] at various concentrations (0.2% to 4%). Peñalver et al. [7] used the broth microdilution method and estimated that the MIC (minimum inhibitory activity) of oregano *EO* against *S. Enteritidis* was 0.25% (v/v).

And the study of Koutsoumanis et al. [8] whose showed that the addition of oregano essential oil to taramasalad resulted in a decline in the number of *S. enteritidis* cells immediately after inoculation with increased death rate. According to our results, a reduction of *Salmonella* was observed and its death rate depended on the pH, the storage temperature and the essential oil concentration.

The antimicrobial effect of oregano and rosemary essential oil shown in this study is due to the compounds presents in the oil. Between the 32 components in the essential oil of oregano, representing 89.5% of the total, the major components of oregano essential oil are carvacrol (61.21%), p-cymene (15.12%) and γ-terpinene (4.80%), terpinolene (3.63%), β-caryophillene (2.62%) and α-terpinene (2.34%). Between the 39 components that were identified in the essential oil of rosemary, representing 89.5% of the total, the major constituents being, α-pinene (36.42%) camphor (15.05%), 1,8-cineole (12.02%) and camphene (11.08%), borneol (4%), β-pinene (3.67%), p-cymene (2.14%)and γ-terpinene (0.18%) [9].

Furthermore, antimicrobial activities of the *EOs* are difficult to correlate to a specific compound due to their complexity and variability. Nevertheless, some researchers reported that there is a relationship between the chemical composition of the most abundant components in the *EOs* and the antimicrobial activity [10-11]. For example, camphor (herein, abundant in rosemary *EO*) is well-known chemicals having antimicrobial potentials [12]. On the other hand, based on a report, α/β-pinene (monoterpene hydrocarbons abundant in *R* or *O EO*) and borneol (one of the major oxygenated monoterpene in Rosemary *EO*) had slight activity against a panel of microorganisms. As a result of these findings, the higher antimicrobial activities of *Rosmarinus officinalis EO* could be attributed to its particular chemotype characterised by its complexity with oxygenated-hydrocarbons as dominant components and the presence of equivalent amounts of monoterpene hydrocarbons and sesquiterpene hydrocarbons (with borneol as major component).

Moreover, many reports mentioned that carvacrol and their precursors (p-cymene and γ-terpinene), are biologically and functionally closely associated [13]. In that context, compared to the *EO* of *R*, p-cymene (2.14%) was more abundant in the *EO* of *O* (15.12%). Meanwhile, the latter *EO* contains a moderately higher level of γ-terpinene (4.80% and 0.18%, respectively).

Regarding the PCR analysis, all the colonies of Salmonella spp. isolates were confirmed by the Salmonella-specific PCR-based methodology. The primer set used for specific amplification of Salmonella genomic DNA fragments has been previously published [14]. For positive samples, an amplified fragment of 284 bp of *invA* gene was clearly visualized in a horizontal agarose gel electrophoresis stained with ethidium bromide (Figure 1).

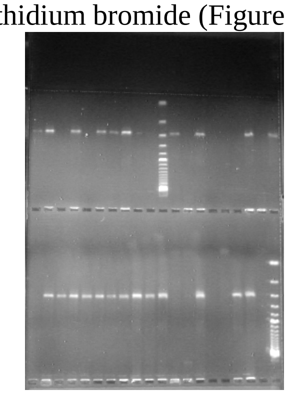

Figure 1. Confirmation of the identity of Salmonella

IV. CONCLUSIONS

This study showed that essential oil of common food spices, particularly rosemary, and oregano at 0.4% are capable inhibiting pathogenetic microorganism in pork burgers. In contrast, the addition of both essential oils at 0.05% did not show any antibacterial activity against *Salmonella*. Of the various essential levels, the 0.4% of rosemary was of particular promise as it exhibited strong effects against *Salmonella*. These herbs are widely used in the food industry and are generally regarded as safe (*GRAS*). Hence, they may be considered as natural and niche preservatives acceptable by the meat science industry.

ACKNOWLEDGMENT

The authors are grateful to Fundación Séneca (Agencia de Ciencia y Tecnología de la Region de Murcia) for the postdoctoral fellowship of Gema Nieto (12494/PD/09).

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