Animal Health Research Institute,
Assiut Regional Laboratory

INHIBITORY EFFECT OF SOME SPICE EXTRACTS ON LISTERIA MONOCYTOGENES IN MINCED MEAT

(With 2 Tables and 3 Figures)

By

A.M. ABD EL-MALEK; H.H. ESSA; N.H. MAKAR and R.S. REFAIE

(Received at 28/5/2006)

التأثير المثبط لمستخلصات بعض التوابل على ميكروب الليستيريا مونوسيتوجينيس فى اللحم المفروم

أشرف محمد عبد المالك ، حمدى حسين عيسى ، نبيل حبيب مقار ، رمضان سيد رفاعى

ميكروب الليستيريا مونوستيوجينيس من الميكروبات الخطيرة التى تؤثر على صحة الإنسان ويزيد من خطورتها قدرتها على البقاء والنمو فى درجة الحرارة المنخفضة لأنها من الميكروبات المحبة للبرودة ولذلك أجريت هذه الدراسة لإختبار تأثير ثلاث مستخلصات من بعض التوابل الشائع إضافتهم فى صناعة الأغذية (مثل الزعتر و السماق و الفلفل الأسود) على نمو ميكروب الليستيريا مونوسيتوجينيس. و قد أتبعت طريقتان لدراسة هذا التأثير لهذه المستخلصات، طريقة agar cup method بإستخدام ٣ تركيزات ٢,٥ ، ٥ و١٠% من مستخلصات التوابل السالفة الذكر و قد وجد أن أقل تركيز فى تأثيره المثبط أعطى منطقة مثبطة (١٥مم) هو مستخلص السماق و الزعتر تركيز ٢,٥% على نمو ميكروب الليستيريا مونوسيتوجينيس. الطريقة الثانية لدراسة هذا التأثير food model قد أجريت على اللحم المفروم بإستخدام نفس التركيزات السابقة و تم حفظ العينات عند درجة ٣ م لمدة سبعة أيام وقد أظهرت النتائج أن أقل تركيز أعطى تأثير مثبط على نمو ميكروب الليستيريا مونوسيتوجينيس هو مستخلص السماق ٥ % حيث أدى إلى تناقص عدد هذا الميكروب بعد ٧ أيام من الحفظ بنسبة تثبيط ١,٩log .

SUMMARY

Listeria monocytogenes is of great concern to the food industry, especially in foods stored under refrigerated conditions where, unlike most food-borne pathogens, *L. monocytogenes* is able to multiply so this investigation was conducted to study the inhibitory effect of some spice extracts namely thyme, sumac and black pepper commonly used in food

industry on the growth of this pathogen. Three different concentrations (2.5, 5 and 10%) of the spice extracts were used. Two different procedures were carried out to evaluate the inhibitory effect of these spice extracts, agar cup method and food model. In, agar cup method, the obtained results showed that the lowest concentration which produced inhibitory effect on the growth of this pathogen with inhibition zone of 15mm were of sumac and thyme 2.5%. In the second procedure on food model (minced meat) stored at 3 °C for 7 days, the results revealed that the lowest concentration which exhibited a great decline in counts of *L. monocytogenes* after 7 days of storage by log 1.9 cfu/g was sumac 5% (in comparison to control).

Key words: *listeria monocytogenes, inhibitory effect, thyme, sumac and black pepper*

INTRODUCTION

Listeria monocytogenes is a Gram-positive asporogenous coccobacillus which gained increasing attention as a pathogen of public health importance owing to large numbers of food-borne outbreaks of listeriosis and of great concern to the food industry, especially in foods stored under refrigerated conditions where, unlike most food-borne pathogens, *L. monocytogenes* is able to multiply (Juntilla *et al.*, 1988). Consequently, refrigeration should not be relied upon as the sole method for the control of *L. monocytogenes* but should be incorporated with another means of preservation. One possible option is the use of plant extracts. Because of negative consumer perception of chemical preservatives, attention is shifting towards natural alternatives. Particular interest has been focused on the potential application of plant essential oils (EOs) and other extracts (Rasooli *et al.*, 2006).

Spices and herbs have been long used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the last 19th century have documented the antimicrobial properties of some spices, herbs and their components (Shelf, 1983 and Zaika, 1988).

Selected spices and their EOs have been studied with the aim of inhibiting the growth of *L. monocytogenes* in foods. Cloves, cumin, garlic powder, thyme, paprika, red and black pepper, rosemary, mace, marjoram and pimento have given good results in terms of their

capability of reducing the number of these organisms (Aureli *et al.*, 1992; Ting and Deibel, 1992; Hefnawy *et al.*, 1993; Pandit and Shelef, 1994 and Hao *et al.*, 1998).

Sumac (Rhus coriaria L., family Anacardiaceae) grows wild in the region extending from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and the Southeastern Anatolian Region of Turkey. The name derived from "sumâqâ," meaning red in Syriac. The spice, produced by grinding the dried fruit with salt, is used as condiment and sprinkled over kebabs and grilled meat as well as over salads that often accompany these dishes. It has a sour taste (pH 2.5) that is derived from the citric and malic acids found in its juice. In folk medicine, it is used for treatment of indigestion, anorexia, diarrhea, hemorrhagia and hyperglycemia (Wetherilt and Pala, 1994). Sumac is commonly used as spice in the Mediterranean region especially in meat and fish dishes.

Among the aromatic plants belonging to the Lamiaceae family, the genus Thymus is noteworthy for the numerous species and varieties of wild-growing plants. Many of these are typical of the Mediterranean area. The plants are extensively used (fresh and dried) as a culinary herb. The EO is utilized as flavour ingredients in a wide variety of food, as well as in perfumery. Because of its antiseptic, antispasmodic and antimicrobial properties is also used for medicinal purposes (Van Den Brouke and Lemli, 1981 and Panizzi *et al.*, 1993).

It is well documented that for most spices and plant materials the most active constituent against microorganisms was found to be the essential oil fraction (Aktuğ and Karapinar, 1986 and Zaika, 1988). As sumac contains very low quantities of essential oil, i.e., 0.02-0.03% (Brunk *et al.*, 1993), as well as thyme and pepper contain very low quantities of essential oil, which is difficult to collect separately and because essential oils are generally alcohol soluble, studies with alcohol extracts were conducted.

Therefore, this study was conducted to study the inhibitory effect of some spice extracts namely thyme, sumac and black pepper commonly used in food industry on the growth of *L. monocytogenes* in minced meat stored under refrigerated conditions.

MATERIALS and METHODS

Bacterial strain:

L. monocytogenes strain was obtained from Institute für Milchhygiene ünd Milchtechnologie, Vet. Med. Univ., Vienna, Austria.

A fresh culture was prepared by inoculating 10 ml of tryptic soy broth (TSB) with 0.6 yeast extract (TSBYE) with a loopful of the stock culture and incubating the inoculated tube at 32° C for 18-20h (Thongson *et al.*, 2005).

Spices used: sumac powder, thyme powder and black pepper powder, which purchased from a local market in Assiut city.

Extraction procedures:

Extraction of active constituents of sumac powder, thyme powder and black pepper powder using maceration technique (Abd El-Mawla, 1996): 10 gm of each spice were soaked in 50 ml alcohol 70%, left for complete extraction then filtration in air until complete evaporation then diluted in Tween 80 to obtain 10, 5 and 2.5% concentrations.

The Cup Method:

The method described by Zaika, (1988) was applied. Fifty milliliter Nutrient Agar (NA) cooled to 50 °C after autoclaving at 121 °C for 15 min, were inoculated well with 0.5 ml of an overnight (12-18h) *L. monocytogenes* culture, mixed well and poured into standard Petri plates. After setting of medium after about 1 h, cups of 1cm diameter were prepared. The base of each cup was sealed with 50ul of sterilized molten NA. The cups were filled by adding 300 ul of spice extracts (2.5, 5 and 10%) concentrations while Tween 80 was added in one cup as a control. The plates having cups were incubated for 48h at 37 °C. After incubation the growth inhibition zones around every cup (including cup) were measured with a caliper and recorded.

Food model (Ceylan *et al.*, 1998)**:**

1000 g of fresh meat was purchased from a local butcher. The samples were minced and divided into groups; the first group was divided into three equal portions each of 100 g in sterile plastic bags. The 1st, 2nd and the 3rd bags received 2.5, 5 and 10% thyme extracts. Also, the second group was divided into three equal portions each of 100 g in sterile plastic bags, then 2.5, 5 and 10% sumac extracts were added into the 1st, 2nd and the 3rd bags Moreover, the third group was divided into three equal portions, the 1st, 2nd and the 3rd bags received 2.5, 5 and 10% black pepper extracts, respectively, whereas, the fourth group was considered as control sample which had no spice. *L. monocytogenes* then was added to these mixtures to obtain 1X 10^7 cfu/g initial inoculum level. Both the bacterial inoculua and spice extracts were distributed in the minced meat by stomacher for 2 min. All plastic bags were

refrigerated at 3 °C and examined at the 0, 2nd, 5th and 7th days to evaluate the viable cell counts of *L. monocytogenes*.

Evaluation of *L. monocytogenes* in the inoculated samples:

Ten gm of the inoculated ground beef sample were transferred into sterile 250-stomacher bag together with 90 ml of sterile 0.1-peptone water. The sample was thoroughly homogenized by using a stomacher for 2 min. Serial dilutions of the homogenate were prepared by using 0.1-peptone water as diluents. 0.1 ml portions of three consecutive dilutions were spread-plated on tryptic soy agar (TSA) with 0.6 yeast extract (TSAYE) (Thongson *et al.*, 2005). The plates were incubated at 37° C for 24h.

RESULTS

Results were demonstrated in Tables 1&2 and Figures 1-3.

Table 1: Growth inhibition zones (mm) by different conc. of thyme, sumac and black pepper extracts on the growth of *Listeria monocytogenes*.

Type of extracts	Control	Conc. of thyme extracts			Conc. of sumac extracts			Conc. of black pepper extracts		
		2.5%	5%	10%	2.5%	5%	10%	2.5%	5%	10%
Inhibition Zones (mm)	10	15	17.1	26.2	15	17.9	28	10	10.5	13.7

Table 2: Inhibitory effect of different conc. of thyme, sumac and black pepper extracts on the growth of *Listeria monocytogenes (log cfu/g)*.

Days	Control	Thyme			Sumac			Black pepper		
		2.5%	5%	10%	2.5%	5%	10%	2.5%	5%	10%
0 time	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
After 2 days	7.3	6.4	6.3	4.2	6.6	6.3	4.2	7.2	6.5	6.2
After 5 days	7.8	6.3	6.2	5.6	6.3	6.2	5.3	6.3	6.3	6.3
After 7 days	8.9	6.3	6.2	5.3	5.7	5.3	6.3	6.3	6.3	6.3

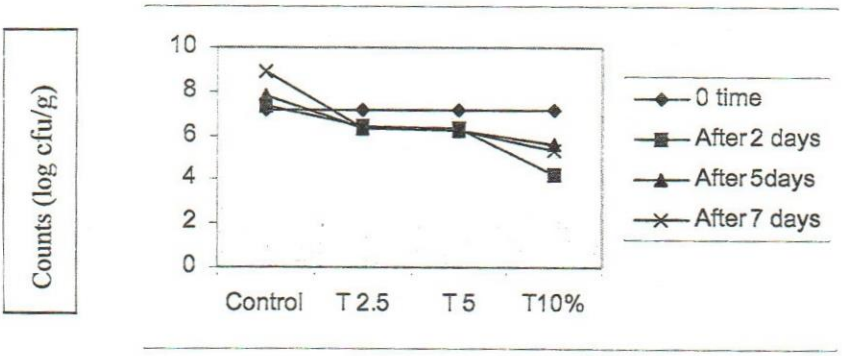

Fig. 1: Effect of thyme extracts (T) on counts of *L. monocytogenes*

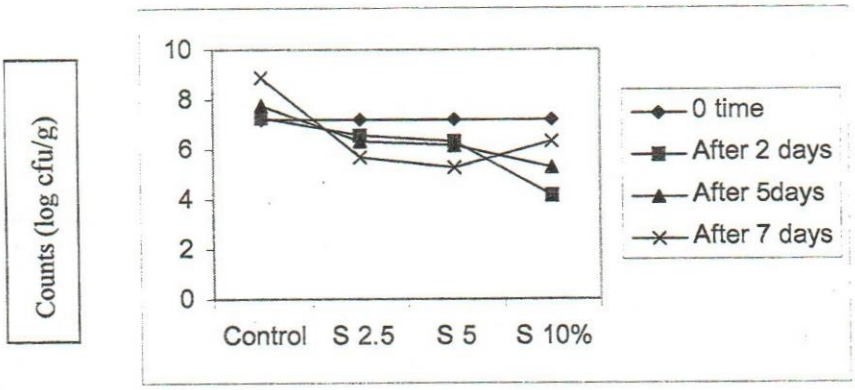

Fig. 2: Effect of sumac extracts (S) on counts of *L. monocytogenes*

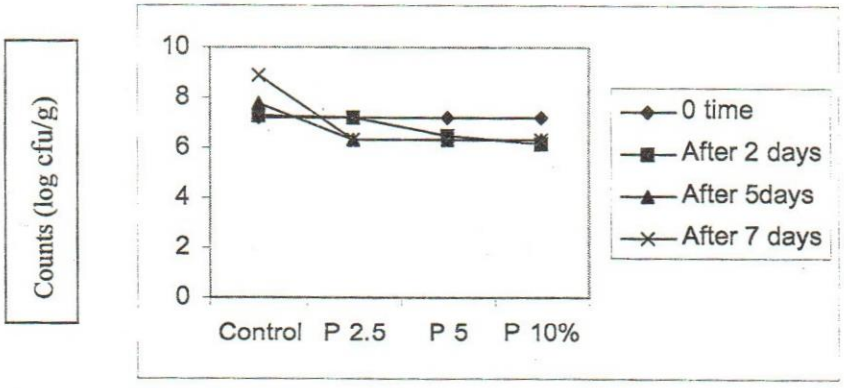

Fig. 3: Effect of black pepper extracts (P) on counts of *L. monocytogenes*

DISCUSSION

L. monocytogenes has been recognized as one of the major food-borne pathogen due to its ability to survive in adverse conditions.

The current interest in the use of compounds derived from spices as antimicrobial agents was sparked in the 1980s by changes in consumer attitudes toward the use of preservative agents such as nitrates and NaCl in foods (Shelef, 1983).

The inhibitory effect of different concentrations of thyme, sumac and black pepper extracts on the growth of *L. monocytogenes* tested by agar cup method is represented in Table 1. The obtained results showed that the most inhibitory extract which gave the greatest zone of inhibition (28mm) on the growth of *L. monocytogenes* was sumac 10% followed by 26.2mm by thyme 10%, whereas, the lowest concentration (2.5%) of sumac and thyme caused a zone of inhibition of 15mm. On the other hand, different concentrations of black pepper extract had no inhibitory effect on the growth of *L. monocytogenes* (Table 1).

From the results outlined in Table 2 and Figures 1, 2 and 3 it could be observed that thyme and sumac extracts (10%) had the highest inhibitory effect on the growth of *L. monocytogenes* in minced meat stored at 3 °C at the 2nd day of storage with reduction of 3 log cfu/g, whereas, the lowest concentrations which gave the most inhibitory action on the growth of this pathogen by log 1.9 cfu/g was sumac 5% after 7 days of storage. These obtained results were in agreement with those previously published by Aureli *et al.*, (1992); Nasar-Abbas and halkman, (2004); Abu-Shnab *et al.*, (2005) and Rasooli *et al.*, (2006).

Aureli *et al.* (1992) found that minced meat with thyme oil reduced *L. monocytogenes* population over the first week of storage.

Alcohol extract of sumac was found to be effective against all the tested organisms (6 Gram-positives and 6 Gram-negatives). Among the Gram-positives, Bacillus species (*B. cereus*, *B. megaterium*, *B. subtilis* and *B. thuringiensis*) were found to be the most sensitive, surviving up to only 500 mg/L of the spice, followed by *Staphylococcus aureus* (1000 mg/L), and then by *L. monocytogenes* (1500mg/L). Of the Gram-negative bacteria, *Salmonella enteritidis* and *Escherichia coli* type 1 were found to be more resistant, surviving up to 3000 mg/L of the spice (Nasar-Abbas and halkman, 2004).

Sumac is of Semitic origin and appears to derive ultimately from an Aramaic adjective summaq "dark red" (Semitic root SMQ or ŚMQ "to be red"); compare Modern Hebrew sumak. The name was

transported to European languages via Arabic summaq [سماق] "sumac" (Wetherilt and Pala, 1994).

Sumac is a very popular condiment in Turkey and Iran, where the ground fruits are liberally sprinkled over rice. Mixed with freshly cut onions, it is frequently eaten as an appetizer. The well-known Turkish fast food specialty döner kebap is sometimes flavoured with sumac powder (Nasar-Abbas and halkman, 2004).

In Palastine, Sumac (Rhus coriaria L.) is a well known spice, popular and has been utilized extensively in many different meals, such as in Zatar (dukka) which is a blend of sumac, thyme and citric acid with seasame seeds; almusakhan which is composed from fragmented chicken, small fragments of onions and sumac, as well as in salads and others (Abu-Shnab *et al.*, 2005).

Regarding black pepper as shown in Table 2 and Figure 3, it was found that black pepper at maximum conc. (10%) produced weak or very small effect in populations of *L. monocytogenes* by log 0.9 cfu/g after 7 days of storage at 3°C. This result was in agreement with those obtained by Ting and Deibel (1992) who emphasized that black pepper of concentrations up to 3% had no effect on *L. monocytogenes*. Also, Hefnawy *et al.* (1993) tested two strains of *L. monocytogenes* (strain Scott A and strain V7) for their response to spices (including black pepper) in a liquid medium (TSB) held at 4°C for 7 days, and they found that black pepper had no effect on *L. monocytogenes* strain V7, whereas, it reduced but did not completely inactivate the population of strain Scott A.

While numerous in vitro studies have demonstrated the effectiveness of spices, herbs, or plant extracts and their active ingredients against pathogens, few studies have addressed the use of plant-derived antimicrobial to inhibit pathogenic or spoilage organisms associated with meat (Cutter, 2000).

Many factors in foods could be responsible for the reduction of antimicrobial activity of spices and spice extracts while applied on different types of food. This observation was recorded by many investigators such as Ismaiel and Pierson, (1990) who reported that antimicrobial activity of spices and oils diminished in food as a result of solubilization of the antimicrobial agents into the food's lipid fraction.

The results of the present study indicate the existence of the antimicrobial activity in the extracts of sumac and thyme.

It can be concluded that ethanolic extracts such as sumac and thyme extracts, which inhibited the growth of *L. monocytogenes* at low

concentrations, could be considered as preservative materials for some kinds of foods; they could find an application as additives to foodstuffs in storage to protect them from listerial contamination.

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