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ORIGINAL ARTICLE

Effect of Sumac (*Rhus coriaria*) and Rosemary (*Rosmarinus officinalis*) water extracts on microbial growth changes in ground beef meat

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Abstract
The present study investigated the effect of sumac (SWE) and Rosemary water extracts (RWE) on microbial growth in ground beef meat during refrigerated storage. The meat samples were explored for changes in TVC, E.coli (0157:H7) and S.aureus populations. Antimicrobial effectiveness of extracts was more pronounced against Gram-positive strain compared with Gram-negative strain. Antimicrobial impact of extracts was also studied on ground beef meat. It was observed that antimicrobial activity of the water extracts was mostly dependent on the types of microorganisms present in meat. The greatest antimicrobial effect of SWE and RWE was found on S.aureus. Furthermore, the antimicrobial activity of RWE was lower than that of SWE. The results indicated the strong potential of SWE and RWE as a natural preservative for ground beef meat.
Keywords: Sumac, Rosemary, Antimicrobial activity, Beef meat, Water extracts

Introduction

Raw meat can be easily contaminated by microorganisms and support the growth of pathogens, leading to serious food-borne illnesses. Refrigeration is the most common preservation method of raw meat and meat products. In order to extend the shelf life time of refrigerated storage, synthetic additives may be added to muscle foods. Since the safety of synthetic additives has been questioned in recent years, consumers increasingly demand the use of natural products as alternative preservatives in foods (Solomakos et al., 2008). It is a major source of high quality protein, vitamins (thiamin, riboflavin, niacin, pantothenic acid, B6 and B12) and minerals such as iron, zinc and phosphorous (Williams, 2007). Meat provides an excellent medium for growth of different microorganisms. Pathogenic bacteria including Listeria monocytogenes, Escherichia coli O157:H7, Salmonella and Staphylococcus aureus as well as spoilage bacteria such as Pseudomonas, Acinetobacter, Moraxella and Enterobacteriaceae can survive and grow in meat (Ghabraie et al., 2016; Smaoui et al., 2016). Molds (Rhizopus, Sporotrichum and Aspergillus) and yeasts (Candida and Torulopsis) may also be found in fresh meat (Comi, 2017). Polyunsaturated fatty acids (PUFAs) in meat are susceptible to oxidation leading to quality deterioration, such as color changes, off-flavors and odors. Furthermore, oxidation of PUFAs results in the formation of lipid oxidation products (LOPs), such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), which might adversely affect human health following their consumption (Hecke et al., 2017). Lipid oxidation represents one of the major causes of the progressive deterioration in the quality of meat products, limiting their storage shelf life. The deterioration in organoleptic characteristics, and the associated loss of nutritional value induced by the oxidative process, can be delayed by the addition of

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antioxidants (Contini et al., 2014). There are various strategies for meat preservation, including refrigeration, radiation, vacuum packaging, heat processing and addition of preservatives but With regard to the problem of biodegradability of plastics and the risk of chemical preservatives, there is an increasing preference for natural preservatives (Davidson et al., 2013; Ekrami and Emam- Djomeh, 2014). Spices and herbs have long been used as food additives. Spices can improve sensory properties (color, flavor and taste) and extend the shelf life of foods by lowering bacterial count and inhibiting lipid oxidation (Van Hecke et al., 2017).

Sumac (*Rhus coraria L.*) is a member of the Anacardiaceac family and it has many applications in different countries. Sumac is a shrub with a long history application in traditional medicine and Iranian cuisine. It is used as medical herb and spices as a condiment and sprinkled over kebobs, grilled meats, soups, and some salads. It is used in traditional medicine for treatment of indigestion, anorexia, diarrhea, hemorrhages, hyperglycemia, ocular trachoma and ear infection .It is grown wild in the region from the Canary Island over the Mediterranean area to Iran and Afghanistan. In Iran sumac is grown in Mazandaran, Azarbayegan, Khorasan, Shiraz, Ghazvin, Ghom and Hamedan (Ahmadi et al., 2017).

Rosemary (*Rosmarinus officinalis*) is a woody, perennial herb with fragrant evergreen needle-like leaves. It is native to the mediterranean region and is a member of the mint family Lamiaceae. Rosemary is extremely high in iron, calcium, and vitamin B6. It has a very old reputation for improving memory, and has been used as a symbol for remembrance in Europe. Carnosic acid, found in rosemary, shields the brain from the free radicals. It grows in most regions in Iran. Because of remedy properties, rosemary uses to treat Parkinson's, Alzheimer, also it has antidiabetogenic, antifungal, antimicrobial, anti-inflammatory, antiplatelet and antioxidant effects (Jalali-Heravi et al., 2011).

The antioxidant and antimicrobial potential of sumac and rosemary extracts has been reported, there are no data on effectiveness of sumac and rosemary water extract in beef meat. However, there is no information about the inhibitory effects of water extracts of sumac and rosemary on microbial growth and lipid oxidation in ground beef meat. Hence, the aims of this study were (1) to investigate of the antimicrobial activity water extracts of sumac and rosemary and (2) to evaluate the effect of these extracts on growth of spoilage microorganisms in ground beef meat during refrigerated storage.

Materials and methods

Preparation of Water Extract of Spices

Ground plant materials (5 g) were added to distilled water (1 L) and refluxed at 60˚C for 16h. Then, the extracts were filtered through whatman filter paper (Sigma-Aldrich, St. Louis, MO) and the filtrates were concentrated on a rotary evaporator and then lyophilized. The lyophilized extracts were placed in sealed bottles and stored at 4C. The extracts were dissolved in distilled water prior to use (Moreno et al., 2006; Nasar-Abbas and Halkman, 2004).

Preparation of Meat Samples

To prepare the meat samples for the first, connective tissue attached to the meat was removed and meat was cut to 5×5cm pieces (100g) using a sharp knife. Then meat was twice grinded by an electric meat grinder (diameter = 4mm). Meat samples were then divided into four parts to compare the following types of sample: control sample and antimicrobial sample (100, 200 and 400 (mg/ml) sumac/ rosemary water extract). For each of these packaging, 100g of ground meat was transferred into sterile plastic Petri dishes. Dishes were kept in a refrigerator at 4±1°C for

10days. In order to prevent changes in microbial growth conditions, which may occur during sampling and handling, four replicates of each package were created, and one of them was used at each sampling time including 0th (the day of producing packages), 3rd, 6th and 10th days.

Preparation of culture media and microbiological tests

Two bacterial strains including E.coli (0157:H7) (ATCC35218) and S.aureus (PTCC1431) were used in order to assess the antimicrobial activity of sumac/ rosemary water extract. Five concentrations of antimicrobial extract were used in broth dilution susceptibility (BDS) test in order to measure both minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). To perform a BDS test, 1ml of standard inoculum of the microorganism (organisms 1×106ml, a 1:500 dilution of a suspension of turbidity equal to a McFarland standard) was added to an equal volume of each concentration of antimicrobial agent, as well as to a tube of the growth medium without antimicrobial agent, which has been served as a growth control. Negative control that contained only antimicrobial agent was used for optical comparison.

Microbiological Analysis

At each sampling day (0, 3, 6 and 10), meat samples (10g) were aseptically transferred into individual stomaching bags containing 90 mL of sterile peptone water solution (0.1%) and homogenized in a stomacher (Stomacher 400, Circulator, Seward) for 1 min. Serial decimal dilutions were prepared by diluting 1 mL of homogenate in 9 mL of peptone water, and 0.1 mL from appropriate dilutions was spread on the surface of specific media as follows: Total viable count (TVC) was determined on plate count agar (Merck, Darmstadt, Germany) at 37C for 24 h. E. coli bacterial strain isolating in lauryl sulfate triptose (Merck KGaA) (37 °C, 48 h); positive results were replicated in MacConkey broth (Merck KGaA) as well as in EC (E. coli) broth (Merck KGaA), with incubation at 37 and 44.5 °C respectively for 48 h. Positive tubes were spread in EMB (Eosyn methylene blue) (Merck KGaA) (37 °C, 48 h). Enumeration of S.aureus was performed on BPA (CHROM agar, Paris, France) at 37C for 24 h.

Disc diffusion assay

A 5- ml portion of this inoculum was placed onto the surface of pre- dried Mueller–Hinton agar (MHA; Oxoid) plates and allowed to remain in contact for 1 min. Excess inoculum was removed using a sterile syringe and the plates were allowed to dry for 20 min at room temperature. Sterile 8 mm filter paper discs (Schleicher and Schuell, Dassel, Germany) were placed on the plates and immediately 20-µl portions of the essential oils were added. Sterile PS was used as control. After allowing 1 h at room temperature for the essential oils to diffuse across the surface, the plates were incubated at 37°C for 24 h. The inhibition zone was measured in millimetre and the assay was carried out three times for each extract (Zaidan et al., 2005).

Agar (cup/well) Diffusion Assay

Muller hinton agar (cooled to 45°C) was poured into sterile standard petri plates (20mL). This was then inoculated with 1mL of pathogenic culture by spread plate technique. After setting, medium cups of 8mm diameter were prepared with the help of a sterile cork borer. The base of each cup was sealed with 100µL of sterilised Muller hinton agar. The cups were filled by adding 60µL of the different spice extracts with varying concentration while sterilized distilled water was used as control. The plates were incubated for 24h at 37°C. After incubation, the

zones of inhibition around each cup were measured (including cup) in millimeter with the help of antibiotic zone measuring scale. All the analyses were applied in triplicates (Irshad et al., 2012; Parekh et al., 2005).

Statistical Analysis

Microsoft Windows Excel 2013 and SPSS software (version 19.0, SPSS, Inc., Chicago, IL) were used to analyze the resulting data. All experiments were conducted with three or more replicates and their mean values were used for statistical analysis. Data were initially evaluated by analysis of variance and then a Duncan multiple range test was employed to detect significant differences ($P<0.05$) among antimicrobial properties of the samples.

Results and discussion

Antimicrobial activity of extracts

The shelf-life of meat and meat products is the storage time until spoilage. The point of spoilage may be defined by a certain maximum acceptable bacterial level, or an unacceptable off odor/ off flavor or appearance. The shelf-life depends on the numbers and types of microorganisms. mainly bacteria, initially present and their subsequent growth (Borch et al., 1996). Table 1 and 2 shows the antimicrobial impact of different concentrations of the sumac and rosemary extracts on against S.aureus. Average halo diameter (mm) of various concentrations of sumac and rosemary extracts using disc diffusion technique against S.aureus were determined between 11.08-14.77 mm and 9.17-13.40 mm, respectively, while for agar (cup/well) diffusion technique, these Average halo diameters were 13.91-16.34 mm and 10.07-12.95 mm, respectively.

Table 3 and 4 shows the antimicrobial impact of different concentrations of the sumac and rosemary extracts on against E.coli. Average halo diameter (mm) of various concentrations of sumac and rosemary extracts using disc diffusion technique against E.coli were determined between 8.19-11.37 mm and 7.24-10.17 mm, respectively, while for agar (cup/well) diffusion technique, these Average halo diameters were 8.58-11.94 mm and 7.54-10.71 mm, respectively.

Table 1. Antimicrobial activity of various concentrations of sumac and rosemary extracts using disc diffusion technique against S.aureus (Average halo diameter (mm))

	0 (mg/ml) Negative control	25 (mg/ml)	50 (mg/ml)	100 (mg/ml)
Sumac	0.00 ± 0.00^{d}	11.08 ± 0.03^{c}	13.49 ± 0.15^{b}	14.77 ± 0.03^{a}
Rosemary	0.00 ± 0.00^{d}	9.17 ± 0.04^{c}	11.11 ± 0.14^{b}	13.40 ± 0.17^{a}

* Means with different superscripts are significantly different ($p<0.05$). Data are means±standard deviation.

Table 2. Antimicrobial activity of various concentrations of sumac and rosemary extracts using agar (cup/well) diffusion technique against S.aureus (Average halo diameter (mm))

	0 (mg/ml) Negative control	25 (mg/ml)	50 (mg/ml)	100 (mg/ml)
Sumac	0.00 ± 0.00^{d}	13.91 ± 0.05^{c}	15.53 ± 0.03^{b}	16.34 ± 0.07^{a}
Rosemary	0.00 ± 0.00^{d}	10.07 ± 0.05^{c}	11.55 ± 0.04^{b}	12.95 ± 0.02^{a}

* Means with different superscripts are significantly different ($p<0.05$). Data are means±standard deviation.

No growth was observed for the negative control sample, which confirms the sterile condition of the experiments. According to the results, E.coli and S.aureus was more sensitive to sumac extract as compared with rosemary extract.

Table 3. Antimicrobial activity of various concentrations of sumac and rosemary extracts using disc diffusion technique against E.coli (Average halo diameter (mm))

	0 (mg/ml) Negative control	25 (mg/ml)	50 (mg/ml)	100 (mg/ml)
Sumac	0.00±0.00^{d}	8.19±0.02^{c}	9.66±0.04^{b}	11.37±0.04^{a}
Rosemary	0.00±0.00^{d}	7.24±0.05^{c}	9.47±0.03^{b}	10.17±0.06^{a}

* Means with different superscripts are significantly different ($p<0.05$). Data are means±standard deviation

Table 4. Antimicrobial activity of various concentrations of sumac and rosemary extracts using agar (cup/well) diffusion technique against E.coli (Average halo diameter (mm))

	0 (mg/ml) Negative control	25 (mg/ml)	50 (mg/ml)	100 (mg/ml)
Sumac	0.00±0.00^{d}	8.58±0.06^{c}	10.14±0.08^{b}	11.94±0.07^{a}
Rosemary	0.00±0.00^{d}	7.54±0.03^{c}	9.95±0.05^{b}	10.71±0.03^{a}

* Means with different superscripts are significantly different ($p<0.05$). Data are means±standard deviation.

Microbial analysis of meat samples

As can be seen from Fig 1 and 2 the initial value for TVC of ground beef meat was 3.26–3.30 log cfu/g representative of good quality (Soldatou et al., 2009). According to International Commission on Microbiological Specifications for Foods (ICMFS), the upper microbiological limit of acceptability for meat is 7 log cfu/g (Ridell and Korkeala, 1993). In the present study, TVC reached 7 log cfu/g on days 6 for control samples, day 6 for samples containing 100 mg/ml SWE and RWE and day 10 for samples containing 200 and 400 mg/ml SWE and RWE. Treatment with SWE and RWE significantly ($P<0.05$) decreased the TVC of samples compared with control samples. In samples treated with 400 mg/ml SWE, the TVC were reduced by 0.4, 0.5 and 3.5 log cfu/g on days 3, 6 and 9, respectively (Fig 1), as well as, In samples treated with 400 mg/ml RWE, the

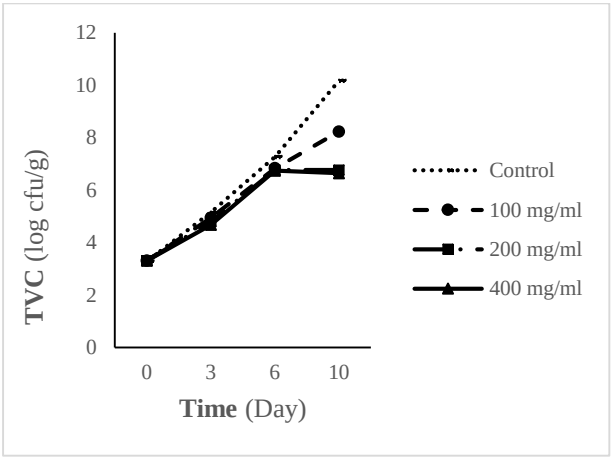

Fig 1. Total viable bacterial count of ground beef meat incorporated without and with sumac extract during storage at 4°C for 10 days

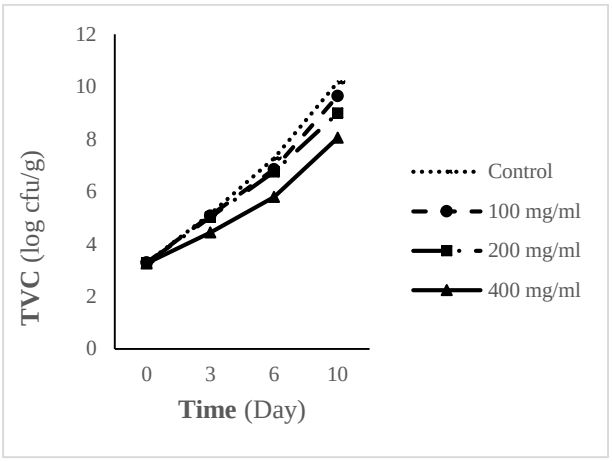

Fig 2. Total viable bacterial count of ground beef meat incorporated without and with rosemary extract during storage at 4°C for 10 days

a

TVC were reduced by 0.7, 1.47 and 2.12 log cfu/g on days 3, 6 and 9, respectively (Fig 1).

In the initial days RWE was more effective ($P<0.05$) than SWE in suppressing microbial (TVC) growth but and then SWE was more effective ($P<0.05$) than RWE. Growth of Total viable count was significantly increased over the time in all samples, while for antimicrobial samples, substantial change was observed in growth rate. By the end of experiment day, however, a slight increase was observed in growth rate for antimicrobial samples with RWE. In other study, the antimicrobial effect of water extract of sumac against some foodborne bacteria has been evaluated (Abdullah et al., 2015; Abu-Reidah et al., 2014; Aliakbarlu and Mohammadi, 2015; Naim and Mushtaq, 2017; Raeisi et al., 2017; Yadolahi- Baghloei et al., 2017). It was found that E. coli was a resistant bacteria, whereas S.aureus had the least resistance. Another study showed that the water extracts of sumac and rosemary had the strongest antimicrobial activity.

Conclusions

This study showed that the water extracts of sumac and rosemary could inhibit the microbial growth in ground beef meat during refrigerated storage. Generally, SWE was more effective than RWE in inhibiting microbial growth in ground beef meat during refrigerated storage. Because both sumac and rosemary are commonly used as seasoning ingredients in muscle foods (kebab, chicken meat meal and so on), the application of their extracts in these foods not only can improve sensory characteristics but also extend the shelf life. Meanwhile, these extracts might have health-promoting effects on the consumers due to their potential antimicrobial activities.

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