Efficacy of sumac spice incorporation in Egyptian kofta against *Staphylococcus aureus* and *Enterobacteriaceae*

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**Abstract**

Egyptian kofta is considered one of the processed meat products with high nutritional value with an economic price, suiting a wide range of consumers. So, it requires protection from microbial contamination during their preparation, storage, and distribution. Sumac (*Rhus coriaria*) spice extract can be considered as herbal antimicrobial shown to have their activities against many foodborne pathogens. The antibacterial activities of different concentrations of sumac spice extract (1%, 2.5% and 5%) were tested in vitro against *Staphylococcus aureus* and *Enterobacteriaceae* using the agar disc diffusion method. The average value of total *S. aureus* count (CFU/g) in untreated samples (control) was $1.1 \times 10^4 \pm 7.6 \times 10^2$ CFU/g at the 6th day during chilling storage which significantly (p<0.05) decrease at the 9th day of storage to $7 \times 10^3 \pm 3.4 \times 10^3$, $9 \times 10^3 \pm 1.1 \times 10^3$ and $8 \times 10^3 \pm 2.9 \times 10^3$ CFU/g in treated samples with 1%, 2.5% and 5% sumac spice extract respectively. The average value of total *Enterobacteriaceae* count in the 6th day was $1 \times 10^4 \pm 3.5 \times 10^2$ CFU/g in the untreated kofta samples which significantly (p<0.05) decrease in the 9th day of storage to $1.1 \times 10^3 \pm 1 \times 10^3$, $9 \times 10^3 \pm 1.7 \times 10^3$ and $8.1 \times 10^3 \pm 7.7$ CFU/g in treated samples with 1%, 2.5% and 5% sumac spice extract respectively. Our findings suggested the possibility of using the fruit of Sumac as a novel source of natural antimicrobial for the food industries.

1. Introduction

Meat and meat products are very popular food in Egypt as well as throughout the world. No wonder since they are delicious as considered as good and cheap sources of protein characterized by good flavor and easily digested. The increase of human population and the great progress of various aspects of life make the consumer to use meat products in different forms for their ease preparation such as kofta (Saad et al., 2018).

Kofta is a family of meatball which consists of minced or ground meat, usually beef, mixed with spices or onion. It can be exposed to several ways of contamination through improper preparation, bad handling of foods and improper storage which constitute the most direct and harmful source of microbiological contamination (Hassan et al., 2015).

A major problem in food hygiene is the fecal contamination of meat product with Enterobacteriaceae such as *Salmonella* spp., *Escherichia coli*, *Proteus*, and *Klebsiella* species. The bacterial contamination and hygienic measures during meat production can be measured using the aerobic plate count, total Enterobacteriaceae and total coliforms counts (McEvoy et al., 2004).

*Staphylococcus aureus* plays a great role in bacterial contamination of cooked meat. Staphylococcus can carry on human hands, nasal passage or throats, so workers play as major role of *S. aureus* contamination during preparation, processing, or even through post cooking contamination by touching cooked meat that are usually eaten without further cooking or heating. Most foodborne illnesses of *S. aureus* outbreaks are a result of production of heat stable toxins in the meat which may lead to severe food poisoning outbreaks (United Stated Department of Agriculture, Food Safety and Inspection Service, 2003)

Therefore, new preservation techniques needed to improve the quality and safety of meat products, with maintaining their good nutritional and organoleptic...
properties due to the growing demand of consumers for safe and natural products (Burt, 2004). Thousand years ago, many spices had been used as preservatives due to their antimicrobial and antioxidant effect beside being flavoring and coloring agents (Srinivasan, 2005).

Sumac (*Rhus coriaria* and related to family-*Anacardiaceae*) is a very popular spice in countries of the Middle East, where it is widely used in meat dishes. Sumac is used in most Arab countries, the term sumac is derived from the Arabic root, *summaq*, meaning red, referring to the colour of sumac fruit (Figure 1). Sumac belongs to the genus *Rhus*, found in subtropical and temperate regions throughout the world. Sumac plants are large shrubs or small trees, reaching a height of 3–10 m, with pinnately compound leaves. They bear greenish white flowers in dense panicles and red drupaceous fruits (also called sumac bobs), from which the spice (Figure 2) is derived (Ravindran *et al*., 2012).

**2. Materials and methods**

**2.1 Collection of plant material**

The ripened (reddish-brown), native sumac spice (*Rhuscoriaria*) bought from local markets in Ismailia Governorate.

**2.2 Preparation of plant extract**

The sumac ethanol extract was prepared as described by Abd El-Mawla (1996), with some modification. The extracted prepared in Pharmacology Department in Suez Canal University by maceration technique as the following: 250 g of sumac spice macerated in 750 mL of absolute ethanol 99.9% then allowed to complete exhaustion. The final extract (bright reddish mixture) filtered and the filtrate was concentrated in rotatory evaporator (R-100 Buchi– Switzerland) under reduced pressure until complete evaporation. The yield after complete evaporation of ethanol was 53 g of the sumac-dried extract for each 250 g of spice. The sumac dried extract emulsified by sterile propylene glycol to give final concentration of 1%, 2.5% and 5%; these concentrations were according to Nasar *et al*. (2004). The extract kept in sealed containers and refrigerated at 3±1˚C until used in bacteriological evaluation. Propylene glycol commonly used as an excipient in a variety of drugs and it authorized in food products and cosmetics (European Medicines Agency, 2013) and it gave antibacterial activity at 100% concentration (Nalawade *et al*., 2015).

**2.3 Preparation of Egyptian kofta**

Fresh beef meat sample purchased from local market at Ismailia Governorate (Egypt) on the day of preparation. It cut and minced with grinder through a 4 mm plate diameter then 17 g of common salt added for each one kg of meat. Then it was divided into four groups, first group was a control group with 0% sumac spice extract, 2nd group was mixed with 1% sumac spice extract while 3rd and 4th groups were mixed with 2.5%, and 5% sumac spice extract respectively. Then each group divided to 30 samples (each about 50 g). Each sample formed into meatballs (kofta), then wrapped with saran wrap, and placed in a chiller at 3±1˚C for zero, third, sixth, ninth, eleventh and fifteenth days. Five samples (from control and each concentration) were removed for bacteriological evaluation periodically.

**2.4 Determination of antimicrobial activity of the prepared extract**

The antimicrobial activity of the prepared extracts was tested in vitro against *S. aureus* and Enterobacteriaceae using the agar disc diffusion method according to National Committee for Clinical Laboratory...
Standards (1993).

2.5 Bacteriological evaluation

Samples homogenate and serial decimal dilutions were prepared following the recommendation of Downes and Ito (2001). The serial dilutions of each sample examined for evaluation the effect of the added extracts on *S. aureus* count (Downes and Ito 2001) and total *Enterobacteriaceae* count (ISO, 2004).

2.6 Statistical analysis

All values presented as mean ± standard error. Data analysis was performed by using SPSS statistical software program (SPSS, 2007). Data analyzed for the significant differences between fresh and cooked offal. Data subjected to one-way analysis of variance (ANOVA). Any significant differences (p<0.05) were analyzed by the multiple comparison procedure of LSD (least significant differences), using a level of significance of alpha = 0.05.

3. Results and discussion

The emergence of natural food preservatives against the currently used chemical agents is on the raise and requires worldwide consideration. Food investigators on the use of plants as antimicrobials will not only authenticate their use in preservatives of meat products but will provide future promises in the discovery of new drugs with antimicrobial potential.

3.1 Determination of antimicrobial activity of the prepared extract

There is an interest in discovering new natural antimicrobials such as spices, herbs and their extracts. The sumac extract displayed a variable degree of antimicrobial activity on different bacteria (Nasar et al., 2004).

The result obtained in Table 1 revealed that the inhibition zone (mm) of sumac spice extract against *S. aureus* and *Enterobacteriaceae*. For *S. aureus* it was 13, 16 and 17 mm at concentration level 1%, 2.5% and 5% while the control positive Norfloxacin10_{necg} gave 24 mm. These results were nearly similar to Nimri et al. (1999), Nasar et al. (2004), Moshtaghi et al. (2013), Aliakbarlu et al. (2014) and Khalkhali and Noveir (2018), while high records were reported by Abu-Shanab et al. (2005), Ali et al. (2014), Gabr et al. (2014) and Mahdi et al. (2016) meanwhile lower results recorded by Ertürk (2010) and Qadir et al. (2013).

These results showed that there is an inhibitory effect of sumac against *S. aureus* and *Enterobacteriaceae*, which increase with concentration. Sumac extract gave higher inhibition zone against *S. aureus* comparing to *Enterobacteriaceae* that agreed with Saxena et al. (1994), Fazeli et al. (2007), Raodah et al. (2014) and Abou-Reidah et al. (2014) whose results cleared that Gram-positive bacteria were more sensitive than Gram-negative bacteria when treated with sumac. This may be due to the differences in cell wall structures between Gram-positive and Gram-negative bacteria. The presence of lipopolysaccharides in the outer membrane of Gram-negative bacteria makes their membrane act as a barrier against many natural substances.

3.2 Bacteriological evaluation

Plants are able to produce different compounds that used to protect themselves against different types of pathogens. These compounds like phenolic acid that has antimicrobials action as it interact with the proteins (Cowan, 1999).

3.2.1 Effect of sumac spice extract on *S. aureus* count

Results given in Table 2 showed the effect of sumac spice extract on total *S. aureus* count (CFU/g) in the treated kofta samples. In zero-day, the mean value ± standard error in group 1, group 2, group 3 and group 4 was $5.3 \times 10^3 \pm 5.6 \times 10^4$, $9.1 \times 10^2 \pm 37.9$, $5.2 \times 10^2 \pm 41.6$ and $5.2 \times 10^2 \pm 36.1$, respectively. While in the 3rd day were $8 \times 10^3 \pm 4.9 \times 10^4$, $5 \times 10^3 \pm 3.5 \times 10^4$, $3 \times 10^3 \pm 57.7$ and $2 \times 10^3 \pm 2.9 \times 10^4$, respectively. On the other hand, in the 6th day the count reach to $1.1 \times 10^4 \pm 7.6 \times 10^5$, $7 \times 10^3 \pm 58.4$, $5 \times 10^3 \pm 4.6 \times 10^3$ and $4 \times 10^3 \pm 59.3$, respectively. In
Table 2. Effect of sumac spice extract on S. aureus count (CFU/g) in the treated kofta samples

| Days   | Group 1         | Group 2         | Group 3         | Group 4         |
|--------|-----------------|-----------------|-----------------|-----------------|
|        | Mean SE         | Mean SE         | Mean SE         | Mean SE         |
| Zero-day | 5.3×10³ ± 5.6×10² | 9.1×10³ ± 37.9 | 5.2×10² ± 41.6 | 5.2×10² ± 36.1 |
| 3rd day | 8×10³ ± 4.9×10² | 5×10³ ± 3.5×10² | 3×10³ ± 57.7 | 2×10³ ± 2.9×10² |
| 6th day | 1.1×10⁴ ± 7.6×10² | 7×10³ ± 58.4 | 5×10³ ± 4.6×10³ | 4×10³ ± 59.3 |
| 9th day | S S             | 7×10⁶ ± 3.4×10³ | 9×10⁶ ± 1.1×10³ | 8×10⁶ ± 2.9×10³ |
| 12th day | S S             | S S             | 5.7×10³ ± 2.9×10⁴ | 3.1×10³ ± 2×10⁴ |
| 15th day | S S             | S S             | 5.1×10⁶ ± 9.9×10³ | 3.3×10⁶ ± 4.9×10³ |

Mean values in the same row that are not followed by the same letter are significantly different (p<0.05). S: spoiled, SE: Standard error.

9th day group 1 spoiled, while group 2, group 3 and group 4; Mean value ± standard error was 7×10⁴± 3.4×10³, 9×10³± 1.1×10³ and 8×10³± 2.9×10², respectively. In addition, the 12th day Mean value ± standard error of treated groups were 1.1×10⁵ ± 5.7×10², 2.9×10⁴ ± 3.1×10³ and 2×10⁴ ± 4.9×10², respectively. Finally, in the 15th day group 2 spoiled, while group 3 and group 4; Mean value ± standard error was 5.5×10⁶ ± 9.9×10³ and 3.3×10⁶ ± 9.7×10², respectively. In the Zero-day, there were a significant difference (p<0.05) between group 1, group 2 while there was not significant difference between group 2, group 3 and group 4. Meanwhile, in the 3rd day and 6th day there were significant differences between group 1, group 2 and group 3 while there was not significant difference between group 3 and group 4. In the 9th and 12th day there were a significant difference (p<0.05) between group 2 and group 3 while there was no significant difference between group 3 and group 4. Finally, in the 15th day there were no significant difference (p>0.05) between group 3 and group 4.

Sumac spice extract significantly (p<0.05) decreased the total S. aureus count of treated samples compared with control samples that spoiled at the 6th day. These obtained results agreed with those reported by Shabir (2012) and Yadolahi et al. (2017).

S. aureus count were gradually increased during refrigeration for all treated groups (2, 3 and 4) in different ratios depending on the sumac concentration but still lower than that group 1. According to EOS (2005) group 1 (untreated) were exceeding the permissible limits 10² but the treated samples agreed with this limit in the zero-day.

The inhibitory effect of sumac is due to the high citric and malic acid contents (Wetherilt and Pala 1994), which may cause a change in pH (Sumac pH value is 2.5), this may be a reason for inhibition the bacterial growth.

The results obtained in Table 3 showed the reduction percent of S. aureus count (CFU/g) in the treated kofta samples with sumac spice extract at concentrations 1%, 2.5% and 5%. At concentrations 1%, the reduction percent was 82.8% in zero-day then decreased to 37.5% and 36.4% in the 3rd day and 6th day, respectively while at concentrations 2.5%, the reduction percent was 90.2%, 62.5% and 54.5% in the zero-day, 3rd day and 6th day, respectively. At concentrations 5%, the reduction percent of S. aureus count at zero-day, 3rd day and 6th day was 90.2%, 75% and 63.6% respectively.

These previous results revealed that the highest reduction percent of S. aureus count were achieved by sumac spice extract at concentrations 5% (group 4) followed by concentrations 2.5% (group 3) and the lowest effect was sumac spice extract at concentrations 1% (group 2).

3.2.2 Effect of sumac spice extract on Enterobacteriaceae count

Results in Table 4 showed the effect of sumac spice extract on total Enterobacteriaceae count (CFU/g) in the treated kofta samples. In zero-day, the mean value ± standard error in group 1, group 2, group 3 and group 4 was 2×10³ ± 1.7×10², 6.3×10³ ± 26.7, 2.9×10³ ± 43.3 and 2.1×10² ± 15.3, respectively. While in the 3rd day were 6×10³ ± 2×10², 5.2×10³ ± 2.2×10², 2.9×10³ ± 88.2 and 2.1×10³ ± 2.4×10², respectively. On the other hand, in...
the 6th day the count reach to $1 \times 10^4 \pm 3.5 \times 10^2$, $9.1 \times 10^3 \pm 1.7 \times 10^2$, $8 \times 10^3 \pm 5.7 \times 10^2$ and $7.1 \times 10^3 \pm 1.7 \times 10^2$, respectively. In 9th day group 1 spoiled, while group 2, group 3 and group 4; Mean value ± standard error was $1.1 \times 10^4 \pm 1 \times 10^3$, $9 \times 10^3 \pm 1.7 \times 10^2$ and $8.1 \times 10^3 \pm 77.7$, respectively. In addition, the 12th day Mean value ± standard errors of treated groups were $3.9 \times 10^4 \pm 1.4 \times 10^3$, $1.1 \times 10^4 \pm 7 \times 10^2$ and $9.1 \times 10^3 \pm 1.7 \times 10^2$, respectively. Finally, in the 15th day group 2 spoiled, while group 3 and group 4; Mean value ± standard error was $4 \times 10^4 \pm 1.4 \times 10^3$ and $5 \times 10^4 \pm 1.4 \times 10^3$, respectively. In the Zero, there were a significant difference (p<0.05) between group 1, group 2 and group3, while there was no significant difference between group 3 and group 4. Meanwhile, in the 3rd day there were significant differences between all groups. In the 6th day there were a significant difference (p<0.05) between group 1, group 2 and group 4, while concerning to group 3 no significant appeared. In the 9th day there were significant differences (p<0.05) between group 2 and group 4, while concerning to group 3 no significant appeared. In the 12th day there were a significant difference (p<0.05) between group 2 and group 3 while there were no significant differences between group 3 and group 4. Finally, in the 15th day there were no significant differences (p>0.05) between group 3 and group 4.

Total Enterobacteriaceae count significantly (p<0.05) decreased in treated samples with sumac extract compared with untreated one that spoiled at the 6th day, so generally sumac with different concentrations can effectively act against Enterobacteriaceae. The obtained results agreed with those reported by Vatansever et al. (2018) and Langroodi et al. (2018).

It could be observed that group 1 (control) had the highest Enterobacteriaceae count at any time of refrigeration compared to other treated groups that count gradually increased during refrigeration for all treated groups (2, 3 and 4) but still lower than that group 1.

This results may be due to sumac contains phenolic compounds and tannic acids that have anti-bacterial effect (Langroodi et al. 2018).

The results in Table 5 showed the reduction percent of Enterobacteriaceae count (CFU/g) in the treated kofta samples with sumac spice extract at concentrations 1%, 2.5% and 5%. At concentration 1%, the reduction percent was 68.7% in zero-day then decreased to 14.35% and reach to 12% in the 3rd day and 6th day, respectively while at concentration 2.5%, the reduction percent was 85.2%, which is the highest percentage, followed by 50.8% then 22.3% in the zero-day, 3rd day and 6th day respectively. At concentrations 5%, the reduction percent of Enterobacteriaceae count (CFU/g) in the treated samples at zero-day, 3rd day and 6th day was 89.5%, 64.6% and 31.4% respectively.

It's appeared from previous results that the highest reduction percent of Enterobacteriaceae count were achieved by sumac spice extract at concentrations 5% (group 4) followed by concentrations 2.5% (group 3) and the lowest effect was sumac spice extract at concentrations 1% (group 2).

### 4. Conclusion

Incidence of food poisoning bacteria in kofta constitute a public health hazard and considered as indicator of poor production, post processing contamination or fecal contamination where the most

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#### Table 4. Effect of sumac spice extract on total Enterobacteriaceae count (CFU/g) in the treated kofta samples

| Days   | Group 1 | Group 2 | Group 3 | Group 4 |
|--------|---------|---------|---------|---------|
|        | Mean SE | Mean SE | Mean SE | Mean SE |
| Zero-day | $5.3 \times 10^3$ | $5.6 \times 10^3$ | $9.1 \times 10^3$ | $37.9$ | $5.2 \times 10^4$ | $41.6$ | $5.2 \times 10^3$ | $36.1$ |
| 3rd day | $8 \times 10^3$ | $5 \times 10^3$ | $3.5 \times 10^2$ | $3 \times 10^3$ | $57.7$ | $2 \times 10^3$ | $2.9 \times 10^2$ |
| 6th day | $1.1 \times 10^4$ | $7.6 \times 10^2$ | $7 \times 10^3$ | $5 \times 10^3$ | $4.6 \times 10^3$ | $4 \times 10^3$ | $59.3$ |
| 9th day | S | S | $7 \times 10^6$ | $3.4 \times 10^3$ | $9 \times 10^3$ | $1.1 \times 10^2$ | $8 \times 10^3$ | $2.9 \times 10^2$ |
| 12th day | S | S | $1.1 \times 10^4$ | $5.7 \times 10^3$ | $2.9 \times 10^4$ | $3.1 \times 10^3$ | $2 \times 10^4$ | $4.9 \times 10^3$ |
| 15th day | S | S | S | S | $5.5 \times 10^4$ | $9.9 \times 10^2$ | $3.3 \times 10^4$ | $9.7 \times 10^2$ |

Mean values in the same row that are not followed by the same letter are significantly different (p<0.05). S: spoiled, SE: Standard error.

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#### Table 5. Reduction percentage of Enterobacteriaceae count (CFU/g) in the treated kofta samples with sumac spice extract at concentrations of 1%, 2.5% and 5%

| Day       | Group 1 | Group 2 (1%) | Group 3 (2.5%) | Group 4 (5%) |
|-----------|---------|--------------|----------------|--------------|
|           | Mean    | Mean Reduction | %    | Mean Reduction | %    | Mean Reduction | %    |
| Zero-day  | $2 \times 10^3$ | $6.3 \times 10^3$ | 0.8670 | 68.7 | $2.9 \times 10^2$ | 0.852 | 85.2 | $2.1 \times 10^2$ | 0.895 | 89.5 |
| 3rd day   | $6 \times 10^3$ | $5.2 \times 103$ | 0.1435 | 14.35 | $2.9 \times 10^3$ | 0.508 | 50.8 | $2.1 \times 10^3$ | 0.646 | 64.6 |
| 6th day   | $1 \times 10^4$ | $9.1 \times 10^3$ | 0.12 | 12 | $8 \times 10^3$ | 0.223 | 22.3 | $7.1 \times 10^3$ | 0.314 | 31.4 |
important food poisoning pathogens associated with products are S. aureus and Enterobacteriaceae. The availability of many herbs and spices nowadays makes the use of their extracts an effective alternative to synthetic preservative which poses a public health hazard. Sumac spice extract can be used in kofta by different concentrations during processing to increase the shelf life and reduced the microbial load of S.aureus and Enterobacteriaceae in examined kofta samples during storage.

Conflict of interest
The authors declare no conflict of interest.

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