The Effects of Autoclaving, Salt and Protein on Antimicrobial Activities of Iranian Sumac

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Abstract

Although many compounds have already approved for use in food as antimicrobials, research for finding greater number of these compounds are still interesting because most currently approved food antimicrobials have limited applications due to food compound interactions. Finding new food antimicrobials requires expensive investigations. Traditional herbal antimicrobial agents like sumac can play an important role. If it is demanded to promote sumac to the rank of a food antimicrobial agent, its stability and interactions must be evaluated. The evaluation of sumac heat stability was done by measuring MICs and MBCs of the extract against some food- related bacteria in extreme condition of autoclaving. The main components of sumac extract are tannic compounds. Tannins have interactions with salt and proteins, and they become sediment in such conditions. Antimicrobial activities of the extract were measured by well diffusion method in the presence of salt and protein and compared with the activities of pure extract. The results show that sumac is heat stable but has interactions with salt and proteins that reduce its activity against Gram- negatives.

Keywords: Iranian sumac, Autoclaving, Food interactions, Minimum inhibitory concentration (MIC), Minimum bactericidal concentrations (MBC)

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Introduction

One of the major advances in human history is ability to preserve food and inhibit food spoilage by preservation techniques, namely food antimicrobials. Food antimicrobials are compounds added to or present in foods that retard microbial growth or kill microorganisms. Although many compounds have already approved for use in food as antimicrobials, research for finding greater number of these compounds are still interesting because most of the traditional, currently approved food antimicrobials have limited applications due to food compound interactions. Interaction with food components makes food antimicrobials less available to inhibit microorganisms of food products and a good food antimicrobial agent should have least such interactions. It should be also non-toxic, non- allergenic, cheap, and stable to any processes to which it is exposed [1]. Finding new antimicrobial food preservative with such characteristics requires many time consuming and expensive investigations. For instance, a novel food antimicrobial agent should pass generally very strict toxicological tests to be approved by international regulatory agencies.

Recent studies showed that herbal antimicrobials can play an important role. Because one way of indicating non- toxicity of antimicrobials is their continuous consumption as a food over a long period, spices can be good candidates for investigating food antimicrobials [1]. Sumac is an Iranian spice used vastly in Iranian cuisine. Antimicrobial activities of this spice were reported in our previous studies [2, 3]. But if it is demanded to promote sumac to the rank of a food antimicrobial agent, its stability and interactions must be evaluated.

This study tries to assess stability of antimicrobial effects of sumac during autoclaving. Also, regarding this issue that most of the food products have -more or less- some amounts of salt or protein, this research seeks possible interactions of these components with sumac.

Materials and methods

Microorganisms and growth conditions

Two Gram-negative and one Gram-positive standard and one clinical isolated food-related bacteria were used in the experiments. *Staphylococcus aureus* 6539-P, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027 were stocked in the Department of pharmaceutical and Food Control, School of Pharmacy, Medical Sciences/ University of Tehran while *Salmonella typhi* was a clinical strain isolated and identified in the microbiological control laboratory of the department. Stock cultures of bacteria were kept in 20% glycerol PBS (phosphate buffered saline) at −70 °C. Active cultures were generated by inoculating 100 µl of the thawed microbial stock suspensions into 5 ml nutrient broth (Merck, Germany) followed by overnight incubation at 37 °C. Freshly synchronized cultures of bacterial strains were prepared by successively transferring 100 µl of the vegetative cells into Muller Hinton broth and incubating for 24 h at 37 °C. The cells were harvested by centrifugation at 1600*g* for 10 min, washed with PBS, spun at 1600*g* again and diluted in sterile water to obtain 10^8 cfu/ml as estimated by the surface plate counting method [4].

Sample preparation

Iranian sumac which is the ground unsalted epicarps of *Rhus coriaria* L. is available in the local market in two forms of brown (ripened) and brown red (semi ripened). The brown ripened sumac used in this study was

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purchased from the local botanical market and were identified by the Herbarium of the School of Pharmacy (TEH), Tehran University of Medical Sciences. Water extracts of the specimen was prepared by maceration method using 40°C sterile distilled water. Extract was concentrated in a vacuum rotary evaporator (Buchi, Switzerland) and were dried by incubation in 40°C.

Stability determination during autoclaving

500 mg of the sumac dry extract was diluted in sterile distilled water to make a concentration of 50% (w/v). The extract was divided into two parts. One part was filter sterilized and the other was exposed to autoclaving condition (121°C and 1 atm) for 15 minutes. To determine the minimum inhibitory concentration (MIC), serial dilutions of the samples were prepared between 0.03 to 3.75% (w/v) in Muller Hinton broth. Final concentration of bacteria in individual tubes was 10^6 cfu/ml. Control tubes contained no extract. After overnight incubation at 37°C, the test tubes were examined for possible growth and MIC of each part of the extract was determined as the lowest concentration that ended with no growth. Tubes containing concentrations above the MICs were streaked onto Muller Hinton agar plates to achieve minimum bactericidal concentrations (MBC) of individual samples against the tested strains.

Determination of possible interactions with salt and gelatin

The main components of sumac extract are tannic compounds. Tannins have interactions with salt and proteins [5]. To evaluate the effects of these interactions in antimicrobial activities of the extract, 1 ml of sumac extract (20%) was added to 2 ml of a gelatin solution (1%) or a saline solution (10%) separately and consequently, nearly all of the tannic compounds of the extract were separated in the form of sediment. After centrifuging for 10 minutes at 5000g, 100 µl of the transparent and tannin-less supernatant was examined against *Staphylococcus aureus* and *Pseudomonas aeruginosa* by well diffusion method. After overnight incubation at 37°C, the inhibition zones around wells were measured in millimeter using a caliper.

Results

Determination of the stability of sumac antibacterial activity in high temperature was done through evaluating MIC and MBC of the extract before and after autoclaving. The results show that antimicrobial activity of sumac extract on all of the test bacteria except *P. aeruginosa* is stable and heat-resistant (Table 1).

Table 2 shows the effect of food/ sumac interaction on antibacterial activity of the extract. Food/ sumac interaction has no destructive effect on anti-Gram positive activities of the extract in spite of ruinous effect on its anti-Gram negative activities.

Discussion

Ideally, a good antimicrobial preservative should have a strong antimicrobial activity, no interaction with food components, no negative effect on food taste and appearance, and also should be non-toxic, non- allergenic, cheap, and stable to any processes to which it is exposed [1]. Antimicrobial effects of sumac extract were evaluated in our previous studies [2, 3]. In this research, the stability of antimicrobial effects of sumac extract during high temperature was evaluated using extreme conditions of autoclaving. The results show that antimicrobial activity of sumac extract on all of the test bacteria except *P. aeruginosa* is stable and heat-resistant (Table 1). This virtue

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Table 1. Antibacterial activity of sumac before and after autoclaving

	S. aureus		*E. coli*		*P. aeruginosa*		*S. typhi*	
	MIC[a]	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Before autoclaving	0.10	0.95	0.45	0.95	0.45	0.95	0.45	0.95
After autoclaving	0.10	0.95	0.45	0.95	0.95	0.95	0.45	0.95

[a] Results are given in %(w/v).

Table 2. The effect of food/ sumac interaction on antibacterial activity of sumac.

	Extract	**Extract + Gelatin**	**Extract + NaCl**	**Gelatin**	**NaCl**	**sediment**
S. aureus	21[a]	21	21	----	----	12
P. aueruginosa	20	14	16	----	----	10

[a] Results of well inhibition zone are given in millimeter.

of the extract is very important because combination use of preservation techniques is usual and therefore, the extract can be used in combination with thermal methods. Moreover, spices usually have microbial burden that if it be high, spices should be considered as a source of contamination instead of food antimicrobials. Like others, sumac has microbial burden and with eliminating the contaminations, autoclaving will guarantee the safety of using sumac in food processes [6].

Another purpose of this study was to assess possible adverse effects of food/ sumac interaction on antimicrobial activities of sumac extract. The main compounds of sumac, tannins, have interactions with salt and proteins that make tannins biologically inactive [5]. As mentioned in Table 2, the interactions have no destructive effect on anti-Gram positive activities of the extract in spite of ruinous effect on its anti-Gram negative activities. Reduction in anti- Gram negative activities of sumac is a lack point because most of the food-born bacteria are Gram negatives. More investigation on the effect of food/ sumac interaction on antimicrobial activities of sumac extract is recommended.

To evaluate the effects of interaction, this study suffices for preliminary antimicrobial tests (well diffusion method) because more quantitative test like determining the MIC of tannin-less extract did not be comparable with MIC of tannin-containing extract, because of changing in ratios after separation of tannins.

According to the results, unlike Smooth Sumac (*Rhus glabra* L.), a similar species, non- tannic compounds may be responsible for some antibacterial activities of Iranian sumac (*Rhus coriaria* L.) [7]. The reduction in the activity of the extract against *P. aeruginosa* shows that tannic compounds could be in charge with anti- Gram negative activities of sumac.

Besides all the antimicrobial advantages and disadvantages, sumac has some other characteristics. It can enhance taste and appearance of food products. As a representative, in Iranian cuisine sumac is used as a flavor and color additive. Furthermore, some studies showed antioxidant effects of

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sumac [8, 9]. More investigation on capability of being sumac as a food preservative and additive is one of our future works.

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