The Effect of Arak Stems Extracts on Chemical Characteristics, Bacterial Activity and Sensory Evaluation of Beef Sausage Products.

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***Abstract*—**The use of natural antimicrobial agents, a safe and ecological approach to increase the shelf life and enhance food safety, has gained increasing attention in recent years. The antibacterial activity of aqueous stem extracts of *Salvadora persica* L. were evaluated on the microbial growth of different bacterial lines by determining inhibition ratio. In addition, these aqueous extract was tested as natural preservative agent in beef sausage by determining the total bacterial count and sensory quality characteristics of products. The extract was prepared by soaking arak stem in distilled water (10g/100ml, w/v) for 48 hrs. the tested extract exhibited effectiveness for preventing growth of some spoilage bacteria, and exhibited the strongest inhibitory effects against the bacterial growth of (Streptococus mitis, Streptococcus salivarius, Streptococcus mutans, Staphylococcus aureus, Bacillus sabtilis, Pseudomonas earuginosa, E. coli, Salmonella typhimurium, and Candida albicans). The inhibition ratios were ranged from 50-100% depending on bacterial cultivar. Also, total viable plate count and coliform group count were lower than control sample. Moreover, the organoleptic results of beef sausage products revealed that there are no significant differences between samples in all sensory attributes. This study proves the effectiveness of arak as antibacterial agent and a bio preservative agent and recommends its potential employing in acceptable meat processed products where spoilage is caused mainly by microbial activity.

***Keywords*—** Antibacterial plants, Arak, Beef sausage products, Natural preservative, *Salvadora persica*.

I. Introduction

FOOD quality deterioration due to a many physical, chemical, enzymatic and microbiological reactions. The various forms of spoilage and food poisoning caused by micro-organisms are preventable to a large degree by a number of preservation techniques, most of which act by preventing or slowing microbial growth. The survival and/or growth of infectious pathogenic bacteria or the growth of toxinogenic ones [1]. Preservatives are natural or synthetic substances that add to prepare food items, cosmetics and pharmaceuticals in order to increase their shelf life and maintain their quality and safety by inhibiting, retarding or arresting their fermentation, acidification, microbial contamination and decomposition [2]

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In the recent years, consumers have become more concerned about the processed food they eat. Synthetic preservatives, which have been used in foods from long periods, may lead to the injury of many diseases [3]. Besides, the use of synthetic compounds have significant drawbacks, such as increasing cost, handling hazards, concerns about residues on food and threat to human environment [4]. Therefore, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. Those are, in the extracts and essential oils of spices and herbs [5]. Arak (*Salvadora persica* L.) is a desert plant which grows from north-western India to Africa [6]. Leaves make good animal feed and are rich in minerals, the fresh leaves are eaten as salad [7]. Branches and roots of *Salvadora persica* are widely used as a teeth cleaning stick [8]**.** Arak contains important phyto-constituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts as chlorides [9]**,** sulphur [10]**,** organic sulphur compounds [11] and lignan glycosides [12]. Pharmacological studies indicated that *S. persica* L. plant possess anti-microbial, anti-plaque, aphrodisiac, alexiteric, analgesic, anti-inflammatory, anti-pyretic, astringent, diuretic and bitter stomachic activities [13], [14]**,** anticonvulsant ,and Antiulcer activity [15]**,** [16], hypoglycemic effect and it reduced body weight [17]. Arak has great medicinal use in the treatment of stomachache, toothache, treat hook worm, to lower cholesterol plasma levels, and reestablishment of the components of gastric mucosa [18].

Salvadora persica and its derivatives can also be used as antimicrobial agents; alcoholic and aqueous extracts have strong antibacterial activity on *Streptococcus mutans, Lactobacillus acidophilus, Aggregati bacter, actinomycete mcomitans, Porphyromonas gingivalis*, and *Haemophilus influenzae* [19]**,** and *Candida albicans* [20]. Furthermore, antifungal, antibacterial, and antioxidant activities of this plant have also been reported by [21], [22]. Despite, the importance of the various activities of this plant related to food safety and preservation, but it has not received much attention as a natural food preservative agent. Therefore, the present study demonstrates the possibility of using the aqueous stem extract of this plant as antibacterial and a natural food preservative agent as well as its effects on beef sausage microbial profile and organoleptic characteristics.

II. Materials And Methods

A. Plant Material

Dried stems of *S. persica* were purchased from a local market in Almadinah Almonawarah city, KSA, were cut in 2014-2015 season. Aqueous Arak Stem Extract (AASE) Preparation Air dried stem of arak was cut into small pieces and grounded with a grinding machine into powder. Two quantities, each ten grams of powdered dried stem arak (dry weight) were macerated in 100ml of sterile de-ionized water (ratios 10%, w/w) in sterile screw capped bottles at 40^{o}C for 48hr to obtained (AASE). The extracts were centrifuged at 3000 rpm for 15min. Then, supernatants were sterilized by passing through filter paper (0.45µm pore size). Then it kept in refrigerator at 4^{o}C until used within one week [23].

B. Microrganisms

A total of nine microbial strains including *Staphylococcus mitis, Streptococcus salivarius, Streptococcus mutans, Staphylococcus aureus, Bacillus sabtilis, Pseudomonas earuginosa, E. coli, Salmonella typhimurium* and *Candida albicans,* were used in the presented study. These strains were obtained from the culture collection of Department of Microbiology, Faculty of Pharmacy, Tanta University, Egypt.

C. Determination of the Minimum Inhibitory Concentrations of Arak Extracts

The minimum inhibitory concentrations (MICs) of the aqueous arak extract was determined by the agar diffusion method [24]. Agar plates containing medium composed of double strength Muller Hinton plus one of the following concentrations of each extract (15%, 30% and 50%). Sterile de-ionized water was used to adjust the final concentrations. The tested bacterial strains (10^4/spot) were inoculated onto the surfaces of the agar plates by using the multipoint inculcator. The plates were then incubated at 37^{o}C for 24hr before defining the MICs.

D. Beef Sausage Preparation

Spices were obtained from local markets from Giza, Egypt. Each spice was powdered in the laboratory in an electric mill. Spices mixture was prepared according to [25] as follow (beef lean 680g, beef fat 150g, ice 100g, sodium chloride 18g, skimmed milk 43g, powdered rusk 0.4g, sodium tripolyphosphate 11g, fresh garlic 0.3g, sodium glutamate 1g, ascorbic acid 1g, and powder spices mixture 9.3g). Beef lean were minced twice with 10% water or arak stem extract as ice flakes, aiming to keep the mixture smooth as well as to minimize temperature rise and microbial growth during shopping. The other ingredient were added and mixed together, then meat mixture was ground for 10 minutes using a meat grinder. The obtained emulation was than stuffed into previously cleaned and prepared natural mutton casings. All sausages were packed in polyethylene bags, placed in cooler 4 to 5^{o}c for 6 hours then part of sausage was examined (zero time analysis), while the rest of samples were frozen at -20^{o}c for different time before analysis.

* Powdered spice mixture (fennel 59.76, coriander 27.99, cubeb 3.42, black pepper 3.42, clove 3.42, laurel leaves 1.99%)

E. Cooking Methods

Frozen beef sausage were thawed at 5^{o}C in refrigerator and cooked by fraying individually in little amount of sunflower oil at 165^{o}C for 5min/side according to the method described by **Cannel *et al*. (1989)** [26].

F. Sensory Evaluation for beef sausage

Beef sausage containing aqueous arak extract with different concentrations were subjected to sensory evaluation by ten trained panelists from the staff members of the national research center, Giza, Egypt. According to the procedure of [29], the sensory evaluation was carried out for color, taste, odor, texture and overall acceptability of produced beef sausage.

G. Statistical Analysis

The obtained results of sensory evaluation were statistically analyzed using SPSS statistical package (Version 9.05) according to [30]. Analysis of variance (ANOVA), Duncan's multiple range test and least significant difference (LSD) was chosen to determine any significant difference among various treatments at $p \leq 0.05$.

III. Results And Discussion

A. Antibacterial Activity of Aqueous Arak Stem Extract (AASE)

Data in table 1 exhibited the antimicrobial activity of aqueous arak stems extract (AASE). The obtained results demonstrated that (AASE) was more effective than by concentrate increasing to all examined microorganisms.

TABLE 1
THE PERCENTAGE OF MINIMAL INHIBITORY CONCENTRATIONS FOR AQUEOUS ARAK STEM'S EXTRACTS (AASE) AT DIFFERENT LEVELS

Microorganisms types	15% extract	30% extract	50% extract
Streptococcus mitis	50	100	100
Streptococcus salivarius	60	100	100
Streptococcus mutans	60	100	100
Staphilococcuse aureus	70	100	100
Bacillus sabtilis	30	100	100
Pseudomonas earuginosa	20	40	50
E. coli	100	100	100
Salmonella typhimurium	60	100	100
Candida albicans	40	95	100

The highest growth inhibition was obtained for *Streptococcus* strains with a ratio of inhibition (50-60), 100 and 100% at 15, 30 and 50% concentration of arak extract, respectively. The most resistant bacteria strain was *Pseudomonas earuginosa* which was low affected with all treatments exhibiting 20, 40 and 50% growth inhibition at 15, 30 and 50% concentration of arak extract, respectively. These results are in harmony with those concerning the essential oils of *S. persica* which have a considerable effect on several

aerobic bacteria as reported by [18], [21], [31], [32]. Also, it was reported that the volatile oil of Jordanian *S. persica* exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria [18]. The current and reported results clearly showed that both water soluble and fat soluble constituents of arak have antibacterial activity.

These antimicrobial properties of *S. persica* may be attributed to various chemicals in the extracts such as sodium chloride, potassium chloride, vitamin C, salvadourea, salvadorine, saponins, silica sulfate compounds, isothiocyanate, tannins, tannic acid, benzyl isothiocyanate, alkaloids, terpenoids, oleic, linoleic and stearic acids, chloride, sulphate, thiocyanate, nitrate and resin [33], cyanogenic and lignin glycosides [12].

B. Determination of Gross Chemical Composition of beef sausage Products

The percentage of gross chemical components; moisture, crude protein, crude fat, ash and carbohydrates content of beef sausage samples treated with (AASE) levels were determined and the obtained results are tabulated in Table 2. A linear relationship between chemical composition values and the increasing of aqueous arak stem extract concentrations as showed in table 2.

TABLE 2
PROXIMATE ANALYSIS OF BEEF SAUSAGE TREATED WITH AQUEOUS ARAK STEM EXTRACT (AASE)

Components %	Treatments			
	Control sample	15% arak extract	30% arak extract	50% arak extract
Moisture	65.31	65.49	65.74	65.91
Crude protein*	61.28	60.71	60.22	59.60
Crude fat*	20.00	19.19	18.65	18.29
Ash*	9.5	10.21	11.02	11.61
Carbohydrates*	7.23	7.15	6.92	6.88

*On dry weight basis; **Values present the means of triplicate determinations

Crude protein, crude fats, and carbohydrates content of the treated beef sausage were decreased. On the other hand, moisture and ash were increased. This may due to the high content of aqueous arak extract of minerals such as sodium chloride, potassium chloride and silica sulfate and low content of other components as crude protein, crude fats and carbohydrates as reported by [33,34].

C. Microbiological experiments

The total count of bacteria, detection of microorganisms and total coliform bacteria of treated beef sausage were carried out and the obtained results are tabulated in tables 3, 4 and 5.

1) *Total Count of Bacteria in Beef Sausage Products:*

Data in table 3 revealed that the increasing of concentration levels of aqueous arak stem extracts in the beef sausage decreased the growth rate of total viable count compared with control sample. The most effective treatment was at 50% of (AASE).

TABLE 3
TOTAL VIABLE PLATE COUNT (log10 CFU/g SAMPLE) OF BEEF SAUSAGE INCORPORATED WITH DIFFERENT LEVELS OF AQUEOUS ARAK STEM EXTRACT (AASE)

Treatments / Assay time	Control	15% of (AASE)	30% of (AASE)	50% of (AASE)
Zero time	6.22	6.21	6.21	6.22
3 days	6.25	6.24	6.24	6.22
6 days	6.48	6.45	6.41	6.33
9 days	6.71	6.60	6.49	6.38
12 days	6.85	6.72	6.59	6.41
15 days	7.18	7.02	6.85	6.65

This could be attributed to the increasing amount of antibacterial agents of arak by increasing the extract concentration and maceration time. These results were in agreement with that reported by [21,34].

2) Detection of microorganisms in produced beef sausage:

Results in table 4 exhibits that all samples were free from microorganisms such as *E. coli*, *Salmonila* and *Staphylococcus aureus.* On the other hand, colifom bacteria group was detected in all samples, this may be due to a contamination of meat with coliform bacteria group by water during washing after sludge.

3) *Total coliform bacteria in produced beef sausage:*

The following results as per inhibition were thus obtained. All the examined aqueous arak stems extracts against coliform bacteria were found effective as bacterial suppressant. The interesting observation was found that as the concentration level of (AASE) increased the growth rate of colifom bacteria group was decreased as showed in table 5.

TABLE 4
DETECTION OF MICROORGANISMS IN BEEF SAUSAGE TREATED WITH DIFFERENT LEVELS OF AQUEOUS ARAK STEM EXTRACT (AASE)

Treatment	E. coli	Salmonila	Staphylococcus aureus	Coliform group
Control	N.D	N.D	N.D	D
15% of (AASE)	N.D	N.D	N.D	D
30% of (AASE)	N.D	N.D	N.D	D
50% of (AASE)	N.D	N.D	N.D	D

D. Sensory Quality Characteristics for Beef Sausage As Affected By the Incorporation of Aqueous Arak Stem Extract (AASE)

The effect of addition of aqueous arak stems extract (AASE) on sensory quality characteristics, juiceness; tenderness; odor; flavor; texture; color and general acceptability of beef sausage, and the obtained sensory judging scores were listed as in table 6.

TABLE 5
TOTAL COLIFORM BACTERIA (log10 CFU/g SAMPLE) OF BEEF SAUSAGE INCORPORATED WITH DIFFERENT LEVELS OF AQUEOUS ARAK STEM EXTRACT (AASE)

Treatment	First week	Second week	Third week
Control	2.82	3.51	6.14
15% of (AASE)	2.95	3.07	3.91
30% of (AASE)	2.91	3.00	3.44
50% of (AASE)	2.88	2.92	2.96

From the obtained results in (Table 6), it could be illustrated that the sensory scores of the most evaluated organoleptic quality characteristics of cooked beef sausage slightly decreased or were not affected with increasing concentration level up to 50% of (AASE). The produced beef sausage had good sensory quality and acceptability. On the other hand, cooked beef sausages containing 50% of (AASE) exhibited a slightly significant reduction in the judging scores of the organoleptic quality characteristics; especially odor, flavor and color. This may be attributed to the herbal flavor.

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TABLE 6
INFLUENCE OF AQUEOUS ARAK STEM EXTRACT (AASE) ON SENSORY CHARACTERISTICS OF PRODUCED BEEF SAUSAGE BATCHES

Treatment	Juiciness	Tenderness	Odor	Flavor	Texture	Color	General acceptability
Control	9.8±0.21[a]	9.8±0.31[a]	10.0±0.00[a]	10.0±0.01[a]	9.8±0.20[a]	9.9±0.12[a]	9.8±0.16[a]
15% of (AASE)	9.4±0.26[a]	9.7±0.25[a]	10.0±0.01[a]	10.0±0.01[a]	9.8±0.22[a]	9.9±0.11[a]	9.5±0.31[a]
30% of (AASE)	9.5±0.30[a]	9.8±0.11[a]	9.80±0.11[a]	9.90±0.11[a]	9.7±0.28[a]	9.8±0.14[a]	9.3±0.35[a]
50% of (AASE)	9.7±0.19[a]	9.8±0.18[a]	9.30±0.24[b]	9.40±0.20[b]	9.8±0.31[a]	9.4±0.20[b]	9.1±0.41[b]
L.S.D**	NS	NS	0.5	0.42	NS	0.33	0.5

*Mean of sensory characteristics score: mean of each organoleptic characteristic score obtained from 10 panelists; the means within the same column having different superscripts are significantly varied (at p≤5)

IV. CONCLUSION

Arak has been recognized as a potential safe food and pharmaceutical ingredient. Aqueous extract of arak's stems has good antibacterial activity and it may be added to beef sausage components at levels approximately 50% as a natural food preservative. This can be done not only with improving shelf life period of product but also without adverse effects on sensory characteristics. These current findings have shown the potential use of aqueous arak's stems extract as a food bio-preservative and a safety food additive.

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