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The Effect of *Cuminum cyminum* Essential oil on Growth and Survival of *Staphylococcus aureus* during Storage of Hamburger

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Abstract

In recent years, due to transformed resistance form of pathogenic bacteria to chemicals the application of herbal essential oil as natural preservatives has been paid much attention. The objective of this study was to determine the antimicrobial effect of cuminum cyminum essential oil on Staphylococcus aureus in hamburgers. The essential oil was extracted through steam distillation and its components were identified by mass spectrometry – gas chromatography (MS-GC). The extracted essential oil was added in concentrations of 0.03, 0.015 and 0.0075% to hamburgers which had been infected with103 cfu/g S. aureus. The data was statistically analyzed by use of software SPSS 18 with the confidence level being 95%. The results of Gc were identified 23 compounds in 96% of essential oil. The main compounds found in the essential oil were phenyl propanol (28.21%), γ - terpinene (23.9%), β -pinene (14.72%), cumin aldehyde (14.31%) and p- cymene (8.13). The result showed that the number of bacteria significantly decreased as the concentration of essential oil increased. However, no variations were observed in chemical parameters. The best essential oil concentration regarding inhibitory effect against bacterial growth as well as manufacturing hamburgers with savory properties was 0.03% followed by 0.0075 %. The results demonstrated that Cuminum cyminum essential oil at high concentrations holds a great antimicrobial potential, thus it may be used as a substitute for chemical preservatives for controlling Staphylococcus aureus caused poisoning resulting from consuming meat products.

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Introduction

Staphylococcus well Salmonella as as Cholestridium perfringence are among important pathogenic bacteria contributing to food poisoning (Ultee et al., 2002; Sadeghi et al., 2013). Staphylococcus grows in foods at 7-48 °C, pH 4.9 and salt concentration of 7-10% producing enterotoxin. The produced enterotoxin is resistant to heating such that it not eliminated through cooking, pasteurization and drying. It matters in consumption of meat products because the bacterium and its produced toxin have high stability in this type of foods (Jay, 2000). Staphylococcus - caused poisoning is characterized by extraintestinal infections, abdominal pain, nausea, headache etc (Jay, 2000). Since this bacterium is widely distributed in foods and it is difficult to completely eliminate it from some foods, preservatives should be used in order to control its growth (Blackburn and peter, 2002). Herbal essential oil generally recognized as safe (GRAS) have antimicrobial activity against a wide range of microorganisms which not only extend the shelf - life of foods but also they do not hazard the health of consumers due to their natural compounds (Kotzekidou et al., 2008). The mechanism of action of the plant essential oil is partly associated with their hydrophobic attribute as they enter into the lipid fraction of cellular membrane and promote the cellular contents to exit the cell thereby disturbing their structure. The essential oil also affects the enzymatic function as well as the synthesis of cellular constituents (Pol et al., 2002; Burt,2004). Cumin Cuminum L. cyminum belongs to Apiacea family which has long been used as spice and medication in traditional medicine (Khatibi et al., 2008). The main compounds found in Cuminum cyminum essential oil are cuminal, cumming alchol, γ- terpinene, and βpinene all exerting an antimicrobial effect in addition to having a characteristic aroma (Oroojalian et al., 2009; Li, 2009). The results of research have shown that Cuminum cyminum essential oil at low concentrations has comparable effects to the standard antibiotics (Sinh et al., 2002) and play very effective role in inhibiting growth of some important pathogenic bacteria (Iacobelis et al., 2005; Nostro et

al., 2005). However, little information is available concerning the inhibitory effects of this essential oil on certain foods such as meat products. This study thus is aimed at determining the effect of different concentrations of Cuminum cyminum essential oil on chemical parameters and growth of *Staphylococcus aureus* in hamburgers and the generalization of the results if possible, to food industries at large scale.

Materials methods

Materials

Cuminum cyminum plants was purchased from Medicinal Plant Research Institute of Jahad, Staphylococcus aurus ssp. ATCC6538 was obtained from Department of Microbiology, Faculty of Veterinary Medicine, Tehran University. Hamburgers were obtained from a local meat products plant in Tehran and transferred to the laboratory.

Essential oil analysis

The essential oil was extracted from the aerial parts of Cuminum cyminum plants through steam distillation by Clevenger system (Baydar *et al.*, 2004). Then the compounds were identified by use of gas chromatography – mass spectrometry (GC/ MS) of thermo quest Finnegan type. The characteristics of GC/MS were as follows: capillary column of 30 m long, internal diameter of 250 μm , internal layer thickness of 0.25 μm , temperature 50-265°C, gradually increase to ca. 2.5°C, per min and keeping at 265°C, for 30 min. The temperature of injection chamber was 250 °C, with the rate of helium being 1.5 ml/ min, energy of ionization (EI) of detector was 70 eV and the temperature of ionization source was 250°C .

It should be noted that the spectra were identified by using their inhibitory index along with mass spectra of standard compounds and software wiley (Marriott *et al.*, 2001; Addams, 2000).

Preparation of Bacteria

Staphylococcus aurus ssp. ATCC6538 was prepared and tested for sensitivity to lysostaphin, heat – resistant thermo nuclease, and coagulase.

The lyophilized bacterium was cultured in BHI broth at 37°C, for 16-18 h with at least two successive culturing so that bacterial count approximated the inoculation dose. Then it was mixed with sterile glycerine in the ratio of 1:5 of which 500 µl was poured into eppendorf microtubes and kept at -20°C, for later use. The second culture was also prepared (Basti *et al.*, 2007).

Bacterial inoculation

To prepare the bacterial inoculation, the culture stored at -20°C was transferred to BHI broth (merc, germany) and kept at 37°C for 16-18 h. Then another culture from the previously developed culture was prepared on BHI broth at 37°C for 18 h. Next, 5 ml of the broth was added to sterile cuvetts and their absorbance was read at 600 nm by using spectrophotometer (Hewlet Packard). Cuvetts were used for enumeration of bacteria of which the cuvett containing 1×10³ cfu/ml was selected. It should be noted that culturing on agar plate also confirmed the bacterial count. From this cuvett then 10- fold dilutions of 10²-10⁵ cfu/ml were prepared and the cuvett containing 1×10³ cfu/ml was used for inoculation (Sadeghi *et al.*, 2013).

Hamburger preparation

The hamburger samples along with egg yolk suspension were cultured on Baird Parker Agar (BPA; Merck, Germany) and the absence of the bacterium was confirmed. Then, different concentrations of Cuminum cyminum essential oil (0, 0.0075%, 0.015% and 0.03%) were added and 1×103 cfu/g of S. aureus was inoculated and mixed thoroughly. The samples in sterile bags were stored at -12°C at o°C (upon addition of essential oil and bacterial inoculation) as well as 7, 15, 30, 45 and 60 d. All microbial (S. aureus count, total microbial, mold, and yeast count) and chemical (moisture, protein, fat, carbohydrate, ash) tests were performed following the standard procedure (AOAC). It is worthy note that the numbers of visible colonies of S.aureus as an indicator on the repeated B.P.A plates along with selected reservoir for Baird Parker Agar were determined after incubation at 37 °C for 48h. To assess the effect of the essential oil on sensory properties of hamburgers (aroma, taste, and total acceptability) the samples containing different concentrations of the essential oil along with a control sample were fired in oil under the same conditions and then evaluated by a panel consisting of seven trained panelists. In order to determine the aroma and taste characteristics of the samples the palatability test including 1: intolerable, 2: dislike, 3: unattractive, 4: intermediate, 5: like, 6: very like and 7: excellent, was used. 1 meant acceptable and 0 represented unacceptability (Moosavi *et al.*, 2008).

Statistical analysis

To evaluate the effect of the essential oil on microbial and chemical parameters, software SPSS 18 and ANOVA and t-test were used with the significance level being 95%. All experiments were conducted in triplicates and the results were expressed as the mean of data.

Results

The chromatogram obtained from the analysis of GC / MS of the essential oil of cumin is shown in Fig1 and also the results of type and percentage as well as stability time of the compounds are presented in Table 1. The 23 compounds were identified in the essential oil extracted by distillation method which comprised 96.9 percent of the essential oil. The main compounds included phenyl propanol (28.21), γ -terpinene (23.9), β -pinene (14.72%), cumin aldehyde (14.31%), P-cymene (8.13). The carotol showed the smallest amount (0.12) in the essential oil (Table 1). The results of investigation of *S.aurens* growth during different storage periods of hamburgers affected by different concentrations of Cuminum cyminum essential oil are presented in Fig. 2 and Table 2.

The best concentration for inhibition of bacterial growth and manufacture of hamburgers with desirable attributes was 0.03% followed by 0.0075%. As illustrated in Fig 2 concentration of 0.0075% at 0, 15, 30 and 45 days retarded the bacterial growth. Ttest showed that the difference in means between the control and the sample containing 0.0075% essence was significant (p<0.05). For 0.015% a decreasing

trend of bacterial growth was observed from 15 day and log reduction in bacterial count was found at 30 and 40 days.

For 0.03% the growth of bacteria was retarded which continued up until day 60. The decrease was reflected as variation in bacterial log as t-test confirmed the difference in means of control and test groups (p<0.05). The results generally demonstrated that increasing concentration of essence had significant effect on the reduction of S. aureus count. Also the synergistic effects of different forms of essential oil within different storage periods as compared with the control and different studied variables as well as the effect of storage period on the bacterial growth were statistically significant (p<0.05) suggesting an

improvement in the bacterial growth as the storage period extended. Also total microbial, mold, yeast count reduced and no mold and yeast contamination was found. Chemical examination of the samples showed that the contents of protein, fat, moisture, ash and starch (total carbohydrate) did not change following addition of the essential oil. In evaluation of the effect of Cuminum cyminum essential oil on organoleptic properties of hamburgers containing the essential oil and the control (without essential oil) the samples containing 0.0075% essential oil was accepted by the panel as hamburgers with novel aroma competing with the commercial counterparts. All essential oil contained hamburgers were accepted by the panel (Table 3).

Table 1. Results of GC/MS used for Cuminum cyminum essential oil.

No.	Compound	Percentage	Time (min)	
1	1-phenylpropanol	28.21	16.72	
2	gammaTerpinene	23.9	9.97	
3	BetaPinene	14.72	7.64	
4	cumin aldehyde	14.31	15.20	
5	P-cymene	8.13	8.87	
6	BetaMyrcene	1.49	7.85	
7	l-Phellandrene	1.10	8.23	
8	AlphaPinene	1.3	6.39	
9	Alpha-Thujene	0.55	6.21	
10	GammaCurcumene	0.5	21.01	
11	Beta-Farnesene	0.36	20.48	
12	AlphaTerpinene	0.34	8.54	
13	AlphaTerpinolene	0.32	10.56	
14	Calarene	0.25	18.60	
15	Trans-Caryophyllene	0.2	19.70	
16	Cis-Sabinenehydrate	0.18	10.96	
17	Terpineol	0.18	13.20	
18	Alpha-thujenal	0.16	17.68	
19	p-menthe- 1,4- dien- 7- ol	0.16	17.50	
20	Delta.3-Carene	0.15	8.35	
21	2- (2-Hydroxycyclohexyl) – furan	0.14	14.51	
22	Phellandral	0.13	15.96	
23	Carotol	0.12	23.43	
24	Total Trace Elements	3.1	-	

Table 2. Mean of S. aures (cfu/g) count affected by different concentrations of Cuminum cyminum essential oil and storage.

Concentration of Cuminum cyminum	Time (d)					
	0	7	15	30	45	60
0	1.2 *10 ³	2.0*103	9.0*10 ³	3.0*104	3.0*105	4.7*10 ⁶
0.0075	1.1 *10 ³	2.0*103	4.8*103	9.3*103	2.1*105	4.7*106
0.015	1.2 *10 ³	2.0*10 ³	3.9*103	9.3*103	4.5*10 ⁴	2.4*10 ⁶
0.03	1.2 *10 ³	1.8*103	2.9*103	3.0*103	3.04*104	4.4*105

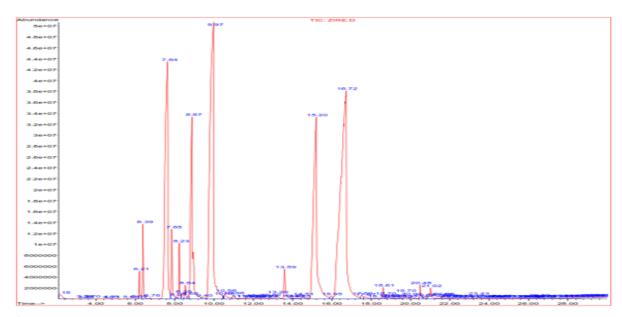
Table 3. Comparison of organoleptic characteristics of hamburgers containing different oncentrations of Cuminum cyminum essential oil1.

Concentrations cyminum (%)	of	Cuminum Taste	Aroma	Total acceptability
0		5.42 ^a	^a O 5.7	a 0.99
0.0075		5.85 ^a	6.42 ^a	a 1.00
0.015		^b 4.14	5.75 ^a	a 0.97
0.03		^c 4.00	5.14 ^a	^a 0.95

a-c significant difference within column at confidence level of p < 0.05.

Discussion

The results of the study on the chemical compounds of Cuminum cyminum essential oil in this work is partly in consistent with other research (oroojalian *et al.*,2009; Sadeghi *et al.*,2013; Iacobellis *et al.*,2005; Bettaieb *et al.*,2010).



Time

Fig. 1. Chromatogram obtained from the analysis of GC / MS of the essential oil of cumin (time chart horizontal axis and the vertical axis is frequency).

In most of these studies the main constituent of cumin essential oil are cumin aldehyde, γ - terpinene, β - pinene and cymene. It should be noted that herbal essences may be varied depending on geographical region, variety, plant age, and method of extraction (Bagamboula). Antimicrobial action of the essential oil is influenced by their chemical composition and antimicrobial activity. Lacobellis *et al.*, showed that cumin aldehyde in the amount of Ca. 16% in Cuminum cyminum essential oil has potent antibacterial effects against gram – positive bacteria. Our results suggest that the amount of this compound is largely in agreement with the results obtained by Iacobellis. Some research has shown that the essential

oil containing phenolics such as carvacrol, thymol, γ-terpinene and P-cymene hold a great antimicrobial potential which is in consistent with our results. Also in the present study, derivatives of γ- terpinene in the amount of 23.9% were among the main constituents of the essential oil. Despite the promising results of our study burt *et al.*, reported that the antagonistic effect of γ- terpinene against bacteria was weak (Burt,2004). The studies on food – borne pathogens also have shown that gram – positive bacteria are more susceptible to essential oils than gram – negative ones (Karaman Iand *et al.*, 2003; Burt, 2004) This may be due to the fact that lipopoly saccharide cell was of gram – positive bacteria

prevent the hydrophobic lipid from penetrating and accumulating into cell membrane (Bajpai et al.,2008). In general the studies conducted on plant essential oil have revealed that the essential oil prolong bacterial lag phase and retard the growth in log phase. Their action is more associated with their accumulation in lipid bilayer of cell membrane and its decomposition (Valero and Giner, 2006; Tassou et al.,2000). Another part of findings revealed that hamburger may be a proper food model bearing Cuminum cyminum essential oil. The Cuminum cyminum essential oil at different concentrations significantly retarded the growth of S.aureus and prevented the bacterium from growing to reach its toxic dose (106 cfu/g). Given the significant decrease in bacterial count the required amount of essential oil for development of desirable sensory variations in foods does not meet the requirements for controlling the growth of S.aureus. Many studies have been conducted on the antimicrobial effects of medicinal plants on foods because of development of bacterial resistance in recent years along with growing public knowledge about the hazards of preservatives to human health (bakkali et al., 2008; Bonjar,2004; Voravuthikunchai al.,2005). Oroojalian and coworkers studied the effects of Cuminum cyminum essential oil on four bacterial including Staphylococcus aureus obtained promising results. In their study, minimum inhibitory concentration of essential oil was estimated 0.37-3 mg/ml (Oroojalian et al., 2009). Sheikh et al., (2010) investigated the antibacterial activity of cumin on 10 gram - positive and gram - negative bacterial species and determined that cumin may be used as a novel antibacterial source against human pathogens. The findings of their study medicinal plants support the results obtained by the authors. Also Jirovetz et al., studied the effect of Cuminum cyminum essential oil on different gram - positive species including S. aureus was incapable of inhibiting the growth of S. aureus showing weak antimicrobial effect. Our results are not in agreement with the findings of Jirovetz et al., However, Kamali and Gackkar found a potent antimicrobial activity of Cuminum cyminum essential oil extracted by water distillation against S.aureus being consistent with our results (El-kamali *et al.*,2009; Gachkar *et al.*,2007). In the study carried out by Ozcan and Erkmen, on the antibacterial activity of the essential oil of nine medicinal plants including Cuminum cyminum against different bacteria including *S.auresus* at three concentrations of 1, 10 and 15%, different essential oil showed different effects. In this study, the essential oil individually or in combination with each other was effective in inactivation of spoilage bacteria (Ozcan and Erkmen). Our findings suggest antimicrobial activity of Cuminum cyminum essential oil being in agreement with other research.

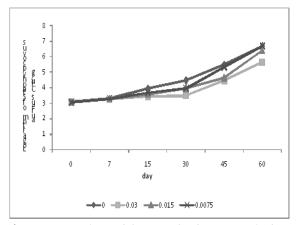


Fig. 2. Comparison of the growth of *S. aureus* in four groups with and without Cuminum cyminum essential oil in hamburgers.

Conclusion

Given the results obtained from the present study it may be concluded that Cuminum cyminum essential oil could be used as a natural preservatives flavoring and antibacterial agent along with other natural protective ways against bacteria in many food products such as hamburgers and meat products in order to reduce or substitute chemical and synthetic preservatives.

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