European Online Journal of Natural and Social Sciences 2014;
Vol.3, No.4 pp. 950-957
ISSN 1805-3602
www.european-science.com

Antimicrobial Effect of Dill Seed Oil Essence on Growth of *Staphylococcus Aureus* in Hamburgers

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Received for publication: 14 June 2014.
Accepted for publication: 17 October 2014.

Abstract

In the present study, antimicrobial effect of dill seed's oil in 6 levels (0.003-0.006-0.0125-0.025-0.05-0.1 percents) and 4 time period of 10, 20, 30, and 40 days on the growth of staphylococcus aures and total amount of microorganisms in preserved hamburger at the temperature of -18±1 °C was considered. Also, to find out about the effect of dill seed essence addition to hamburger on sensuous characteristics of the product, sensuous evaluation based on a 5-point hedonic scale was performed in three sections of taste, odor, and total reception. Results obtained from microbial experiments showed reduced staphylococcus aureus amount and total amounting, and these changes were significant in concentrations over 0.0125 ($P<0.05$). Among essence concentration levels, the least and most effects were determined to be those of 0.003 and 0.1, respectively. Sensuous evaluation results revealed that as dill essence concentration increased to 0.0125 percent, it had a positive effect on sensuous characteristics of hamburger. In higher concentration, however, it reduced sensuous characteristics of the product. These variations were significant in the concentration of 0.1 compared with other treatments ($P<0.05$). According to obtained results, dill seed's oil essence had a harnessing and lethal impact on staphylococcus of hamburger, and it can be used as a natural preserver in meat products especially hamburger.

Key words: Staphylococcus aureus, dill seed essence, antimicrobial activity, and hamburger

Introduction

Meat and meat products constitute one of the most important food resources in individuals' daily diet, its usage being affected by different factors. Using such products has had eye-catching increase due to ease of use and lack of need for cooking most of them, thus providing a considerable part of nutritional needs of society, especially for youths and adolescents (jime'nez. F et al., 2001). On the other hand, in recent years people have shown much interest in using functional foods, especially in cases where meat has been used in the mixture, for example in meat products like hamburger could be mentioned. However, increasing number of reports concerning diseases transmitted via food and especially secondary contamination of food products during post-processing stages brings up the issue of food material health that has led to consumers' concern as well as that of producers and other operators involved in food industries (Reji & Aantrekker., 2004). Hamburger is a meat product that has popular usage due to different reasons such as ease of use, use of meat in mixture and it pleasant taste. Given the fact that hamburger is a raw product till it is consumed, it is necessary to control the microbial quality of this meat product (Fernan'dez et al., 2005). One of the most important pathogen microorganisms in meat products like hamburgers is

staphylococcus aureus which causes staphylococcal poisoning, threatening general health for the community (Hall, 1997). Propagation of such diseases in connection with food along with social and economic issues thereby brings forward the necessity of producing healthier food materials and, therefore, using new compositions that are as much as possible non-synthetic and natural. Nutritional infections control has become possible since the discovery of chemical drugs and anti-microbial compounds. However, some bacteria are resistant to compounds mentioned above (Essawi & Srour, 2000). In addition, there are concerns regarding side effects of chemical preservers on consumer health that have focused attentions to use natural antimicrobial materials and replace chemicals with them (Mitscher et al., 1987). Meanwhile extensive researches have been conducted with the aim of finding antimicrobial properties of herbal extracts on a broad spectrum of bacteria, yeast, and fungi e.g. herbal essences of cinnamon (Farag et al., 1989), mountain pennyroyal (Zaika & Kissenger,1981), mint and thyme (Aktug & Karapinar, 1986) that could be mentioned as the most important of these studies. Dill with the scientific name of *anethum graviolens* is a member of the *Apiaceae* family. Dill was first planted in Palestine and is has probably been transferred from the Ancient Rome to other European amountries (Omidbaigi, 2001). Dill is planted extensively in Iran, Caucasia, Ethiopia, Egypt, India, England, Spain, Italy, and Hungary. Its geographical diversity in Iran has been mentioned, naturally, in different regions of Tabriz, Khorasan, and Tafresh. Dill is a one-year plant, with whole growing stalk of it containing essence, though its amount differs in different limbs. The color for dill essence is clear and its odor is relatively acute like that of cumin (Zargari, 1997). Anti-fungous and anti-bacterial effect of dill seed essence (Delaquis et al., 2002) and its acetone extract (Singh et al., 2005) has been proved. Thus, this study was conducted with the aim of finding anti-microbial effect of dill seed essence on the growth of staphylococcus aureus in one of the most popular meat products i.e. hamburgers to make antimicrobial effect of this herbal essence more clear.

Materials and methods

Preparation

Dill seed's oil essence : Essence used was obtained from *Giah Esans* Company located in Gorgan Amounty.

Hamburgers: 90% hamburgers were obtained from *Kaleh Food Industries Factory* located in Amol Amounty and transferred to laboratory.

Microbial genealogy under study: The standard staphylococcus aureus ATCC-29737 required for addition to hamburgers were obtained in the form of lyophilized ampoules from the collection of bacteria and fungi of the Iranian organization for scientific-industrial studies.

Activating microbial genealogy

According to the instruction of the manufacturer, the lyophilized ampoule containing standard genealogy of staphylococcus aureus was scratched from above the cotton pile. Then the area around the ampoule was disinfected completely using sterile gauze dampened with 70 degree-alcohol. The ampoule wrapped by sterilized gauze was broken from the scratch point. Cotton pile was removed with the help of a sterilized forceps; in sterilized conditions under a hood and using a sterilized Pasteur's pipette of 0.5 ml, 0.3—0.4 ml from culture area of sterilized liquid of Merck was added to existing dry material in the ampoule and after achieving consistency, a microbial suspension was obtained. The resulting suspension was mixed via sterilized anise, and part of that was transferred for the purpose of creating mother culture on nutritional agarics environment (Difco, Laboratories), and culturing was done. Next, for staphylococcus aureus to grow, culture environments containing germs were kept in stove for 24 hours at 37 °C. After the duration, another culture was obtained on nutritional agarics as preserved culture to be used in subsequent stages.

Producing 0.5 standard solution of McFarland

One method to determine bacteria amount in liquid environments is indirect amounting. In the turbidity measurement method which is one of the most popular methods for indirect amounting turbidity of the liquid environment in which the bacteria grew is compared via a standard whose turbidity is proportional to a certain number of bacteria. Standard turbidity can be created with certain compositions of chemicals one sample of which is barium sulfate, whose intensity has been evaluated via McFarland with an estimated bacteria amount. McFarland standards are obtained by adding a certain volume of 1% sulfuric acid solution and 1.75% choleric barium to get barium sulfate solution with certain light density, and usually the 0.5 standard McFarland, produced by adding 9.95 ml of pure sulfuric acid of 1% volume (36% normal) to 5% ml of barium choleric of 1.75% (48% molar), is mostly popular. McFarland's 0.5 standard creates turbidity equal to that of an equivalent bacterial suspension.

Preparation of microbial suspension of 0.5 McFarland

For each series of experiments, fresh 24-hour culture of staphylococcus aureus is needed. So a culture was obtained from spare culture (sub-master) and young fresh 24-hour culture, whose microorganisms are in their active phase, was used in work. So 24 hours before experiment, insemination was done from the spare culture to nutritional agar steep culture environment and was kept in stove for 24 hours at 37 °C. To make microbial suspension after growing related culture, colonies of its surface was rinsed with normal saline solution of 0.9% and a thick suspension of microbes was obtained. Then a small amount of this thick microbial suspension was poured in sterilized spiral pipes using a sterilized Pasteur pipette, and then by adding the normal saline solution, the thick suspension was thinned to an extent that, compared with the 0.5 standard McFarland, suspension absorption (turbidity) in wavelength of spectrometer's 530 nm was equal to absorption (turbidity) of the 0.5 McFarland solution. Therefore, a suspension with an approximate thickness of 1.5×10^8 cfu/ml (10 microorganisms in each ml) is obtained, which was used in insemination. Next by doing necessary computations, it was determined that 3.5 ml of the abovementioned suspension is required to achieve final thickness of 5×10^5 for staphylococcus aureus. Using hamburger compound on a Baird-Parker agar environment (Merck, Rahaway, NJ) with yolk suspension (Merck, Rahaway, NJ) and plate amount agar (Laboratorries Difco) culture was done and 1000 grams of hamburger was immediately mixed with 3.5 ml of suspension containing staphylococcus aureus. From the abovementioned contaminated compound, culture was done to control staphylococcus thickness in mentioned environments.

In next stage, under sterilized conditions, ten 20-gram samples was weighed from resulted compound in a sterilized plastic container and were chosen as control samples. After that, 100 grams of the contaminated hamburger was weighted and 0.1 ml of dill seed's oil essence was added to that and completely mixed. Then five 20-gram samples of this hamburger was weighed and filled, under sterilized conditions, into plastic disposable containers with a 40 ml capacity and refrigerated at -18 °C. In other treatments also, 0.05, 0.025, 0.0125, 0.006, and 0.003 ml of dill seed's oil essence were added to hamburgers respectively, and after weighing in sample sizes of 20 grams, they were filled in intended plastic containers and refrigerated.

Over time periods of 0 (1^{st} day of work and after research), 10, 20, 30, and 40 days, two samples were picked from each treatment, and to measure growth of staphylococcus aureus and get total amount, surface culture and compound culture were used in Baird-Parker agar and Plate amount agar environments, respectively. They were then kept in stove for 24 hours at 37 °C. To amount and identify microorganisms, amounter colony model of *Funker-Gerbe* and model BH2 of the *Olympus* electronic microscope were used. Different treatments have been specified in table 1 with described symbols.

Microorganisms total amount (TC) test

Amountable colonies in this test consist of bacteria amount, yeast amount, and mould amount. Suspension preparation and thinning this microbial test were done according to standard 356 and related standards as follows:

After preparing the sample for consistent distribution of microorganisms, the sample container was stirred completely before test. To get 10^{-1}tenuity from each hamburger sample, 10 grams of hamburger was completely blended under sterilized conditions with 90 ml of sterilized physiological serum and then to get a 20^{-2}tenuity, one milliliter of tenuity 10^{-1} was added with sterilized pipette to 9 milliliters of sterilized physiological serum, and it was stirred to get consistent. To get a 10^{-3} tenuity one milliliter was picked from tenuity 10^{-2} as before and poured into 9 milliliters of sterilized physiological serum. This was done for lower tenuities as well. For all samples to get consistent, all pipes were placed in shaker for 15 seconds.

Next, one milliliter of tenuities 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, etc. obtained above was transferred to two series of plates. Culture environment of plate amount agar melted and cooled at 45±1 °C was poured into sterilized plates in 15 milliliter amounts, with all plates getting stirred 5 times clockwise, 5 times amounter clockwise, and 5 times horizontally on desktop surface near flame so that the culture environment was consistently mixed with the sample. After solidifying culture environments, plates were inverted and were incubated at 30±1 °C for 72 hours. After this duration elapsed, the plates were removed from incubator and all colonies developed in the culture environment was accurately amounted using a colony amounter. After doing total amount of microorganisms, plates with 2 sequential tenuities having at least 15 and at most 300 colonies were chosen for amounting, and their mean was calculated using the following formula.

$$colonies\ count\ mean = \frac{total\ colonies\ of\ two\ sequential\ tenuities}{volume\ inseminated\ suspension\ in\ culture\ environments \times tenuity\ coefficient}$$

Table 1. Types of treatments and their abbreviations

Abbreviations	Type of treatment
C	Hamburger contaminated with staphylococcus aureus without essence (control)
G1	Hamburger contaminated with staphylococcus aureus+0.003 % of essence
G2	Hamburger contaminated with staphylococcus aureus + 0.006 % essence
G3	Hamburger contaminated with staphylococcus aureus + 0.0125% essence
G4	Hamburger contaminated with staphylococcus aureus + 0.025 % essence
G5	Hamburger contaminated with staphylococcus aureus + 0.05 % essence
G6	Hamburger contaminated with staphylococcus aureus + 0.1 % essence

Sensuous evaluation

Evaluation of sensuous characteristics was done based on 5-Point Hedonic Scale. First 0.1-0.05-0.025-0.0125-0.006-0.003 % of dill seed essence were added to hamburger samples and after 72 hours of preservation, burgers were fried at 180 °C for 10 minutes, and the sample without essence was used as the control sample. Burger samples were coded. Then required questionnaire was obtained and given to the qualified panelist. 15 lab students and experts were chosen as panelists. Panelists rated the samples in terms of taste, odor, and general acceptance by choosing among choices of *very good=5, good=4, medium=3 (neither good nor bad), bad=2*, and *very bad=1*.

Statistical analysis

All experiments were conducted in three iterations separately, with analysis of data being done by SPSS™ 18. Also, means comparison was done with LSD test in statistical level ($P<0.05$). By the way, data in tables and figures have been presented as mean plus standard deviation. Also, all curves and charts were drawn using Excel™ 2007.

Results and discussion

As shown in table 2, regarding the effect of different dill seed essence concentrations on amount of staphylococcus aureus in burgers during preservation, amount of staphylococcus aureus in the control sample has increased till the 20th day, with the amount fixed and slightly changed between 20th and 40th day. It is explained that from the 1st day to 10th day of preservation growth had a quicker intensity. This trend of early increase was caused by presence of water, fat, protein, and minerals in food material under experiment. It is obvious that hamburgers contain a considerable amount of these nutritional materials which increase microbial resistance. Another cause can be considered to be the resistance of staphylococcus aureus to freezing. Difference between control sample and treatment G1 during preservation was not significant at all experiment time points, and no considerable conflicts were seen ($P<0.05$). In treatments G3 and G2, a considerable effect was not seen on amount of staphylococcus aureus, but after tenth day this difference grew with a significant different emerging on 20th day. After 20th day until end of preservation amount of staphylococcus aureus declined. Regarding treatment G4, it is seen that it has controlled staphylococcus amount with a significant difference so that staphylococcus amount did not grow considerably until the end of preservation. This decrease in amount was so that on final day microorganism growth was investigated and these treatments approximately remained on their initial state and their amount was not increased. This state does apply for treatment G5 as well, where staphylococcus aureus did not grow and this concentration was not able to control completely amount of this microorganism, and decrease their amount after 20th day on, so that it caused decreased microorganism amount with a significant difference compared with the control sample. The Best performance in connection with inhibiting staphylococcus aureus growth in hamburgers concerns treatment G6 i.e. concentration 0.1 % essence of dill seed in the hamburger, where essence not only inhibited growth of staphylococcus amount but also decreased its number and growth from early days ($P<0.05$).

Table 2. Mean of staphylococcus aureus in different treatments in hamburgers during preservation (Log cfu/g)

Day					
treatments	0	10	20	30	40
C	$5.22^{a}\pm0.02$	$5.62^{ab}\pm0.11$	$6.51^{a}\pm0.08$	$6.41^{a}\pm0.04$	$6.46^{a}\pm0.21$
G1	$5.22^{a}\pm0.02$	$5.69^{a}\pm0.07$	$6.43^{a}\pm0.05$	$6.37^{a}\pm0.10$	$6.27^{a}\pm0.14$
G2	$5.22^{a}\pm0.02$	$5.51^{bc}\pm0.01$	$6.12^{b}\pm0.14$	$5.92^{b}\pm0.08$	$5.71^{b}\pm0.03$
G3	$5.22^{a}\pm0.02$	$5.46^{bc}\pm0.06$	$5.91^{c}\pm0.15$	$5.86^{b}\pm0.11$	$5.41^{c}\pm0.09$
G4	$5.22^{a}\pm0.02$	$5.52^{bc}\pm0.09$	$5.6^{d}\pm0.01$	$5.62^{c}\pm0.06$	$5.18^{d}\pm0.14$
G5	$5.22^{a}\pm0.02$	$5.39^{cd}\pm0.11$	$5.51^{d}\pm0.06$	$5.35^{d}\pm0.03$	$5.09^{de}\pm0.03$
G6	$5.22^{a}\pm0.02$	$5.29^{d}\pm0.11$	$5.24^{e}\pm0.06$	$5.16^{e}\pm0.06$	$4.99^{e}\pm0.05$

Difference in the amounts shows a significant difference between numbers of each column ($P<0.05$). The most and the least effect of dill seed essence on decreased staphylococcus amount in hamburger concerns concentrations 0.003 and 0.1 respectively. It is worthy of note here that in

0.0125 treatments and beyond it, staphylococcus aureus amount on 40th day approximately was equal to initial amount and sometimes less than initial amount of mentioned microorganisms. This exhibits one of unique traits for dill seed essence.

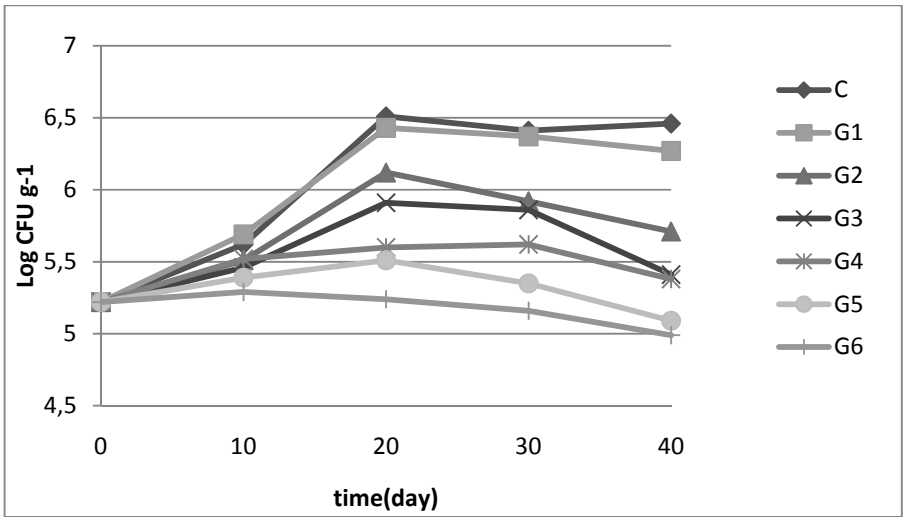

Figure 1. Staphylococcus aureus amount change in different treatments over 40 days of preservation

Table 3. Mean and total amount of staphylococcus aureus in different treatments in hamburgers during preservation (Log cfu/g)

Day					
treatments	0	10	20	30	40
C	6.32^{a} ±0.02	6.54^{a} ±0.11	6.81^{a}±0.08	6.87^{a}±0.04	6.9^{a}±0.21
G1	6.32^{a}±0.02	6.41^{b}±0.07	6.68^{b}±0.05	6.67^{b}±0.10	6.51^{b}±0.14
G2	6.32^{a} ±0.02	6.39^{b}±0.01	6.4^{c}±0.14	6.31^{c}±0.08	6.11^{bc}±0.03
G3	6.32^{a}±0.02	6.07^{d}±0.06	6.16^{d}±0.15	6.09^{d}±0.11	5.67^{c}±0.09
G4	6.32^{a}±0.02	6.12^{c}±0.09	5.98^{e}±0.01	5.88^{e}±0.06	5.5^{c}±0.14
G5	6.32^{a}±0.02	6.01^{e} ±0.11	5.79^{f}±0.06	5.68^{f}±0.03	5.34^{c}±0.03
G6	6.32^{a}±0.02	5.98^{e}±0.11	5.64^{g}±0.06	5.51^{g}±0.06	5.21^{c}±0.05

Different numbers denote significant difference between numbers in each column (P<0.05).

As mentioned earlier, to find out about the effect of adding dill seed essence with different concentrations on taste, odor and general acceptance, sensuous evaluation was done with 5-Point Hedonic Scale. According to table 4, no significant difference percent has been seen concerning taste by increasing the essence to concentration of 0.0125, and essence addition affected positively taste, thus improving it (P<0.05). Of course this difference was not significant but as essence concentration increased from 0.0125 to 0.1 percent, it significantly decreased satisfaction level in terms of taste of hamburgers (P<0.05). As regards the effect of essence addition on hamburger odor, results showed that as essence increased to concentration of 0.003 percent, product odor improved and results differences revealed that these changes were significant in odor (P<0.05). Similar to the factor of taste, a reverse effect is observed as essence concentration increased so that in treatment 0.1 percent, it considerably led to aggravated odor of the product. Regarding general acceptance, the results were approximately similar to factors of taste and odor, so that concentrations of 0.003 and

0.006 had the best sensuous traits. In general, it can be stated that as essence amount increased it positively affected sensuous traits so that in lower concentrations (0.003 and 0.006 percent) this amount is more than that of the control sample, but it had reverse effect as essence amount increased, thus reducing sensuous traits intensively.

Table 4. Evaluation of dill seed essence addition with different concentrations in hamburgers

Treatments	taste	odor	general acceptance
C	4.2^{a}	3.9^{b}	4.1^{b}
G1	4.3^{a}	4^{bc}	4.2^{b}
G2	4.3^{a}	4.2^{a}	4.4^{a}
G3	4^{a}	3.7^{c}	3.9^{c}
G4	3.8^{b}	3.5^{d}	3.9^{c}
G5	3.5^{c}	3.4^{d}	3.6^{d}
G6	3.2^{d}	2.8^{e}	3.1^{e}

Differences in the amount denote significant difference between numbers in each column ($P<0.05$)

Conclusion

The greatest antimicrobial effect of dill seed essence regards concentration of 0.1 percent. As essence concentration decreased, antimicrobial effect decreased so that in the 0.003 percent concentration no significant difference was observed ($P<0.05$). Also as essence concentration increased to 0.0125 percent, sensuous traits of the product slightly improved. However as essence concentration in hamburgers grew to 0.1 concentration, these characteristics decreased intensively so that in these treatments significant differences were observed compared with other treatments ($P<0.05$). Given the consumer tendency and emphasis of WHO on using natural preservers for food materials and this study's results, dill seed essence can be used as a natural additive with antibacterial traits. Also, effects of dill seed essence on other microbes such as mould and yeast can be investigated.

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