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Evaluating the Antimicrobial Effect of *Zataria multiflora* Essential Oil on *E. coli* 0157:H7 in MDM (Mechanical Deboned Meat) on Different Days of Storage in Refrigerator

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Due to the fact that the *Zataria multiflora* essential oil has antimicrobial effects on some food pathogenic microbes, the aim of the study is to seek to determine the effects of different *Zataria multiflora* essential oil concentrations of (0, 0.005, 0.015 and 0.03%) on *E. coli* 0157:H7 growth and survival in MDM (mechanical deboned meat) on different days (10 days) storage in refrigerator. The plants essential oil was prepared using the distilled water and the Essential oil analysis was performed by gas chromatograph connected to mass spectrograph (GC/MS). The antimicrobial effects of different *Zataria multiflora* essential oil concentrations were evaluated for the bacterium growth in a sample culturing condition in a laboratory. The results showed that by increasing the essential oil concentration, the bacterium growth rate in the storage period would take a downward trend and decrease; and the concentration level of %0/03 had the highest antibacterial effects which was statistically significant ($P<0.01$). It was also stated that the *E. coli* 0157:H7 bacterium population in MDM (mechanical deboned meat) with different *Zataria multiflora* essential oil concentrations kept at 4 ^{o}C showed a higher degree of reduction compared with the 10 ^{o}C which is considered to be unfavorable temperature condition and shows the storage key role for maintaining a better condition.

Key Words: *Zataria multiflora*, Essential oil, *E. coli* 0157:H7, Chicken meat dough.

Regarding the contamination of the slaughtered chicken and lack of sanitation observation in slaughterhouses and low carrying temperature and insanitary bone removal, these kinds of meat are encountered with higher loads of microbe load ingression. The removed bones used in the MDM (mechanical deboned meat) are highly contaminated. Chicken meat used as fillers is remarkably used in meat products, but it causes general health threats due to its high contamination (Vermozy, 2002). Refrigerating is a way to store and preserve the products in which for increasing the storing duration, the antioxidants are used all of which are synthetic (Imaida, 1983).

The plant essential oil significance is in the fact that besides they provide a better flavor and taste in the food, their effective materials have antimicrobial phenol effects, which make people,

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go for these materials rather than using the chemical ones (Burt, 2004). The *Zataria multiflora* belongs to the Lamiaceae family which grows in Iran, Pakistan and Afghanistan and has gone under so many researches and studies on its components and its essential oil inhibitory features on pathogenic bacteria or food poisoning agents (Basti *et al*., 2007: Ebrahimzadeh *et al*., 2003: Fazali *et al*., 2007: Moosavy *et al*., 2007 and shatifi far *et al*., 2007). And the Carvacrol is considered to be one of the most significant antimicrobial phenol compounds of the *Zataria multiflora* and it also has Tanoan, Phelovnoied, Saponin and other bitter products (Burt, 2004). *E. coli* 0157:H7 is a warm anaerobe bacterium and is considered to have less infective activity (50-5 organism) and is highly pathogenic (Betts, 2000) and cause hemorrhagic colitis, hemolytic anemia due to uremia syndrome (HUS) and Thermbosytopatic purpura , which can lead to death (Karmali, 1998).

For the practical application of the plant essential oils as preservatives in foods, evaluating their antimicrobial effects single-handedly and in combination with other factors (such as storage temperature, etc.) will be important and required in the food-borne pathogens growth and food poisoning microorganisms in vitro and food models condition (Lemay *et al*., 2002). The aim of the study is evaluating the antimicrobial effect of the *Zataria multiflora* essential oil on *E. coli* 0157:H7 in chicken bone paste on different days of storage in refrigerator.

MATERIALS AND METHODS

The *Zataria multiflora* was taken from the Fars province and the name was scientifically approved by a botanist and plant expert at Jihad medicinal plant institute, then, the plants were dried in the sun shade and dried plant shoots were distilled using Clevenger apparatus for 3 hours.Then, the essential oil analysis was performed by gas chromatograph connected to a mass spectrograph (GC/MS).

The Microorganism under study and its preparation process for inoculation

E.coli 0157: H7 (Atcc 25922) bacterium provided and donated by the Veterinary Microbiology Department at Islamic Azad University of Tehran, Science and Research branch was prepared. A colony of these bacteria was activated in two consecutive times in brain heart infusion broth at 35 °C for 18 hours. After the second 18 h culturing, different amounts of the were added to Covet tubes containing 5 ml sterile BHI broth and the light absorption at 600 nm wavelengths was measured and determined. Then, The Covet tubes containing 1×10^8 bacteria were determined the confirmation of which was done through bacterial linear culturing, therefore, 0.1 ml of the prepared dilutions was transfer to dual plates containing Cyphixim Telluride Sorbitol MacConkey agar condition (CT-SMAC) which contained 0.5 mg Cyphixim and 2.5 mg/l of potassium Telluride.

After 24 h of storage in the warm chamber at 35 °C, Sorbitol-negative colonies (colorless) were counted and 5 to10 colonies were selected for authorization by biochemical tests of Simon Citrate agar, TSI agar, SFM agar and MR-VP broth. The *E.coli* 0157: H7 properties in these tests such as glucose, Andol and methyl were positive, but Citrate, SH2, CO2 and VP were negative. Finally, to determine the Serotype, the agglutination test with specific *E.coli* 0157: H7 anti-serum was used. Several 10 dilutions were prepared from the tube each of which was used to obtain the 10^3 inoculation doses used in this experiment (Bouchra; 2003, Meng *et al*, 2001 and Rasavilar, 1998).

MDM (mechanical deboned meat) preparation

The bones in 20 kg of chicken was removed of which 8.89 kg of separated bones were prepared and was converted into MDM (mechanical deboned meat) by Buder weighing around 5.001 kg, then, they were sterilized into 25 g pieces and were put in sterile plastic in sterile conditions into on which the microbial culturing was used to approve the sterilization.

Bacterium inoculation and essential oil addition to the MDM (mechanical deboned meat)

25 g of the MDM (mechanical deboned meat) with different predetermined concentrations of the *Zataria multiflora* and the bacterium doses were put into the sterilized bags (bag mixer) of 35× 19 cm and the sterile bags were transferred into the Stomacher (interscienceAooVw) and were kept for 1.5 minutes in room temperature to reach the uniform distribution of bacteria and essential oil in the MDM (mechanical deboned meat) then the

closed the Stomacher (interscienceAooVw) are placed at 4 and 10 degree Celsius condition (proper and improper refrigeration temperature) for 10 days and microbial counting were performed every 2 days from day zero to day 10.

Microbial analysis

At the desired times, 225 ml of 0.1% peptone water sterile water was added to (25 g) MDM (mechanical deboned meat) in the Stomacher (interscienceAooVw) bags in sterile condition and were uniformed and homogenized at room temperature by the Stomacher (interscienceAooVw) and then further dilutions were prepared using tubes containing 0.1% peptone water and were cultured in agar plates containing the brain and heart infusion broth (BHI) medium and were placed in warm chamber at 35 °C for 24 h. It is worth mentioning that this experiment was performed in 3 replicates and the results were recorded (Thomas, 2004).

Statistical analysis

Different concentration effects of *Zataria multiflora* essential oils on The *E.coli* 0157: H7 bacterium growth was performed using one-way ANOVA using SPSS Version 16 software and the TUKEY test (Post Hoc LSD) was used to compare the means.

RESULTS

As it can be seen, Table 1 shows the results of *Zataria multiflora* essential oils compounds analysis using GC-MS instrument. The highest compound was the Carvacrol with 59.72.

The results of the inoculated *E.coli* 0157: H7 analysis) 10^3 cfu/g) in MDM (mechanical deboned meat) with different concentrations of (0, 0.005, 0.015 and 0.03%) of the *Zataria multiflora* essential oil after 10 days of storage at 4 and 10 degree Celsius were given in tables 2 and 3.

Table 1. The results of *Zataria multiflora* essential oils compounds analysis using GC-MS instrument

Compound name	Inhibitory index	Percentage (%)
α-Thujene	932	0.29
α-Pinene	938	3.73
Octanol	995	0.28
Octanone	999	0.27
β-Pinene	1006	0.46
β-Myrcene	3101	1.52
δ-Caren	1017	1.49
Para - Cymene	1024	8.01
Dihydrocarveol	1036	0.99
γ-terpiene	6810	7.04
Linalool	4110	1.91
4-Terpineol	1176	0.74
α-Terpineol	2119	0.85
Cumucaldehyde	1197	0.86
Thymol methyl ether	1235	0.70
Carvacrol methyl ether	4012	1.94
Carvacrol	1297	59.72
Terans-caryophyllene	1418	0.36
β-Caryophyll ene	6142	1.73
Aromadendrene	1439	0.82
Spathylenol	1571	0.41
Caryophyll ene	1582	0.39
Total	-	94.51

Table 2. Effect of *Zataria multiflora* essential oils on *E.coli* 0157: H7 growth in MDM (mechanical deboned meat) after 10 days of storage at 10 °C.

Day	Number of bacteria (Log cfu.g)[A] Control group	*Zataria multiflora* concentrations		
		0.005	0.015	0.03
0	3.15±0.11	3.09±0.04	3.00±0.02	3.00±0.04
2	4.51±0.22	3.99±0.06	3.15±0.21	2.98±0.02
4	5.53±0.29	4.24±0.21	3.32±0.32	3.07±0.21
6	6.16±0.72	4.98±0.19	3.74±0.41	3.38±0.44
8	7.39±0.41	5.61±0.47	4.45±0.42	3.47±0.63
10	8.14±0.09	6.27±0.23	4.97±0.15	3.54±0.19

Table 3. Effect of *Zataria multiflora* essential oils on *E.coli* 0157: H7 growth in MDM (mechanical deboned meat) after 10 days of storage at 4 degree

Day	Number of bacteria (Log cfu.g)[A] Control group	*Zataria multiflora* concentrations		
		0.005	0.015	0.03
0	3.01±0.35	3.05±0.11	3.03±0.07	3.02±0.06
2	4.24±0.11	3.69±0.24	3.05±0.08	3.03±0.07
4	5.17±0.04	4.14±0.03	3.12±0.04	3.01±0.06
6	5.33±0.07	4.71±0.05	3.31±0.13	3.04±0.06
8	6.76±0.08	5.14±0.94	4.12±0.11	2.69±0.07
10	7.89±0.06	6.12±0.06	4.78±0.1	3.43±0.1

DISCUSSION AND CONCLUSION

The study shows that the highest concentration of the *Zataria multiflora* of 0.03% at 4 degree Celsius will decrease the bacterium growth if we stretch the storage time ($P<0.01$). The number of *E.coli* 0157: H7 bacterium in the experimental groups with different concentrations of *Zataria multiflora* compared with that of the control group at 4 and 10 degree Celsius showed a significant difference ($P<0.05$).

At the 0.03% concentration level of the *Zataria multiflora* the number of the *E.coli* 0157: H7 bacterium on day 8th reached 2.69 (Log cfu/g) at 4 degree Celsius which remained fixed up to the end of the storage time ($P<0.01$). At 10 degree Celsius the bacterium population in control group showed a permanent upward trend at the time of the storage which reached 8.14±0.09 log cfu/g at the end of the experiment but at the same time at 4 degree Celsius the population density of the bacterium showed to be 3.54±0.19 log cfu/g that represent the positive effect of the *Zataria multiflora* concentrations ($P<0.01$)

Recent years have witnessed a growing interest in studies on using the natural preservatives in food preparation and processing instead of the chemical ones. The primary reason for using these kinds of plant besides having their effective material is their medicinal effects. It should also be noted that plants essential oils improve the food tastes and flavors which make people prefer these materials over the chemical ones (Sharififar *et al.*, 2007). So far, many studies have been conducted on the synergistic effect of various components of plants essential oils in foods to increase their antimicrobial strengths so that the antimicrobial effect of Carvacrol and Thymol in *Zataria multiflora* essential oil reported to have more impact if used simultaneously than each of them in isolation (Basti *et al.*, 2009)

Based on the result of the study, the 0.005% concentration level of the *Zataria multiflora* had less inhibitory effect on the bacterium growth. As it was reported in studies by Friedman *et al* (2002), Marilena *et al* (1999), Mohammadi *et al* (2011), Noori *et al* (2011) and Sagdic (2003) that the plant essential oils had antimicrobial effect on the *E.coli* 0157: H7. It has been reported in many studies that the antimicrobial effect of the essential oils is heavily dependent on the dose amount (Burt, 2004 and Sagdic, 2003) with which the current study reached the same conclusion.

Ozkan *et al* (2003) showed that the *Zataria multiflora* at 0.2 concentration level did not have any antibacterial effect on *E.coli* 0157: H7 using the disc technique while it shows a high activity at 0.4 concentration level. Burt *et al* (2003) found that the *Zataria multiflora* at low concentration of 0.12% and 0.25% have bacteristatic and bactericide effects. Moreover, Sagdic *et al* (2002) reported that the *Zataria multiflora* at 0.5, 1.5 and 2% concentration levels had dose-related bactericide effect on *E.coli* 0157: H7 at nutrient broth for 7 days storage time at 37 degree Celsius. Carvacrol is the most important phenol component of the *Zataria multiflora*, which is revealed to have antimicrobial effects by the

researchers. (Akgul, 1988; Bagamboula, 2004; Bouchra, 2003; Chami, 2004; Lpez, 2005; Didry, 1994; Periago, 2001).

Considering the current study, the coefficient correlation of the essential oil concentration with bacterium count logarithm was negative at 4 and 10 degree Celsius. The coefficient negative nature shows that if we increase the essential oil concentration, the bacterium growth at the storage time will decrease. It was also revealed that the different concentration effects on the bacterium growth rate was significant ($P<0.01$). Based on the results of the study the significant effect of the Zataria essential oil on the given bacterium showed that the inhibitory effect of the essential oil at 4 degree Celsius experienced a major increase at ($P<0.01$) which is congruent with the research findings of many researchers (Blackbum, 2002; Fujikawa, 2006).

In the present study, statistically speaking, the storage temperature and time and also the concentration of the *Zataria multiflora* had a significant effect on the bacterium growth rate ($P<0.01$).Therefore, it can be stated that the given plant essential oil can serve as a natural preservative and an antibacterial product on warm-negative bacterium such as *E.coli* 0157: H7 in MDM (mechanical deboned meat).

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