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Inhibition of *Escherichia coli* O157: H7 in Çemen with Different Garlic Levels

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Abstract: In order to determine the effect of garlic in çemen on reduction of *Escherichia coli* O157:H7, çemen with different garlic levels (0, 7.5, 10.0, 15.0, 20.0 and 25%) were prepared. Each batch of çemen was contaminated with *E. coli* O157:H7 (10^7 cfu g⁻¹) and stored at 4°C for 60 days and 20°C for 60 days in sterile glass jars. *E. coli* O157:H7 counts and pH were determined at various stages of storage. It was determined that both garlic levels and storage temperatures had significant effect on *E. coli* O157:H7 count. *E. coli* O157:H7 in çemen that was stored at 4°C were slowly inhibited than that stored at 20°C. During storage at 4°C, *E. coli* O157:H7 count dropped to below detectable level (<100 cfu g⁻¹) at 30 days in çemen containing 25% garlic while at 45th days in çemens containing 10, 15 and 20% garlic. At 20°C, it dropped below detectable level (<100 cfu g⁻¹) on the 10th days in çemens containing 10, 15, 20 and 25% garlic. Investigating with immunomagnetic separation (IMS), it was determined that the samples with below detectable level (<100 cfu g⁻¹), contained *E. coli* O157:H7.

Key words: *Escherichia coli* O157:H7, çemen, garlic

Introduction

Çemen is a seasoning mixture which is prepared by mixing water with flour *Trigonella foenum graecum* seed, smashed fresh garlic, paprika and red pepper (Aksu, 1999; Tekinşen and Doğruer, 2000; Aksu *et al.*, 2005; Aksu and Kaya, 2005). Çemen is used for pasting of pastirma which is a dry cured meat product. Çemen is rubbed during the curing, pressing and drying meat steps, and contribution a typical color, taste, flavour and aroma into the pastirma. Furthermore, çemen is utilized to protect against putrefaction, spoilage and mould growth by avoiding contact with pastirma (Kök, 1985; Çetin *et al.*, 1997; Aksu and Kaya, 2001, 2002). Also, garlic which is present in çemen prevents to mould growth in pastirma (El-Khateib *et al.*, 1987). Çemen is not only used to the production of pastirma but is also consumed as a breakfast supplement.

Garlic is the main component in çemen and has protective properties Garlic (*Allium sativum*) belongs to the *Liliaceae* family. Garlic bulbs are a rich source of carbohydrates and proteins. Garlic contains from 61 to 64% moisture, 31% carbohydrate, 5 to 6% protein, 0.2% lipid, 3.9 to 4.6 mg g⁻¹ phosphorus, 1.0 to 1.2 mg g⁻¹ potassium and 0.5 to 0.9 mg g⁻¹ calcium (Kaufman *et al.*, 1999). Garlic also contains ascorbic acid, nitrate, nitrite and a hydroxy radical compound [allixin (allicin), diallyl sulfide, allyl sulfide and propylsulfide] (Aguirrezebal *et al.*, 1998, 2000; Susheela, 2000; Milner, 2001; Shukla and Taneja, 2002). Garlic has antimicrobial, antioxidant, antifungal and antiviral activity

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(Iberl *et al.*, 1990; Artık and Poyrazoğlu, 1994). Fresh and grounded garlic has been reported (Siegers *et al.*, 1999; Shukla and Taneja, 2002) to inhibit cancer, caused by polycyclic aromatic hydrocarbons and nitrosoamines.

E. coli O157:H7, the most important member of enterohemorrhagic *E. coli* (EHEC) group, is amongst the most important food borne pathogen (Philips, 1999). It was emphasized that pathogenicity of *E. coli* O157: H7 depended on production of verotoxin and the presence of colonization factors (Bülte, 1995). It is reported that death rate due to the infections caused by *E. coli* O157:H7 is higher than food borne diseases caused by *Camphylobacter* and *Salmonella* although the utter is seen more sparsely (Philips, 1999). Since *E. coli* O157: H7 identification as a human pathogen in 1982 (Boyce *et al.*, 1995), it has been considered to be an important pathogen that can cause serious illness (Eribo and Ashenafi, 2003; Carney *et al.*, 2006). Also, acid adaptation of *E. coli* O157:H7 can enhance its survive in acidic foods (Eribo and Ashenafi, 2003; Bachrouri *et al.*, 2006; Lekkas *et al.*, 2006). Çemen without garlic is a suitable food for pathogen bacteria growth, and it may store at 2-4°C for 2-3 months; therefore, pathogen bacteria growth is a major problem during storage and in the market place. To preserve the quality characteristics of çemens in markets, garlic must be added and the product must be packaged and stored at low temperature. The object of the present study was to evaluate the effect of different levels of garlic (0, 7.5, 10.0, 15.0, 20.0 and 25%), storage time (60 days) and storage temperature (at 4 and 20°C) on the inhibition of *E. coli* O157:H7 in çemens.

Materials and Methods

Preparation of Çemen

Flour from *Trigonella foenum graecum* seed, fresh garlic, paprika and red pepper was utilized as the raw material to produce çemen. Çemens were prepared by mixing of 500 g of flour *Trigonella foenum graecum* seed, 75 g paprika, 75 g red pepper and 1200 mL water (Aksu, 1999). Addition of 0, 7.5, 10.0, 15.0, 20.0 and 25.0% levels of garlic with three replicates were utilized in this research. The çemens were contaminated with a culture of 10^7 cfu g⁻¹ levels *E. coli* O157:H7 for 24 h and was stored for 60 days at 4°C and 30 days at 20°C. The prepared semens was flowed in sterile jars and analyzed over time (0, 5, 10, 15, 30, 45 and 60 days).

Contamination of çemens was with *E. coli* O157:H7 (ATCC 35150, VT-1 and VT-2 positive).

pH Analysis

The pH values of çemen was measured at 0, 5, 10, 15, 30, 45 and 60 days during storage. The pH values of çemens were given by the method described by Gökalp *et al.* (2001) with a pH meter (SCHOTT L 6880, Lab Star pH).

E. coli O157:H7 Analysis

Sorbitol MacConkey Agar (SMAC, Merck) was used for counting of *E. coli* O157:H7. Enumerated of colorless colonies which have growing at the end of incubation for 24 h at 37°C at aerobic conditions. Assays of indole and latex agglutination (*E. coli* O157 Test, Oxoid) were applied to typical colonies (Kofoth *et al.*, 1996). The Immunomagnetic Separated (IMS) method were used in samples which were under the detectable level (<100 cfu g⁻¹) for counts of *E. coli* O157:H7.

Results and Discussion

In Table 1 and 2, it is presented that, the counts of *E. coli* O157:H7 of çemens which were produced by adding different level of garlic and stored at different temperatures. It was determined that levels of garlic have important effects on the *E. coli* O157:H7 count. The increasing level of garlic in çemens, increased inhibition of *E. coli* O157:H7. Also, It was concluded that the temperature of storage have important effects on *E. coli* O157:H7 counts. The inhibition of *E. coli* O157:H7 was lower in çemens stored at 4°C than at 20°C (Fig. 1 and 2). At 4°C, count of *E. coli* O157:H7 decreased under the detectable level (<100 cfu g⁻¹) in 30 days with çemen containing 25% of garlic while decreasing to 45 days in çemen which contained 10, 15 and 20% levels of garlic. In the control groups, reduction was 2.25 log unit during 60 days. However, in çemen containing 7.5% level of garlic, reduction for 3.80 log unit in the end of 60 days was determined (Table 1). As seen is Table 2 and Fig. 1, counts of *E. coli* O157: H7 of the control group samples increased during the first five days of storage. However, reduction of *E. coli* O157: H7 in all samples with add of garlic was obtained. At 20°C, it was also found that the count of *E. coli* O157: H7 decreased under the detectable level (<100 cfu g⁻¹) at 10 days in çemen produced by adding 10, 15, 20 and 25% levels of garlic (Fig. 2). Samples that were under the detectable level (<100 cfu g⁻¹) of *E. coli* O157:H7 were examined by IMS and it was determined that the samples of çemen included *E. coli* O157: H7. In studies with çemens for pastirma, count of microorganisms belong to the family of *Enterobacteriaceae* were <100 cfu g⁻¹ according to El-Khateib *et al.* (1987) and were <10 cfu g⁻¹ according to Kotzekidou and Lazarides (1991). The main component, which has antimicrobial property in garlic, is allixin. Allixin (allicin) is present at the levels of 0.6-1.21% in dried garlic. Formation of allixin affects the allinase (allixin alcyle sulfinate liyase) enzyme which is activated when the cloves of garlic are broken (Iberl *et al.*, 1990; Akgül, 1993; Artik and Poyrazoglu, 1994). In a study done by Rees *et al.* (1993) concentrations of garlic related to microorganisms growth was determined. According to this research, a minimum concentration for

Table 1: *E. coli* O157:H7 (log cfu g⁻¹) counts for çemen with different garlic levels stored at 4°C

Levels of garlic (%)	Time of storage (Days)						
	0	5	10	15	30	45	60
0	6.85	6.30	6.22	6.38	5.30	5.04	4.60
7.5	6.50	6.07	5.74	5.30	4.52	4.07	2.70
10	6.24	6.00	5.85	5.60	3.62	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾
15	6.50	6.08	5.64	5.32	3.00	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾
20	6.46	6.00	5.60	4.48	2.30	<2.00 ⁽⁺⁾	*
25	6.52	6.11	5.11	3.90	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾	*

The results of analysis were shown as mean values of three replicates, *: Analysis wasn't done. ⁽⁺⁾: IMS analyses were found positive

Table 2: *E. coli* O157:H7 (log cfu g⁻¹) counts for çemen with different garlic levels stored at 20°C

Levels of garlic (%)	Time of storage (Days)				
	0	5	10	15	30
0	6.85	8.40	7.70	6.78	4.00
7.5	6.50	5.26	3.00	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾
10	6.24	3.80	2.00	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾
15	6.50	3.62	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾	*
20	6.46	3.34	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾	*
25	6.52	2.52	<2.00 ⁽⁺⁾	<2.00 ⁽⁺⁾	*

The results of analysis were shown as mean values of three replicates, *: Analysis wasn't done. ⁽⁺⁾: IMS analyses were found positive

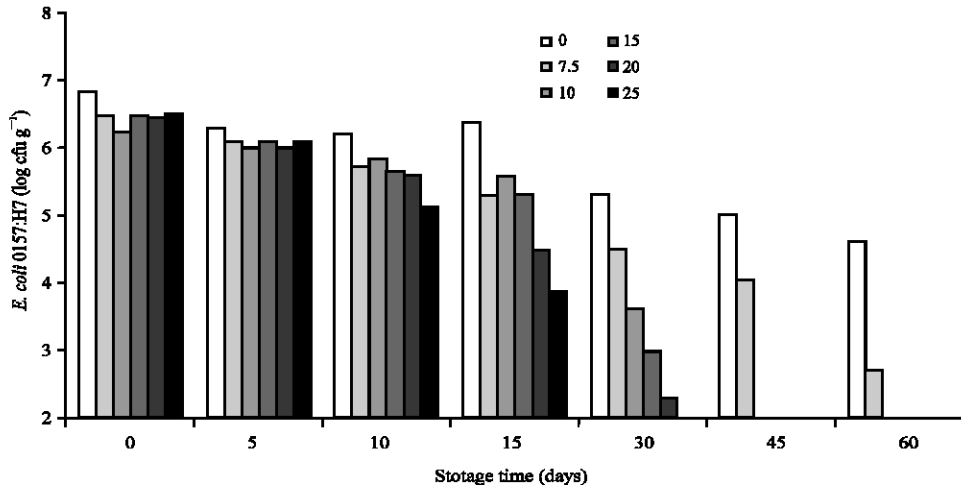


Fig. 1: Effect of storage time on *E. coli* O157: H7 counts of çemen samples stored at 4°C

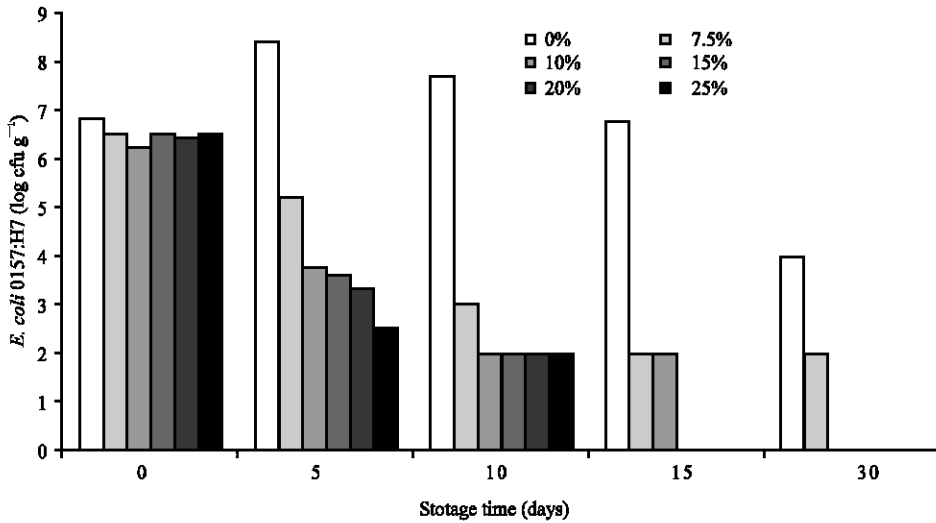


Fig. 2: Effect of storage time on *E. coli* O157: H7 counts of çemen samples stored at 20°C

inhibition of *E. coli* was from 0.6-2.5 mg mL⁻¹. Tekinsen *et al.* (1996) also determined that as the level of garlic decreased, activities of microorganisms increased in çemen. Öz (2000) and Öz *et al.* (2002) determined that counts of *E. coli* O157:H7 decreased in sucuk containing garlic.

It was shown that by increasing the levels of garlic the pH increased of 0 day. The pH value decreased until 45 days of storage in çemen without garlic (0%) stored at 4°C but increased later in storage (Table 3). The pH value was decreased during storage at 20°C in çemen containing 0, 7.5 and 10% garlic. In çemen stored at 20°C, the pH value decreased until the end of 10 days and increased later (Table 4). In a study done by Guraya *et al.* (1998), it were shown that *E. coli* O157: H7 continued

Table 3: pH values for çemen with different garlic levels stored at 4°C

Levels of garlic (%)	Time of storage (Days)						
	0	5	10	15	30	45	60
0	5.83	5.63	5.66	5.81	5.81	5.76	5.90
7.5	5.87	5.78	5.78	5.95	5.92	5.81	5.86
10	5.90	5.83	5.81	5.97	5.96	5.82	5.97
15	5.95	5.88	5.87	6.03	6.00	5.88	6.02
20	5.98	5.92	5.93	6.08	6.05	5.91	*
25	6.01	5.95	5.95	6.13	6.06	5.93	*

The results of analysis were shown as mean values of three replicates, *: Analysis wasn't done

Table 4: pH values for çemen with different garlic levels stored at 20°C

Levels of garlic (%)	Time of storage (Days)				
	0	5	10	15	30
0	5.83	5.13	4.54	4.38	4.19
7.5	5.87	5.64	4.56	4.05	4.06
10	5.90	5.70	5.65	4.46	*
15	5.95	5.77	5.70	5.79	*
20	5.98	5.82	5.70	5.92	*
25	6.01	5.84	5.77	5.96	*

The results of analysis were shown as mean values of three replicates, *: Analysis wasn't done

vitality even with fallen values of pH, a_w and temperature. Again, it were defined that *E. coli* O157:H7 could grow is an environment of 4.5 to 9.0 pH in Trypticase Soy Broth with pH adjusted with HCl but it could not growth under a pH of 4.5 (Glass *et al.*, 1992).

Conclusions

The results from the present study indicate that use of garlic in the çemen production significantly related the *E. coli* O157:H7 growth. Increasing levels of garlic in çemen, caused a reduction of *E. coli* O157:H7 growth. But, it was determined that decreased samples under the detectable level (<100 CFU/g) for *E. coli* O157:H7 count still countered *E. coli* O157:H7 by IMS analyses. However, complete elimination of *E. coli* O157:H7 in çemen even containing 25% garlic was impossible. *E. coli* O157:H7 count were inhibited at a greater amount in çemen stored at 20°C compared to that stored at 4°C. These results confirmed that the garlic has antimicrobial affect on pathogen microorganism such as *E. coli* O157:H7

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