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SENSITIVITY OF THREE PATHOGENIC BACTERIA TO TURKISH CEMEN PASTE AND ITS INGREDIENTS

H. Yetim[1] O. Sagdic[1] M. Dogan[1] And H. W. Ockerman[2]

[1]*Erciyes University, Dept. Food Engineering, 38039, Kayseri, Turkey*
[2]*The Ohio State University Dept. Anim. Science Columbus, OH*

Introduction

Meat spoils readily unless kept at temperatures in the proximity of 0 ^{o}C or is processed immediately. Therefore traditional methods of meat preservation, such as salting and drying, still continue to play an important role in many of the world's less developed countries (Norman and Corte, 1985). Salted and dried meat products with specific textural and eating quality characteristics could be produced to have a useable shelf-life of 3-5 months without noticeable deterioration with unique taste and flavor which makes them a delicacy (Leistner 1987).

Pastirma is one of the salted and partially fermented dry cured meat product which is pasted (outside covered with paste which makes it different from its counterparts) and stored for several months without refrigeration, and it is highly regarded and very popular in most of the Middle East countries (El-Khatib et al. 1987; Tekinsen and Dogruer 2000; Aksu and Kaya 2001). The end product is highly tasty and nutritious, and it contains an average of 45% moisture, 30% protein, 15% fat, 5% carbohydrate (from paste) and 5% ash under normal conditions and may be consumed either raw or cooked. The paste, called cemen used to cover pastirma is prepared from following herbs; 50% fenugreek (ground), 35% garlic (fresh and ground), and 25% red pepper (ground) are mixed with water to make a slurry like paste. It is then placed on the surface of the meat block, approximately 0.3-0.5 cm thick (Gokalp et al. 1999).

There have been some studies for the microbiological stability of pastırma because microbiological contamination may occur during the pastirma production, thereby the risk of spoilage or public health would increase. Cemen paste is the first hurdle to protect the product, but there was very limited research on the cemen paste and its ingredients for their antibacterial activity. For example, El-Khatib et al. (1987) stated that cemen paste had about $1.0x10^{7}$ total bacteria and $<10^{2}$ Enterobacteriacea per gram sample. Nevertheless there is no information on the survival of some well known pathogens in the cemen paste.

One of the main ingredient of the cemen is fenugreek (*Trigonella foenum-graecum*) which is a leguminous plant grown in northern Africa, the Mediterranean, European, western Asia and northern India. Fenugreek seed have been used medicinally and as a spice (food) for many years (Billaud and Adrian 2001). Many research reports have indicated that fenugreek seed reduced the total serum cholesterol levels and lowered blood glucose and peroxidation lipids in plasma (Anuradha and Ravikumar 1998). There have also been many reports about antibacterial effects of garlic (Harris et al. 2001; Sallam et al. 2004; Fernandez-Lopez et al. 2005). The objective of the present study was to evaluate the effects of cemen paste and its basic ingredients for their potential to inhibit some common pathogenic

bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica*) encountered in foods.

Material and Methods

Plant materials: Basic cemen ingredients; fenugreek (Trigonella foenum-graecum), garlic (Allium sativium) and red hot pepper powder (Capsicum annuum) were purchased from retail markets in Kayseri, Turkey.

Preparation of test bacteria: Three bacterial species including Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 2392 and Yersinia enterocolitica ATCC 1501 were used to determine the antibacterial activity of each sample. Bacterial species were obtained from stock cultures and were grown in nutrient broth. E. coli and S. aureus were incubated at 37°C for 18 h, Y. enterocolitica was grown in nutrient broth and incubated at 22°C for 18 h. Broth cultures were prepared overnight in nutrient broth with final cell concentrations of approximately 106-107 cfu/mL.

Preparation of cemen paste: Experimental cemen was made of fenugreek, garlic, red hot pepper powder (RHPP) and water. The 29.1% of fenugreek, 20.4% of garlic, 8.7% of red hot pepper powder and 41.8 of fresh drink water were homogenously mixed and the mixture was aseptically kneaded. Then they were packaged and stored at 4 oC during the analysis.

Chemical analyses of cemen paste: Ten g of cemen was mixed with 10 mL of distilled water, and the pH was measured using a pH meter (Hanna Ins., Italy). Total solid (%) contents were determined by heating at 105 oC to a constant weight. Colour of cemen paste were measured by colorimeter (Lovibond, UK) as L, a and b values.

Determination of inhibitory effects: The inhibitory effects of cemen paste and basic ingredients against bacterial species were measured using serial dilution method. The fenugreek, garlic and red hot paper powder (RHPP) were prepared at the 29.1%, 20.4%, 8.7% concentrations in nutrient broth, respectively. Then, these samples were sterilized in an autoclave. Additionally, the 58.2% (CP1) and 29.1% (CP2) concentrations of cemen paste were mixed in nutrient broth to provide basic nutrients of the bacteria and then they were autoclaved. All the samples were separately inoculated of %1 of fresh bacterial culture (E. coli, S. aureus and Y. enterocolitica). Flasks inoculated with E. coli and S. aureus were incubated at 37 °C for 4 days, Y. enterocolitica was incubated at 22 °C for 4 days. Every day, the number of colonies in the tubes was counted using serial dilution method in nutrient agar. The growth inhibition level (%) caused by each the ingredient of the cemen and the cemen paste on test bacteria was determined according to the following equation:

$$\text{Growth inhibition level}\,(\%) = \frac{\text{control population - treated population}}{\text{control population}} \times 100$$

The control flask was prepared as described above except that the flask contained no added ingredients (Sagdic 2003). Bacterial counts were reported as log cfu/g or cfu/mL.

Results and Discussion

In the physicochemical analysis, the color values of cemen paste were L= 36.49, a= 22.26 and b=40.65, and total solids of cemen samples were average 56.51% with 4.83 pH values.

In this study, mainly the effects of cemen paste and its basic ingredients for their potential to inhibit common pathogens *E. coli, S. aureus and Y. enterocolitica* were evaluated. The growth inhibition levels of cemen ingredients and cemen pastes inoculated with the three pathogenic bacteria are shown in Table 1. In the study, the CP1 (full cemen paste), CP2 (50% diluted cemen paste) and garlic had strongly antibacterial activities against all the bacteria tested. The fenugreek and especially red hot pepper powder (RHPP) had a small inhibitory effect on the test bacteria (Table 1). The effectiveness of the inhibitor materials followed the sequence: CP1>CP2>garlic> fenugreek>RHPP.

Table 1. Growth inhibition levels (%) of cemen paste and its ingredients against some pathogenic bacteria

Cemen ingredients and cemen paste	*E. coli* time (days)				*S. aureus* time (days)				*Y. enterocolitica* time (days)			
	1	2	3	4	1	2	3	4	1	2	3	4
Fenugreek	18	33	54	66	26	54	72	100	34	51	69	78
Garlic	50	74	100	100	82	100	100	100	40	62	87	100
RHPP	10	10	11	12	11	14	13	22	10	10	12	7
CP1	63	100	100	100	61	100	100	100	52	73	100	100
CP2	60	86	100	100	42	85	100	100	47	70	100	100

RHPP: Red hot pepper powder, CP1: full cemen paste, CP2: 50% diluted cemen paste

All the samples had a low or high inhibitory effect against all test bacteria during the 4 days. In general, fenugreek and RHPP had a bacteriostatic effect when compared to the control treatment while the CP1, CP2 and garlic showed bactericidal effect. Again, the CP1, CP2 and garlic samples completely inhibited the tested bacteria in one of the storage time (Table 1). The CP1 was the most active on all bacteria in serial dilution method. Additionally, *S. aureus* was the most sensitive bacterium to the cemen paste or its ingredients while *Y. enterocolitica* was the most resistant bacterium against those samples.

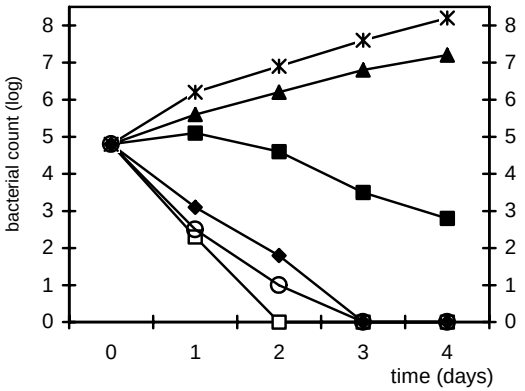

Figure 1. The inhibitory effect of fenugrek, garlic, RHPP, cemen paste against *E.coli*

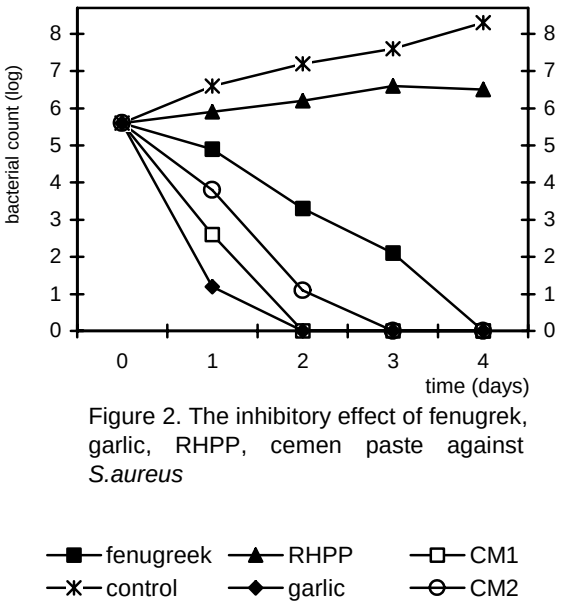

Figure 2. The inhibitory effect of fenugrek, garlic, RHPP, cemen paste against *S.aureus*

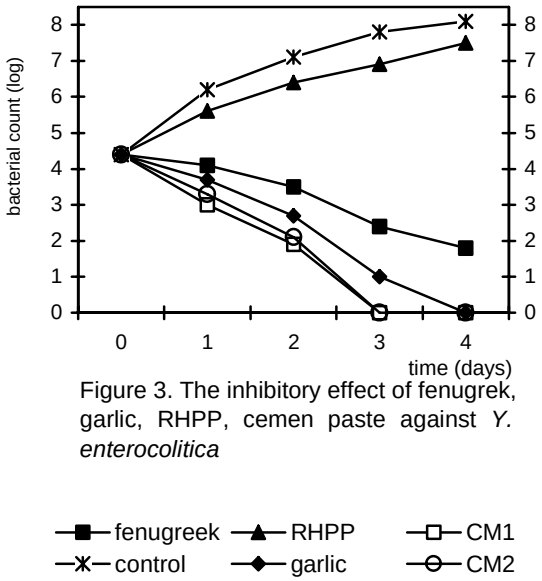

Figure 3. The inhibitory effect of fenugrek, garlic, RHPP, cemen paste against *Y. enterocolitica*

The antibacterial activities of the cemen ingredients and cemen paste against *E. coli* are shown in Fig. 1. Among the tested materials, cemen paste had the highest inhibitory effect against *E. coli*. While CP1 had a bactericidal effect against *E. coli* at the second day, garlic and CP2 had bactericidal effect at the third day for this bacteria (Fig. 1). The inhibitory effect of cemen paste against *E. coli* was higher than that of garlic, this result might be due to the synergistic effect of cemen ingredients. The fenugreek and RHPP showed a slightly bacteriostatic effect to *E. coli* (Fig. 1).

Figure 2 shows the effects of the cemen ingredients and cemen paste against *S. aureus*. The antibacterial activities of cemen paste and garlic were about the same against *S. aureus*. While CP1 and garlic showed bactericidal effect to *S. aureus* at the second day, CP2 and fenugreek had bactericidal effect at the third and/or fourth day (Fig. 2). *S. aureus* was the most sensitive bacterium against cemen paste and its ingredients. Fenugreek had bactericidal effect against *S. aureus* but it had bacteriostatic effect to *E. coli* and *Y. enterocolitica*.

The inhibitory effects of the cemen paste and its ingredients against Y. enterocolitica are shown in Fig. 3. Y. enterocolitica was the most resistant bacterium against the cemen paste and its ingredients. Cemen paste had the highest inhibitory effect against Y. enterocolitica. A significant observation was that the garlic had bactericidal effect to Y. enterocolitica at the fourth day while CP1 and CP2 showed some effect at the third day (Fig. 3). The inhibitory effect of cemen paste against Y. enterocolitica was higher than that of garlic, again this might be resulted from the synergistic effect of cemen ingredients.

As the fenugreek had strong bacteriostatic effect against *Y. enterocolitica*, RHPP showed a quite poor bacteriostatic effect to *Y. enterocolitica*.

Some researchers indicated that garlic had antibacterial activity (Harris et al. 2001; Sallam et al. 2004; Fernandez-Lopez et al. 2005). According to Sagdic et al. (2003) the extract of red pepper had no activity against some pathogenic bacteria. However, there has been a very limited research on the antibacterial effects of the cemen paste and fenugreek. For example, Mansouri (1999) reported that fenugreek had no inhibitory effect against *S. aureus* using ethanolic extract. Current results were different from previous reports for the fenugreek and red pepper (Mansouri 1999, Sagdic et al. 2003). It has been well known that the antimicrobial effects of the cemen ingredients and cemen paste are depended on the species and concentrations of the ingredients, regional conditions and test bacterium strains. Additionally the method used to test antibacterial activity could have an effect on the results, and the differences between the previous reports might be due to these variations.

Based on all of these results, cemen paste on the three pathogens showed the strongest inhibitory effect compared to the cemen ingredients alone. Dogruer et al (1998) reported that cemen paste had a positive effect on the microbiological quality of pastrami, and this resulted from the antimicrobial effects of garlic. El-Khateib et al. (1987) indicated that cemen paste inhibited the growth of moulds on Turkish pastirma. However, antimicrobial activities of cemen paste on pathogenic bacteria were missing.

4. Conclusion

Based on the results of this study, cemen paste and garlic appeared to inhibit the growth of the three pathogens tested. In general, the inhibitory effect of cemen paste was higher than that of garlic. This might be resulted from the synergistic effect of

cemen ingredients. Cemen paste and garlic had completely inhibitive effect on the bacterial growth in broth culture. The results of this study confirmed the protective effect of cemen paste and garlic in food preservation, especially Turkish pastirma a traditional dry cured meat product and sauce. Antibacterial effects of the cemen paste against E. coli, S. aureus and Y. enterocolitica suggest microbiological safety for public health; also low pH (4.83) value would be another hurdle for organism growth. It can be suggested that similar studies should be conducted on actual meat systems to confirm above findings.

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