Inhibition Of E. coli, Salmonella And Staph. aureus Bacteria By Onion And Garlic Extracts In Refrigerated Minced Beef

Abdelfattah M E , Hanan M T Elawendy, Fatma El-S Gaber*

Food Hygiene Unit. Animal Health Research Institute, Egypt.

*Toxicology Unit, Chem. Dept. Animal Health Research Institute, Egypt.

ABSTRACT

Escherichia coli O157:H7, *Salmonella typhimurium* and *Staph. aureus* are of great concern to the food industry, especially in foods stored under refrigerated conditions where, unlike most food-borne pathogens are able to multiply, so this investigation was conducted to study the inhibitory effect of two spice extracts namely onion and garlic commonly used in food industry on the growth of these pathogens. Two different concentrations (1 and 4%) of the spice extracts were used on food model (minced beef) stored at 5°C for 14 days. The results revealed that concentration of 1% of onion extract was less inhibitory than 4%.

However, with garlic extract, high inhibitory effect was observed for two concentrations. *Staph. aureus* showed less sensitivity towards onion extract inhibition, however, the three tested pathogenic microorganisms were significantly inhibited by garlic extract.

INTRODUCTION

Spices are plants with intensive and distinct flavours and aromas used in fresh or dry form. Different spices have been added to various food products for centuries, mainly to contribute to the characteristic flovor of the end product. The earliest report on use of spices as preservative was around 1550 BC., when the ancient Egyptians used these substances not only for food preservation, but also for embalming their dead *(1)*. Spices include leaves (bay, mint, rosemary, coriander, laurel, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander, cinnamon) rhizomes (ginger) and other plant parts *(2)*. The growth of microorganisms in meat products may cause spoilage or food-borne disease. Therefore, the developmental application using natural products with antibacterial activities in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food-borne disease *(3)*. Multiple techniques have been used to investigate antimicrobial activities and the primary data varied considerably in quality and quantity of different spices. Garlic and onion were found to be the most potent spices *(4)*.

Refrigeration is often the main and frequently the only factor to control food-borne pathogens in these types of foods. Hence, temperature abuse of such foods can result to food-borne illness. In addition, some psychrotrophic pathogens can grow in refrigerated food with little or no obvious change of sensory characteristics. Addition of antimicrobial ingredients in combination with refrigeration may compensate for a lack of a terminal heating step and provide additional protection to low temperature alone *(5)*. The antiseptic potential of spices resides in the essential oils. One of the most potent spices is garlic, in which several antimicrobial components are present, the principle active substance was identified as allicin *(6)*. Essential oil fractions of garlic are efficient against Gram-positive as well as Gram-negative food-borne pathogens *(7)*.

Therefore, this work was conducted to study the inhibitory effect of some spices extracts namely onion and garlic on the growth of food-borne bacterial pathogens; *E. coli* O157:H7, *Salmonella typhimurium* and *Staph. aureus* in minced meat stored under refrigerated conditions.

MATERIAL AND METHODS

Bacterial strain: E. coli O157:H7 ATCC43888 was obtained from Candiar Research Institute for Food Safety (CRIFS)'s culture collection.

Salmonella typhimurium and *Staph. aureus* were obtained from microbiological Resources Center, Cairo Mircen-Egypt, the Egyptian Microbial Culture Collection.

Preparation of bacteria

Bacterial cultures were maintained in culture agar at 5°C, then grown in tryptic phosphate broth at 30°C at least twice for 24 h periods to being used in the experiment. Twenty-four hour culture of each bacterium was sedimented by centrifugation and plates were resuspended in 0.1 M potassium phosphate buffer (pH 7.0), the cell suspensions were serially diluted to an approximate final concentration of 10^5 CFU/g .

Antimicrobial plant extracts

The antimicrobial plant extracts used were onion and garlic. All extracts were obtained from commercial chemical and flovor vendors.

All extracts were dissolved in ethyl alcohol (to obtained 20% solution), which allowed better distribution of extracts in the minced beef samples *(8)*.

Treatment

Minced meat samples were prepared *(9)*. Meat samples were purchased from local supermarket aseptically in sterile polyethylene bags, minced by a sterile glass blender jar and divided aseptically into 6 groups; each of the first 3 groups were divided into three portions each of 100 g in sterile plastic bags. The 1st and 2nd bags received 1 and 4% onion extracts and spread in the minced meat with a sterile glass road. The 3rd bags were considered as control samples to which 0.1 ml ethyl alcohol was applied. Garlic extracts were applied to the second 3 groups by the same previously mentioned method and concentrations.

All samples were kept in a laminar flow biologically safety cabinet for 15 minutes for alcohol to be evaporated before being inoculated with tested pathogens.

The treated samples were then inoculated with 0.1 ml of 10^6 CFU/ml populations of *E. coli* O157:H7, *Salmonella typhimurium* and *Staph. aureus*. Inoculated samples were held undisturbed for 30 minutes to allow residual moisture to be evaporated or absorbed. Samples were then placed into sterile polyethylene bags and kept at 5°C , the population of each inoculated microorganism after 0, 4, 8 and 12 days were detected in duplicates *(5)*.

Bacteriological examination

Enumeration of *E. coli* O157:H7 ***(10)*** and *Salmonella typhimurium* ***(11)*** , and *Stpah. aureus* ***(12)*** was carried out.

Statistical analysis

Statistical analysis were done using SAS *(13)*.

Table 1. **Inhibitory effect of different concentrations of onion extract on the growth of *E. coli* O157:H7, *S.typhimurium* and *Staph. aureus* (Log_{10} CFU/g) in treated minced beef stored at 5°C**

Time/day	*E. coli* O157:H7			*S.typhimurium*			*Staph.aureus*		
	Control	1%	4%	Control	1%	4%	Control	1%	4%
0	f 5.25	f 5.25	f 5.25	g 5.23	g 5.23	g 5.23	f 5.23	f 5.23	f 5.23
4	d 6.23	e 6.08	g 4.25	e 6.30	f 6.00	i 4.55	d 6.18	d 6.13	g 4.90
8	b 7.33	c 7.05	h 3.33	c 7.13	d 6.85	h 4.68	b 7.23	c 7.10	e 5.90
12	a 8.20	a 8.10	i 2.18	a 8.23	b 8.10	j 3.88	a 8.18	a 8.10	h 4.03

Log_{10} CFU/g have the same letters(a-j) aren't significant ($P<0.05$).

Table 2. Inhibitory effect of different concentrations of garlic extract on the growth of *E. coli* O157:H7, *S.typhimurium* and *Staph. aureus* (Log_{10} CFU/g) in treated minced beef stored at 5°C

Time/day	*E. coli* O157:H7			*S.typhimurium*			*Staph.aureus*		
	Control	1%	4%	Control	1%	4%	Control	1%	4%
0	d 5.20	d 5.20	d 5.20	d 5.23	d 5.23	d 5.23	d 5.23	d 5.23	d 5.23
4	c 6.20	e 4.55	f 3.38	c 6.20	e 4.55	f 4.08	c 6.18	e 4.45	f 3.25
8	b 7.15	g 2.52	h 2.18	b 7.13	g 3.68	h 3.15	b 7.15	f 3.35	g 3.03
12	a 8.18	i 1.75	j 1.28	a 8.20	i 2.63	j 1.35	a 8.15	h 2.45	i 2.13

Log_{10} CFU/g have the same letters (a-j) aren't significant ($P<0.05$).

DISCUSSION

Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. Historically, garlic has been used worldwide to fight bacterial infections. Allium vegetables, particularly garlic (*Allium sativum*) exhibited a broad-spectrum antibiotic against both Gram-positive and Gram-negative bacteria.

Garlic inhibits the growth of microorganisms as well as toxin production. More research must be done to assess the value of garlic as an alternative to chemical food preservatives, especially in foods in which the garlic flavor would be added bonus. There is also potential use for garlic by itself or in combination with other herbs and spices to extend the shelf life of raw meat products *(14)*.

The data present in Table 1 show that the effect of different concentrations of onion on *E. coli* O157:H7, *Salmonella typhimurium* and *Staph. aureus* in treated minced beef stored at 5°C. The effect of 4% onion decreased the numbers of *E. coli* O157:H7 by 1.92 and 3.07 orders of magnitude comparable to zero time and 4 and 6.02 orders of magnitude comparable to control in the 8^{th} and 12^{th} days of storage respectively. This concentration decreased the number of *S. typhimurium* by 0.55 and 1.35 orders of magnitude comparable to zero time and 2.45 and 4.35 orders of magnitude comparable to control in 8^{th} and 12^{th} days of storage respectively. On the other hand, this concentration decreased the number of Staph. aureus by 1.20 orders of magnitude comparable to control in the last day of storage. No significant differences were observed in the concentrations of 1% onion on 4^{th} and 12^{th} days of storage on *Staph. aureus* and on the last day of storage on *E. coli* O157:H7. Nearly, similar findings were reported ***(15-17)***.

The effect of garlic extract on *E. coli* O157:H7, *S. typhimurium* and *Staph. aureus* are recorded in Table 2, where the concentrations of 1% and 4% of garlic decreased the number of E. coli O157:H7 by 3.45 and 3.92 orders of magnitude comparable to zero time and by 6.43 and 6.90 orders of magnitude comparable to control in the last day of storage, respectively. While the numbers of S. typhimurium decreased by2.60 and 3.88 log cycles at the concentrations of 1 and 4% comparable to zero time and by 5.57 and 6.85 log cycles as compared with control in the last day of storage, respectively.

On the other hand, these concentrations decreased the number of *Staph. aureus* by 2.78 and 3.1 orders of magnitude comparable to zero time and 5.70 and 6.02 orders of magnitude comparable to control in the last day of storage respectively.

Significant differences were observed between the two (1 and 4%) concentrations of garlic extracts on all the days of storage on all pathogens. Previous studies reported similar results ***(15-19)***

Antimicrobial activity of spices depend on several factors which includes: i) kind of spice, ii) composition and concentration of spice, iii) microbial spice and its occurrence level, iv) substrate composition and v) processing conditions and storage ***(20)***. Garlic extract (1% w/v) showed bacteriostatic effect towards E. coli O157:H7 ***(21)***. The antimicrobial activity of garlic extracts on human pathogenic bacteria including *Escherichia coli, S. typhimurium* and *Staph. aureus* were sensitive to ground garlic and its extracts *(22)*. One of the most potent spices is garlic, in which several antimicrobial components are present; the principle active substance was identified as allicin. Extensive studies have been performed to determine its inhibitory properties on many food-born pathogens, both Gram-positive and Gram-negative bacteria, have been shown to be inhibited by garlic.

The main bacteriological effect of allicin is its rapid reaction with thiol-containing protein, and since it can freely permeate through phospholipid bilayers, it can interact with intracellular thiol-containing proteins *(6)*. The antimicrolial substances in the onion extracts, which are mainly phenolic compounds such as carvacrol, eugenol and thymol. These compounds exhibit wide range of

antimicrobial properties. The mode of action is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport and/or coagulation of cell contents *(7)*.

In this study we can conclude that onion and garlic exhibited marked antibacterial activity, with onion extract showing the lowest inhibition and the garlic the highest. Comparatively, concentration of 1% of onion extract was less inhibitory than 4%. However, with garlic extract, high inhibitory activity was observed for the two tested concentrations. *Staph. aureus* showed less sensitivity towards onion extract inhibition, however, the three tested pathogenic microorganisms were significantly inhibited by garlic extract.

Conclusively, where onion and garlic extracts can be used as natural antimicrobial activity incorporating in various kinds of food products.

REFERENCES

1. ***Oiye S O and Muroki N M (2002):*** Use of spices in foods. J. Food Technol. In Africa, 7 (4-6): 39-44.
2. ***Singh G , Marimuthu P , Murall H S and Bawa A S (2005):*** Antioxidative and antibacterial potentials of essential oils and extracts isolated from various spice materials, J. Food Safety, 25 (5): 130-145.
3. ***Fernández-López J , Zhi N , Aleson-Carbonell L , Pérez-Alvarez J A and Kuri V (2005):*** Antioxidant and antibacterial activities of natural extracts, application in beef meatballs. Meat Science, 69: 371-380.
4. ***Kumral A and Sahin L (2003):*** Effects of some spice extracts on Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Yersinea enterocolitica and Enterobacter aerogenes. Annals Microb., 53 (4): 427-435.
5. ***Enshrah K I M (2002):*** Inhibitory effect of some plant extracts on *Aeromonas hydrophila* and *Listeria monocytogenes* in refrigerated cooked Egyptian beef and chicken. J. Egypt. Vet. Med. Assoc., 62 (6c): 103-111.
6. ***Vertuyten J , Leroy F and Vuyst L (2004):*** Effects of different spices used in production of fermented sausages on growth of and curvacin a production by Lactobacillus curvatus LTH1174. Appl. Environ. Microbiol., 70 (8): 4807-4813.
7. ***Seydim A C and Sarikus G (2006):*** Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Research International, 39: 639-644.
8. ***Hao Y Y , Brackett R E and Doyle M P (1998a):*** Inhibition of *Listeria monocytogenes* and *Aeromonas hydrophila* by plant extracts in refrigerated, cooked beef. J. Food Protect., 61: 307-312.
9. ***Hefnawy Y A , Moustafa S I and Marth E H (1993):*** Behaviour of *Listeria monocytogenes* in refrigerated and frozen ground beef and sausage and both with and without additives. Lebensm. Wiss. U. Technol., 26: 167-170.
10. ***APHA "American Public Health Association" (1992):*** Compendium of methods for the microbiological examination of food. 3rd Ed., Washington D.C., USA.
11. ***Mulder R W A W (1977):*** Inactivation of salmonella on chilled and deep frozen broiler carcasses by irradiation. J. Appl. Bact., 42: 179-185.
12. ***FAO "Food and Agriculture Organization" (1992):*** Manual of food quality control. 1- Microbiological analysis-Staphylococcus count. pp. 131.
13. ***Littell R C , Freund J and Spector P C (1991):*** SAS system for linear models. 3rd ed. SAS series in statistical applications. SAS Institute Inc. Cary. NC. USA.
14. ***Sivam G P (2001):*** Protection against *Helicobacter pylori* and other bacterial infection by garlic. J. Nutrition, 131: 1106-1108.
15. ***Benkeblia N (2004):*** Antimicrobial activity of essential oil extracts of various onions

(*Allium cepa*) and garlic (*Allium sativum*). Lebensmittel- Wissenschaft und Technologie, 37 (3): 263-268.

16.Sarma N (2004): Can garlic (*Allium sativum*) be used as a meat preservative? Transactions of the Kansas Academy of Sci., 107 (2-3): 148-154.

17.Al-Delaimy K S and Ali S H (2006): Antimicrobial action of vegetable extracts on the growth of pathogenic bacteria. J. of the Science of Food and Agriculture, 21 (5): 110-112.

18.Gomaa N F and Hshish M H (2003): The inhibitory effect of garlic (Allium sativum) on growth of some microorganisms. J. Egypt. Public Health Assoc., 78 (5-6): 361-372.

19. Ekwenye U N and Elegalam N N (2005): Antimicrobial activity of ginger (*Zingiber Officinale Roscoe*) and garlic (*Allium sativum*) extracts on *Escherichia coli* and *S. typhi*. Int. J. Mol. Med. Adv. Sci., 1 (4): 411-417.

20.Farag R S , Daw Z Y , Hewedi F M and El-Baroty G S A (1989): Antimicrobial activity of some Egyptian spices essential oils. J. Food Protec., 52: 665-667.

21.Leuchner R G K and Zamparini J (2002): Effect of spices on growth and survival of *Escherichia coli* O157 and *Salmonella enterica* serovar enteridis in broth model systems and mayonnaise. Food Control, 13: 399-404.

22.Arora D and Kaur J (1999): Antimicrobial activity of spices. International J. of Antimicrobial Agents, 12: 257-262.

الملخص العربى

تثبيط ميكروب إيشريشيا كولاى والسالمونيلا والعنقود الذهبى بمستخلص البصل والثوم فى اللحم البقرى المفروم المبرد

محمد السيد عبد الفتاح ،حنان مصطفى طه اللاوندى ، فاطمة السيد جابر*

وحدة صحة الأغذية – معهد بحوث صحة الحيوان – مصر

*وحدة السموم- قسم الكيمياء - معهد بحوث صحة الحيوان – مصر

يحدث التسمم الغذائى إثر تناول الغذاء أو الشراب الملوث بميكروبات ممرضة أو سمومها. وهناك أنواع من الميكروبات مرتبطة بحالات شديدة من التسمم الغذائى نتيجة تناول لحوم الابقار المفرومة وغير المطبوخة أو المطهية جيداً ، منها ميكروب الايشريشيا القولونية O157:H7 ، ميكروب السالمونيلا تايفيميوريم والمكور العنقودى الذهبى والتى تؤدى إلى حدوث فشل كلوى وخاصة فى الأطفال.

وقد أجريت هذه الدراسة لاختبار تأثير مستخلصين من بعض التوابل الشائع إضافتهم فى صناعة الأغذية مثل (البصل و الثوم) على نمو هذه الميكروبات السابق ذكرها فى اللحم المفروم وتم حفظ العينات عند درجة حرارة ٥ درجة مئوية لمدة ١٤ يوم باستخدام تركيزين ١% و ٤% من مستخلصات التوابل سالفة الذكر.

وقد اظهرت النتائج أن التأثير المثبط لتركيز ١% من مستخلص البصل أقل من تركيز ٤% لنفس المستخلص على الميكروبات الثلاث. وكان ميكروب المكور العنقودى الذهبى أكثر مقاومة للتأثير المثبط لمستخلص البصل. فى حين أن التأثير المثبط لتركيز ١% أو ٤% لمستخلص الثوم كان عالى ومعنوى على جميع الميكروبات المستخدمة فى التجربة.