発酵肉における香辛料の抗菌効果

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Antibacterial Effects of Spices on Fermented Meat

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Summary

Garlic and coriander were examined to determine their antibacterial and promotional activity against various bacteria. By paper disc diffusion, was admitted some inhibition of spoilage and pathogenic bacteria. Garlic revealed its antibacterial activity against in order of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas fragi.* While coriander demonstrated an antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas fragi* in orderly. Neither spice showed any inhibitory effect against *Lactobacillus plantarum* and *Pediococcus pentosaceus.* In liquid medium, they promoted the growth of both Lactic acid bacteria cultures, but there was inhibition of the growth of some spoilage and pathogenic bacteria. In beef patties, the addition of both spices resulted in the inhibition of some spoilage bacteria, however, there was activation of the growth of Lactic acid bacteria. The results indicate that the use of garlic and coriander in fermented meat could encourage an optimal fermentation process and depress the presence of spoilage and pathogenic bacteria.

Introduction

Recently, there have been many investigations into the application of Lactic acid bacteria starter cultures for development of the preservative qualities of meat and traditional meat products[7,10,21]. The lactic acid produced during the fermentation process lowers the pH and imparts a tangy flavor. A very important function of starter cultures on meat, is the inhibition of growth of spoilage and pathogenic bacteria[4,6,7,15-17].

Spices have been traditionally used in meat products because of the contribution to flavor development. The importance of spices can be found not only in their flavoring, but also in their medicinal, preservative and antioxidant properties. Azzous[1] used spices as an antifungal agent, Bullerman[3] studied the effect of spices on aflatoxin production, Farbood[9] reported the effect of spices on the growth of bacteria in meat and Huhtanen[11] investigated the inhibition of *Clostridium botulinum* using some spices. The effects of spices on Lactic acid bacteria activity were investigated by Zaika[22,23], who reported that some spices have no effect on the acid production of starter cultures. Shelef[18] also reviewed that Lactic acid bacteria was less sensitive to spices with antimicrobial activity than other gram positive organisms and that the minimum inhibitory concentration ranged from 1 to 5 %.

Since garlic and coriander are widely used in meat products, especially in traditional fermented or non fermented meat products, and in order to achieve an optimal activity

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of Lactic acid bacteria in the development of such products, it is very important to evaluate the effects of spices on the growth of Lactic acid bacteria while inhibiting the growth of non starter flora.

This paper reports on the relative inhibitory activities of garlic and coriander and the effect of these spices on the growth of some spoilage and pathogenic bacteria and on the growth of Lactic acid bacteria in liquid medium and in meat.

Meterials and Methods

The spices of garlic and coriander were obtained from Department stores as spice powder (House Co., Ltd.)

The bacteria examined in this study were *Lactobacillus plantarum* IAM 1216, *Pediococcus pentosaceus* IAM 12296, *Staphylococcus aureus* IAM 1011, *Escherichia coli* RB, *Bacillus subtilis* IFO 3025 and *Pseudomonas fragi* IFO 3458. The strains were incubated in Yeast extract Peptone Glucose (YPG) broth which contained yeast extract, 5 g; peptone, 10 g; glucose, 10 g; Tween 80, 1 g; L-cystein, 0.1 g in distilled water 1000 ml, pH 6.8[15].

The antibacterial activity of spices against some spoilage bacteria and Lactic acid bacteria cultures. Five precent of each spice in 0.1 M buffer phosphate pH 7.0 was extracted by boiling water bath at 100℃ for 15 min and cooled at room temperature, centrifuged at 8000 rpm and filtered-sterilized. The antimicrobial activity was tested by agar diffusion technique, using bacterial cultures. Standard plate count agar was inoculated with 1 % of an active YPG broth culture of test bacterial cultures. Sterilized spice extracts were taken, 0.1 ml by volume, to a sterilized filter paper disc of 13 mm diameter. The disc was placed on a seeded agar surface. The plates were left at room temperature for 1 h to allow the test material to diffuse into the agar, then incubated at 30℃ for 16 h. The plates were examined for zones of growth-inhibition of test bacterial culture around the disc by measuring the diameter of the zones.

The effect of spice-extract of garlic and coriander on the growth of bacterial cultures in YGP broth media. Each was cultured in a series of tubes containing a broth medium with 0 %, 2 %, 3 % and 5 % of spice extract. All bacteria were incubated at 30℃, growth was measured as turbidity by spectrophotometer at 660 nm.

The effect of spices on the growth of some spoilage bacteria in meat. Fresh beef was purchased from a local market on the day of preparation. Meat was cut and minced with a meat grinder through a 4 mm plate diameter. Each 10 g sample of the minced meat was mixed with either 0 %, 2 %, 3 %, or 5%, of garlic or coriander. Each sample was then wrapped with saran wrap, and placed in an incubator at 30℃. During incubation for 0, 12 and 24 h, each sample was homogenized with 10 portions of sterile buffered saline and was used for microbial analyses.

One ml of each homogenate sample was aseptically diluted stepwise through a series of tubes containing 9 ml sterile buffer saline. Appropriate diluents of each tube were placed on the following 6 media in duplicate; Plate Count Agar (PCA) (Difco Co., Ltd.) supplemented with 10 % NaCl for Micrococci[20], Crystal Violet Terazolium (CVT) agar (Eiken Ltd.) for Gram-negative bacteria[17], Vogel Johnson agar (Eiken Ltd.) for Staphylococci, Bacto OF Basal medium (oxidation/fermentation medium) (Difco Co., Ltd.)

Table 1 Antiimicrobial activity of garlic and coriander against some bacterial cultures

Test organisms	Antimicrobial activity[a)]	
	Garlic 5 %	Coriander 5 %
	zone (mm)	
Escherichia coli	16.0	15.5
Staphylococus aureus	18.5	16.5
Pseudomonas fragi	16.0	15.5
Bacillus subtilis	18.0	17.5
Lactobacillus plantarum	13.0	13.0
Pediococcus pentosaceus	13.0	13.0

a) disc diameter is 13.0 mm

which contains : OF Basal medium, 9.4 g ; yeast extract, 2.0 g ; Tween 80, 1 g ; 13 g in 1000 ml distilled water for Pseudomonads, Desoxycholate agar (Nissui Co., Ltd) for Coliform[2)] and De Man Rogosa Sharp (MRS) agar, to which was added 0.02 % sodium azide for Lactic acid bacteria[13)].

MRS and PCA duplicate pour plates were incubated at 30℃ for 48 h, Vogel Johnson duplicate surface plates were incubated at 35℃ for 48 h. CVT surface plates were incubated at 30℃. OF Basal medium surface plates were incubated at 25℃ for 48 h and all white colonies were counted[2)]. For Coliform, duplicate pour plates were made of each dilution using 1 ml in Desoxycholate agar. After the pour plates solidified, they were covered with an additional 5 ml or more of Desoxycholate agar, incubated at 30 ℃ for 24 h, and the red colonies were counted[2)].

Results and Discussion

The antibacterial activity of garlic and coriander extracts against some bacterial cultures was assayed using filter paper disc agar diffusion technique as shown in Table 1. The data for the inhibition zones indicate that garlic and coriander had an effect against *Escherichia coli* (3 and 3.5 mm), *Staphylococcus aureus* (5.5 and 3.5 mm), *Pseudomonas fragi* (3 and 2.5 mm) and *Bacillus subtilis* (5 and 4.5 mm). The inhibitory effect of garlic appeared higher than coriander especially against *Staphylococcus aureus,* expect on the inhibition of *E. coli.*

Neither the garlic nor coriander had any effect against *Lactobacillus plantarum* and *Pediococcus pentosaceus* starter culture (0 mm). These data correspond with some researchers that showed *Lactobacillus plantarum* resistance toward antimicrobial activity of spices[22,23)]. This result indicates that garlic and coriander had an inhibitory effect against some spoilage and pathogenic bacteria, but no effect on the Lactic acid bacteria.

Figures 1 and 2 describe the effects of garlic and coriander extracts on the growth of bacterial cultures in liquid media. The growth of *E. coli, S. aureus, P. fragi* and *B. subtilis* were inhibited by the presence of garlic. *E. coli* and *P. fragi* showed more resistance than the other test microorganisms, they grew well at 3 % but were inhibited at the 5 % level of garlic. *L. plantarum* and *P. pentosaceus* were not inhibited by the presence of garlic, rather their growth was stimulated by its presence. Coriander also showed to inhibit the

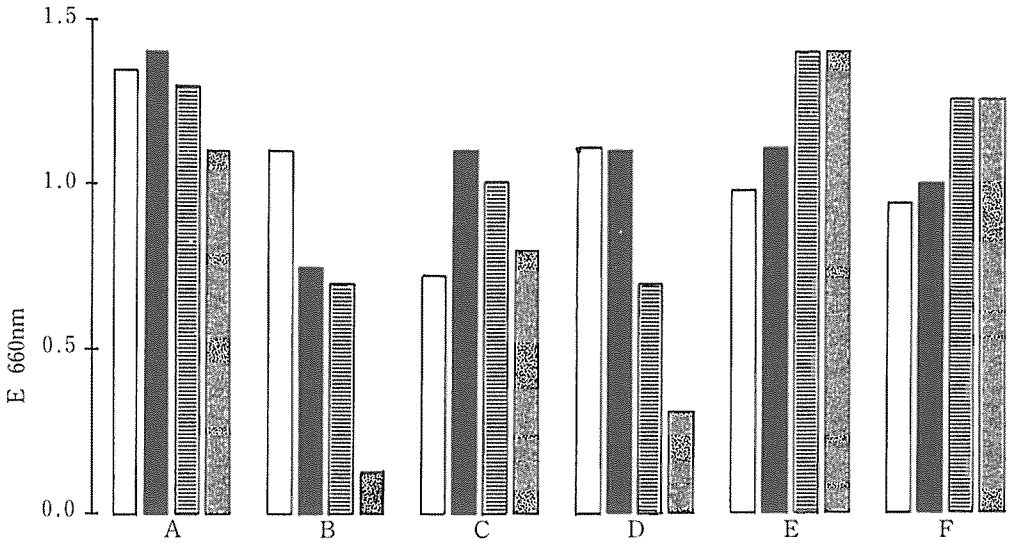

Fig. 1 Effect of garlic on the growth of some bacterial cultures in liquid medium after incubation at 30℃ for 24 h. Garlic content;□ :0%, ■ :2%, ▤ :3%, ▩ :5%
Bacteria cultures; A : *Escherichia coli* B : *Staphylococcus aureus*
C : *Pseudomonas fragi* D : *Bacillus subtilis*
E : *Lactobacillus plantarum* F : *Pediococcus pentosaceus*

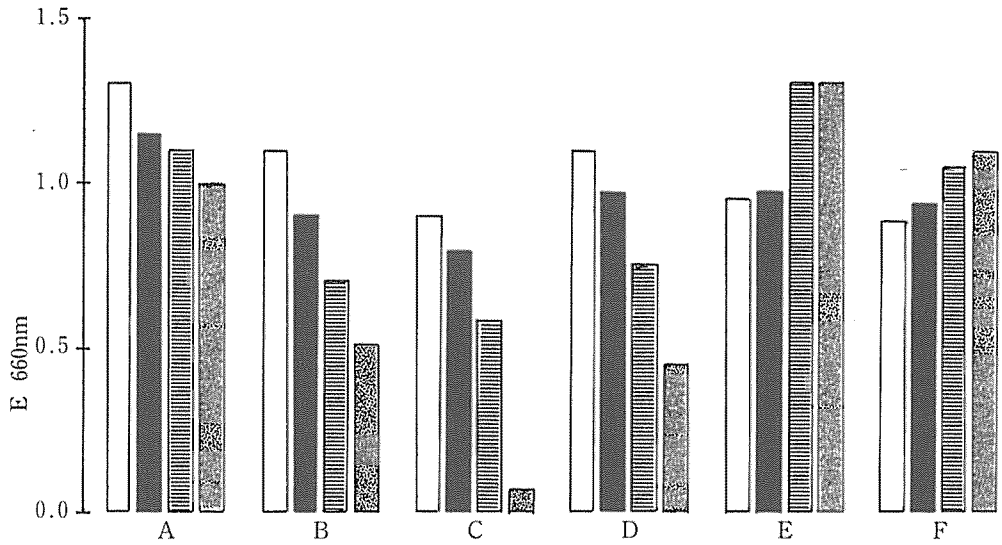

Fig. 2 Effect of coriander on the growth of some bacterial cultures in liquid medium after incubation at 30℃ for 24 h. Coriander content;□ :0%, ■ :2%, ▤ :3%, ▩ :5%
Bacteria cultures; A : *Escherichia coli* B :*Staphylococcus aureus*
C : *Pseudomonas fragi* D : *Bacillus subtilis*
E : *Lactobacillus plantarum* F : *Pediococcus pentosaceus*

growth of bacterial cultures, except *L. plantarum* and *P, pentosaceus.* The inhibition of some spoilage cultures was greater with an increase of spice content. These data showed that both garlic and coriander stimulate the growth of Lactic acid bacteria, and the inhibitory effect of coriander against *E. coli* and *P. fragi* was higher than garlic.

The effect of garlic and coriander on the growth of some spoilage bacteria and Lactic

Table 2 Effect of garlic on the growth of some bacteria in meat during storage at 30℃

Storage time (h)	Bacteria	Concentration of garlic (%)			
		0	2	3	5
		log CFU/g			
0	Staphylococci	3.23	3.15	3.08	3.93
	Pseudomonads	3.81	3.79	3.75	3.34
	Gram(−)bacteria	4.40	4.38	4.05	3.98
	Coliform	3.60	3.59	3.38	3.06
	Micrococci	4.17	4.15	3.97	3.89
	LAB[a)]	4.55	4.61	4.80	4.78
12	Staphylococci	5.00	4.62	4.32	3.51
	Pseudomonads	5.50	4.80	4.38	3.52
	Gram(−)bacteria	5.99	4.85	4.69	3.86
	Coliform	5.58	4.97	4.85	3.75
	Micrococci	6.90	5.91	5.38	4.64
	LAB[a)]	6.95	6.93	6.96	7.01
24	Staphylococci	6.60	5.75	4.82	3.74
	Pseudomonads	5.82	4.96	3.94	2.88
	Gram(−)	7.69	6.94	6.43	5.74
	Coliform	8.92	7.86	7.89	6.84
	Microcci	6.44	5.98	5.00	4.49
	LAB[a)]	8.67	8.61	8.74	8.76

a) Lactic acid bacteria

Table 3 Effect of coriander on the growth of some bacteria in meat during storage at 30℃

Storage time (h)	Bacteria	Concentration of coriander (%)			
		0	2	3	5
		log CFU/g			
0	Staphylococci	3.32	3.28	3.25	3.15
	Pseudomonads	3.79	3.78	3.71	3.64
	Gram(−)bacteria	4.36	4.34	4.30	3.27
	Coliform	3.61	3.62	3.62	3.50
	Micrococci	4.23	4.20	4.20	3.15
	LAB[a)]	4.50	4.47	4.53	4.55
12	Staphylococci	5.23	5.16	4.87	3.93
	Pseudomonads	5.46	5.25	4.70	3.94
	Gram(−)bacteria	5.94	5.93	4.97	4.08
	Coliform	5.43	5.38	5.00	4.74
	Micrococci	6.88	6.81	5.99	5.42
	LAB[a)]	6.97	6.97	6.97	6.99
24	Staphylococci	6.62	6.49	5.85	4.64
	Pseudomonads	5.80	6.49	5.85	4.43
	Gram(−)	8.70	7.63	6.86	5.98
	Coliform	8.91	8.46	7.94	6.99
	Microcci	6.50	6.00	5.81	5.41
	LAB[a)]	8.65	8.67	8.79	8.83

a) Lactic acid bacteria

acid bacteria in meat are shown in Table 2 and Table 3. Both the garlic and coriander inhibited the growth of some spoilage bacteria, and did not stimulate their growth. They showed no inhibiting effect for Lactic acid bacteria, rather stimulated its growth.

The primary constituents of spices' essential oils are known for their antimicrobial effects. Shelef[18] reported that Allicin, the essential oil substance isolated from garlic, inhibited gram-negative bacteria and pathogenic fungi in culture media, also discovered that most of the antimicrobial substances were phenol compounds such as eugenol, thymol eugenol and thymol carvacrol. The spices, besides containing antimicrobial substances, were also reported to contain a substance which stimulates the growth of Lactic acid bacteria. Zaika and Kissinger[24] reported that the enhancement of fermentation has been identified with the presence of an appropriate concentration of manganese ion in spices. Coventry and Hickey[5] also investigated wether manganese in spices could stimulate the growth and acid production of Lactic acid bacteria.

In conclusion, the spices garlic and coriander could be used for developing the qualities of fermented-meat products because of their inhibitory action against spoilage bacteria and their stimulatory action on Lactic acid bacteria as starter.

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発酵肉における香辛料の抗菌効果

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香辛料のガーリックおよびコリアンダーが発酵肉中の細菌におよぼす抑制作用および増殖作用を比較した。ガーリックおよびコリアンダーはペーパーディスク拡散法で腐敗菌，病原菌の抑制作用が認められた。ガーリックは *Staphylococcus aureus, Bacillus subtilis, Pseudomonas fragi, Escherichia coli* の順に，そしてコリアンダーは *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas fragi* の順に抑制した。しかし，スターターとしての乳酸菌 *Lactobacillus plantarum* および *Pediococcus pentosaceus* は抑制されなかった。液体培地において，ガーリックおよびコリアンダーは腐敗菌および病原菌を抑制したが，乳酸菌に対しては増殖作用が認められた。牛肉にガーリックおよびコリアンダーを添加すると腐敗菌，病原菌は抑制され，乳酸菌は抑制されなかったので，発酵肉に有用な作用があることが認められた。