Antimicrobial Effect of Extracts of Cruciferous Vegetables

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The cruciferous vegetables cauliflower, broccoli, cabbage, Chinese radish, Chinese kale, and Chinese kitam were used in this study to prepare water-soluble and methanol-water extracts. Crude protein extracts were also obtained by diethylaminoethyl (DEAE) anion exchange chromatography. Water-soluble polysaccharides were prepared by ethanol precipitation followed by ultrafiltration. The antimicrobial effects of all these extracts were evaluated against Gram-positive bacteria, Gram-negative bacteria, and yeast. Crude protein extracts exhibited the greatest antimicrobial activity in monoculture experiments. The antimicrobial effects of cruciferous vegetables were also studied by steeping beef, carrot, and celery in chlorine (10 ppm) or citric acid solution (1%) containing the crude protein extract (500 ppm) for different time periods. Total aerobic plate counts and coliform counts on these foods decreased significantly after 10 minutes in all steeping solutions ($p < 0.05$).

Key Words: cruciferous, extracts, crude protein, water-soluble polysaccharide, antimicrobial effect
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Many plants and vegetables contain compounds with antimicrobial activity. For example, shepherin (a peptide) in the root of shepherd's purse inhibits Gram-negative bacteria and fungi [1]. Steroid and non-methylated fatty acids extracted from *Diplotaxis harra* Forsle inhibit fungi, yeast, and Gram-negative and Gram-positive bacteria [2]. An isothiocyanate (6-methyl sulfinylhexyl isothiocyanate) in Japanese horseradish may inhibit aggregation of human platelets [3]. Some spices and herbs inhibit the growth of *Shigella sonnei* and *Shigella flexneri* [4].

Cruciferous vegetables such as cauliflower, cabbage, mustard, Chinese cabbage, carrot, Chinese kale, and turnip are edible plants that are low in calories, high in fiber, rich in vitamins and minerals, and have physiologic effects in humans [5,6]. Some important enzymes such as chitinase, glutathione transferase, and epoxide hydrolase are also found in cruciferous vegetables [7,8]. Indole and isothiocyanate, enzymatic products of myrosinase from glucosinolate found in cauliflower and cabbage, lower the incidence of tumor formation and have an antioxidative effect [9]. There are also antifungal components such as iprodione [10], chitosan [11–13], and a peptide [14] in cruciferous vegetables.

The aim of this research was to study the antimicrobial activities of cruciferous vegetables. Various components from crucifers, such as those in water extracts, methanol-water extracts, and crude protein extracts (CPEs) and water-soluble polysaccharides (WSPS), were prepared and tested for their ability to inhibit microorganisms associated with food spoilage and illness. The results of these analyses could be useful in selecting some of these extracts as natural

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disinfectants in food preparation. To further understand the practical applications of these extracts, their antimicrobial effects on the surfaces of foods were also studied.

Materials and Methods

Microorganisms

The following microorganisms were used in this study: *Bacillus cereus* CCRC 14665 (Culture Collection and Research Center, Taiwan), *Bacillus subtilis* CCRC 14199, *Enterobacter aerogenes* CCRC 10370, *Escherichia coli* CCRC 11634, *Listeria monocytogenes* CCRC 14845, *Proteus vulgaris* CCRC 12153, *Pseudomonas aeruginosa* CCRC 10773, *Pseudomonas fluorescens* CCRC 13902, *Salmonella enterica* serotype Typhimurium CCRC 12497, *S. sonnei* CCRC 10773, *Streptococcus faecalis* CCRC 10066, *Staphylococcus aureus* CCRC 11863, and *Candida albicans* CCRC 21538. Bacteria were cultured in nutrient broth (Difco, Sparks, MD, USA) at 37°C for 18 hours. Yeasts were cultured in Sabouraud medium (Difco) at 28°C for 40 hours.

Vegetable preparation

Six different kinds of fresh cruciferous vegetables including cauliflower (*Brassica oleracea* var. *italica*), broccoli (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), Chinese radish (*Raphanus sativus*), Chinese kale (*B. oleracea* var. *alboglabra*), and Chinese kitam (*Brassica rapal*) were purchased from a local market in Ping Tung, Taiwan.

After rinsing in running tap water, the vegetables were freeze-dried (600 g, dry weight) and then macerated in methanol (Merck, Darmstadt, Germany) overnight. The methanol extracts were filtered and concentrated under vacuum. The residue was extracted with boiling distilled water (1:10 wt/vol) for 15 minutes and then centrifuged (4,000*g*) for 10 minutes. The supernatant obtained was lyophilized to provide a methanol-water extract.

Water extracts and CPEs were obtained by blending lyophilized vegetables in distilled water (1:10 wt/vol) in a blender (Osterizer, Sunbeam Products Inc, Boca Raton, FL, USA). The homogenate was maintained at 20°C for 10 hours and filtered through filter paper (Whatman No. 1, Whatman plc, Brentford, Middlesex, UK). Part of the filtrate was lyophilized to give the water extract. The remainder was subjected to ammonium sulfate precipitation. Ammonium sulfate was added to the filtrate to a final concentration of 50% and the precipitate collected by centrifugation at 12,400*g* at 4°C for 15 minutes. The precipitate was dissolved in 0.02 M Tris-HCl (pH 8.2) and applied to a diethylaminoethyl (DEAE)-Sepharose (Amersham Pharmacia Biotech AB, Uppsala, Sweden) anion-exchange column (40 cm × 3 cm). The column was washed stepwise with 0.02 M Tris-HCl (pH 8.2) solution containing 0.1, 0.3, and 0.8 M NaCl, respectively. The eluate with the highest protein content was collected, dialyzed overnight at 4°C and then lyophilized. The lyophilized sample was designated the CPE [15]. Protein content was determined by the Bradford assay [16].

WSPS were prepared by adding distilled water (1:10 wt/vol) to the vegetables and heating at 60°C for 20 minutes, then filtering through Whatman No. 1 filter paper. Ethyl alcohol (95%) was added to the filtrate, followed by 75% alcohol to precipitate polysaccharides [17]. The precipitate was collected by centrifugation and dissolved in distilled water and filtered (Militan system, ELO 04; Millipore Co, Billerica, MA, USA) to collect the components with molecular weight below 10^5 Da. These components were concentrated by lyophilization and then redissolved in deionized water (pH 7.0). The content of the WSPS was determined using the phenol-sulfuric acid method [18].

Antimicrobial activity of cruciferous vegetables

A preliminary disk diffusion test was used to investigate the antimicrobial activities of the vegetable preparations [19,20]. To determine antibacterial activity, melted medium (45°C, nutrient agar for bacteria, Sabouraud medium for yeast) containing 10^6 colony-forming units (CFU)/mL was poured onto solidified medium on a plate. The solidified medium was plate count agar (Difco) for bacteria and YM agar (Difco) for yeast. A paper disk (Adventec, Toyo Roshi Kaisha Ltd, Tokyo, Japan), 8 mm in diameter and 0.85 mm thick, containing 30 μL of sample solution (0.5 μg/μL) was placed in the center of the plate after the top agar had solidified. Bacterial plates were incubated at 37°C for 14 hours while yeast plates were incubated at 28°C for 24 hours [21]. The antimicrobial activity was assessed by the diameter (mm) of the inhibition zones, measured using calipers.

Combined effect of CPE and other chemicals on foods

Simulated foods including fresh beef (8 × 8 × 0.8 cm), celery and carrot (5 × 1.5 × 0.5 cm) dices were individually steeped in 100 mL solution at a final concentration of 500 ppm CPE from cabbage alone or with either 1% citric acid or 10 ppm chlorine. The steeped solutions were incubated at 20°C for 5, 10, or 30 minutes.

After steeping, the foods were placed into 100 mL of

0.1 M potassium phosphate buffer (pH 7.0) and homogenized with a stomacher (Type BA 7021, Seward Medical, London, UK) for 1 minute. The homogenates were collected and total aerobic plate counts (TPC) and coliform counts were measured using Petrifilm [22,23]. The antimicrobial effect was expressed in terms of lethality, defined as the logarithmic ratio of microbial counts before and after treatment [24].

Statistical analysis

All treatments were conducted in three samples and duplicate tests were run for each treatment. Data from each treatment underwent analysis of variance. Duncan's multiple range test was used to determine significant difference ($p < 0.05$) between treatments.

Results

Antimicrobial effect of extracts

The antimicrobial effects of the four extracts of cruciferous vegetables are shown in the Table. Water extracts from cabbage had the highest antimicrobial activity, especially against Gram-negative bacteria, and of all bacteria examined, *E. aerogenes* was the most inhibited by cabbage extracts. Water extracts from all six kinds of cruciferous vegetables also inhibited *C. albicans*, the only fungus used in this experiment. The inhibition of *C. albicans* by the six water extracts was greater than that of *B. cereus* and *B. subtilis*, but lower than that of other Gram-positive bacteria. None of the water extracts was able to inhibit *P. vulgaris*.

Methanol-water extracts of cabbage, Chinese kale, and Chinese kitam had antimicrobial effects against two species of *Pseudomonas*. Only the cabbage methanol-water extract inhibited the two *Bacillus* spp. Methanol-water extracts of broccoli, Chinese kale, and Chinese kitam exhibited greater inhibition of *C. albicans* than did their water-based counterparts.

WSPS from all six kinds of cruciferous vegetables had greater inhibitory effects against Gram-negative bacteria, especially *E. coli* and *Salmonella enterica* serotype Typhimurium, than against Gram-positive bacteria. Cabbage WSPS had greater effects against the two Gram-positive cocci than against *Bacillus* spp. ($p < 0.05$). The inhibition of *S. sonnei* by WSPS from Chinese radish was not significantly different from that by WSPS from Chinese kale ($p > 0.05$).

CPEs from cauliflower, broccoli, cabbage, and Chinese kitam inhibited *P. vulgaris*, although water and methanol-water extracts from these vegetables did not. All CPEs exhibited significantly greater antimicrobial effects against *S. sonnei* than water extracts ($p < 0.05$). CPEs had greater inhibitory effects against Gram-negative bacteria than against Gram-positive bacteria, as shown by the significantly larger inhibition zones observed in all four Gram-negative cultures with CPEs, including *E. aerogenes*, *E. coli*, *S. enterica* serotype Typhimurium and *S. sonnei*, than in Gram-positive cultures ($p < 0.05$).

Combined effect of CPE from cabbage and other chemicals on foods

Both TPC and coliform counts on foods were greatly reduced by steeping longer in CPE (Figures 1 and 2). There were significant differences in lethality between 5 and 10 minutes' steeping in CPE ($p < 0.05$), but there were no significant differences in lethality between 10 and 30 minutes' steeping in CPE ($p > 0.05$). Among all tested samples, lethality, in terms of coliform counts and TPC, was greatest when carrot was steeped in CPE alone. The lethality for the same kind of foodstuff in the triplicate experiments (data not shown) did not differ significantly ($p > 0.05$) at the same time point.

Discussion

Antimicrobial effect of extracts

Many plants are rich in water-soluble compounds, such as tannins, terpenoids, alkaloids, and flavonoids, which have been shown *in vitro* to have antimicrobial properties [25]. Of all the water extracts from cruciferous vegetables tested in this study, water extracts from cabbage had the highest antimicrobial activity, especially against Gram-negative bacteria. Water extracts from the vegetables tested in this study showed the greatest inhibitory effect against *E. aerogenes* among all of the tested microorganisms.

The methanol-water extracts of cruciferous vegetables also demonstrated antimicrobial effects in this study, although they were significantly lower than the antimicrobial effects of the water extracts. Results from previous studies have also shown that some analogous methanolic and water-soluble compounds in mushroom and many other plants have antimicrobial effects [26,27].

Antimicrobial effects of WSPS from the six kinds of cruciferous vegetables were lower than those of the water extracts, but higher than those of methanol-water extracts. CPEs from the six different kinds of cruciferous vegetables, especially cabbage, showed greater inhibitory effects than those of other extracts, except for water extracts from Chinese

Table. Antimicrobial effect of extracts of cruciferous vegetables*

Tested microorganisms	Water extract						Methanol-water extract						Water-soluble polysaccharide						Crude protein extract					
	CF	BC	CB	CR	CK	CT	CF	BC	CB	CR	CK	CT	CF	BC	CB	CR	CK	CT	CF	BC	CB	CR	CK	CT
Gram-positive																								
Bacillus cereus	11.1	11.0	12.6	10.5	—	9.6	—	—	10.2	—	9.5	9.9	11.1	12.2	11.1	12.1	11.4	11.1	14.3	14.0	16.2	12.6	11.9	12.9
	D	E	FG	D		D			CDE		DE	DE	C	CD	D	CD	C	D	CD	C	DE	EF	F	DE
Bacillus subtilis	—	9.2	11.5	9.3	—	9.5	—	—	9.6	—	8.9	9.4	—	11.3	11.0	12.0	—	10.9	11.0	12.4	14.3	12.2	—	12.5
		F	G	D		D			E		E	E		D	D	CD		D	E	CD	E	F		DE
Streptococcus faecalis	11.5	12.3	13.5	—	9.6	9.6	10.0	10.7	10.3	10.1	10.5	10.6	12.9	14.1	15.0	—	11.8	12.1	16.2	17.4	20.6	17.9	14.0	16.4
	D	CDE	EF		E	D	C	BC	CDE	BC	BCD	CDE	B	B	C		C	D	BC	B	BC	C	E	C
Staphylococcus aureus	14.4	15.6	16.2	16.0	15.2	15.7	9.7	10.4	11.5	10.0	10.9	11.1	13.3	14.1	15.3	—	—	—	17.3	18.0	22.4	20.4	19.1	19.8
	C	B	CD	B	C	B	CD	CD	ABC	BC	ABC	BCD	B	B	C				B	B	B	B	C	B
Listeria monocytogenes	11.3	12.0	13.1	9.7	10.6	10.9	9.1	9.5	11.3	9.2	9.1	10.7	10.4	10.6	11.8	—	—	—	13.8	14.2	16.5	12.0	12.4	12.2
	D	DE	EFG	D	E	D	D	D	ABCD	C	E	CDE	C	D	D				D	C	DE	F	EF	DE
Gram-negative																								
Proteus vulgaris	—	—	—	—	—	—	—	—	—	—	—	—	—	11.3	11.3	—	—	11.5	10.6	11.2	11.2	—	—	10.8
														D	D			D	E	D	F			E
Pseudomonas aeruginosa	11.2	13.1	15.8	12.5	14.2	15.1	—	—	10.0	—	10.6	10.6	—	—	12.3	11.2	11.1	11.6	16.1	17.4	20.1	15.1	16.5	17.4
	D	CD	CD	C	CD	BC			DE		BCD	CDE			D	D	C	D	BC	B	BC	D	D	C
Pseudomonas fluorescens	9.7	13.7	14.9	12.2	13.1	14.2	—	—	10.5	—	9.8	10.0	—	—	12.5	11.7	11.1	12.1	13.1	14.2	18.2	12.1	12.4	13.1
	E	C	DE	C	D	C			BCDE		CDE	DE			D	CD	C	D	D	C	CD	F	EF	D
Enterobacter aerogenes	20.5	20.7	21.9	19.3	18.9	18.9	10.2	11.6	12.2	10.7	11.6	12.5	13.5	13.1	15.8	13.1	11.8	12.3	23.0	25.1	25.2	24.0	22.7	23.2
	A	A	AB	A	A	A	BC	AB	A	AB	AB	AB	B	BC	BC	C	C	D	A	A	A	A	A	A
Escherichia coli	19.1	19.5	20.2	18.2	17.3	18.6	11.2	11.7	11.7	10.0	10.5	12.6	17.0	17.7	20.0	19.1	18.1	19.2	23.2	25.3	26.6	21.2	20.4	21.7
	B	A	B	A	B	A	A	AB	AB	BC	BCD	A	A	A	A	A	A	A	A	A	A	B	BC	AB
Salmonella serovar	18.3	19.8	20.4	18.0	17.1	18.6	11.2	11.8	12.5	11.2	12.0	12.2	17.3	17.5	20.3	18.0	17.4	17.7	24.7	25.5	27.4	24.2	21.7	23.1
Typhimurium	B	A	B	A	B	A	A	A	A	A	A	AB	A	A	A	AB	A	B	A	A	A	A	AB	A
Shigella sonnei	18.2	20.2	22.6	—	—	15.7	—	—	9.6	—	9.1	10.2	15.9	16.8	17.5	17.2	14.9	14.2	25.1	26.5	27.5	21.3	21.2	22.9
	B	A	A			B			E		E	DE	A	A	B	B	B	C	A	A	A	B	AB	A
Yeast																								
Candida albicans	11.2	11.6	16.8	12.5	10.3	10.9	11.0	12.2	12.4	11.2	10.6	11.8	11.2	10.6	11.7	11.1	11.3	11.5	14.5	14.9	18.1	14.4	12.4	13.6
	D	DE	C	C	E	D	AB	A	A	A	BCD	ABC	C	D	D	D	C	D	CD	C	CD	DE	EF	D

*Inhibition zone in mm (including 8 mm disk) as the mean of triplicate of experiments.
Capital letters A–G: within the same column, values denoted by different capital letters are significantly different at $p < 0.05$.
CF = cauliflower; BC = broccoli; CB = cabbage; CR = Chinese radish; CK = Chinese kale; CT = Chinese kitam; — = no inhibitory activity.

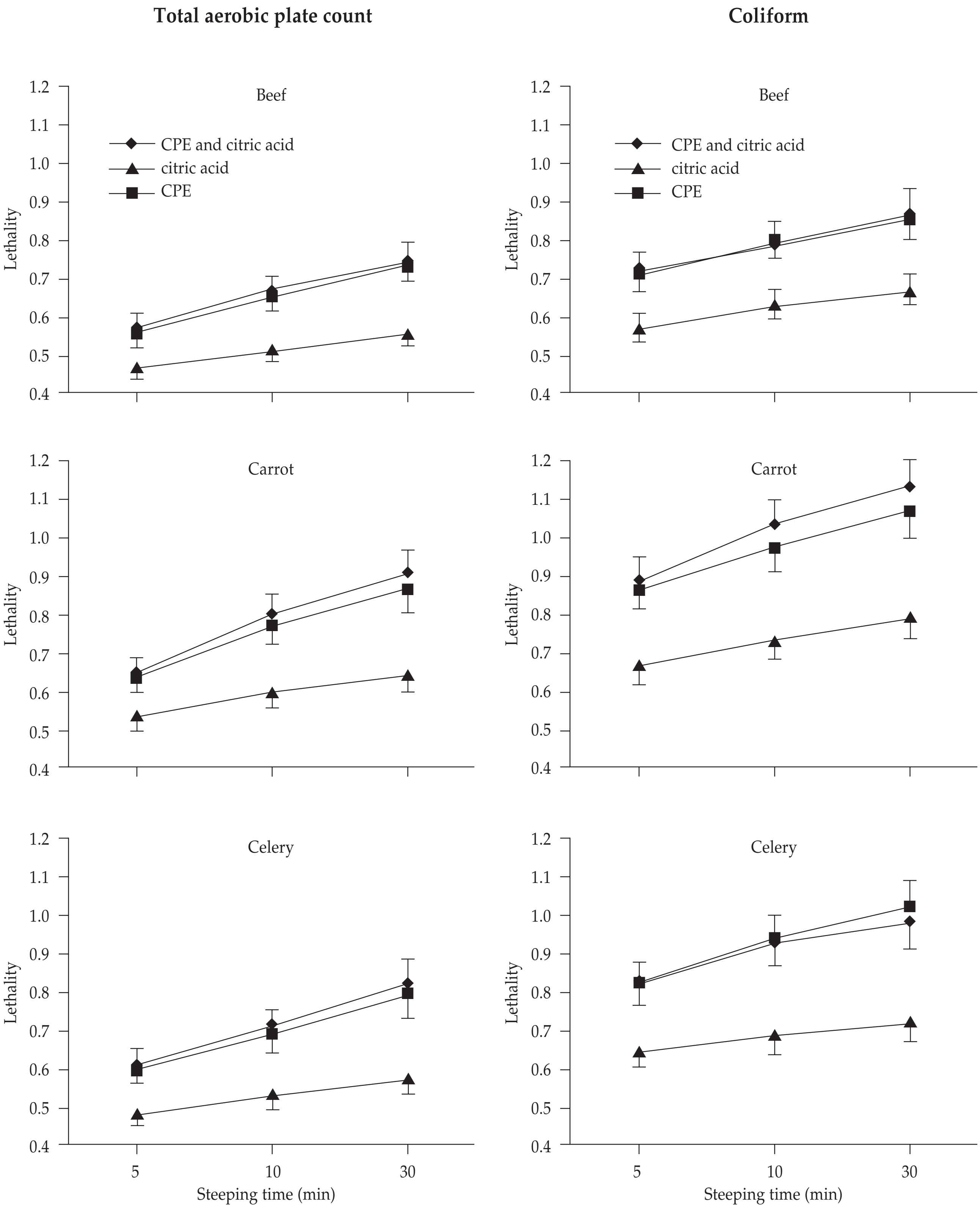

Figure 1. *Combined effect of crude protein extract (CPE) and citric acid on handling foods. Beef, carrot, and celery were steeped in a final concentration of 500 ppm CPE in the presence of 1% citric acid for 5, 10, or 30 minutes. The data are represented in terms of lethality, defined as the logarithmic ratio of microbial counts before and after treatment.*

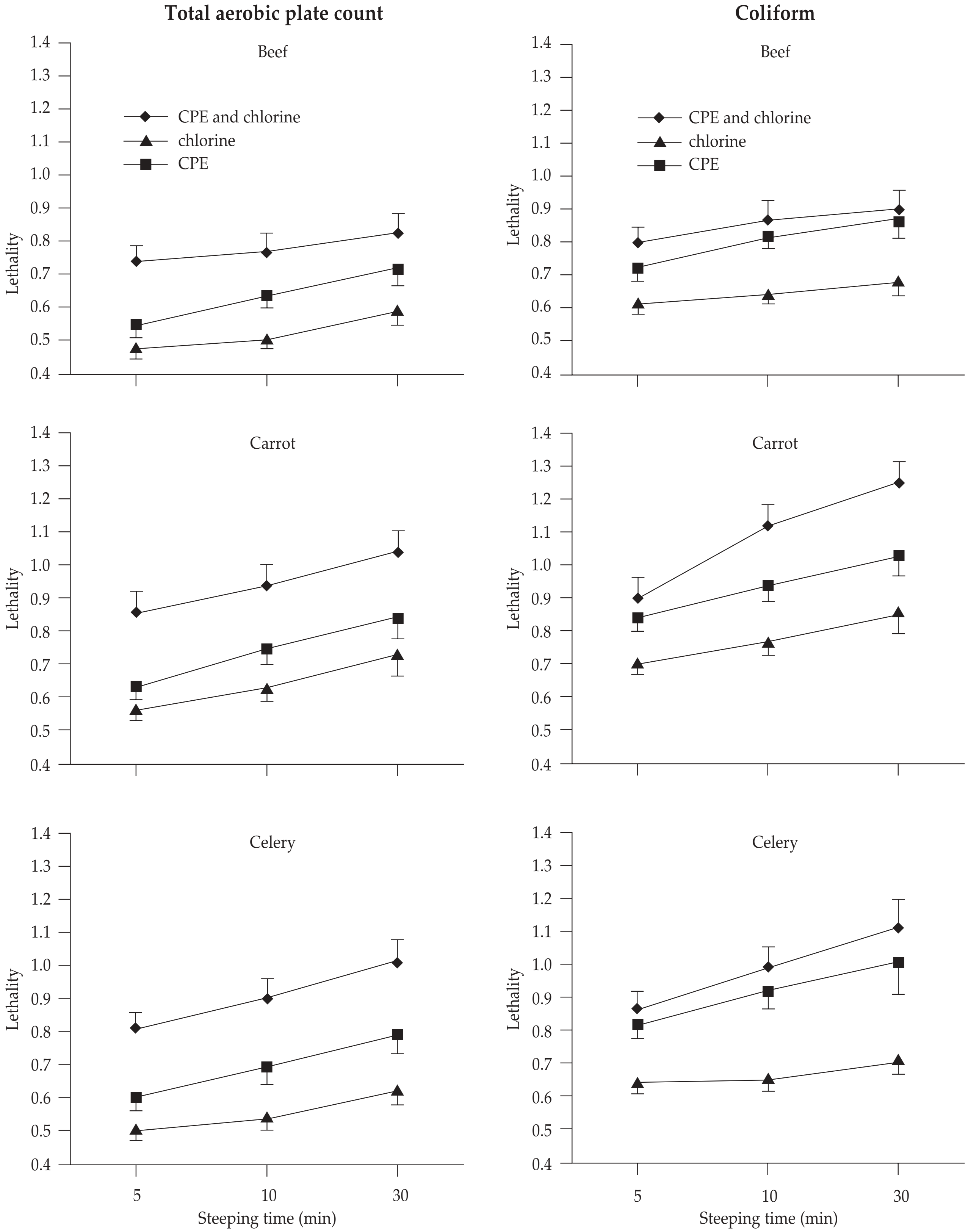

Figure 2. *Combined effect of crude protein extract (CPE) and chlorine on handling foods. Beef, carrot, and celery were steeped in a final concentration of 500 ppm CPE in the presence of 10 ppm chlorine for 5, 10, or 30 minutes. The data are represented in terms of lethality, defined as the logarithmic ratio of microbial counts before and after treatment.*

radish, Chinese kale, and Chinese kitam against *P. fluorescens*. Proteins or peptides isolated from various bioresources have previously been reported to have antimicrobial effects [14,25,28]. The antimicrobial activities of proteins may be associated with their three-dimensional structures [29]. Our results showed that CPEs from cruciferous vegetables have greater antimicrobial effects than those of other extracts. The mechanisms involved in these greater antimicrobial effects of CPEs are worthy of further investigation.

Combined effect of CPE from cabbage and other chemicals on foods

Generally, salad vegetables and meats are cleaned by steeping these foodstuffs in water for a short period of time (e.g. 5 minutes). Preliminary tests conducted in this study indicated that steeping carrot, celery, and beef in water for 5 minutes only resulted in a decrease in viable coliform counts of 21%, 22%, and 27%, and in TPC of 42%, 38%, and 24% on carrot, celery, and beef, respectively. In order to improve the efficiency of disinfection, the steeping water was supplemented with CPE from cabbage alone and selective combinations of CPE with citric acid or chlorine.

The antimicrobial effect of combined chemicals is much greater than that with one chemical alone [24]. Francis and O'Beime found that treating ready-to-use vegetables with various reagents such as chlorine, citric acid, and ascorbic acid helped to reduce the counts of both *Listeria innocua* and *E. coli* on vegetables [30]. Some plant extracts have also been used to treat livestock disease in South Africa [31]. The shelf-life of guava juice can be extended by adding combined extracts of corni fructus, cinnamon, and Chinese chive [21]. In this study, the lethality of citric acid and CPE with beef was slightly greater than that of CPE alone with beef after the same duration of treatment (Figure 1). This means that citric acid does not increase the lethality of CPE. Similar results were obtained for carrot and celery. The combination of CPE and chlorine had the greatest lethality, in terms of coliform counts and TPC, followed by CPE alone and chlorine alone (Figure 2). This means that chlorine enhances the lethality of CPE, especially in terms of TPC. The antimicrobial effect of citric acid might be related to its acidity, while that of chlorine might be related to its ability to denature protein [24]. These results indicate the feasibility of using CPE for food treatment.

Direct application of CPE or water extracts to food is recommended for sanitizing purposes. Pure active antimicrobial compounds isolated from food products have been recommended as food preservatives and for use in antiseptic processes [14]. The combination of vanillin, ascorbic acid, and adjustment of water activity may be promising for natural strawberry preservation [32]. Bagamboula et al also reported that various combinations of low temperature, pH, and sodium chloride inhibited *Shigella* spp. [4]. These naturally occurring, active antimicrobial compounds may help to alleviate the pressures associated with long-term use of antimicrobial chemicals, such as the emergence of drug-resistant strains [33,34], and they can be used in combination with physical treatments such as pH, temperature control, and regulation of water activity in the food manufacturing process to extend the shelf-life of food products [35].

Conclusions

This study demonstrated that different extracts from cruciferous vegetables have different degrees of antimicrobial activity. Four different extracts from each of the six cruciferous vegetables exhibited greater inhibitory effects against Gram-negative bacteria than against Gram-positive bacteria. CPEs exhibited the greatest antimicrobial effects compared with other extracts. Both TPC and coliform counts on the surfaces of beef, carrot, and celery decreased significantly after steeping in CPE. This hints at the possibility that naturally occurring compounds can be used as food preservatives and might be applicable in antiseptic processes.

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十字花科植物萃取物之抗菌效用

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本實驗利用幾種十字花科植物，包括白花椰菜、綠花椰菜、高麗菜、白蘿蔔、芥藍及青江菜，提取水及甲醇抽出物，並利用陰離子交換層析法抽取蛋白質，以酒精沉澱及超過濾法抽取水溶性多醣類等四種成分，檢測對幾種革蘭氏陽性菌、革蘭氏陰性菌及酵母菌之抗菌活性，發現對單一試驗菌之抗菌效果以蛋白質抽出液最佳。利用蛋白質抽出液、或與 **10 ppm** 氯液或 **1% citric acid** 之混合液浸泡牛肉、胡蘿蔔及芹菜，在浸泡不同時間後，以浸泡 **10** 分鐘後對三種試驗食材表面之 **total aerobic plate count** 及 **coliform** 會有顯著下降效果 ($p < 0.05$)。

關鍵詞：十字花科植物，萃取物，粗蛋白質，水溶性多醣類，抗菌效用

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