P-02-09

Antimicrobial activity of peptic hydrolysate fractions derived from desalted duck egg white on foodborne pathogens in pork chops (#96)

Rommanee Thammasena, Deng C. Liu

National Chung Hsing University, Department of Animal Science, Taichung, Taiwan

Introduction

Chemical additives are widely used to prevent the growth of foodborne pathogens and extend meat self-life during refrigerated storage. But consumers' concern of the chemical additives safety has risen in recent years and the demand of natural preservatives in foods has increased (Govaris *et al.,* 2010). Several bioactive peptides derived from enzymatic hydrolysates have been demonstrated against some selected foodborne pathogens (Almas *et al.,* 2011). Therefore, the aim of the study was to evaluate the antimicrobial activity of peptic fractions from hydrolysates of desalted duck egg white on 4 selected food pathogens in pork chops during storage at10℃for 3 days.

Methods

Hydrolysis of desalted egg white solution and fractionation of protein hydrolysates

Desalted duck egg white powder (DDEWP) was prepared according to the method of Fu (2016). A solution was prepared with 15 g DDEWP and 100 mL RO then porcine pepsin was added by E/S ratio of 0.3% (w/v). The hydrolysis was performed at 37 °C for 9 h. then was stopped by heating at 95 °C for 10 min. and the supernatant of hydrolysate was collected by centrifugation. The supernatant of hydrolysate was fractionated by using UF membranes with 100, 30 and 10 kDa MWCO. The fraction with Mw >100 kDa (F-I), 100-30 kDa (F-II), 30-10 kDa (F-III) and <10 kDa (F-IV) was collected, freeze-dried and stored in the moisture proof cabinet, individually.

Antibacterial activity

Agar diffusion method was performed according to the method of Jemil *et al*. (2014).

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) assay was performed according to the method of Norberg *et al.* (2011).

The preparation of pork chop and treatment

A total of 240 pieces of 1 cm thickness of fresh pork chops was divided into 5 group -control: no treatment; blank: sterile water; DDEWP: 150 mg/mL; F-IV-100: 100 mg/mL; and F-IV-150: 150 mg/mL. The culture of four selected bacteria: *E. coli*, *S. typhimurium*, *S. aureus* or *P. aeruginosa* was inoculated on the top surface of pork chops in each group, individually, and the bacterial level was approximately 10^5 CFU/cm^2. The solution was sprayed on the top surface of pork chop with the final concentration at 150 mg/cm^2 and100 mg/cm^2 for F-IV-100 sample. Then, samples were packed with polyethylene film and kept at 10 °C for 3 days.

Microbial analysis

Microbial incubation and counts were performed according to the method of Northcutt *et al.* (2008).

Results

Bacterial inhibition zone of peptide fraction of DDEW hydrolysate

Four fractions of DDEW hydrolysate exhibited remarkable zone of inhibition in the three selected bacteria except of *E.coli* and the largest zone of inhibition was observed in F-IV (Mw<10 kDa). The zone of inhibition of F-IV for *S. aureus*, *P. aeruginosa* and *S. typhimurium* was 1.07, 1.00 and 0.70 cm /dimeter, respectively. However, F-IV showed ineffective inhibition on the growth of *E.coli* and inhibition zone was 0.59 cm in this research (Table 1).

MIC of peptide fraction of DDEW hydrolysate

The MIC value of four peptidic fraction was shown in Table 1. The MIC value of all fractions for *S. aureus*, *S. typhimurium*, and *E.coli* were 150 mg/mL and significantly higher than that of *P. aeruginosa* (75 mg/mL).

The microbial qualities of pork chops during storage

In this study the count of *S. aureus* in all groups were increased during storage (Table 2). Significantly, F-IV fraction exhibited effective retardation on the growth rate of *S. aureus* (p<0.05), and the count of pork chops were significantly (p<0.05) decreased with the dose increase. At the end of storage, the bacterial counts of F-IV100 and F-IV150 were 5.21 and 5.10 log CFU/cm^2, respectively. In the growth of *E. coli,* the F-IV groups also had lower bacteria counts than other groups during storage. Especially at day 0, the counts of F-IV100 and F-IV150 was 4.75 and 4.53 log CFU/cm^2, respectively, significantly lower than that of the control (5.18 log CFU/cm^2), blank (5.13 log CFU/cm^2) and DSEWP (5.02 log CFU/cm^2). The antimicrobial effects of F-IV for *S. typhimurium* were also showed a similar tendency to *E. coli*. During storage *S. typhimurium* of control significantly increased from 5.56 at 0 day to 5.95 log CFU/cm^2 (p<0.05) at the end. The treatment of F-IV fractions maintained lower count of *S. typhimurium* (p<0.05) compared to the others groups during the storage. In the growth of *P. aeruginosa,* F-IV groups also demonstrated good inhibitory efficiency. Notably, the count of F-IV group was (p<0.05) lower than the control, blank and DSEWP throughout the whole storage. At the initial (day 0), the count of F-IV100 and F-IV150 was <

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4 log CFU/cm^2 and increased to 4.17 and 4.12 log CFU/cm^2 respectively at the end of storage.

Conclusion

F-IV Mw <10 kDa from hydrolysates of DSEW exhibited significant inhibitory efficiency on the selected pathogenic bacteria such as *E. coli*, *S. typhimurium*, *S. aureus* and *P. aeruginosa* due to larger inhibition zone and small MIC value. Furthermore, in application for pork chops the F-IV150 also showed significant (p<0.05) inhibition on the growth of *S. aureus*, *S. typhimurium*, *E. coli* and *P. aeruginosa* during storage.

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Bacteria	Fraction				Amp*	Cef*
	F-I	F-II	F-III	F-IV		
Diameter of inhibition zone (cm)[a]						
S. aureus	0.60±0.02^{D}	0.74±0.04^{C}	0.82±0.03^{C}	1.07±0.03^{B}	1.98±0.03^{A}	-
E.coli	0.25±0.01^{E}	0.32±0.03^{D}	0.43 ±0.03 ^{C}	0.54±0.02^{B}	1.68±0.03^{A}	-
S. typhimurium	0.42±0.02^{D}	0.53±0.03^{C}	0.58 ±0.01 ^{C}	0.70±0.01^{B}	2.27±0.03^{A}	-
P. aeruginosa	0.52±0.03^{E}	0.64±0.03^{D}	0.75 ±0.03^{C}	1.00±0.02^{B}	-	1.28±0.02^{A}
MIC						
S. aureus	150	150	150	150	-	-
E.coli	150	150	150	150	-	-
S. typhimurium	150	150	150	150	-	-
P. aeruginosa	75	75	75	75	-	-

Mean ± S.D., n=3

[a]Diameter of inhibition zone (cm), which minus with the well diameter of 1.2 cm or positive disc diameter of 0.6 cm.

[A-E]: Means within the same row without the same superscript are significantly different (p<0.05).

Amp = Ampicillin (10μg/disc) was used as positive reference standards to determine the sensitivity of *S. typhimurium*, *S. aureus* and *E. coli*.

Cef = Ceftazidime (30μg/disc) was used as positive reference standards to determine the sensitivity of *P. aeroginosa*.

Table 1. Analysis of inhibition zone and minimum inhibitory concentration (MIC) on selected bacteria

Bacteria	Treatment	Time of storage (day)			
		0	1	2	3
		Log CFU/cm^2 ± SD			
S. aureus	Control	5.41±0.03^{Ac}	5.54±0.04^{Ab}	5.54±0.04^{Ab}	5.64±0.06^{Aa}
	Blank	5.41±0.05^{Ab}	5.43±0.04^{Bb}	5.42±0.04^{Bb}	5.56±0.08^{Aa}
	DSEWP	5.18±0.04^{Bc}	5.26±0.07^{Cb}	5.32±0.04^{Bab}	5.36±0.05^{Ba}
	F-IV100	5.02±0.06^{Cc}	5.10±0.10^{Dbc}	5.13±0.10^{Cab}	5.21±0.06^{Ca}
	F-IV150	4.83±0.05^{Db}	4.91±0.11^{Eb}	5.04±0.13^{Ca}	5.10±0.08^{Da}
E. coli	Control	5.18±0.09^{Aa}	5.24±0.08^{Aa}	5.24±0.05^{Aa}	5.25±0.05^{Ba}
	Blank	5.13±0.10^{Ac}	5.14±0.06^{Abc}	5.24±0.08^{Ab}	5.44±0.07^{Aa}
	DSEWP	5.02±0.04^{Bc}	5.14±0.07^{Ab}	5.32±0.09^{Aa}	5.38±0.05^{Aa}
	F-IV100	4.75±0.03^{Cb}	4.83±0.14^{Bb}	5.03±0.09^{Ba}	5.07±0.07^{Ca}
	F-IV150	4.53±0.06^{Dc}	4.56±0.04^{Cc}	4.91±0.10^{Cb}	5.11±0.08^{Ca}
S. typhimurium	Control	5.56±0.04^{Ab}	5.59±0.17^{Ab}	5.67±0.09^{Bb}	5.95±0.10^{Aa}
	Blank	5.30±0.10^{Bb}	5.36±0.14^{Bb}	5.72±0.04^{Ba}	5.79±0.13^{ABa}
	DSEWP	5.14±0.13^{Cc}	5.28±0.10^{Bb}	5.86±0.05^{Aa}	5.89±0.04^{Aa}
	F-IV100	4.66±0.05^{Dc}	4.73±0.11^{Cc}	5.03±0.10^{Cb}	5.71±0.09^{Ba}
	F-IV150	4.51±0.10^{Ec}	4.62±0.08^{Cc}	4.85±0.07^{Db}	5.19±0.18^{Ca}
P. aeruginosa	Control	4.16±0.09^{ABc}	4.21±0.06^{ABc}	4.39±0.09^{ABb}	4.97±0.07^{Ba}
	Blank	4.08±0.11^{Bc}	4.09±0.11^{Bc}	4.29±0.09^{Bb}	5.18±0.11^{Aa}
	DSEWP	4.21±0.10^{Ac}	4.27±0.09^{Ab}	4.49±0.07^{Ab}	5.00±0.10^{Ba}
	F-IV100	3.75±0.06^{Cc}	3.95±0.08^{Cb}	4.00±0.09^{Cb}	4.17±0.10^{Ca}
	F-IV150	3.74±0.13^{Cb}	3.75±0.15^{Db}	3.85±0.16^{Db}	4.12±0.13^{Ca}

Mean ± S.D. n=9

[A-D]: Means within the same column without the same superscript are significantly different (p<0.05).

[a-c]: Means within the same row without the same superscript are significantly different (p<0.05).

Table 2. Changes in S. aureus, E. coli, S. typhimurium and P. aeruginosa counts in pork chop

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