PE8.16 Antioxidative properties of various acid hydrolyzed chitosan solutions and its effect on the pork qualities during refrigerated storage 95.00

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Abstract—**the objective of this study was to evaluate antioxidative properties of various acid hydrolyzed chitosan solutions and its effect on the pork qualities during refrigerated storage for 5 days. In this study, solutions of 4% chitosan (CS) with or without 0.2% phosphoric acid (PA), 0.5% acetic acid (AA) or 1.0% lactic acid (LA) were prepared. The results showed that the DPPH radical scavenging activities decreased during storage. AA+CS and LA+CS had significantly higher DPPH radical scavenging activities. PA and LA had significantly higher ferrous ion chelating abilities. PA+CS and LA+CS had ferrous ion chelating abilities of 41.26-63.25% during storage. Combination of various acids and CS did not significantly influence the L*, a* and b* values. After stored for 5 days, samples that had CS and PA, AA or LA had significantly lower TBARS values than the samples that only had acids added. After stored for 5 days, samples of PA+CS and AA+CS had significantly lower VBN values than PA and AA, respectively. Synergistic effect of acids and CS led to lower total plate counts and psychrotrophic bacteria counts of samples. Samples with combination of CS and acids tended to have better sensory results.**

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***Index Terms*—antioxidative, chitosan, pork, quality.**

I. INTRODUCTION

CHITOSAN (poly-b-1, 4-linked glucosamine) is a cationic polysaccharide made from alkaline N-deacetylation of chitin. Chitosan has been applied in some meat and meat products to improve qualities [1-4]. Some acids, such as lactic acid, acetic acid and etc., have been used to disinfect carcasses and meat products [5]. In addition, many of those acids have been recognized as generally recognized as safe (GRAS) [6]. Therefore, the objective of this study was to evaluate antioxidative properties of various acid hydrolyzed chitosan solutions and its effect on the pork qualities during refrigerated storage.

II. MATERIALS AND METHODS

A. Chitosan solution and pork sample preparation

In the preparation of various chitosan solutions of PA+CS, AA+CS and LA+CS, 4 g of chitosan (MW 250 kDa and 96% degree of deacetylation) was dispersed in 1000 ml of distilled water, to which phosphoric acid (2 g), glacial acetic acid (5 ml) or lactic acid (1 ml) was add individually to dissolve chitosan. Solutions at the same conc. but without additions of chitosan were also prepared with accordingly as PA, AA and LA. The pH of each solution was adjusted to pH 6.0 by adding 1N NaOH. A sample without any dipping treatment was used a control. Fresh porcine longissimus muscles were cut into cubes. After dipping in the solutions for 5 min, samples were drained gently with tissues, placed in plastic bags and stored at 4°C and analyzed accordingly.

B. DPPH scavenging activity and ferrous ion chelating ability

According to the method of Kivrak *et al*. (2009), DPPH scavenging (%)=[1 - (absorbance $_{sample}$ /absorbance $_{blank}$)]×100 at wave-length of 517 nm. According to the method of Kim and Lee (2009), ferrous ion chelating (%)=[1 - (absorbance $_{sample}$ /absorbance $_{blank}$)]×100 at wave-length of 562 nm.

C. Instrumental color measurement

Ground samples were placed in a measuring container, and then the L*, a* and b* values of samples were measured with a colorimeter (Nippon Denshoku Ze 2000, Japan).

D. Thiobarbituric acid (TBARS) and volatile basic nitrogen (VBN)

TBARS values of the samples were determined according the methods described by Faustman *et al.* (1992) and expressed as mg malonaldehyde/kg meat. Volatile basic nitrogen was determined according to CNS (1982) by the Conway micropipette diffusion method.

E. Microbial evaluation

Eleven g samples with 99 ml sterile distilled water were homogenized for 1 min. Serial dilutions were then made. Plate count agar was used for enumeration of total plate count and psychrotrophic bacteria count, and using pour plate method to enumerate bacteria. Total microflora and psychrotrophic bacteria were incubated at 37ºC for 48 h and 7ºC for 5 days, respectively.

F. Sensory evaluation

Samples were evaluated by a 10-member panel. Sensory attributes including color, off-aroma and overall acceptance was conducted using a 1 to 7 point hedonic scale test, with 1 representing light color, less off-aroma and less overall acceptance.

G. Statistical analysis

Data were analyzed using the general linear model (GLM) of SAS with a 5% level of significance. Means were separated using the least square means.

III. RESULTS AND DISCUSSION

The results showed that AA+CS and LA+CS had significantly ($P<0.05$) higher DPPH radical scavenging activities (41.33 and 43.53%) than AA and LA (8.36 and 7.72%) at day 1 (Table 1). The DPPH radical scavenging activities decreased during storage. At day 5, samples of AA+CS and LA+CS still had significantly higher DPPH radical scavenging activities. The results showed that PA and LA had significantly higher ($P<0.05$) ferrous ion chelating abilities (93.20-97.88%) during storage. When combining with chitosan, PA+CS and LA+CS had ferrous ion chelating abilities of 47.75-63.25% during storage.

There was no significant difference of L* values between treatments during storage (Table 2). Combination of various acids and chitosan did not significantly influence the L*, a* and b* values of samples between treatments. TBARS values increased during storage. After stored for 5 days, samples with chitosan and PA, AA or LA had significantly lower TBARS values than the samples that only had acids added (Table 3). Table 3 illustrates that VBN values increased during storage as expected. After stored for 5 days, samples of PA+CS and AA+CS had significantly lower VBN values than the samples with only acids added (PA and AA), respectively.

The results showed that the combination of acids and chitosan lowered the total plate count (TPC) of samples (Table 4). Samples had AA+CS had the significantly lowest TPC among treatments. Similarly, synergistic effect of acids and chitosan also led to lower psychrotrophic bacteria counts of samples.

Table 5 illustrates that samples with combination of chitosan and acids had significantly higher sensory colors than the ones with only acids added and LA+CS had the highest color scores. Samples off-aroma increased during storage. After stored for 5 days, samples with the combination of chitosan and acids tended to have mathematically lower off-aroma scores than the ones with only acids added even though without significant difference. After stored for 5 days, sample with the combination of chitosan and acids tended to have mathematically higher overall sensory acceptance.

IV. CONCLUSION

In conclusion, the combination of chitosan and acetic acid or lactic acid had significantly higher DPPH radical scavenging activities while the combination of chitosan and phosphoric acid had significantly higher ferrous ion chelating ability. Combination of chitosan and various acids improved the physico-chemical, microbial, and sensory qualities of fresh pork during refrigerated storage.

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Table 1. DPPH scavenging activities and Fe^{2+} chelating abilities of various acid hydrolyzed chitosan solutions

Treatment	DPPH scavenging activity (%) Storage time (day)			Fe^{2+} chelating ability (%) Storage time (day)		
	1	3	5	1	3	5
PA	12.03^{bx}	9.02^{by}	2.64^{bz}	97.88^{ax}	93.20^{ay}	95.49^{ay}
PA+CS	ND*	ND	ND	63.25^{cx}	59.83^{by}	55.34^{bz}
AA	8.36^{cx}	5.85^{cx}	4.36^{by}	46.96^{ex}	35.41^{dy}	30.60^{dz}
AA+CS	41.33^{ax}	34.99^{ay}	9.18^{az}	6.59^{fx}	3.25^{ey}	3.95^{ey}
LA	7.72^{cx}	7.40^{bcx}	3.63^{by}	95.57^{bx}	94.68^{ax}	94.19^{ax}
LA+CS	43.53^{ax}	34.16^{ay}	9.91^{az}	49.99^{dx}	47.77^{cy}	41.26^{cz}

*Not determined. CS: chitosan (0.4%); PA: phosphoric acid (0.2%); AA: acetic acid (0.5%); LA: lactic acid (1.0%). Footnotes are applied to all tables.

$^{a-f}$ Means within a column for the same test with different superscripts differ significantly ($P<0.05$).

$^{x-z}$ Means within a row for the same test with different superscripts differ significantly ($P<0.05$).

Table 2. Effects of various acid hydrolyzed chitosan solutions on the instrumental colors of fresh pork during storage at 4°C

Treatment	L* value Storage time (day)			a* value Storage time (day)			b* value Storage time (day)		
	1	3	5	1	3	5	1	3	5
Control	60.15^{ax}	60.01^{ax}	61.56^{ax}	8.32^{cx}	8.22^{bx}	7.05^{dy}	14.38^{abx}	14.50^{abx}	14.69^{ax}
PA	59.44^{ax}	59.33^{ax}	61.40^{ax}	9.26^{bx}	8.71^{abx}	7.22^{cdy}	14.87^{ax}	14.98^{abx}	14.56^{ax}
PA+CS	59.84^{ax}	60.17^{ax}	62.81^{ax}	9.18^{bx}	9.18^{ax}	7.90^{bcdy}	14.52^{abx}	14.72^{abx}	14.23^{ax}
AA	59.90^{ax}	60.68^{ax}	61.04^{ax}	8.86^{bcx}	8.44^{abx}	7.95^{bcx}	14.20^{bx}	14.53^{abx}	14.37^{ax}
AA+CS	59.08^{ax}	60.30^{ax}	60.53^{ax}	9.00^{bcx}	8.50^{abx}	8.19^{bx}	14.17^{bx}	14.27^{bx}	14.57^{ax}
LA	59.19^{ax}	59.86^{ax}	61.00^{ax}	9.42^{abx}	8.77^{abx}	7.85^{bcdy}	14.50^{abx}	15.04^{abx}	14.65^{ax}
LA+CS	60.12^{ax}	60.62^{ax}	61.48^{ax}	10.15^{ax}	8.86^{aby}	9.21^{ay}	14.30^{abx}	15.16^{ax}	14.66^{ay}

Table 3. Effects of various acid hydrolyzed chitosan solutions on the TBARS and VBN values of fresh pork during storage at 4°C

Treatment	TBARS Storage time (day)			VBN Storage time (day)		
	1	3	5	1	3	5
Control	0.49^{ax}	0.56^{ay}	0.71^{az}	6.00^{ax}	9.24^{ay}	12.24^{az}
PA	0.45^{bx}	0.54^{aby}	0.64^{bz}	5.77^{abx}	8.54^{aby}	11.09^{abz}
PA+CS	0.47^{abx}	0.49^{cx}	0.59^{cdy}	5.54^{abx}	7.16^{bcy}	9.24^{cz}
AA	0.50^{ax}	0.51^{bcx}	0.60^{cy}	5.54^{abx}	8.09^{abcy}	10.63^{bz}
AA+CS	0.47^{abx}	0.50^{cx}	0.55^{dy}	5.31^{abx}	6.93^{cy}	8.09^{cz}
LA	0.47^{abx}	0.51^{bcy}	0.64^{bz}	5.54^{abx}	8.08^{abcy}	11.09^{abz}
LA+CS	0.38^{cx}	0.42^{dy}	0.56^{dz}	5.08^{bx}	6.70^{cy}	10.16^{bz}

Table 4. Effects of various acid hydrolyzed chitosan solutions on the microbial counts of fresh pork during storage at 4°C

Treatment	Total microflora count (CFU/g) Storage time (day)			Psychrotrophic bacteria count (CFU/g) Storage time (day)		
	1	3	5	1	3	5
Control	3.54^{ax}	3.70^{ax}	3.74^{ax}	2.48^{x}	3.06^{ay}	3.30^{ayz}
PA	3.08^{bx}	3.20^{bx}	3.56^{aby}	$< 2^{*}$	2.95^{ax}	3.02^{abx}
PA+CS	2.67^{cx}	2.88^{cdx}	3.10^{cxy}	< 2	< 2	2.87^{bcx}
AA	3.05^{bx}	3.21^{bx}	3.45^{bxy}	< 2	< 2	2.84^{bcx}
AA+CS	$< 2^{*}$	2.87^{dx}	2.74^{dx}	< 2	< 2	2.50^{dx}
LA	3.15^{bx}	3.25^{bx}	3.54^{aby}	< 2	< 2	3.10^{abx}
LA+CS	2.89^{bcx}	2.98^{bcxy}	3.07^{cy}	< 2	< 2	2.72^{cdx}

* No 30-300 colonies growth found in samples diluted to 10^{-1}.

Table 5. Effects of various acid hydrolyzed chitosan solutions on the sensory evaluation of fresh pork during storage at 4°C

Treatment	Sensory color Storage time (day)			Off-aroma Storage time (day)			Overall acceptance Storage time (day)		
	1	3	5	1	3	5	1	3	5
Control	5.40^{abx}	5.05^{bx}	4.40^{by}	1.25^{ax}	2.50^{ay}	2.95^{ay}	5.20^{abx}	4.45^{bcy}	3.85^{bcz}
PA	4.15^{dx}	4.30^{dx}	3.65^{cx}	1.10^{ax}	2.15^{aby}	2.35^{by}	4.70^{bx}	4.05^{cy}	3.60^{cy}
PA+CS	4.90^{bcx}	4.78^{bcx}	4.50^{bx}	1.15^{ax}	1.95^{bcy}	2.10^{by}	4.75^{bx}	4.45^{bcx}	4.35^{abcx}
AA	4.33^{dx}	3.98^{dx}	4.05^{bcy}	1.30^{ax}	1.80^{bcy}	2.50^{abz}	4.90^{bx}	4.55^{bcx}	3.80^{bcy}
AA+CS	4.98^{bcx}	4.50^{cdx}	4.60^{bx}	1.10^{ax}	1.50^{cx}	2.45^{aby}	5.20^{abx}	4.70^{bx}	4.40^{abx}
LA	4.68^{cdx}	4.30^{cdx}	3.65^{cy}	1.20^{ax}	1.95^{bcy}	2.55^{abz}	5.10^{abx}	5.00^{bx}	4.05^{bcy}
LA+CS	5.93^{ax}	5.65^{ax}	5.65^{ax}	1.10^{ax}	1.75^{bcy}	2.00^{by}	5.65^{ax}	5.60^{ax}	4.80^{ay}

Based on a 1 to 7 scales (1=light color, less off-aroma and less overall acceptance).