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Changes in Total Plate Counts and Quality of Pig Small Intestine by Different Washing and Packaging Methods

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OPEN ACCESS

Received August 14, 2018

Revised November 15, 2018

Accepted November 20, 2018

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Abstract Pig small intestine not only is used as food but also for sausage casings production in many countries worldwide. However, it is well recognized that the small intestine is important source of spoilage and pathogenic bacteria. The present study aimed at investigating the effects of different washing and packaging methods on the changes of microbial levels and physicochemical characteristics of pig small intestine. After collecting and trimming off of visible fats, the pig small intestine samples were treated with; (i) different packaging methods: aerobic packaging (AP), skin packaging (SP), and vacuum packaging (VP); and (ii) washing with different concentrations of acetic acid. The treated samples were then stored at 4°C for 1, 4, 7, and 10 d. At 1-d storage, higher pH value was found in the AP-treated samples, however, after 7 to 10 days the samples treated with SP had higher values compared to the ones treated with AP and VP ($p < 0.05$). Thiobarbituric acid reactive substances values were higher in the AP-treated samples than those of the SP- and VP- treated samples at 7-d storage ($p < 0.05$). At 10th d, total plate counts (TPC) were higher in the control than in the acetic acid-washed samples ($p < 0.05$). Additionally, the TPC was lower in the SP- and VP-treated samples than the AP-treated samples at 7-d storage ($p < 0.05$). These obtained results suggest that the applications of washing with acetic acid solution and/or SP and VP methods could be an effective way to extend the shelf-life of pig small intestine during cold distribution.

Keywords pig small intestine, total plate counts, packaging, acetic acid

Introduction

Meat by-products may constitute a valuable resource if they are utilized properly to produce into value-added products (Toldrá and Reig, 2011; Zhang et al., 2010). It has been reported that the efficient utilization of meat by-products may arise up to 11.4%

and 7.5% of the gross income for the beef and pork production sector, respectively (Jayathilakan et al., 2012). There is a large variety of meat by-products but in general, most of them contain good amounts of nutrients such as essential amino acids, minerals and vitamins (Aristoy and Toldrá, 2011; Honikel, 2011; Kim, 2011). It should be taken into account that certain meat by-products can be considered as foods of interest depending on the country and local traditions while, in other places they can be considered as inedible foods (Ockerman and Basu, 2004a). In fact, some meat by-products with high nutritional value like blood, liver, lung, heart, kidney, brain, spleen and tripe which constitute a part of the diet and culinary recipes in many countries worldwide (Nollet and Toldrá, 2011).

In Korea, meat by-products are distributed from slaughterhouses to the final consumers through meat processors or wholesale markets, wholesalers, retailers and butcher shop (Kang et al., 2014). The distribution from the slaughterhouses to the wholesaler takes place in two channels: meat processors (65.8%) and the wholesale markets (34.2%). The wholesalers supply the retailers or chain stores. The retailers then supply the chain stores, butcher shop, and restaurants. “*Sundae*” (Korean blood sausage) is a kind of Korean traditional food made from pig by-products. The food is usually manufactured from pig large intestine and blood. Some parts such as boiled pig heart, liver, lung, small intestine etc. are also served with the “*Sundae*”. Pig heads are also used to make into some dishes like “*Pyeonyuk*” (slice of boiled meat) and widely consumed in the country. Majority of pigs’ feet are used to make “*Jokbal*” dish and being commonly sold. Thus, in Korea, most parts of pig by-products are consumed after making into some ready-to-eat dishes as mentioned above. While, for the other remaining parts of pork by-products such as small intestine, is considered as the most valuable material, and is usually used to make the roasted small intestine dish. On the other hand, the pig small intestine is used as the important material for production of sausage casings in many countries (Guerrero-Legarreta, 2011). However, it should be noted that the pig small intestine often carries a large number of unwanted bacteria which may cause the spoilage and discoloration if it is not properly handled during distribution period (Drosinos et al., 2011; Guerrero-Legarreta, 2011). The pig small intestine, therefore, should be properly washed and treated before using in order to guarantee the microbiological safety. Kang et al. (2014) suggested that prior to chilling storage a careful washing process is needed for pork by-products in order to maintain their quality, hygiene, and safety.

Furthermore, packaging is one of the effective ways to protect meat and meat products from undesirable impacts on quality such as microbiological and physio-chemical changes (Lindh et al., 2016). Nowadays, many packaging techniques (aerobic, overwrap, vacuum, and skin packaging (SP) etc.) have been applied for most meat products, however, the protection effects differ among them (McMillin, 2017). Thus, the objective of this study was to investigate the effects of different washing and packaging methods on the quality (pH and color) and microbial levels of pork small intestine.

Materials and Methods

Pig small intestine samples collection and treatment

The small intestine samples from crossbred pigs (about 105 to 110 kg body weights) collected at a commercial slaughterhouse (Korea) were used in the present investigation. The slaughters of the pigs were carried out under the commercial slaughtering process including: electrical stunning, bleeding, scalding, dehairing, washing, skinning and evisceration. Immediately after slaughter, the small intestine (duodenum and jejunum parts) samples were collected, trimmed off of visible fats and connective tissues and washed with tap water to remove food remnants and feces. Thereafter, the small intestines (about 100 kg) were cut into fragments (approximately 1 m in length) which were then used for packaging and acetic acid washing treatments.

For packaging treatments; the samples (approximately 16 kg each) were used for each packaging method; aerobic packaging (AP), skin packaging (SP) and vacuum packaging (VP). In each packaging method, the assigned samples were divided into small equal portions (1 kg each) which were then packaged by the above mentioned methods. After packaging, the packaged samples were divided into different storage groups (1, 4, 7, and 10 d, about 4 kg samples/group). The storage was done in a cooling room at 4°C.

For washing treatment; the samples (12 kg each) were rewashed with acetic acid solutions at different level (0%, 0.5%, 1%, and 2% v/v). The washing was done by immersing the samples in the acetic acid solution (5 liters per each kg sample) and washed for 2 min. Thereafter, the samples were rinsed with tap water for 1 min and absorbed with wiper papers, placed into plastic pouches (about 1 kg sample/bag), sealed and stored at 4°C for 1, 4, 7, and 10 d as shown the Table 1.

Total plate counts (TPC)

The total plate counts (TPC) in pig small intestine were determined following the procedures of Korea Food & Drug Administration (KFDA) Food Code (2008). Twenty-five grams of pig small intestine were taken and added with 225 mL of peptone water (1 g/L peptone), and were then homogenized for 1 min in a stomacher (400 VW, Bag Mixer, France). A serial dilution was made for the homogenized samples using peptone water. The diluted samples (1 mL each) were plated onto the Petrifilm Aerobic Plate Counts (3M Health Care, MN, USA) and incubated at 37°C for 48 h in an incubator. All the red colonies appearing on the plates were counted as the TPC and expressed as log colony forming units/g (CFU/g).

pH measurement

pH values were determined by homogenizing 3 g of each sample with 27 mL distilled water using a homogenizer (T25basic, IKA, Malaysia). The pH values were measured using a pH-meter (S20K, Mettler Toledo, Swiss).

Instrumental color

The surface color of pig small intestine was measured using a chromameter (CR-400, Minolta, Japan) standardized with a white plate ($Y=93.5$, $X=0.3132$, $y=0.3198$). Five measurements were taken on different locations of the outer surfaces of the samples and results were expressed according to the Commission International de l'Eclairage (CIE) system and reported as CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness).

Lipid oxidation measurement

Thiobarbituric acid reactive substances (TBARS) content was determined to evaluate the lipid oxidation levels in samples, following the procedure of Buege and Aust (1978). The samples (5 g each) added with distilled water (15 mL), saturated

Table 1. Treatment methods of small intestine with different washing process after pig slaughter

Treatment	Description	Washing ratio
C	Tap water washing 1 time (3 min)	
T1	0.5% (v/v) acetic acid washing (2 min)+tap water washing 1 time (1 min)	Washing solution (5 liters) : pig small intestine (1 kg)
T2	1% acetic acid washing (2 min)+tap water washing 1 time (1 min)	
T3	2% acetic acid washing (2 min)+tap water washing 1 time (1 min)	

butylated hydroxyanisole (50 µL) and 20 mL of thiobarbituric acid (0.02 M)/ trichloroacetic acid (15% w/v) (TBA/TCA at 1:1 ratio) were homogenized at 11,000 rpm for 15 s using an Ultra-Turrax T25B. The volume of the sample homogenate was adjusted to 50 mL with the TBA/TCA solution and immediately placed on ice. The tube containing homogenate was immersed in a 90°C-water bath for 15 min. After removal from the water bath, the sample tubes were immediately placed on ice to cool for 20 min and centrifuged at 3,000×g for 10 min using an Avanti J-E centrifuge (Beckman Coulter Inc., CA, USA). About 1.5 mL of supernatant was taken and the absorbance was measured at 531 nm using an UV– visible spectrophotometer (ProteomeLab Du-800, Beckman Coulter, Inc., USA). The TBARS content was calculated on the sample weight basis and expressed as mg malondialdehyde/kg (MDA/kg) sample. Three repetitions were applied for each sample in each treatment.

Statistical analysis

All experiments were repeated thrice. Data was collected for the determination of pH, color, TPC, and thiobarbituric acid reactive substances. The data was analyzed using a one-way ANOVA of SAS software (SAS Institute Inc., Cary, North Carolina, USA) followed by Duncan's multiple range tests to determine significant difference between the treatments ($p < 0.05$).

Results and Discussion

Total plate counts (TPC)

The changes in TPC of pig small intestine washed with different concentrations of acetic acid during cold storage are presented in Table 2. The TPC in all the treatments were significantly ($p < 0.05$) increased with increased storage days. The TPC were significantly ($p < 0.05$) higher in the control as compared to those in the treatments at the end of storage (10th d). However, no significant differences in the TPC occurred among treatments with acetic acid solutions. The results showed that prior to the storage, washing the pig small intestine with 0.5% acetic acid solution was more effective in inhibiting the growth of TPC.

The effect of packaging methods on the TPC in the pig small intestine during cold storage are presented in Table 3. The TPC in all the samples significantly ($p < 0.05$) increased as increasing the storage time. The TPC was significantly ($p < 0.05$) higher in the AP-treated samples than in the SP- and VP-treated samples after 7 d storage. However, there were no

Table 2. Changes in total plate counts (Log CFU/g) of pig small intestine by different washing process methods during cold storage at 4°C

Treatment	Storage days				SEM
	1	4	7	10	
C	2.17 ^b	3.29 ^{ABb}	3.3 ^{Bb}	5.18 ^{Aa}	4.23
T1	2.35 ^b	3.75 ^{Aa}	3.31 ^{Bab}	3.67 ^{Bab}	2.92
T2	1.56 ^b	2.76 ^{Bb}	3.45 ^{Bb}	4.54 ^{Ba}	3.65
T3	1.11 ^b	2.32 ^{Bb}	4.31 ^{Aa}	3.49 ^{Bb}	3.36
SEM	1.56	2.91	3.36	4.23	

^{A,B} Means with different superscripts within a same column differ significantly ($p < 0.05$).

^{a,b} Means with different superscripts within a same row differ significantly ($p < 0.05$).

C, tap water washing 1 time; T1, 0.5% acetic acid washing+tap water washing 1 time; T2, 1% acetic acid washing+tap water washing 1 time; T3, 2% acetic acid washing+tap water washing 1 time. The washing ratio of all treatments was washing solution of 5 liters vs. small intestine of 1 kg (volumes /weight).

Table 3. Changes in total plate counts (Log CFU/g) of pig small intestine by different packaging methods during cold storage at 4°C

Treatment	Storage days				SEM
	1	4	7	10	
AP	3.51 ^b	3.82 ^b	4.13 ^{Ab}	5.12 ^a	4.13
SP	3.55 ^b	3.02 ^b	3.56 ^{Bb}	5.13 ^a	4.07
VP	3.58 ^b	3.87 ^b	3.45 ^{Bb}	4.85 ^a	3.81
SEM	2.79	3.29	3.2	4.25	

^{A,B} Means with different superscripts within a same column differ significantly ($p < 0.05$).

^{a,b} Means with different superscripts within a same row differ significantly ($p < 0.05$).

AP, aero packaging; SP, skin-pack packaging; VP, vacuum packaging.

significant differences in the TPC among all treatments after 10 d storage. These results suggest that the use of SP or VP method was more effective in improving the microbiological safety for the fresh pig small intestine during cold distribution.

It is suggested that the internal organs of animals should be removed within 30 min after bleeding. Bijker (1981) illustrated a drastic increase in the bacterial growth curve when products were stored at 4°C compared to that of similar products stored at 2°C. Even at 2°C, bacterial counts in beef, pork, and lamb organs were altered during 5 d of storage (Hanna et al., 1982). The freezing of liver, kidney, and heart for 4 d did not significantly decrease the bacterial counts in these products (Hanna et al., 1982). Kang et al. (2014) suggested that small intestine is required a lot of washing times to remove blood, indigestible feed, and feces, etc. These authors have demonstrated that the reduction of initially high bacterial numbers is very important for shelf-life extension and freshness maintenance of pig small intestine.

Shelf-life can be defined as the period of time a product can be stored without becoming sensorial unacceptable or becoming a health risk. The predominant reason for meat spoilage is microbial activity, oxidation and enzymatic autolysis (Dave and Ghaly, 2011). In some cases, spoilage is caused by one specific microorganism (Gram and Dalgaard, 2002; Kalchayanad et al., 1993), but spoilage mainly depends on the composition of a heterogeneous microflora. However, other reasons for spoilage exist, since even sterile vacuum-packed meat has a limited shelf-life and becomes bitter over time, probably due to the proteolytic deterioration of meat proteins by intrinsic enzymes (Meinert et al., 2009). Odorous impression (scale anchored to the extremes 'fresh' and 'spoiled') is suitable attribute for describing spoilage of pork, beef (Meinert et al., 2009), and chicken (Franke et al., 2017), because the development of deviating raw meat odor and psychotropic growth follows the same pattern (Meinert et al., 2009).

Preservation of meat and meat products is essential for the meat industry. Several thermal and non-thermal methods are known and have been tested for decontaminating effects on bacteria in packed products to obtain an increased shelf-life and safety (Aymerich et al., 2008). In the present study, however, our treatment methods (packaging under different conditions and washing with acetic acid solutions) partly showed the beneficial effects on improving shelf-life and safety of pig small intestine during cold storage.

pH

Table 4 shows the changes in pH values of pig small intestine as affected by different packaging methods during cold storage. The pH values of the AP-treated samples significantly ($p < 0.05$) decreased with increased storage time whereas, no constant tendency was found in the SP- and VP- treated samples with increased storage time. The SP-treated samples had significantly ($p < 0.05$) higher pH value than the SP- and VP-treated samples during storage. After 7 d of cold storage, the pH value of VP-treated samples was lower than those of the AP- and SP-treated samples ($p < 0.05$). Therefore, the VP method seemed to be more

Table 4. Changes in pH of pig small intestine by different packaging methods during cold storage at 4°C

Treatment	Storage days				SEM
	1	4	7	10	
AP	6.82 ^{Aa}	6.65 ^{ABb}	6.52 ^{Bc}	6.57 ^{Bbc}	0.03
SP	6.75 ^{ABa}	6.62 ^{Bb}	6.61 ^{Ab}	6.73 ^{Aa}	0.02
VP	6.65 ^{Ba}	6.69 ^{Aa}	6.47 ^{Cb}	6.46 ^{Cb}	0.02
SEM	0.03	0.01	0.01	0.03	

^{A-C} Means with different superscripts within a same column differ significantly ($p < 0.05$).

^{a-c} Means with different superscripts within a same row differ significantly ($p < 0.05$).

AP, aero packaging; SP, skin-pack packaging; VP, vacuum packaging.

effective in maintaining the pH environment of small intestine during cold distribution. Kang et al. (2014) demonstrated that after 7 d of cold storage, pH levels of white viscera with small and large intestine were favorable for microbial growth. From this reason, the small intestine requires a more careful washing process than the other pork by-products.

Color and TBARS

Table 5 shows the changes in color traits of pig small intestine as affected by different packaging methods during cold storage. The treatment and storage time did not cause any changes in CIE L* values. The samples treated with AP and SP had significantly ($p < 0.05$) higher CIE a* and CIE b* values than the ones treated with VP after 7 d storage. These results suggest that lower oxygen content in the VP treated samples made them become paler in color.

The changes in TBARS of pig small intestine by different packaging methods during cold storage are shown in Table 6.

Table 5. Changes in color pig small intestine by different packaging methods during cold storage at 4°C

Items	Treatment	Storage days				SEM
		1	4	7	10	
CIE L*	AP	66.75	62.11 ^B	64.03 ^A	64.55	0.82
	SP	64.74 ^b	69.61 ^{Aa}	60.08 ^{Bc}	61.98 ^c	0.76
	VP	65.44 ^a	62.62 ^{Bab}	60.57 ^{Bb}	64.86 ^a	0.59
	SEM	0.61	0.96	0.64	0.91	
CIE a*	AP	17.59 ^a	15.26 ^{Bb}	17.59 ^{Aa}	16.62 ^a	0.39
	SP	13.41 ^b	12.35 ^{Cb}	18.11 ^{Aa}	17.71 ^a	0.47
	VP	13.15 ^c	16.8 ^{Aa}	15.03 ^{Bb}	17.99 ^a	0.38
	SEM	0.24	0.43	0.31	0.34	
CIE b*	AP	8.01 ^{Ac}	11.24 ^a	10 ^{Ab}	9.75 ^{Bb}	0.26
	SP	8.06 ^{Ac}	10.5 ^b	9.91 ^{Ab}	12.31 ^{Aa}	0.29
	VP	6.61 ^{Bc}	10.62 ^a	7.96 ^{Bb}	10.02 ^{Ba}	0.32
	SEM	0.18	0.25	0.24	0.3	

^{A,B} Means with different superscripts within a same column differ significantly ($p < 0.05$).

^{a-c} Means with different superscripts within a same row differ significantly ($p < 0.05$).

AP, aero packaging; SP, skin-pack packaging; VP, vacuum packaging.

Table 6. Changes in thiobarbituric acid reactive substances of pig small intestine by different packaging methods during cold storage at 4°C

Treatment	Storage days				SEM
	1	4	7	10	
AP	1.31 ^{Bb}	1.59 ^{Aa}	1.22 ^b	1.17 ^b	0.04
SP	1.18 ^{Bb}	1.3 ^{Bab}	1.31 ^a	1.23 ^{ab}	0.02
VP	1.62 ^{Aa}	1.42 ^{Bab}	1.23 ^b	1.18 ^b	0.05
SEM	0.07	0.04	0.03	0.03	

^{A,B} Means with different superscripts within a same column differ significantly ($p < 0.05$).

^{a,b} Means with different superscripts within a same row differ significantly ($p < 0.05$).

AP, aero packaging; SP, skin-pack packaging; VP, vacuum packaging.

There were no changes in TBARS values for all the treatments as increasing the storage time. However, the TBARS values in the AP-treated samples were significantly ($p < 0.05$) higher than those of the SP- and VP-treated samples after 7 days storage. These results suggest that the SP and VP methods were effective in preventing the lipid oxidation in the small intestine during storage. In the meat industry, adding value to meat by-products by applying the technological and scientific innovations to make the low valued by-products into the products with highly economic profitability is necessary to support the meat production sector (Ockerman and Basu, 2004a; Ockerman & Basu, 2004b; Pearl, 2004).

Conclusion

Washing with 0.5% acetic acid solution had the beneficial effects on the enhancement of microbial safety and appearance color stability of pig small intestine. Also, SP or VP was found more effective in inhibiting bacterial growth and lipid oxidation than the AP during cold storage. Based on the results obtained in the present study, it is concluded that the application of washing with 0.5% acetic acid solution and/or VP and SP methods is necessary for maintaining the quality of pig small intestine during distribution under cold condition.

Conflicts of Interest

The authors declare no potential conflict of interest.

Acknowledgments

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of storage and distribution technology for meat by-products, Project No. 90697403)” Rural Development Administration, Korea.

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