Bruises in beef cattle at slaughter in Mexico: Implications on quality, safety and shelf life of the meat

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Abstract In emergent economies and developing countries of Africa, Asia and Latin America, the major cause for carcass rejection from the international market is bruising; nevertheless many of these carcases are destined to local markets and meat processing industries for human consumption. Therefore, the aim of the present study was assess the effect of bruised meat on pH, microbiologic count and biogenic amines (BA) profiles along 21 days of ageing (sampling 1st, 7th, 14th and 21st day) with two packaging method (plastic bag vs vacumm) at 4° C. A total of 50 bruised carcasses were sampled from 1000 young bulls (Brown Swiss X Zebu) of 18-24 months old and an average live weight of 450±66 kg. The results showed significant differences between packaging systems and bruised vs non-bruised meat. The bruised meat caused higher biogenic amine concentrations than non-bruised meat. We conclude that bruised meat favoured increments of biogenic amine concentrations, even more than non-bruised meat. The plastic bag + vacuum system limited the increments of BA concentration during storage time therefore it improved shelf life of meat. These results emphasized the importance of implementing best management practices during pre-slaughter operations of cattle in order to reduce a possible risk factor for bruised meat.

Keywords: Meat safety; Bruised meat; Biogenic amines; Meat pH; Meat microbiology; Animal welfare

Introduction

Animal welfare is considered an important attribute of an overall 'food quality concept', and there is a growing realisation of a link between animal welfare and food safety (Miranda-de la Lama et al. 2014). Improper handling and transportation are also responsible for stress-induced meat quality problems, such as shrinkage of the carcass, higher pH, DFD meat and damage to the carcass through bruising (Chandra and Das, 2001). A bruise is a focal discolouration of the carcass surface, caused by an extra-vascular collection of blood and a trauma on the body caused by the impact of a blunt instrument (Strappini et al. 2009). Bruises are indicators for detecting basic pre-slaughter logistic chain failures, because they help to identify the source of problems, such as electric prod usage, projecting objects in facilities and trucks, and animals falling, abusive stockman-ship, social mixing, rough edges or drop gates (Miranda-de la Lama et al., 2012). In developed countries, a high incidence of bruising in cattle has been observed in industrial slaughterhouses, e.g. Namibia (90%; Hoffman and Lühl, 2012), Brazil (84%; Andrade et al. 2008), Mexico (97%; Miranda-de la Lama, et al. 2012), Uruguay (60%; Huertas et al. 2015), Colombia (37.5%; Romero et al. 2013), although much lower rates have been reported in some countries with standards on animal welfare as Chile (9–21%, Strappini et al. 2010).

Shelf life of fresh meat is influenced by stress during slaughter, packaging system, storage time and microbial growth (Li et al. 2014). Biogenic amines (BA) are low-molecular-weight organic bases showing biological activity and could be used as indicators of shelf life in the meat (Lorenzo et al. 2007). Formation and accumulation of BA in meat is the result of the enzymatic amino acids decarboxylation due to microbial enzymes and to tissue activity; therefore, the determination of these compounds is of a great interest, not only for their potential risk on human health, but also because they could be considered indicators of meat quality and freshness, being the BA associated to the degree of food fermentation or degradation by microorganisms (Favaro et al. 2007). Biogenic amines are reported as heat stable compounds and cooking or prolonged exposure to heat will not eliminate the toxin (Naila et al. 2010). The main BA found on meat are putrescine, cadaverine, and histamine, and they could be used as indicators of *Enterobacteriaceae*, *Pseudomonas* spp. *Lactobacilli*, *Enterococci* and *Staphylococci* activity (Galgano et al. 2009).

Currently, in developed countries and some emergent economies bruised carcasses are condemned under meat hygiene regulations (Hoffman and Lühl, 2012). However, the case of emergent economies and developing

countries of Africa, Asia and Latin America, the major cause for carcass rejection from the international market is bruising; nevertheless many of these carcases are destined to meat processing industries and local markets for human consumption (Jibat et al. 2008; Miranda-de la Lama et al. 2012; Regassa et al. 2013). Previous research indicated that bruised beef was microbiologically and technologically sound and therefore suitable for use in processed meat products (Rogers et al. 1992; 1993). In this context, the use of bruised beef with is common in minced meat and meat products due to low cost and easy availability of bruised meat. Whence bruising are of vital interest not only in animal welfare and product quality perspective, but also in on One Health concept. Although it is generally accepted that bruises have a negative impact on the meat quality, there is no significant research that investigates the evolution of bacterial and biogenic amines in bruised carcass. Therefore, the aim of the present study was assess the effect of bruised meat on pH, microbiologic count and biogenic amines (BA) profiles along 21 days of ageing (sampling 1st, 7th, 14th and 21st day) with two packaging method (plastic bag vs vacumm) at 4° C.

Materials and methods

The study was carried out in the State of Mexico from September 2013 to April 2014. The samples were collected at a private slaughterhouse located at "La Paz" municipality (19°21′38″N, 98°58′48″W), at 2260m. The climate is temperate, mean annual temperature and rainfall of 15.9 °C and 686 mm, respectively. The slaughterhouse fulfilled the requirements of the Official Mexican Norms about animal care, humanitarian slaughter and slaughterhouse regulations (NOM-009-ZOO-1994; NOM-024-ZOO-1994; NOM-030-ZOO-1995; NOM-033-ZOO-1995; NOM-194-SSA1-2004).

Selection of bruised carcasses

A total of 50 carcasses were selected and sampled from 1000 inspected commercial young bulls (Brown Swiss X Zebu) of 18-24 months old and an average live weight of 450±66 kg. The animals were raised in farms and feedlots in the states of Queretaro, México, and Morelos (Central Mexico). The average driving time from the farms or feedlots to the slaughterhouse was 3.5±1.0 h. The characteristics of the potbelly trailers did comply with the requirements of the Official Mexican Norms for animal transport. The trailers' characteristics were as follow; 16-

tons capacity with aluminium rigid chassis of five-compartment, passive ventilation and double-decked. The timetable of the slaughterhouse was 600 to 1700 hours (Monday to Saturday) with a slaughtering capacity of 225±35 animals/day at a rate of 33±4 animals/hour. The concrete unloading ramps had nonslip floors that were about as wide as the livestock trailers and they were connected to a lairage area that had nonslip concrete floor and 70 m^2 of roof. Within the slaughterhouse, the animals from different livestock trailers were not mixed with other animals; therefore the animals were housed in different pens that had access to water *ad libitum* but without feed. An electric goad was the instrument used to herd the animals during the stay in the slaughterhouse. A linear passageway starting from the lairage area guided them to the stunning area that did not have a head fixation system. Access to the box was through a guillotine door and a revolving iron exit door. The slaughtering method consisted in a stunning animal phase by means of a captive bolt gun (model USSS-1 JARVIS®) after that, animals were suspended by a hind leg and then the animal's throat was cut with a very sharp knife in order to drain the blood immediately. After, the animals were transferred to the production line to begin with the process of head separation, feet, skin, viscera, and the quartering of the carcasses. The protocol for the post-mortem evaluation was based on the carcass bruising score proposed by Romero et al. (2013). Bruised carcasses for this study were selected according causality only bruises originated in transport and pre-slaughter operations using four standards:

1. *Anatomical affected zone:* Carcasses with bruises on their back and hip anatomical regions were selected at the primary meat inspection point.
2. *Bruise size:* Bruised area between 8 cm and 12 cm in diameter that did not present any sign of infection or abscess was selected for the study. The size of the bruises was selected based on experience of the slaughterhouse workers; the suggested criterion was that the inspectors normally not remove bruises between 6 and 12 cm.
3. *Bruise severity:* It was rated by the observer according to Romero et al. (2013): grade 1: subcutaneous tissue affected; grade 2: as grade 1, plus the muscle tissue affected; grade 3: as grades 1 and 2, plus the presence of broken bones. For this study only carcasses grade 2 were selected.
4. *Bruise colour:* The bruised samples were selected using following criteria: L^* 26.34±5, a^* 15.12±5 and b^* 3.38±5. These measurements were carried out using a Hunter Lab colorimeter (model D25-PC2, Chroma Meter CR-200, Tokyo, Japan); the calibration was carried out using a White tile (L = 94.5, a = 1.0, b = 1.9). The

sample was rotated 90° after each reading, therefore the average of four readings of each sample was presented. *L*, *a* and *b* coordinates were transformed to polar coordinates: hue = $\tan^{-1}(b/a)$ and chroma = $(a^2 + b^2)^{1/2}$. These analyses were carried out in duplicate.

Treatments

Two adjacent samples per carcass of approximately 400 g each (non-bruised and bruised), were collected at the cutting room of the slaughterhouse (Rogers et al. 1992). The samples were collected from the bruised and non-bruised areas using a destructive method, i.e. pieces of beef were removed from the muscles using aseptic techniques. All samples were kept in sterile plastic bags and transferred to the Meat Laboratory keeping the cold chain. In the laboratory, each sample was divided in four pieces of 100 g and 2 cm thickness and the subsamples of bruised and non-bruised meat were packed using two systems; plastic bag (PB) and plastic bag + vacuum (PBV), therefore the treatments were PB with bruised meat (PWB), PB with non-bruised meat (PNB), PBV with bruised meat (VWB), PBV with non-bruised meat (VNB). The plastic bag material was polyethylene and vacuum pack material was nylon/binding layer L.LDPE 300x250 mm, 0.07 mm thick. All samples were stored at 4±1 °C (simulating retail conditions at supermarkets in a refrigeration chamber) during 1, 7, 14 and 21 days. The chamber was illuminated by a standard supermarket fluorescent lamp. The samples in the chamber were rotated every 24 h to minimise light intensity differences and possible temperature variations on the surface of the meat.

pH measurements

The pH of the samples was recorded at day 1, 7, 14 and 21, post-mortem using a portable thermometer and pH-meter (Hanna HI 99163, HANNA Instruments®, USA). Before measurements, the probe was calibrated with standard buffer solutions of pH 4 and 7.

Microbiological counting

Samples were identified and prepared aseptically for microbiological analysis. In order to homogenize the samples,

the following method was used: 10 g of meat were homogenized with 90 mL of peptone water (0.1%), and then the samples were serially diluted 10-fold (1 mL of the homogenates in 9 mL of peptone water). For the total plate count, all suitable serial dilutions were plated following the pour plate method. The Violet Red Bile Glucose Agar (VRBGA) was used for the quantification of *Enterobacteriaceae* using the standard plate count (Covenin 1086). Purple colonies and colonies surrounded by a purple area were counted. The results were expressed as U.F.C./g. Lactic acid bacteria were determined with MRS agar (de Man, Rogosa and Sharpe), the pH was adjusted to 5.6 with glacial acetic acid and using double layer agar incubated at 37 ºC for 48 h.

Determination of biogenic amines

The stock solutions of biogenic amines (BA); each containing 10 g/L were prepared by dissolving 18.24 mg of putrescine dihydrochloride ($C_4H_{12}N_2$ 2HCl), 17.14 mg of cadaverine dihydrochloride ($C_5H_{14}N_2$ 2HCl) and 16.57 mg of histamine dihydrochloride ($C_5H_9N_3$ 2HCl) from Sigma-Aldrich® (St. Louis, MO, USA), in 1 mL of 0.1 N HCl solution respectively and stored at 4°C. The solutions at concentrations of 0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25, 50 and 100 mg/L were obtained by suitably diluting the respective stock solutions and injecting three times onto the HPLC. Linear calibration curves, the regression equation and the determination coefficient (r^2) were calculated for each biogenic amine. The extraction and derivatization procedures were carried out according to Lázaro et al. (2013). Briefly, meat was extracted with perchloryc acid 5%, neutralized with NaOH (pH > 12) and derivatized with addition of benzoyl chloride (40 µL). The reaction was stopped with 5M NaCl, and then, the mixture was extracted with diethyl ether and evaporated to dryness under a stream of nitrogen. Finally, the residue was dissolved in 1000 µL of mobile phase (acetonitrile:water) and stored at 4±1 °C.

The samples were analysed with a HPLC system (Hewlett Packard® series 1100) with a column C_{18} of reverse phase (ACE Excel Super®, 250 mm x 4.6 mm and 5 µm of particle size) protected with a pre-column (ACE Excel C18, 4.6 x 20 mm) and a gradient pump which included a G1311A Quaternary Pump, G1315A Diode Array Detector, G1313A Auto sampler, G1322A, Vacuum Degasser (Agilent Technologies, Santa Clara), a Waters UV-Vis detector and a computer including Agilent software. The mobile phase for gradient elution consisted of two solvent systems: solvent A (Acetonitrile) and solvent B (MilliQ water). Gradient elution was carried out as follows: 50% of solvent A was used by 14 min, then the percentage of solvent A was increased to 100% during 7 minutes

and finally returned to 50% during 4 minutes. The flow rate was 1 mL/min and the column temperature was 25°C, the injection volume onto the column was 20 µL. The eluent was monitored with a UV detector set at 254 nm.

Statistic analyses

Data were analysed as a factorial design with randomized complete-blocks; the general model was:

$$Y_{ij} = \mu + T_i + \alpha_j + T_i * \alpha_j + \beta_3 + e_{ij}$$

Where Y is the mean response in the ith and jth factor, μ represented the mean response, T_i is the bruised meat and the non-bruised meat, α_j is the storage method (plastic bag and plastic bag + vacuum), $T_i * \alpha_j$ is the interaction of T_i and α_j, β_3 is the storage time for 1, 7, 14 and 21 days, that were used as blocks and e_{ij} represent the error term. The PROC MIXED command implemented in SAS 9.2 was used. The Tukey test was used when statistical differences were detected at $P \leq 0.05$. The Pearson correlation test was carried out in pH, concentration of biogenic amines and storage time.

Results

Shelf life evaluation of the meat samples, stored at 4°C for 21 days and packed with plastic bag (PB) and plastic bag + vacuum (PBV) showed significant differences between packaging systems and bruised vs non-bruised meat for pH, microbiological count and chemical parameters. Table 1 shows the significant increments ($P \leq 0.05$) of pH mean values of meat as a function of storage time and packaging systems. The pH values increased significantly ($P \leq 0.05$) from day 1 to 21 in all treatments (0.21 to 0.6 units) in both non-bruised meat and bruised meat. Table 2 shows the values of the total plate count, *Enterobacteriaceae and* lactic acid bacteria counts. The results indicated that all the carcasses sampled had detectable levels of lactic acid bacteria and *Enterobacteriaceae* ($P \leq 0.05$).

Table 3 shows the results of biogenic amines (BA) concentrations on bruised meat and non-bruised meat in the course of storage time, it is observed that concentrations of BA increased from day 1 to 21. When BA concentrations were compared between PB with non-bruised meat (PNB) and PBV with non-bruised meat (VNB), the lowest concentrations of putrescine and cadaverine were detected in VNB, mainly from day 1 to 14 while

183 histamine showed low concentrations from day 14 to 21; note that no significant differences ($P \geq 0.05$) were observed
184 at day 14 either VNB or PBV with bruised meat (VWB). The concentration of histamine in both PB with bruised
185 meat (PWB) and PB with non-bruised meat (PNB) was 21 times more than meat into PBV. The putrescine contents
186 in VWB increased significantly ($P \leq 0.05$) 14 and 13 times more than VNB at day 7 and 14. On the other hand,
187 histamine concentration in PWB increased 17 times more than PNB at day 7. There were significant ($P \leq 0.05$)
188 differences in cadaverine content between non-bruised and bruised meat, the later showed an increment of 1 mg/kg
189 of meat. The cadaverine concentration increased more than twice ($P \leq 0.05$) for PNB and VNB at day 7 and 14. An
190 unexpected finding was that putrescine concentrations in VWB were higher than PWB (18.4 vs 13.5, respectively)
191 from day 1 to 21 and as expected, cadaverine and histamine concentrations increased from day 1 to 21 for both PWB
192 and VWB.

193 Finally, Table 4 shows the Pearson correlation for all variables. The relationship between pH and BA was
194 influenced by storage time. The pH was negatively correlated ($P \leq 0.05$) with cadaverine indicating that pH
195 increments will limit cadaverine concentration at day 14. On the other hand, pH was positively correlated ($P \leq 0.05$)
196 with both histamine and putrescine at day 1 and 7, respectively for VNB and VWB. Putrescine was negatively
197 correlated ($P \leq 0.05$) with cadaverine at day 1 and 7 for PNB, cadaverine with histamine for VNB at day 21. On the
198 other hand, positive correlations were observed between histamine and cadaverine for PWB and putrescine with
199 histamine for VNB at day 14, being the strongest correlation ($r=0.40$; $P \leq 0.01$) observed.

200

201 **Discussion**

202

203 At commercial conditions the final pH measurement is one of the most important reference values for measuring
204 meat quality. Our results indicated that pH on day 1 in PWB and VWB had a pH > 5.7, and the trend were to
205 increase significantly at day 7, 14 and 21 post-mortem. Our results showed a relationship between bruised meat and
206 high pH values, this finding concurs with McNally and Warriss (1996) who reported that 48% of bruised carcasses
207 had pH values greater than 5.8. This is consistent with the fact that bruised areas in the meat would also decompose
208 and spoil more rapidly if the bruises are not removed as the bloody areas could promote bacterial growth.
209 Furthermore, it is hypothesized that stressed or bruised animals would have an abnormally high pH because of
210 glycogen depletion and the subsequent lower production of lactic acid in the muscles of stressed animals (Hoffman

et al. 2010a). Correlation analysis supported the finding that BA (cadaverine, putrescine and histamine) were present simultaneously across all treatments and these were reliant on the acid-pH. The higher pH could also be attributed to the buffer properties of the blood accumulated in the muscle tissue (Quintavalla et al. 2001). The presence of bruises is a reflection of transportation problems and when animals are stressed, glycogen reserves are depleted and higher pH can be obtained. Meat with normal pH, the environment restricts bacterial growth and only the lactic acid bacteria grow to a population capable of causing spoilage. However if muscle pH is higher, other organisms may grow and cause more rapid spoilage (Vimiso and Muchenje, 2013).

The meat is generally packed with air, under vacuum or in protective atmosphere, and the packaging system can contribute to discriminate the type of micro-flora and the type of BA found in the product. Under air, E*nterobacteriaceae* become the dominant spoilage bacteria, while under vacuum the lactic acid bacteria contribute significantly to the meat microflora (Galgano et al. 2009). Our study showed significant differences in BA concentrations between bruised and non-bruised meat, independently of packaging system used. These differences are mainly attributable to the presence of blood and the damaged tissue and how affected the microbial growth as well as their decarboxylase activity during the storage conditions.

The bruised meat represented an ideal medium for E*nterobacteriaceae* and lactic acid bacterial growth (Hoffman et al. 2010b), therefore high concentrations of putrescine were related with microbial growth (Bover et al. 2000), and tenderness of meat (Rogers et al. 1993). Our results indicated that low putrescine concentrations were obtained when meat was packed with PBV from day 1 to 21, however putrescine concentration in PWB and VWB increased 13.5 and 18 times, respectively from day 1 to 21. In this sense, Kaniou et al. (2001), quantified 3.9 µg/g up to 36.3 µg/g of putrescine in beef vacuum packed, from day 1 to 35. Therefore, the results presented in our study and the latter coincide in which; lower putrescine concentrations were detected in meat vacuum packed than in plastic bag only.

Putrescine is toxic at high quantities, the benefits of the existent polyamines in the human diet, nonetheless it is important to have in mind that polyamines are potential precursors of nitrosamines (compounds with carcinogenic action) that reacts with nitrate compounds, one of the potential polyamine precursors is the putrescine, since it affects the enzymes that metabolise the BA, it inhibits the monoamine oxidase, diamine oxidase and hydroxymethyltransferase, as a result, enteric illnesses have been observed in humans (Soufleros et al. 2007). The increment of cadaverine in PWB and VWB was observed between day 7 and 21, the increment was fold compared

with non-bruised meat, cadaverine alike putrescine could be related with the amount of *Enterobacteriaceae* that possess decarboxylase of lysine due to these microorganisms have been reported as a source of cadaverine in meat products coinciding with Eerola et al. (1996) who reported high counting of cadaverine, putrescine and tyramine in fresh meat related with high concentration of *Enterobacteriaceae* and lactic acid bacteria.

The bruised meat, placed in both, plastic bag and vacuum packed presented increments of 1 mg/ kg compared with non-bruised meat. The concentrations of putrescine were higher than cadaverine, these findings agreed with results reported by Bover et al. (2001), the highest concentrations could be related with high levels of microflora specialized in arginine decarboxylation hence it generates high levels of putrescine. Nevertheless, Halász et al. (1994) stated that cadaverine concentration is higher than putrescine, in beef meat. Alike putrescine, there is no scientific evidence about minimum doses of cadaverine for causing damage in humans. However, it is well known that cadaverine, agmatine, and putrescine are non-toxic per se, but they can limit the action of amine oxidase enzymes, hence they contribute to increase toxicity of histamine and tyramine (Ruiz-Capillas et al. 2010).

The latter highlights the importance of histamine quantification in bruised meat and non-bruised meat stored in either, PB or PBV. It was observed that histamine concentration was higher in PWB than VWB (13.70 mg/kg), up to day 21 (24.22 mg/kg), hence it was suggested that lack of oxygen is an important issue since it would limit bacterial growth and hence the decarboxylation of amino acids. The presence of histamine in meat is of paramount importance since the intake of food with high levels of this BA has been related with different symptoms in consumers; such as discomfort, nausea, respiratory problems, hot flashes, sweating, palpitations, migraines, itchy eyes, stomach and intestinal problems and pseudo-allergic reactions. Histamine is vasoactive and psychoactive, in addition it is a mediator of allergic illness, and therefore the consumption of meat with histamine may show the same symptoms of allergic processes, being confused sometimes (Püssa 2013). The histamine concentration observed in our study was under the level that causes damage in human health. Indeed, though the incidence of histamine is worldwide-reported and extensively discussed in scientific works, at present, a specific legislation concerning the maximum concentrations of histamine in food is still lacking (Russo et al., 2010). While for fish products there are clear limits for histamine, for example the European Union established as limit 100 mg/kg in fish belonging to the *Scombridae* and *Clupeidae* family, on the other hand the FDA (Food and Drug Administration) established as limit 50 mg/kg of histamine. It is important to note that there is no maximum levels established for

putrescine, cadaverine and histamine in fresh meat, since it must be taken into account that intake of BA is the sum of all biogenic amines present in all foods.

We conclude that bruised meat favoured increments of biogenic amine concentrations, even more than non-bruised meat. The plastic bag + vacuum system limited the increments of BA concentration during storage time therefore it improved shelf life of meat. These results emphasized the importance of implementing best management practices during pre-slaughter operations of cattle in order to aminorate a possible risk factor for bruised carcasses. Our study contributed to support that proper handling of the carcasses is of prime importance, since non-bruised meat showed lower concentrations of biogenic amines, either placed in plastic bag or vacuum packed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for care and use of animals were followed.

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Table 1. Least square mean (±SE) values of pH in fresh meat under different storage conditions.

Treatments	*Storage time (days)*			
	1	*7*	*14*	*21*
Plastic bag non-bruising (PNB)	5.59 ± 0.04^{cC}	5.73 ± 0.07^{cB}	$5.75\pm0.11b^{cB}$	5.84 ± 0.09^{cA}
Plastic bag with bruising (PWB)	5.73 ± 0.13^{aD}	5.85 ± 0.04^{aC}	6.10 ± 0.29^{aB}	6.32 ± 0.24^{aA}
Vacuum non-bruising (VNB)	5.54 ± 0.09^{dC}	5.57 ± 0.03^{dB}	5.74 ± 0.03^{cA}	5.75 ± 0.05^{dA}
Vacuum with bruising (VWB)	5.67 ± 0.04^{bC}	5.77 ± 0.04^{bC}	5.88 ± 0.43^{bB}	6.06 ± 0.15^{bA}

^{a,b,c,d}: Different lower cases at the same row means significant difference within treatments ($P\leq0.05$)
^{A,B,C,D}: Different upper cases s at the same row means significant difference within days ($P\leq0.05$)

Table 2. Least square mean (±SE) values of microbial counts in fresh beef meat under different storage conditions.

Treatments	*Storage time (days)*			
	1	*7*	*14*	*21*
Total Plate Count (log cfu#/g)				
Plastic bag non-bruising (PNB)	2.74 ± 0.15^{cC}	3.89 ± 0.24^{bB}	6.32 ± 0.27^{aB}	6.92 ± 0.26^{aB}
Plastic bag with bruising (PWB)	3.15 ± 0.21^{dA}	5.74 ± 0.16^{cA}	8.49 ± 0.15^{bA}	10.51 ± 0.32^{aA}
Vacuum non-bruising (VNB)	2.82 ± 0.31^{bB}	2.73 ± 0.33^{bC}	3.54 ± 0.21^{aC}	3.73 ± 0.24^{aD}
Vacuum with bruising (VWB)	2.69 ± 0.18^{bB}	3.40 ± 0.40^{bB}	4.07 ± 0.04^{aC}	5.02 ± 0.30^{aC}
Enterobacteriaceae count (log cfu#/g)				
Plastic bag non-bruising (PNB)	2.02 ± 0.21^{dB}	4.43 ± 0.11^{bB}	6.95 ± 0.19^{bB}	8.96 ± 0.17^{aC}
Plastic bag with bruising (PWB)	3.54 ± 0.14^{cA}	6.06 ± 0.22^{bA}	8.39 ± 0.68^{bA}	15.17 ± 0.59^{aA}
Vacuum non-bruising (VNB)	2.05 ± 0.58^{dB}	4.16 ± 0.09^{bB}	4.64 ± 0.17^{bC}	7.52 ± 0.12^{aC}
Vacuum with bruising (VWB)	2.87 ± 0.33^{cB}	5.60 ± 0.74^{bC}	5.91 ± 1.15^{bD}	10.19 ± 2.76^{aB}
Lactic acid bacteria count (log cfu#/g)				
Plastic bag non-bruising (PNB)	3.23 ± 0.47^{cB}	5.62 ± 0.77^{bA}	5.22 ± 0.53^{bB}	7.82 ± 0.04^{aB}
Plastic bag with bruising (PWB)	3.28 ± 0.30^{cdB}	4.67 ± 0.71^{cB}	6.77 ± 0.82^{bA}	9.50 ± 0.68^{aA}
Vacuum non-bruising (VNB)	3.02 ± 0.14^{bB}	3.44 ± 0.54^{bC}	5.71 ± 0.23^{aB}	6.50 ± 0.03^{aC}
Vacuum with bruising (VWB)	3.90 ± 0.44^{cbA}	4.75 ± 0.19^{bB}	7.32 ± 0.22^{aA}	6.88 ± 0.28^{aC}

^{a,b,c,d}: Different lower cases at the same row means significant difference within treatments ($P\leq0.05$).
^{A,B,C,D}: Different upper cases s at the same row means significant difference within days ($P\leq0.05$).

Table 3. Least square mean (±SE) values of biogenic amines in fresh beef meat under different storage conditions.

Treatments	*Storage time (days)*			
	1	*7*	*14*	*21*
Putrescine (mg/kg fresh weight)				
Plastic bag non-bruising (PNB)	1.78 ± 0.27^{cC}	2.43 ± 0.46^{cC}	11.85 ± 1.34^{cB}	47.39 ± 2.16^{cA}
Plastic bag with bruising (PWB)	5.27 ± 0.44^{aD}	18.33 ± 0.49^{bC}	51.19 ± 3.34^{aB}	71.34 ± 5.28^{aA}
Vacuum non-bruising (VNB)	0.65 ± 0.13^{dD}	1.36 ± 0.31^{dC}	2.74 ± 0.20^{dB}	36.73 ± 2.31^{dA}
Vacuum with bruising (VWB)	3.58 ± 0.16^{bD}	18.99 ± 0.74^{aC}	36.73 ± 1.95^{bB}	65.99 ± 2.31^{bA}
Cadaverine (mg/kg fresh weight)				
Plastic bag non-bruising (PNB)	$1.72\pm0.24b^{cC}$	2.03 ± 0.33^{cC}	5.68 ± 0.29^{cB}	27.28 ± 2.17^{bA}
Plastic bag with bruising (PWB)	1.75 ± 0.19^{bD}	4.42 ± 0.39^{bC}	18.41 ± 0.68^{aB}	46.36 ± 2.56^{aA}
Vacuum non-bruising (VNB)	1.58 ± 0.51^{cC}	1.49 ± 0.33^{dC}	5.23 ± 0.49^{dB}	10.30 ± 0.58^{cA}
Vacuum with bruising (VWB)	2.67 ± 0.16^{aD}	5.44 ± 0.38^{aC}	17.17 ± 1.06^{bB}	46.18 ± 2.76^{aA}
Histamine (mg/kg fresh weight)				
Plastic bag non-bruising (PNB)	0.28 ± 0.17^{cC}	0.13 ± 0.04^{dC}	2.67 ± 0.43^{bB}	11.43 ± 1.55^{cA}
Plastic bag with bruising (PWB)	0.69 ± 0.24^{bD}	2.24 ± 0.55^{aC}	3.29 ± 0.47^{aB}	24.22 ± 3.25^{aA}
Vacuum non-bruising (VNB)	0.32 ± 0.22^{cD}	0.60 ± 0.09^{cC}	1.93 ± 0.10^{cB}	4.42 ± 0.55^{dA}
Vacuum with bruising (VWB)	0.99 ± 0.08^{aC}	1.41 ± 0.32^{bC}	1.97 ± 0.46^{cB}	13.70 ± 1.75^{bA}

^{a,b,c,d}: Different lower cases at the same row means significant difference within treatments ($P\leq0.05$).
^{A,B,C,D}: Different upper cases s at the same row means significant difference within days ($P\leq0.05$).

Table 4. Correlation coefficients of pH, biogenic amines in fresh meat under different storage conditions.

		1st		7th		14th		21st
		Cadaverine	Histamine	Putrescine	Cadaverine	Cadaverine	Histamine	Histamine
		Day						
PNB	pH					-0.31*		
PWB	Putrescine	-0.30*			-0.35*			
	Histamine					0.37**		
VNB	pH		0.29*					
	Putrescine						0.40**	
	Cadaverine							-0.30*
VWB	pH			0.35*				

PNB: Plastic bag non-bruising; PWB: Plastic bag with bruising; VNB: Vacuum non-bruising; VWB: Vacuum with bruising
*P≤0.05; **P≤0.01