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Effects of Marination with Black Currant Juice on the Formation of Biogenic Amines in Pork Belly during Refrigerated Storage

Abstract

The effect of marination with black currant juice (BCJ) was investigated for their effects on meat quality and content of biogenic amines (BAs) (putrescine (PUT), cadaverine (CAD), histamine (HIM), tyramine (TYM), and spermidine (SPD)) in pork belly during storage at 9℃. BCJ was shown to have antibacterial activities against *Escherichia coli* and *Pseudomonas aeruginosa*. Additionally, the pH of pork belly marinated with BCJ (PBB) was significantly lower than that of raw pork belly (RPB) during storage. No significant difference in microorganisms between RPB and PBB was observed at day 0 of storage. However, at days 5 and 10 of storage, volatile basic nitrogen (VBN) was significantly decreased in PBB compared to RPB, and PBB also demonstrated significantly lower numbers of bacteria associated with spoilage (Enterobacteriaceae and *Pseudomonas* spp.) at these time-points. PBB was also associated with significantly reduced formation of BAs (PUT, CAD, TYM, and total BAs) compared to RPB at days 5 and 10 of storage. These results indicated that BCJ can be regarded a natural additive for improving meat quality by preventing increased pH, VBN, bacterial spoilage, and inhibiting BAs formation during refrigerated storage.

Key words: Pork belly, Food safety, Biogenic amine, Marination, Black currant

Introduction

Meat is an important source of nutrition for human health due high amounts of protein, minerals, and various bioactive compounds (Kim et al., 2018; Kim et al., 2019). In particular, pork is one of the most popular and widely-consumed forms of meat in Korea. According to the Organization for Economic Cooperation and Development (OECD), in Korea, the annual pork consumption (31.6 kg per capita) was higher than that of beef (11.9 kg per capita) and poultry (18.8 kg per capita) (OECD, 2021). Pork belly has been indicated as a strong preference among the various cuts of pork available in Korea (Choe et al., 2015; Van et al., 2020). During distribution and storage of pork belly meat, quality can be decreased and various metabolites can be generated (Triki et al., 2018). As one of the metabolites generated during storage, biogenic amines (BAs) is considered as an indicator of freshness of meat (Jairath et al., 2015).

BAs are basic nitrogenous compounds which can be produced by the action of microbial decarboxylases on free amino acids (Fan et al., 2015; Jairath et al., 2015). BAs are mainly produced in foods with high protein content, such as meat, fish, and dairy products (Linares et.al, 2012; Prester, 2011; Vinci & Antonelli, 2002). Generally, the most common BAs in meat and meat products are putrescine (PUT), cadaverine (CAD), histamine (HIM) and tyramine (TYM), and spermidine (SPD) has also been reported as amine present at significant levels in raw meat (Stadnik & Dolatowski, 2010). BAs are formed by spoilage bacteria (Enterobacteriaceae and *Pseudomonas* spp.), gram positive bacteria, and lactic acid bacteria (LAB) (Bover-Cid et al., 2003; Katikou et al., 2006; Ruiz-Capillas & Jimenez-Colmenero, 2005). As high levels of BA intake can result in toxicity, its consumption can be important for human health. Research has indicated that PUT and CAD can potentially be converted to carcinogenic N-nitrosamine as precursor substances (Lee & Kim, 2012), and SPD can also be harmful as a precursor of carcinogenic N-nitrosamines through heating of meat products

(Drabik-Markiewicz et al., 2011). In addition, high intake of TYM and HIM can cause migraine headaches and food poisoning, respectively (Balamatsia et al., 2006).

To inhibit the BAs formation in meat and meat products, various natural substances have been applied in meat. The use of natural substances to meat inhibit not also the formation of potential carcinogens such as BAs (Naila et al., 2010), but also enhance meat flavor and quality (Yusop et al., 2010). For example, green tea, cloves, cumin, and spearmint were reported to be strong inhibitor of BAs production in meat (Cai et al., 2015; Abu-Salem et al., 2011; Naila et al., 2010). According to these research, these natural substances had high antioxidant activity, antibacterial activity due to high phenolic compounds (Cai et al., 2015; Abu-Salem et al., 2011). These natural substances may inhibit the BAs formation by inhibiting the growth of the bacteria producing BAs or inhibiting the biosynthesis of BAs (Mah et al., 2009).

Black currant can be a good candidate as natural substance to inhibit BAs formation in meat, because of its high content of polyphenol (e.g. ferulic acid) and antibacterial activity (Borges et al., 2013; Widén et al., 2015). Black currant (juice or extract) demonstrated effective antibacterial activity against pathogenic and spoilage bacteria such as *Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, Escherichia coli* and *Pseudomonas aeruginosa* (Krisch, 2008; Denev et al., 2014; Widén et al., 2015).

Although black currant extracts has antibacterial activity, its effect on the reduction of BAs contents in meat during storage is limited. Therefore, the aim of this study was to evaluate the effect of black currant marination on the formation of BAs in pork belly during cold storage.

Materials and Methods

Preparation for black currant juice (BCJ) and pork belly

The BCJ was commercial black currant juice used in food industry, and it was purchased from local market. The process of marination and storage of the pork belly are shown in Fig. 1. The pork belly was cut into rectangular slabs of 10 cm × 5 cm × 0.4 cm (length × width × thickness). Pork belly marination was carried out with 95.15% of pork belly, 1.43% of BCJ, 0.57% of salt, and 2.85% of water, selected marinating formula according to previous sensory evaluation (data not shown). The pork belly marinated with BCJ (PBB) was placed into a vacuum pack to ripen for 24 hours at 5℃. The ripened PBB and raw pork belly (RPB) were stored at 9±2℃ for 10 days and analyzed at days 0, 5 and 10 of storage. Each experimental day, pork belly (n=10) was finely ground using a food mixer (HMF-3800SS, Hanil Electric CO., LTD, Seoul, Korea) and used for analysis.

pH of BCJ and pork belly

The pH values of BCJ, PBB and RPB were measured by pH meter (Orion 230A, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Ten grams of each sample were homogenized with 90 mL distilled water (DW) using a homogenizer (PolyTron ® PT-2500E, Kinematica, Lucerne, Switzerland).

Instrumental color of BCJ

The CIE L* (lightness), a* (redness), and b* (yellowness) values of the BCJ was measured with a Minolta chromameter (Model CR-400, Minolta CO., Tokyo, Japan), with the device calibrated using a white calibration plate (L*=93.69, a*=-0.22, b*=4.15).

Antioxidant activity of BCJ

2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical-scavenging activity (ABTS)

ABTS radical-scavenging activity was analyzed by the method of Kim et al. (2019). The stock solution of the $ABTS^+$ radical was made by mixing equal volumes of 14 mM $ABTS^+$ solution and 4.9 mM potassium persulfate solution, and left to react for 12 h at 23±1℃ in the dark. The stock solution was diluted with DW (absorbance of 0.700±0.02 at 735 nm) and assessed using a spectrophotometer (SpectraMax M2, Molecular Devices, CA, USA) at 30℃. The sample (50 μL) was reacted with $ABTS^+$ radical solution (950 μL) at 30°C for 30 min in the dark. The standard curve was established using Trolox (Sigma-Aldrich, St. Louis, MO, USA), and the ABTS values were expressed as mmol Trolox equivalent (TE)/g.

Ferric reducing antioxidant power activity (FRAP)

The FRAP assay was conducted by the method of Kim et al. (2019) with slight modifications. The FRAP working solution was made with acetate buffer (300 mM) of pH 3.6, 2,4,6-tripyridyl-S-triazine in 40 mM HCl (10mM), and $FeCl_3 \cdot 6H_2O$ solution (20 mM) at a ratio of 10:1:1 (v/v/v), respectively. The 25 μL of the sample was reacted with FRAP working solution of 175 μL at 37℃ for 30 min in the dark. The reacted solution absorbance was determined at 590 nm using a spectrophotometer (Molecular Devices). FRAP activity were expressed as mmol TE/g.

1,1-diphenyl-2-pricrylhydrazyl radical scavenging activity (DPPH)

DPPH radical scavenging activity was conducted by the method of Kim and Jang (2021) with slight modifications. The 100 μL of sample was added to wells of a 96-well microplate with 100 μL methanolic solution containing DPPH radicals (0.2 mM). The plate was shaken for 10 s and allowed to react for 30 min at 25℃ in the dark. The absorbance was measured at

517 nm using a spectrophotometer (Molecular Devices). A standard curve was established using Trolox, and DPPH values were expressed as mmol TE/g.

Oxygen radical absorption capacity (ORAC)

The ORAC assay was carried out using the method of Kim et al. (2019). The modified ORAC assay used potassium phosphate buffer (75 mM) of pH 7.4 at 37°C. The 25 μL of the sample was mixed with 150 μL of fluorescein (80 nM) and incubated for 15 min at 37℃. The 25 μL of 2,2'-azobis (2-amidinopropane) hydrochloride (150 mM) was mixed with generate peroxyl radicals. The change in the absorbance of the reacted sample was measured every minute at emission wavelength of 520 nm and excitation wavelength of 480 nm at 37℃ by spectrophotometer (Molecular Devices). A standard curve was established using Trolox, and ORAC values were expressed as mmol TE /g.

Total phenolic content (TPC) of BCJ

TPC was measured using the Folin-Ciocalteu colorimetric method as described by Kim et al. (2021). BCJ was diluted using methanol. 0.5 mL of diluted sample was mixed with 5 mL DW and Folin-Ciocalteu phenol reagent (Sigma-Aldrich), and left for 3 min. After that, the mixture was added to 1 N Na_2CO_3 and reacted for 90 min at 25℃ in the dark. The absorbance of the reacted sample at 760 nm was measured using a spectrophotometer (Molecular Devices). A standard curve was established using gallic acid, and TPC was expressed as mg gallic acid equivalent (GAE)/g.

BAs content of BCJ and pork belly

The BAs content was analyzed according to the method of Eerola et al. (1993). PUT, CAD, TYM, HIM and SPD stock solutions were diluted from 0.078 to 10 μg/mL using 0.4 M

perchloric acid (PCA). Two grams of samples (BCJ, ground raw or cooked pork belly) were homogenized with 10 mL of 0.4 M PCA and centrifuged (1,763×g, 4℃, 10 min). After centrifugation, the homogenate was filtered using Whatman No. 1 filter paper and the remaining pellet was re-extracted using 10 mL of 0.4 M PCA. The filtrated solution was collected, and made to 25 mL with 0.4 M PCA. The extracted solution (0.2 mL) was then mixed with 40 μL of 2 N NaOH, 60 μL of saturated $NaHCO_3$ and 0.4 mL dansyl chloride (10 mg/mL in acetone), and incubated at 45℃ for 40 min. Following the incubation, the solution was mixed with 20 μL of ammonium hydroxide to remove the dansyl chloride from the solution, and incubated in the dark for 30 min. The solution was thereafter mixed with 280 μL of acetonitrile, centrifuged for 10 min at 589 ×g, and filtered using 0.22 μm membrane filter (Rephile Bioscience and Technology, Shanghai, China) for using HPLC analysis.

Quantification of BAs was performed using Agilent 1260 HPLC (Agilent technologies, CA, USA) with Poroshell 120 EC-C18 (4 μm, 4.6 × 150 mm) column (Agilent technologies). The HPLC analysis used a gradient elution program with solvent A (0.1 M ammonium acetate) and solvent B (acetonitrile). The flow rate was 1.0 mL/min. The gradient started with a 50% of solvent B and then, it proceeded linearly for 19 min at 90% of solvent B. This ratio was changed linearly over 5 min to 50% of solvent B and it was kept for 5 min (the total run time was 29 min). The column temperature was 40℃. Samples of 20 μL volume each were injected, and BA amounts were quantified by UV-absorption measured at 254 nm.

Antibacterial activity of BCJ

Bacterial strain

To evaluate its antibacterial activity, BCJ was lyophilized and stored at -20℃ until analysis. The antibacterial activity of BCJ was assessed against *Escherichia coli* (*E. coli*, KCCM

11234) and *Pseudomonas aeruginosa* (*P.* aeruginosa, ATCC 27853). The bacteria strains (*E. coli* and *P. aeruginosa*) were streaked on Mueller Hinton agar (MHA, MB Cell, Seoul, Korea) and incubated at 37℃ for 24 h. A single colony of each test organism from the culture plates were inoculated into 10 mL sterile Mueller Hinton broth (MHB, MB Cell) and incubated at 37℃. Subsequently, the culture was sub-cultured 3 times and used for the paper disc diffusion assay, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) analyses.

Paper disc diffusion assay

The paper disc diffusion assay for evaluating antibacterial activity was conducted according to the method of Ramos et al. (2006) with slight modifications. The lyophilized BCJ powder was dissolved in DW at different concentrations: 30, 20, 10, 5, 2.5 and 1.25 mg/disc. The samples were filter sterilized using a 0.45 μm hydrophobic membrane filter (Rephile Bioscience and Technology). Test organisms were inoculated by transferring a loopful of culture into 10 mL of sterile MHB and incubated at 37℃ for 48 h; following the incubation period, the culture was adjusted to 8 log CFU/mL and incubated in MHA. Sterile 8 mm paper discs (Advantec, Toyo Roshi Kaisha Ltd., Tokyo, Japan) were aseptically placed on MHA surfaces, and each sample was immediately added to disc in volumes of 50 μL. The negative control was 50 μL of DW added to a sterile paper disc, and the positive control was 50 μL of streptomycin added to discs at concentrations of at 0.01 mg/disc for *E. coli* and 0.05 mg/disc for *P. aeruginosa*. The loaded plates were incubated for 24 h at 37℃. After incubation, the inhibition zone diameters (mm) were measured using a digital caliper (A&D Company Ltd., Tokyo, Japan).

MIC and MBC determination

The MIC, which was the least concentration of sample that inhibit microbial growth, was determined by microdilution of MHB in 96-well plates; 130 μL MHB, 20 μL microorganism suspension, and 50 μL sample were loaded to each well. The BCJ was diluted to concentrations of 600, 400, 200, 100, 50, 25 and 12.5 mg/mL, then inoculated with each test bacterial strain. The plates were incubated in a 37℃ incubator for 24 h, and the absorbance of each sample concentration at 600 nm was then measured using a spectrophotometer (Molecular Devices) at 37℃. The MIC was defined as the lowest concentration of BCJ showing no detectable growth.

The MBC, which was the least concentration of sample required to kill microorganisms, was determined by the testing concentrations listed above and performing subcultures on MHA medium. The plates were incubated at 37℃ for 48 h. The MBC was defined as the lowest concentration showing no bacterial colonies in media.

Microorganism analysis of pork belly

To evaluate the total aerobic bacteria (TAB), lactic acid bacteria (LAB), Enterobacteriaceae, and *Pseudomonas* spp. counts, each sample (10 g) was transferred into a sterile stomacher bag with 90 mL of sterile saline solution. Then, samples were homogenized for 40 s using a stomacher (Bag Mixer 400, Interscience, St. Nom, France). A serial dilution was performed using sterile saline solution, and 1 mL diluent was seeded onto petri dish in media. TAB counts were performed on plate count agar (PCA; MB cell) and incubated at 37℃ for 48 h. The man rogosa sharpe (MRS) agar (MB cell) was used for counting LAB as selective media and incubated at 37℃ for 48±2 h in the anaerobic jar. The Enterobacteriaceae and *Pseudomonas* spp. counts were carried out on violet red bile glucose (VRBG) agar (MB cell) and cetrimide (CN) agar (MB cell), respectively, as selective media and incubated at 30℃ for 24±2 h. The numbers of colony-forming units (CFU) per gram of pork belly were calculated.

Volatile basic nitrogen (VBN) of pork belly

VBN content was analyzed by the micro diffusion method, as described by Kim et al (2020). Five gram of ground raw pork belly was homogenized for 30 min in 25 mL DW on a magnetic stirrer, and the homogenate was filtered by filter paper (Whatman No. 1). The filtrate in Conway unit was incubated with H_2SO_4 (0.01 N) at 25℃ for 1 h. Following incubation, 20 μL of Brunswik indicator added to the Conway unit inner chamber, and titrated against NaOH (0.01 N). The VBN value was calculated as follows and expressed as mg/100 g.

$$\text{VBN (mg/100 g)} = 0.14 \times (b–a) \times F/W \times 100 \times 50$$

where a is the volume of NaOH (0.01 N) added to the sample (mL), b is the volume of NaOH (0.01 N) added to the blank (mL), F is the standard factor for NaOH (0.01 N), W is the weight of sample (g).

Statistical analysis

Data were analyzed using the SAS program (ver. 9.2; SAS Institute, Cary, NC, USA). T-test was performed to comparing means of treatment (marination) and a one-way analysis of variance (ANOVA) was performed for data of storage time. Significant differences was determined by Tukey's test ($p<0.05$). Additionally, a two-way ANOVA was carried out to evaluate any interactions between treatment and storage.

Results and Discussion

Characteristics of BCJ

The pH, color, antioxidant activity, TPC, BAs of BCJ were analyzed (Table 1). The pH of

253 BCJ was 3.17 and the CIE L*, CIE a*, and CIE b* of BCJ were 16.81, 0.19, and 0.25,
254 respectively. BCJ showed the antioxidant activity across four traits; ABTS radical scavenging
255 activity: 112.64 mmol TE/g; FRAP activity: 126.46 mmol TE/g; DPPH radical scavenging
256 activity: 104.85 mmol TE/g; ORAC activity: 231.57 mmol TE/g. TPC of BCJ showed 8.58
257 mg GAE/g, which had similar values compared to previously published research (5.80-7.42
258 mg GAE/g black currant extract) (Bakowska-Barczak & Kolodziejczyk, 2011). The BCJ
259 contained four types of biogenic amines: PUT: 4.83 μg/g; CAD: 14.41 μg/g; TYM: 0.50 μg/g;
260 SPD: 1.19 μg/g. The PUT contents of BCJ (4.83 μg/g) was higher than that of other vegetable
261 (Chinese cabbage (1.8 μg/g), Endive (2.8 μg/g), Radicehio (4.3 μg/g)), while the contents of
262 TYM and SPD in BCJ (0.50 and 1.19 μg/g, respectively) was lower than that in Chinese
263 cabbage (1.2 and 15.1 μg/g, respectively), Endive (11.3 and 1.5 μg/g, respectively), Iceberg
264 lettuce (0.9 and 7.8 μg/g, respectively), and Radicehio (2.3 and 11.3 μg/g) (Simon-Sarkadi et
265 al., 1993).
266
267 **Antibacterial activity of BCJ against *E. coli* and *P. aeruginosa***
268 The antibacterial activity of BCJ against spoilage bacteria (*E. coli* and *P. aeruginosa*) was
269 evidence by the presence of inhibition zones, shown in Table 2 and Fig. 2. BCJ at a
270 concentration from 10 to 30 mg/disc showed inhibition zones against *E. coli* and *P.*
271 *aeruginosa*. BCJ had antibacterial activity against *E. coli*, with the range of inhibition zones
272 between 9.71 and 14.87 mm, while an inhibition zone of *P. aeruginosa* of between 8.76 to
273 12.52 mm was clearly observed. The inhibition zones formed by BCJ *against E. coli* and *P.*
274 *aeruginosa* indicated a concentration-dependent effect of BCJ from 10 to 30 mg/disc
275 ($p<0.05$). However, there were no inhibition zones in the concentration range between 1.25
276 and 5 mg/disc.
277 The antibacterial activity of BCJ against *E. coli* and *P. aeruginosa* was also evidenced by

278 the MIC and MBC shown in Table 3. The MIC of BCJ was determined to be 100 mg/mL
279 against both bacterial strains. For the MBC, the inhibitory effect of BCJ was higher in *P.*
280 *aeruginosa* than in *E. coli*. In a previous study, black currant juice, water and methanol
281 extract inhibited the growth of *E. coli* by less than 25% (Krisch, 2008). Meanwhile, a study
282 by Widén et al. (2015) showed that a higher level (10%) black currant juice (pH 4.7)
283 effectively inhibited *P. aeruginosa* growth due to the low pH of the black currant, in
284 concordance with our results (Table 1). According to previous studies, the high antibacterial
285 activity of black currant is resulting from its high levels of phenolic acids such as ferulic acid
286 and chlorogenic acid (Borges et al., 2013; Widén et al., 2015). In particular, the abundance of
287 ferulic acid (113.1 μg/mL) in black currant, and black currant juice, is higher than that of
288 other phenols (Widén et al., 2015). Ferulic acid is one of several phenols with antibacterial
289 activity against pathogenic bacteria. Several reports found that ferulic acid had high
290 antibacterial activity against *E. coli* and *P. aeruginosa* (MIC of *E. coli* and *P. aeruginosa* were
291 100 μg/mL) (Borges et al., 2013; Cho et al., 2017). The possible mechanism for antimicrobial
292 effect of phenolic compounds are known as altering microbial cell permeability, which
293 interfering with membrane function such as nutrient uptake, electron transport, enzyme
294 activity, protein and nucleic acid synthesis (Abu-Salem et al., 2011).
295
296 **pH, microorganism, and VBN of pork belly**
297 Changes in pH value of RPB and PBB during storage were shown in Table 4. The pH
298 values of RPB and PBB significantly decreased on day 10 when compared to day 0 of storage.
299 Of note, the pH values of PBB were low compared to RPB on all storage days ($p<0.05$). In
300 pH, the treatment (marination) and storage had a high degree of interaction with each other
301 ($p<0.0001$). Duffy et al. (2000) previously reported that pH values of vacuum-packed minced
302 beef gradually declined during storage at 0℃ and 10℃ due to predominant growth of LAB

303 in vacuum-packed minced beef. Additionally, Chung et al. (2018) reported that the pH of
304 Hanwoo tteokgalbi treated with black currant powder (pH 5.31) was lower than that of
305 control tteokgalbi (pH 5.40) at day 0 of storage, which agrees with our results. Moreover, an
306 earlier study also reported that black currant contains high level of ascorbic acid (Iversen,
307 1999). Thus, the low pH value of PBB may be affected by the low pH of BCJ (Table 1), and
308 the vacuum conditions may contribute to the predominance of acid-forming microbes during
309 storage.

310 Bacterial contamination is an unavoidable consequence of meat processing and storage. As
311 such, bacterial counts are an important factor in determining the freshness of meat and meat
312 products. To evaluate the antibacterial activity of BCJ on the BCJ-marinated pork belly,
313 microorganism counts in RPB and PBB are shown in Table 4. There were no significant
314 differences in the microorganism abundance between RPB and PBB at day 0 of storage. As
315 the storage period increased, the counts of TAB and LAB were higher in PBB than in RPB,
316 and were evident at both day 5 and 10 of storage ($p<0.05$). On the other hand, amounts of
317 spoilage bacteria (Enterobacteriaceae and *Pseudomonas* spp.) were lower in PBB than in
318 RPB by days 5 and 10 of storage ($p<0.05$), evidencing the antibacterial activity of BCJ.
319 Indeed, in the present study, BCJ demonstrated effective antibacterial activity against *E. coli*
320 (the type species of the type genus of Enterobacteriaceae) and *P. aeruginosa* (the type species
321 of *Pseudomonas*) (Table 3). In addition, the vacuum condition used here can inhibit the
322 growth of *Pseudomonas* spp. and contribute predominance of LAB (Castellano et al., 2008).
323 The low pH of BCJ (pH 3.17) and vacuum packaging can also contribute to providing an
324 optimal environment for LAB growth, while low pH can concurrently inhibit the growth of
325 spoilage bacteria during storage. The treatment (marination) and storage was highly
326 interacted with each other for TAB, Enterobacteriaceae and *Pseudomonas* spp. ($p<0.01$-
327 $p<0.0001$). Therefore, our study demonstrated that PBB can effectively inhibit growth of

spoilage bacteria (Enterobacteriaceae and *Pseudomonas* spp.) due to the antibacterial activity of black currant, and the vacuum conditions.

As well as the number of bacteria, VBN value is also important indicators for determining the freshness of meat. The VBN value of RPB and PBB are shown in Table 4. The addition of BCJ was not associated with a significant difference between the VBN value of RPB and PBB (6.60 and 6.04 mg/100 g, respectively) at day 0 of storage. However, , the VBN value of RPB and PBB increased as the storage period increased, and significant differences were observed from day 5 of storage ($p<0.05$). On days 5 and 10 of storage, the VBN values of PBB (8.35 and 15.10 mg/100 g, respectively) was lower than that of RPB (15.77 and 32.92 mg/100 g, respectively) ($p<0.05$). Moreover, the treatment (marination) and storage had a strong interaction for VBN value ($p<0.0001$). In Korea, the upper limit of the VBN value is 20 mg/100 g for fresh meat (Kim et al., 2020). During storage for 10 days at 9℃, it was observed that the VBN content for PBB did not exceed 20 mg/100 g, in contrast to the RPB. VBN is mainly produced by enzymatic decarboxylation of specific amino acids, which is associated with growth of Enterobacteriaceae and *Pseudomonas* spp. (Li et al., 2019). In the present study, the addition of BCJ significantly reduced the VBN values of PBB from day 5 of storage, which is consistent with the results of microbial analysis (Table 4), indicating that addition of BCJ effectively inhibited the growth of spoilage bacteria in PBB from day 5 of storage.

Effect of marination with BCJ on the formation of BAs in pork belly during storage

BAs are formed by the action of bacterial decarboxylases in meat. Factors involved in the formation of BAs include factors pertaining to the specific raw meat material (such as meat composition, free amino acids, fat content, pH, etc.), microbial growth, processing conditions, and storage conditions (Ruiz-Capillas & Jimenez-Colmenero, 2005). In particular,

353 microorganisms can be an important factor in the formation of BAs; thus, controlling
354 microbial levels using natural antibacterial agents can be a direct strategy for controlling BA
355 content.

356 The effect of BCJ on the formation of BAs in the pork belly during refrigerated storage are
357 shown in Table 5. The PUT, CAD, HIM and TYM contents of RPB and PBB were gradually
358 increased from day 0 to 10 of storage ($p<0.05$). On the other hand, the SPD content of RPB
359 tended to decrease as the storage period increased ($p<0.05$), while the SPD content of PBB
360 showed irregular fluctuation throughout storage. Although there is no criterion for judgment
361 about BAs in fresh meat, according to previous study, total BAs content in fresh pork meat
362 reported in range of 3.8-8.0 μg/g (Kalač, 2006; Favaro et al., 2007; Triki et al., 2018; Min et
363 al., 2007; Ngapo et al., 2017) and even up to 32.8 μg/g (Halász et al., 1994). The addition of
364 BCJ effectively inhibited the formation of BAs in pork belly during the storage period. In the
365 PBB, an inhibitory effect on PUT content was observed from day 0 to 10 of storage ($p<0.05$).
366 Further, a significant inhibitory effect on the formation of CAD and TYM was found on day
367 5 and 10 of storage in PBB. However, HIM was detected only at day 10 of storage, and there
368 was no significant differences between PBB (1.12 μg/g) and RPB (1.45 μg/g). Additionally,
369 no clear reduction of SPD in pork belly was observed in association with addition of BCJ
370 during storage. All BAs contents were strongly affected by BCJ treatment ($p<0.0001$), and
371 treatment and storage had high degree of interaction with each other for PUT, CAD, TYM,
372 Total BAs ($p<0.001$) and HIM ($p<0.05$).

373 PUT, CAD and TYM can be formed by spoilage bacteria in fresh pork, and can therefore
374 be used as spoilage indicators in pork (Li et al., 2014). The formation of PUT and CAD is
375 mainly associated with *Pseudomonas* spp. and Enterobacteriaceae, respectively (Bover-Cid et
376 al., 2003; Geornaras et al., 1995; Katikou et al., 2006; Ruiz-Capillas & Jimenez-Colmenero,
377 2005), while the formation of TYM usually has responsibility with the LAB strains (Bover-

Cid et al., 2001). Prior research has indicated that *Pseudomonas* spp. is also strongly correlated with TYM formation in chilled pork (Li et al., 2014). In the present study, PBB showed low levels of spoilage bacteria (Enterobacteriaceae and *Pseudomonas* spp.) and a low VBN value when compared to RPB. According to Min et al. (2007), VBN values are highly correlated with levels of BAs (PUT, CAD, and TYM) in beef, pork, and chicken during storage. Here, the content of PUT, CAD and TYM were significantly low in PBB compared to RPB; these results may therefore be related to low levels of spoilage bacteria and the low VBN value in PBB during storage. Taken together, our results indicate that addition of BCJ to pork belly can inhibit the growth of spoilage bacteria in pork belly, and can reduce the formation of PUT, CAD and TYM by spoilage bacteria during chilled storage. The lack of difference in HIM content between RPB and PBB could be attributed to the low inhibitory efficiency of black currant on gram-positive bacteria; HIM is formed by decarboxylase of histidine, which is widely distributed in the gram-positive genera (Georgnaras et al., 1995). In a previous study, black currant was shown to include high of ferulic acid, and the level of intracellular content (K^+) release of gram-positive bacteria was lower upon exposure to ferulic acid than that of gram-negative bacteria (Borges et al., 2013; Widén et al., 2015). Thus, BCJ marination would have had a lesser effect on HIM in PBB than its effect on other biogenic amines (PUT, CAD, and TYM) more susceptible to its mechanisms. Additionally, in the present study, the average level of SPD in RPB and PBB was 3.27 and 3.31 μg/g during storage, respectively. A previous study found that the average level of SPD in meat is usually around 3.0 mg/kg (Hernández-Jover et al., 1997), which agrees with our results. During storage, there was an irregular reduction of SPD content associated with BCJ marination. This could be due to different mechanisms of SPD formation. Generally, PUT, CAD, HIM, and TYM are formed on the surface of meat by the activity of bacteria, while SPD is formed naturally in fresh pork meat because it exists as a natural constituent of living cells (Cai et al.,

2015; Hernández-Jover et al., 1997; Paulsen et al., 2006). Therefore, it is reasonable that marination with BCJ would have a lesser effect on SPD. In other studies, a small decline in SPD content has been reported due to the conversion of SPD to PUT (Larqué et al., 2007) or deamination by microbial polyamine oxidase enzymes (Razin et al., 1959) during the storage. Similarly, in our study, the SPD content of RPB and PBB tended to decrease at day 10 of storage than that of day 5 of storage. The total BA content in RPB and PBB gradually increased up to 242.73 and 151.96 μg/g during day 10 of storage time, respectively. At day 0 of storage, there was no significant difference in total BA content between RPB and PBB. However, the inhibitory effect of total BAs in PBB was observable at day 5 and day 10 of storage ($p<0.05$). In particular, the PBB had a higher rate of total BA inhibition (73.16%) at day 5 of storage.

Conclusion

In our study, the addition of BCJ was found to increase shelf life via inhibiting the growth of spoilage bacteria and reduce BAs contents formed during storage in pork belly. Overall, this study suggests that black currant can be used as a natural additive on meat industry and can provide consumer health benefits via reducing BAs in meat products. However, in order to commercially apply black currant to meat, additional studies for the optimal addition concentration of black currant on various meat are needed in consideration of economic efficiency and organoleptic characteristics.

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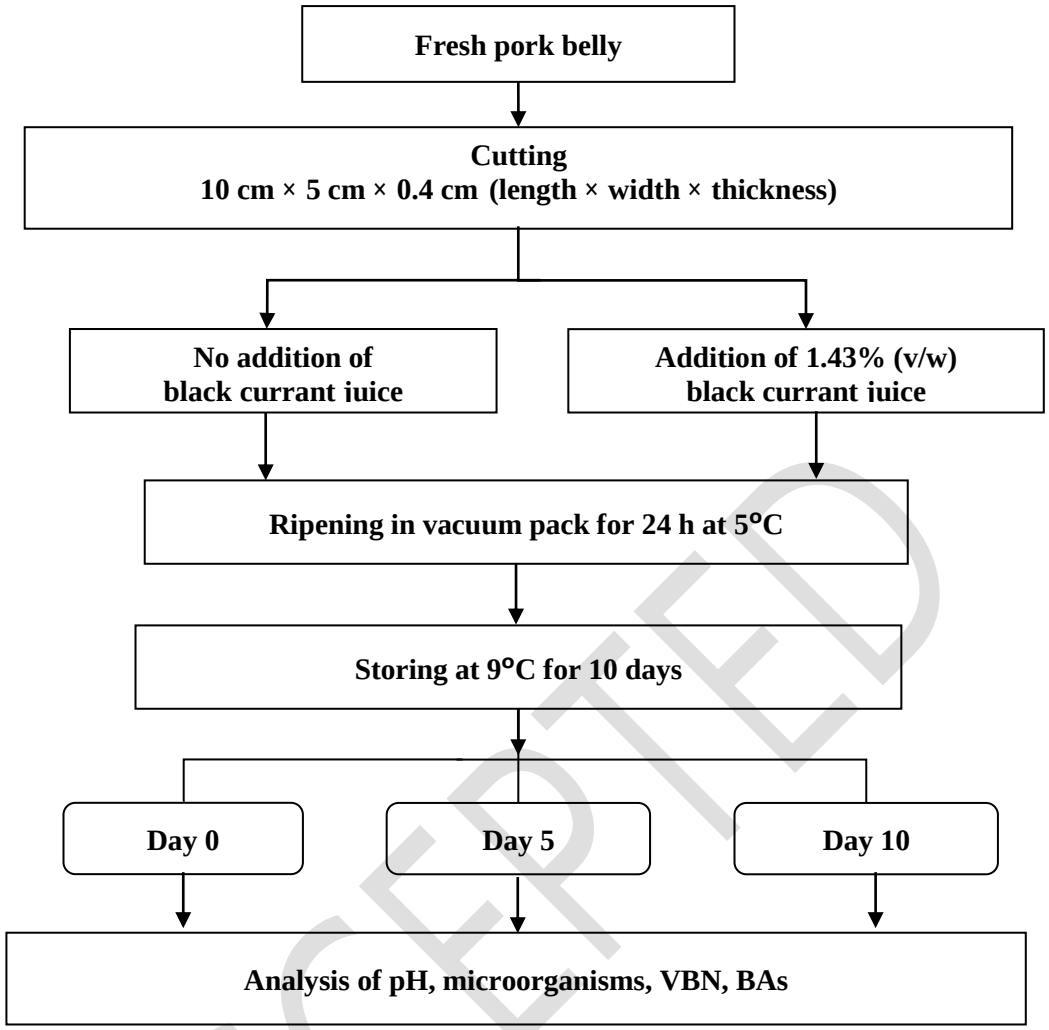
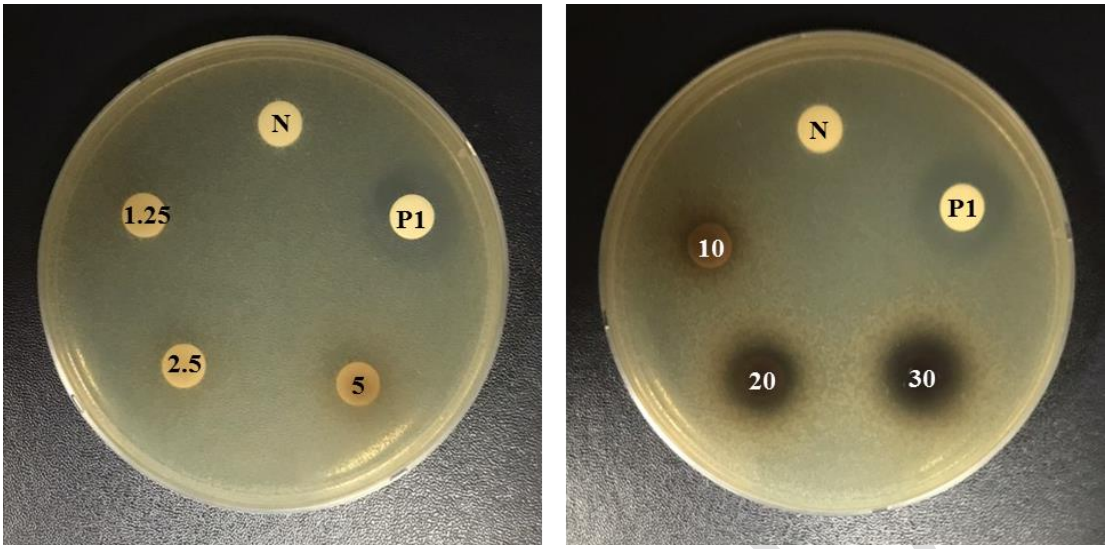
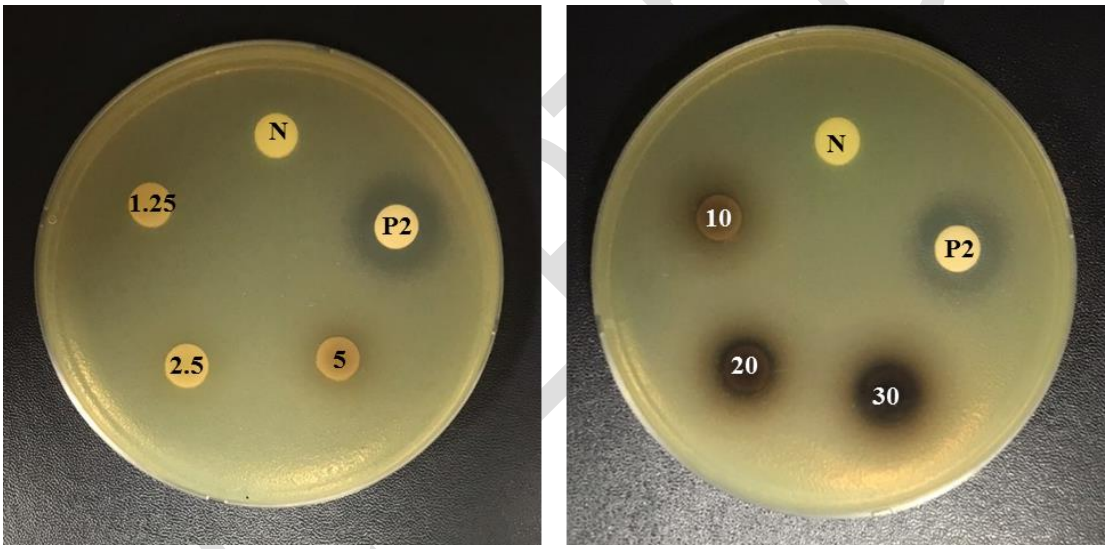


Fig. 1. Diagram of procedure of marinating pork belly

(A)



(B)



571
572 **Fig. 2. Antibacterial activity of black currant juice against *E.coli* (A) and *P. aeruginosa***
573 **using paper disc diffusion assay.** N, Negative control (Distilled water); P1, Positive control
574 (Streptomycin 0.01 mg/disc); P2, Positive control (Streptomycin 0.05 mg/disc); 30, 30
575 mg/disc of sample; 20, 20 mg/disc of sample; 10, 10 mg/disc of sample; 5, 5 mg/disc of
576 sample; 2.5, 2.5 mg/disc of sample; 1.25, 1.25 mg/disc of sample. The 8-mm paper discs
577 were used.
578

579 **Table 1. Characteristics of black currant juice**

Trait		Value
pH		3.17 ± 0.013
Color	L*	16.81 ± 0.036
	a*	0.19 ± 0.015
	b*	0.25 ± 0.006
Antioxidant Activity (mmol TE/g)	ABTS	112.64 ± 0.934
	FRAP	126.46 ± 1.346
	DPPH	104.85 ±3.741
	ORAC	231.57 ± 6.563
Total phenol contents (mg GAE/g)		8.58 ± 0.011
BAs (μg/g)	PUT	4.83 ± 0.291
	CAD	14.41 ± 0.452
	HIM	ND
	TYM	0.50 ± 0.039
	SPD	1.19 ± 0.058
	Total	20.93 ± 0.676

580 Values were expressed as means ± standard deviation.

581 ND, not detected; BAs, biogenic amines; PUT, putrescine; CAD, cadaverine; HIM, histamine;

582 TYM, tyramine; SPD, spermidine.

583

584

585 Table 2. Antibacterial activities of black currant juice against E.coli and P. aeruginosa by
586 paper disc diffusion assay

Treatments	mg/disc	Microorganisms	
		E. coli	*P. aeruginosa*
Negative control (distilled water)		ND	ND
Positive control (streptomycin)	0.05	NT	16.83
	0.01	18.31	NT
Black currant juice	30	14.87^{A}	12.52^{A}
	20	12.73^{B}	11.08^{B}
	10	9.71^{C}	8.76^{C}
	5	ND^{D}	ND^{D}
	2.5	ND^{D}	ND^{D}
	1.25	ND^{D}	ND^{D}
	SEM	0.118	0.038

587 $^{A-D}$ Means within a column with different superscript differ significantly at $p < 0.05$.

588 Unit: mm. NT: not tested. ND: not detected. The diameter of paper disc (8mm) is included.

589

590 **Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal**

591 **concentration (MBC) of black currant juice against *E. coli* and *P. aeruginosa***

Antibacterial activities (mg/mL)	Microorganisms	
	E. coli	*P. aeruginosa*
MIC	100	100
MBC	200	100

592

Table 4. Effects of marination with black currant juice on the pH, microorganism, and VBN value in pork belly during storage at 9±2℃

Traits	Treatment	Storage days (d)			SEM	Significance		
		0	5	10		T	S	T × S
pH	RPB	5.82^{Aa}	5.78^{Ab}	5.59^{Ac}	0.010	****	****	****
	PBB	4.97^{Bb}	5.04^{Ba}	4.71^{Bc}	0.009			
	SEM	0.008	0.012	0.008				
Total aerobic bacteria (Log CFU/g)	RPB	3.57^{Ac}	6.64^{Bb}	7.98^{Ba}	0.030	**	***	**
	PBB	3.29^{Ac}	7.20^{Ab}	8.59^{Aa}	0.098			
	SEM	0.102	0.063	0.035				
Lactic acid bacteria (Log CFU/g)	RPB	2.81^{Ac}	6.31^{Bb}	7.68^{Ba}	0.088	***	****	***
	PBB	2.71^{Ac}	7.03^{Ab}	8.47^{Aa}	0.023			
	SEM	0.107	0.003	0.031				
Enterobacteriaceae (Log CFU/g)	RPB	2.94^{Ac}	6.02^{Ab}	7.34^{Aa}	0.205	***	****	ns
	PBB	2.54^{Ac}	5.51^{Bb}	6.07^{Ba}	0.036			
	SEM	0.243	0.014	0.074				
Pseudomonas spp. (Log CFU/g)	RPB	1.65^{Ac}	4.36^{Ab}	6.97^{Aa}	0.051	****	****	****
	PBB	1.48^{Ac}	3.50^{Bb}	4.31^{Ba}	0.017			
	SEM	0.034	0.021	0.052				
VBN (mg/100 g)	RPB	6.60^{Ac}	15.77^{Ab}	32.92^{Aa}	0.274	****	****	****
	PBB	6.04^{Ac}	8.35^{Bb}	15.10^{Ba}	0.202			
	SEM	0.294	0.187	0.229				

$^{A-B}$ Means within a column with different superscript differ significantly at $p < 0.05$.

$^{a-c}$ Means within a row with different superscript differ significantly at $p < 0.05$.

$^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$.

RPB, raw pork belly; PBB, pork belly marinated with black currant juice.

T, treatment; S, storage day; T×S, treatment × storage day; ns, not significantly.

Table 5. Effect of marination with black currant juice on biogenic amines in pork belly during storage.

BAs (μg/g)	Treatment	Storage days (d)			SEM	Significance		
		0	5	10		T	S	T×S
PUT	RPB	0.56^{Ab}	7.19^{Ab}	63.33^{Ac}	0.010	****	****	****
	PBB	0.50^{Bb}	0.44^{Ba}	21.11^{Bb}	0.009			
	SEM	0.007	0.361	1.152				
CAD	RPB	0.00^{Aa}	59.70^{Ab}	98.28^{Ac}	0.030	****	****	****
	PBB	0.00^{Aa}	12.91^{Ba}	58.34^{Bc}	0.098			
	SEM	0.000	1.805	1.991				
HIM	RPB	0.00^{Ab}	0.00^{Ab}	1.45^{Ab}	0.088	****	*	*
	PBB	0.00^{Aa}	0.00^{Aa}	1.12^{Ab}	0.023			
	SEM	0.000	0.000	0.093				
TYM	RPB	5.23^{Aa}	50.22^{Aa}	76.68^{Ab}	0.205	****	****	****
	PBB	5.18^{Ab}	15.51^{Ba}	68.08^{Bb}	0.036			
	SEM	0.064	1.747	1.501				
SPD	RPB	3.33^{Ab}	3.49^{Ac}	2.99^{Bc}	0.051	****	ns	***
	PBB	3.10^{Bc}	3.52^{Ab}	3.32^{Ac}	0.017			
	SEM	0.029	0.069	0.042				
Total BAs	RPB	9.11^{Ab}	120.59^{Ab}	242.73^{Ab}	0.274	****	****	****
	PBB	8.78^{Ac}	32.37^{Ba}	151.96^{Bb}	0.202			
	SEM	0.088	3.938	4.655				

$^{A-B}$ Means within a column with different superscript differ significantly at $p<0.05$.

$^{a-c}$ Means within a row with different superscript differ significantly at $p<0.05$.

$^{*}p<0.05$, $^{***}p<0.001$, $^{****}p<0.0001$.

BAs, biogenic amines; RPB, raw pork belly; PBB, pork belly marinated with black currant juice.

PUT, putrescine;

T, treatment; S, storage day; T×S, treatment × storage day; ns, not significantly.