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Running Title (within 10 words)	Hanwoo beef quality
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7 **Abstract** The objective of this study was to compare tastes-related components and eating
8 quality properties between Hanwoo steer and cow meats. *Longissimus thoracis* (LT) muscles
9 with same quality grade (1⁺ grade) collected from left sides of the steer and cow carcasses were
10 used for analyses of technological quality traits, free amino acids (FAA), metabolites,
11 nucleotides, fatty acids and sensory attributes. Results unveiled that there were no differences
12 occurring in the chemical composition (fat, protein, moisture and collagen) and technological
13 quality traits (cooking loss, water holding capacity, shear force and color) between the two beef
14 types ($p>0.05$). The cow meat exhibited significantly higher amounts of some FAAs associated
15 with umami (e.g., glutamic acid and lysine), sweetness (e.g., proline and glutamic acid) and
16 saltiness (e.g., histidine and glutamic acid) compared to the steer meat ($p<0.05$). Regarding the
17 nucleotides, no differences occurred in all the identified nucleotides between the two beef types
18 ($P>0.05$). A total of 27 metabolites were identified, however, only some compounds (e.g.,
19 acetate, creatine, creatinine, glucose and inosine etc.) showed their significantly higher
20 amounts in the cow meat compared with those in the steer meat ($p<0.05$). In terms of sensory
21 aspects, the panelists found no differences in scores of all the eating quality traits between the
22 two sex types of beef ($p>0.05$). Overall, excepts some variations in tastes-active compounds,
23 there were no differences in the quality characteristics in general and eating quality in particular
24 between the cow and steer meats when they were in the same quality grade.

25 **Keywords:** Taste, free amino acid, metabolite, nucleotide, eating quality

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29 **Introduction**

30 As Korea's economy has developed quickly, the demand for meats especially palatable beef,
31 has also increased compared to the other countries. This may be evidenced by a larger per
32 capita total meat consumption by 4-16 kg in comparison to China and Japan (Choi, 2016;
33 Eastwood, 2018). Especially, the demand for beef has increased incessantly from 5.3 kg in
34 1990 and 7.8 kg in 2003 to approximately 12 kg in 2017 (Olson et al., 2018). Despite the
35 increased demand, the Korean consumers much prefer the domestic beef (e.g., Hanwoo beef)
36 to imported beef regardless of its several times higher price (Jo et al., 2012). Because the
37 consumers assume that the Hanwoo beef is fresher and more palatable mainly due to its high
38 intramuscular fat content or visual marbling (Chung et al., 2018; Hwang and Joo, 2016).

39 In the Korean beef market, there are three main sex types of Hanwoo beef including steers,
40 cows and bulls. However, the ratio of yearly slaughtered cattle for meat production differs
41 significantly among these sex types. For instance; in 2012, the female cattle accounted for
42 51.90% (corresponding to 436,977 heads) in total slaughtered cattle (718,256 heads), and this
43 ratio was reduced to be 46.80 and 44.60% in 2015 and 2018, respectively (KAPE, 2019). This
44 is probably due to the increased exploitation of the female Hanwoo cattle for reproduction
45 purpose. Contrastingly, the male Hanwoo cattle (steers) slaughtered for meat consumption
46 increased yearly accounted for 41.40, 50.4 and 53.1% in total slaughtered cattle in 2010, 2015
47 and 2018, respectively (KAPE, 2019). It is well recognized that beef cuts from high quality
48 grades (e.g., 1⁺⁺ and 1⁺ grade) are the most desirable by Korean consumers. Nevertheless, the
49 superior grade (1⁺ grades) has predominantly been found in the Hanwoo steers (41.9%)
50 compared to the rate of 18.2% in the cows (KAPE, 2019).

51 To date, although no scientifically verified evidence has been published, Korean

52 consumers generally show their more preference for the female (cows and heifers) Hanwoo
53 meat compared to the male (steers and bulls) meat because they assume that the meat of female
54 cattle is tender and tastier. Consequently, the average market price per kg of cow carcass is
55 generally higher compared to that of the steer carcass with same quality grade (KAPE, 2019).
56 Thus, searching the scientific evidences indicating the eating quality (e.g., tenderness) and taste
57 differences between these two sex types of beef is necessary. Additionally, tenderness and
58 tastes are the most important components of beef eating quality taken into account by
59 consumers (Jo et al., 2012; Joo et al., 2017; Warner et al., 2010). The tastes (sweet, salty, bitter,
60 sour and umami) of cooked meat are contributed by water-soluble constituents such as free
61 amino acids, sugars, nucleotides and metabolites (MacLeod, 1994; Dashdorj et al., 2013;
62 Spanier et al., 2004). While, the tenderness of meat reflected by instrumental shear force value
63 and/or sensorial evaluation score is mainly affected by the amount of connective tissue and
64 intramuscular fat etc. (Warner et al., 2010).

65 Since taste is one of the important eating quality trait of meats (Ramalingam et al., 2019).
66 However, little attention has been paid to the identification of the tastes-related compounds in
67 Hanwoo beef (Dashdorj et al., 2013; Jayasena et al., 2015), especially almost all of these of
68 studies have only focused on meat muscles from steer or heifer whereas, no study was
69 conducted to identify the tastes-active compounds in the Hanwoo cow meat. Thus, the objective
70 of this study was to identify the tastes-related components in Hanwoo cow meat and comparing
71 with those of steer meat.

72

73 **Materials and Methods**

74 **Sample preparation**

75 The LT muscles (10th - 13th rib) with same quality grade (1⁺ grade) from left carcass sides of

76 Hanwoo steers (31-32 months old, average carcass weight of 391 kg, marbling score of 6.8, *n*
77 = 5) and cows (47-53 months old, average carcass weight of 383 kg, marbling score of 6.0, *n*
78 = 5) collected at 24 h postmortem from commercial abattoirs (Jeonju, Korea) were used in the
79 present investigation. After collecting, the LT muscles were vacuum-packaged and then
80 transported to the Meat Laboratory of Animal Products Division for analysis. Following
81 trimming of visual fats, the muscle samples were cut into sub-sample sizes starting from the
82 cranial end of the muscles depending on the type of analysis in the following order: proximate
83 composition (10th rib); fatty acids, free amino acids, metabolites and nucleotides (11th rib);
84 sensory evaluation (12th rib); pH, color, Warner Bratzler shear force (WBSF) and water holding
85 capacity (WHC) and cooking loss (13th rib). The analyses of proximate composition, pH, color,
86 WHC, cooking loss and WBSF were performed on fresh samples (48 h postmortem), while the
87 fatty acids, free amino acids, metabolites, nucleotides and sensory evaluation samples were
88 vacuum-packed and frozen at -20°C until use.

89 **pH measurement**

90 The pH values of the beef samples were measured in triplicate by inserting a stainless steel
91 probe of pH*K 21 meter (NWK-Technology GmbH, Kaufering, Germany) deeply into the
92 muscle tissues. Prior to use, the pH meter was calibrated with standard solutions (pH 4.0 and
93 7.0).

94 **Proximate composition**

95 The protein, moisture, fat and collagen contents were determined using a Food Scan™ Lab
96 78810 (Foss Tecator Co., Ltd., DK) according to the Association of Official Analytical
97 Chemists methods (AOAC, 2007).

98 **Meat color measurement**

99 The color of the meat samples was measured using a Minolta Chroma Meter CR-400 with a

100 D65 illuminant*1 and 2° observer (Minolta Camera Co., Ltd., Osaka, Japan) as described in
101 our previous study (Cho et al., 2020), and expressed as CIE L*(lightness), CIE a*(redness),
102 CIE b*(yellowness), Chroma and hue angle (h°).

103 **Cooking loss and Warner-Bratzler shear force (WBSF) measurement**

104 The cooking loss and WBSF of the beef samples were measured using the protocols as
105 described in our previous (Cho et al., 2017). Briefly, each sample was made into a 2.5-cm thick
106 steak that was placed into plastic bags and cooked in a pre-heated water bath until their internal
107 temperature reached 80°C. After cooling for 30 min under running water (18-20°C), the cooked
108 samples were absorbed with wiping papers to remove the surface water and then their weights
109 were recorded. The cooking loss (%) of each the sample was calculated as the difference
110 between the pre-cooked and post-cooked weights divided by the initial weight. After the
111 cooking loss measurement, the cooked samples were used for the WBSF analysis. Eight cores
112 (1.27 cm in diameter) were prepared parallel to the muscle fiber direction of each the sample
113 using a 0.5-inch metal corer. The WBSF values were obtained by determining the force
114 (kilogram force, kgf) required to cut the cores using a V-shaped shear blade of Instron Universal
115 Testing Machine (Model 4465, Instron Corp, High Wycombe, UK) using a crosshead speed of
116 400 mm/min and a 40 N load cell.

117 **Water holding capacity (WHC)**

118 The WHC of the samples during storage was determined using centrifugation-based method
119 developed by Kristensen and Purslow (2001). Each sample was analyzed in duplicate and the
120 WHC percentage was calculated as a ratio of moisture to the water loss.

121 **Free amino acids (FAA) and nucleotides analysis**

122 The FFA content in the samples was analyzed following the procedure as described by
123 Dashdorj et al. (2013) with suitable modifications. Briefly, 2.5 g of each sample was

124 homogenized with 5 mL distilled water at $1200 \times g$ for 1 min. After filtering through Whatman
125 filter paper (No.4, Whatman Inc., Clifton, NJ, USA), 100 μ L filtrate taken and mixed with 900
126 μ L methanol containing 0.1% formic acid. After centrifuging at $13,000 \times g$ for 10 min at 4°C ,
127 the supernatant was collected and then filtered through a 0.45- μ m filter membrane (Millipore
128 Ltd., Cork, Ireland). The filtrate was derivatized using AccQ-TagTM (Waters Co., Milford, MA,
129 USA) according to the manufacturer's instrument. Finally, 5 μ L of each derivatized sample
130 was injected into a Waters ACQUITY UPLC (model: Xevo TQ-S, Waters Co. Milford, MA,
131 USA) connected to an Intradra Amino Acid column: 2×50 mm, 3 μ m (Imtaka, Uphur St, Suite
132 A, Portland). The eluents and conditions used for separation of FAAs were same as those used
133 in the above-cited reference (Dashdorj et al., 2013). For identification and quantification of the
134 FAAs, the external amino acid standards (Sigma-Aldrich, St. Louis, MO, USA) were used, and
135 the detected FAAs were then expressed as milligram per 100 g meat (mg/100 g meat).

136 Nucleotides were analyzed using the method of Jung et al. (2011) with suitable modifications.
137 For separation, 10 μ L of each sample was injected into Waters ACQUITY UPLC connected to
138 a C18-reverse phase column: 4.6×150 mm, 3 μ m (Cadenza). The mobile phases used were A:
139 40% tetrabutylammonium hydroxide solution-TBA-oH (25 mL), 85% H_2PO_4 solution (500 μ L)
140 and distilled water (1000 mL), and B: 40% TBA-oH (25 mL), 85% H_2PO_4 solution (500 μ L)
141 and methanol (1000 mL). The separation of nucleotides was carried out at 40°C , the detection
142 was monitored at a wavelength of 260 nm and flowing rate of 0.8 mL/min. The solvent gradient
143 set was: initial 100% A, linear change to 95% A for 1 min, linear change to 100% B for 11 min
144 and then 95% A for additional 8 min. The nucleotides were identified by comparing their
145 retention times with those of external standards, and then quantified using internal standard
146 (Purine) (Sigma-Aldrich, St. Louis, MO, USA).

147

148 **Fatty acid profiles**

149 The lipid content in the muscle samples was extracted using a solvent mixture of
150 chloroform: methanol (2:1, v/v) as described by Folch et al. (1957). For the extraction, 10 g of
151 sample was homogenized with 150 mL of the solvent mixture at 300 × g for 3 min using a
152 homogenizer (Polytron, PT-MRC. 2100, Switzerland). After filtration through Whatman filter
153 paper, the filtrate was added with approximately 20 g of Na₂SO₄, thoroughly mixed for 1 min,
154 and then the upper lipid layer was transferred into an Erlenmeyer flask. After drying at 55°C
155 using a rotary evaporator, the lipids layer was reconstituted with 1 mL tricosanoic acid and 1
156 mL of 0.5N NaOH. Finally, the lipid was converted to fatty acid methyl esters following the
157 procedure of Morrison & Smith (1964). The fatty acids were analyzed by a Gas
158 Chromatography (GC, Model Star 3600, Varian Technologies, Palo Alto, CA, USA) connected
159 with a capillary column (30 m × 2 mm × 0.25 μm film thickness). The conditions used for
160 separation, and identification of fatty acids were same as those described in our previous study
161 (Cho et al., 2020). The results were expressed as relative percent (%) of total fatty acids based
162 on total peak area.

163 **Sensory evaluation**

164 The sensorial characteristics of both the beef types were evaluated using the procedure as
165 described in our previous study (Cho et al., 2020). The procedure used for the sensory
166 evaluation was approved by the Institutional Review Board of National Institute of Animal
167 Science (No.11-1390744-000007-01). Briefly, the panel consisted of 7 trained members (at 24
168 to 40 years old) who were the institution's staffs. For evaluation, seven representative strips
169 (50 × 75 × 4 mm) were prepared from each sample using a meat slicer. Two sessions per day
170 were carried out, and each session had 7 panelists in which each the panelist evaluated 7
171 samples. The slices were cooked on an open tin-coated grill at around 160-170°C for

172 approximately 2 min and turned at the start of browning. Immediately after cooking, the cooked
173 samples were placed on individual paper dishes and served to the panelists. The panelists
174 evaluated for tenderness, flavor, roasty flavor, taste (intensity of taste after chewing), juiciness,
175 tenderness and overall acceptability using a 6-point scale. The panelists were asked to refresh
176 their palate with drinking water and unsalted crackers between samples. The sensory evaluation
177 was performed in the sensory panel booth room equipped with white lighting.

178 **Metabolites analysis by NMR Spectroscopy**

179 The cooked LT muscle samples (the samples were cooked under the identical conditions as
180 those used in the sensory evaluation) were used for the metabolites analysis. The analysis of
181 metabolites was performed following the protocol as described by Lin et al. (2007). Briefly,
182 after grinding each the sample (20 mg) was extracted with acetonitrile/water (1:1, v/v) mixture
183 on ice for 10 min. After centrifuging at $3000 \times g$ for 10 min at 4°C , the supernatant was
184 transferred to a new tube which was then freeze-dried. The lyophilized samples were dissolved
185 in $700 \mu\text{L}$ of deuterated water containing 2 mM 3-trimethylsilyl-2,2,3,3-
186 tetradeuteropropionic acid-d₄ (TSP-d₄, Sigma-Aldrich, St. Louis, MO, USA) as an internal
187 standard, and then was transferred into a 5 mm NMR tube for analysis.

188 ^1H -NMR spectra were acquired on a 600 MHz Agilent NMR spectrometer (Agilent
189 Technologies, Palo Alto, CA, USA) equipped with 600MHz 4-mm gHX NanoProbe (Agilent
190 Technologies, Santa Clara, CA, USA) at a ^1H frequency of 599.93 MHz. The ^1H -NMR
191 conditions were set as follows: spinning rate at 2050 Hz and 25°C ; number of scans, 126;
192 acquisition time, 3 s; relaxation delay, 3 s; 90° pulse, 6.35 μs ; total acquisition time per sample,
193 13.9 min. The acquired spectra were phased and then the baseline was corrected and referenced
194 to the TSP-d₄ peak using a Vnmrj (version 4.2, Agilent Technologies, Palo Alto, CA, USA).

195 Metabolites in the ^1H -NMR spectra of the cooked beef samples were tentatively identified

196 using Chenomx 600 MHz library database and Chenomx NMR Suite 7.1 professional.
197 Concentration of each the identified metabolite was calculated by its corresponding peak area
198 divided by the internal standard's peak area multiplied by the known concentration of
199 international standard.

200 **Statistical analysis**

201 The Statistical Analysis System (SAS) package (SAS Institute, Cary, NC, USA, 2015) was
202 applied for analysis of the data. The data were analyzed by using the General Linear Model
203 procedure of the SAS, and the beef type (gender) was considered and fixed as the main effect
204 while the quality traits examined were considered random in the model. Means were compared
205 using Duncan's multiple range test. Significance was set at $p < 0.05$.

206

207 **Results and Discussion**

208 ***Proximate composition***

209 The proximate composition in the LT muscles from the two sex types (cows and steers) is
210 presented in Table 1. The protein, moisture, fat and collagen content were: 18.66 vs 19.49%,
211 61.30 vs 61.45%, 17.82 vs 16.69% and 2.19 vs 2.12% for the steers and cow meats, respectively.
212 No significant ($p > 0.05$) differences occurred in all of these contents between the two beef types.
213 This could be due to the LT samples which were in the same quality grade (1⁺ grade). Studies
214 have reported that the proximate composition (e.g., fat and protein) in beef muscles differ
215 depending on the quality grades (Gajaweera et al., 2020; Jung et al., 2013; Kim and Lee, 2003).
216 Compared with our data, those of Gajaweera et al. (2020) found similar protein, fat, moisture
217 and collagen contents in *longissimus thoracis* muscles from Hanwoo steers with same 1⁺ grade.
218 However, compared with fat content (2.5-5.5%) and marbling scores (2.3-3.7) reported for

219 Hereford cows slaughtered at 4 -12 years old by Galli et al. (2008), or cull Danish Friesian
220 cows (Vestergaard et al., 2007), the cow meat in the present study had several times greater
221 level. This fact could be attributed to the differences in genetics and feeding diet used among
222 the studies.

223 *Technological quality and color traits*

224 Table 2 shows the technological quality and instrument color traits of the LT muscles from
225 both sex types. There were no significant differences occurring in the cooking loss, WBSF,
226 WHC as well as color traits between the two beef types ($p>0.05$). In general, the cooking loss
227 level, shear force values and WHC percent of both the beef types in the present study were
228 almost similar to those reported for Hanwoo steer beef muscles with the same quality grade in
229 literature (Cho et al., 2020; Gajaweera et al., 2020). Interestingly, compared to shear force
230 values (5-6 kg) reported for the same sex type such as Hereford cows slaughtered at 4 -12 years
231 (Galli et al., 2008) and cull Danish Friesian cows (Vestergaard et al., 2007), the cow meat in
232 the present study had approximately a half lower value. This could be attributed to the higher
233 intramuscular fat content in the Hanwoo cow meat compared with those in the meats from other
234 cattle breeds used in these studies. Regarding this, a lot of studies have found a negative
235 correlation between the intramuscular fat and shear force in beef; the higher the fat content the
236 lower the force required to shear and cut the meat (Gajaweera et al., 2018; Park et al., 2000;
237 Wood et al., 2008). The shear force value is considered as an indicator reflecting the tenderness
238 of meats. Compared with WBSF values reported by Obuz et al. (2004) for beef muscles from
239 high USDA quality grade (e.g., choice and select), the WBSF values obtained on the both beef
240 types in the present study were lower. Researchers have found that the sex, mainly by hormonal
241 status, is a vital factor affecting the physicochemical quality and color traits of beef muscles
242 (Panjono et al., 2009; Picard et al., 2019; Sauerwein and Meyer, 1989). Furthermore, research

243 conducted to examine the effect of animal age on meat quality traits of Hanwoo female cattle
244 has shown that increasing the slaughter age resulted in increased shear force, and decreased
245 WHC and lightness of beef longissimus muscle (Cho et al., 2017). Based on the findings
246 reported in these studies, it may be said that sex and slaughter age both have the important
247 effects on the beef quality. In the present study, though the cows were slaughtered at a mature
248 age (47-53 months), and the steers were slaughtered at younger age (31-32 months), no
249 differences occurred in all the technological quality traits examined between these two beef
250 types. This may be explained due to: (i), the beef samples in both the sex types were in the
251 same quality grade (1⁺ grade) group because beef cuts within a same quality grade usually are
252 uniform in quality; (ii), the steers produced after castration (castrated male cattle) usually are
253 changed in their hormonal profiles (Plouzek and Trenkle, 1991) which reduced the action and
254 aggressive behavior whereas, increased the fat deposition in muscle tissues (Destefanis et al.,
255 2003). Other researchers have also shown an increased intramuscular fat content and improved
256 meat quality in castrated male cattle (steers) compared to intact male cattle (bulls) (Silva et al.,
257 2019). Till now, no reports comparing the technological quality traits between the steer and
258 cow meats are available. Corresponding to the present findings, however, Choat et al. (2006)
259 and Mueller et al. (2019) also reported no differences in shear force and color traits such as;
260 lightness and redness between steer and female (heifers) meats.

261 ***Taste –related compounds (free amino acids, nucleotides and metabolites)***

262 The concentrations of FAAs and nucleotides found in the LT muscles of both sex types are
263 presented in Table 3. FAAs are mainly responsible for the tastes of meats, especially sweetness
264 and umami (Dashdorj et al., 2013; Jayasena et al., 2013; Mateo et al., 1996). Based on their
265 similar taste quality, Kato et al. (1989) and Sforza et al. (2001) categorized the FAAs into
266 several classes for instances; glycine, alanine, serine, proline and glutamic acid are associated

267 with sweet taste; glutamic acid, aspartic acid, alanine, serine, lysine and methionine are
268 associated with umami taste; aspartic acid, glutamic acid and histidine are associated with salty
269 taste; valine, leucine, isoleucine, phenylalanine, arginine, proline, tryptophan and methionine are
270 associated with bitter taste. Results show that the most predominant FAAs found in the both
271 beef types were alanine and glutamine which are responsible for sweet taste. Supporting the
272 present results, Cho et al. (2007) and Jayasena et al. (2015) reported similar trend for these
273 amino acids in Hanwoo longissimus muscles. Noticeably, no significant differences occurred
274 in these two major amino acids between the two beef types ($p>0.05$). Other some FAAs
275 associated with umami (e.g., glutamic acid and lysine), sweetness (e.g., proline and glutamic
276 acid) and saltiness (e.g., histidine and glutamic acid), were significantly ($p<0.05$) higher in the
277 cows compared to the steers. Supporting the present findings, Lee et al. (2019) reported higher
278 levels of glutamic acid, leucine, valine and threonine in Hanwoo female longissimus muscles
279 compared to the castrated male cattle.

280 Regarding the nucleotides, inosine-5'-phosphate (IMP) is known as the major breakdown
281 product of adenosine 5- triphosphate (ATP), which is then degraded into hypoxanthine and
282 inosine in meats after slaughter (Nishimura et al., 1988). These breakdown products of 5'-
283 nucleotides are important components contributing and enhancing the taste especially umami of
284 cooked meats (Jayasena et al., 2013; Ichimura et al., 2017; Nishimura et al., 1988). Nucleotides
285 such as IMP is mainly responsible for the tastes of meat, especially sweetness and umami
286 (Dashdorj et al., 2013; Mateo et al., 1996). Results showed that there were no differences in all
287 the detected nucleotides between the cow and steer meats ($p>0.05$), implying a similar
288 conversion rate of ATP to the IMP and others between the two sex types. Compared with our
289 data, those of Dashdorj et al. (2013) found higher amounts of IMP and hypoxanthine in Hanwoo
290 heifer longissimus muscles after 7 and 14 days of chiller ageing. These contrasting results could

291 be attributed to the different sampling time between the studies because increasing post-mortem
292 ageing usually results in increased amounts of IMP and hypoxanthine in fresh meats (Tikk et al.,
293 2006).

294 By using the NMR Spectroscopy technique, 27 metabolites were detected and identified
295 in the cooked LT muscles from both the beef types (Table 4). In general, the NMR profiles
296 displayed a broad range of compounds comprising amino acids, peptides, carbohydrates and
297 nucleotides etc. Among them, alanine, glutamine, glycine, leucine, methionine, tyrosine and
298 valine have also been found in beef *longissimus lumborum* muscles (Frank et al., 2020). All of
299 these amino acids have also found in the raw LT muscle of both the sex types (Table 3). Out
300 of the amino acids, however, only glycine and tyrosine showed a significant ($p < 0.05$)
301 difference between the two sex types. Furthermore, the most predominant amino acid found
302 was alanine whose amount was similar in both the beef types ($p > 0.05$). Alanine is associated
303 with sweet taste of cooked meat (Kato et al., 1989). AMP and inosine were two unique
304 nucleotides found in both the beef types. They are known as the products degraded from ATP
305 by endogenous enzymes in muscle tissues after slaughter as mentioned above. The amount of
306 inosine was about 0.13 mmol/kg greater in the cow meat compared to that in the steer meat.
307 The other metabolites identified such as; acetate, betaine, lactate, carnosine, creatine and
308 creatinine have previously been reported in raw beef muscles (Frank et al., 2020; Kim et al.,
309 2016). Of which, carnosine (an antioxidant dipeptide) was found at an abundant level but no
310 differences occurred in its amount between the two beef types ($p > 0.05$). Carnosine is a
311 metabolite generated from the proteolysis by muscle enzymes (Frank et al., 2020), and
312 contributes significantly to red meat taste (Cambero et al., 2000). Creatine, a lipid metabolism-
313 derived compound, was found at a high level in both the beef types, which agrees with finding
314 of Frank et al. (2020). While, creatinine is known as a breakdown product of creatine from the

315 muscle protein metabolism. Results show that the concentrations of creatine and creatinine
316 were greater in the cow meat compared to that of the steer meat ($p < 0.05$). However, the role of
317 these two metabolites in development of cooked meat taste has not been characterized. In
318 general, the results indicating the variations in levels of some metabolites could be partly
319 related to the sex difference that affects the protein and lipid metabolisms in muscle tissues as
320 well as the proteolysis/breakdown process in meat after slaughter. However, the differences in
321 concentrations of metabolites between the two beef types apparently are negligible.

322 *Fatty acid profiles*

323 The proportion of fatty acids in the LT muscles from both sex types are presented in Table
324 5. Fatty acids not only reflect the nutritional value but also considerably affect the development
325 of cooked meat flavor (Mottram, 1998). Excepting palmitic acid (C16:0), vaccenic acid
326 (C18:1n-7) and oleic acid (C18:1n-9), all the other remaining fatty acids, total saturated fatty
327 acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) did
328 not differ between the two sex types of beef ($p > 0.05$). Studies have found that castrated cattle
329 (steers) are usually changed in their hormonal profiles which directly affects body fat
330 deposition (Plouzek and Trenkle, 1991) and distribution of fatty acids in muscle tissues as well
331 (Lee et al., 2009). This may be the main reason explaining why most of the fatty acids in the
332 steer meat were not different from those of the cow meat. The current findings would agree
333 with those of Mueller et al. (2019), who did not find differences in most of fatty acids between
334 steer and heifer meats whereas, the significant differences only were found between bull and
335 heifer or bull and steer meats.

336 In general, it was observed that both the sex types of beef had the fatty acid profile
337 characteristic of high concentrate-fed animals, being indicated by a high level of MUFAs such
338 as C18:1n9 and n-6/n-3 fatty acids ratio, and a lower level of n-3 PUFAs such as linolenic acid

339 (C18:3n-3) (De Smet et al., 2002). Our results align with those of Jayasena et al. (2015), Lee
340 et al. (2019) who reported similar trends for most of the fatty acids in beef muscles from
341 Hanwoo steers and heifers. Likewise, compared to pastured Australian Angus beef,
342 concentrates-fed Hanwoo beef is lower in C18:3n-3 content and higher in n-6/n-3 fatty acid
343 ratio (Cho et al., 2005). Out of the fatty acids identified, C18:1n-9 was the most abundant fatty
344 acid present in both the beef types, followed by palmitic acid (C16:0), which agrees with those
345 of Jayasena et al. (2015) and Cho et al. (2020). Interestingly, the level of C18:1n-9 was
346 significantly higher in the steer meat (47.86%) compared to that in cow meat (41.48%) ($p < 0.05$).
347 Our results are in accordance with those of Lee et al. (2019): C18:1n-9 content was higher in
348 Hanwoo steer meat compared with that in female cattle meat. Previous studies have found that
349 production system (feeding diet and regimes etc.) significantly affects the fat deposition and
350 fatty acid profiles in beef muscles (Schor et al., 2008; Wood et al., 2008). Therefore, the result
351 indicating the variations in the fatty acids between the two beef types may be mainly attributed
352 to these factor's effects. Unfortunately, the information regarding the diets and feeding regimes
353 for the both sex types of beef in the present study was not recorded.

354 *Eating quality attributes*

355 Sensory evaluation of meat is of utmost important to ascertain the virtual merits in the meat-
356 producing practices as well as the impacts of chemical constituents on the precise sensorial
357 attributes of meat. The results of sensory evaluation for the two beef types are presented in
358 Table 6. On the 6-points scale, the tenderness, juiciness, taste and overall acceptability scores
359 given by the panelists were: 4.22 vs 4.36, 3.78 vs 3.93, 3.56 vs 3.62 and 4.02 vs 4.06 for the
360 steer and cow meat, respectively. There were no statistical differences occurring in all of these
361 sensory traits between the two sex types of beef ($p > 0.05$). Similar to our results, those of Choat
362 et al. (2006) showed that cattle sex (steer and heifer) had no effects on juiciness or flavor,

363 tenderness and overall tenderness scores of *longissimus* muscles. Likewise, research conducted
364 to examine the effects of sex on eating properties of Angus cattle beef has found no differences
365 in flavor, aroma, juiciness and overall acceptability between steer and heifer (Mueller et al.,
366 2019). The result indicating no differences in the taste score could be related to the taste-
367 related components (free amino acids and metabolites) whose variations in amount might not
368 be large enough for the consumers to discriminate these two beef types. On the other hand, the
369 results indicating no differences in the other eating quality traits such as tenderness, juiciness
370 and roasty flavor etc. could be attributed to the intramuscular fat (IMF) content that was similar
371 in both the sex types of beef (Table 1). Supporting the present findings, Mueller et al. (2019)
372 also found no differences in eating quality traits between steer and heifer *longissimus thoracis*
373 muscles that contained a same IMF level. Till now, it is well known that marbling degree or
374 IMF content is the vital factor determining the eating quality of beef since the level of IMF has
375 been found positively correlated to all the eating attributes (Chung et al., 2018; Jo et al., 2012;
376 Joo et al., 2017). In contrast to the current findings, Gajaweera et al. (2018) found higher
377 tenderness, juiciness and overall-liking scores for meat of steers compared to cow meat. These
378 authors also found a positive correlation between the IMF content with the sensory attribute
379 scores in that the significantly higher eating quality scores in the steer meat was due to its
380 double greater IMF level compared to that of cow meat. From the results/observations of our
381 investigation it could be said that the cattle sex apparently had no effects on the eating quality
382 when the quality grade (e.g., marbling or intramuscular fat) was adjusted to a common level.

383

384 **Conclusion**

385 Our results unveil that there were no differences in the chemical composition (e.g., IMF)
386 and technological quality traits such as water holding capacity, cooking loss, shear force and

387 color between cow and steer LT muscles. The marbling score and fat content in the cow meat
388 in the present study were several times greater compared to those reported for other cow breeds
389 (cull dairy and suckling cows) in literature, indicating its better quality and more importance
390 in term of economic value. Out of the fatty acids identified, only C16:0, C18:1n-7 and C18:1n-
391 9 showed significant differences between the two beef types. The cow meat showed greater
392 amounts of some free amino acids and metabolites compared to the steer meat. No differences
393 were found in all the eating quality attributes between the two beef types, this is likely related
394 to: (i) the same IMF content, and (ii) the tastes-related components whose variations in amount
395 might not be large enough for the consumers to discriminate. Considering all the parameters
396 examined in the present study, it may be concluded that excepts some tastes-related compounds,
397 the quality characteristics in general and eating quality in particular were similar for the cow
398 and steer meats when their quality grade was same. Further study is needed to compare the
399 quality characteristics and tastes-related compounds between the steer and heifer meats.

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ACCEPTED

558 **Table 1.** Proximate composition of 1⁺grade LT muscles from Hanwoo cows and steers

Item	Steer	Cow
Protein (%)	18.66±0.28	19.49±0.36
Moisture (%)	61.30±0.22	61.45±0.88
Fat (%)	17.82±0.55	16.69±1.16
Collagen (%)	2.19±0.15	2.12±0.11

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593 **Table 2.** Technological quality and meat color of 1⁺grade LT muscles from Hanwoo cows and
 594 steers

Item	Steer	Cow
Cooking loss (%)	23.79±2.32	24.42±0.93
Warner Bratzler shear force (kg)	2.81±0.17	2.65±0.18
Water holding capacity (%)	61.24±1.49	63.52±1.34
<i>Meat color</i>		
Lightness (L*)	37.53±0.99	37.10±1.16
Redness (a*)	21.18±0.94	21.23±1.82
Yellowness (b*)	10.19±1.06	10.75±1.47
Chroma (c*)	23.52±1.30	22.49±2.31
Hue (h*)	25.41±1.36	26.35±1.47

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Table 3. Free amino acid profiles and nucleotides in 1⁺ grade LT muscles from Hanwoo cows and steers

<i>Items</i>	Steer	Cow
<i>Free amino acids (mg/100g)</i>		
Glycine	0.21±0.13	1.08±0.36
Alanine	25.24±1.65	28.73±1.42
Serine	4.45±0.78	6.17±0.53
Proline	2.35±0.09 ^b	3.17±0.05 ^a
Glutamic acid	3.18±0.60 ^b	5.70±0.82 ^a
Valine	6.13±0.82 ^b	9.22±0.95 ^a
Threonine	3.09±0.38 ^b	4.71±0.39 ^a
Leucine	7.60±1.32 ^b	11.90±1.25 ^a
Isoleucine	3.77±0.63 ^b	6.55±0.72 ^a
Aspartic acid	0.37±0.07	0.43±0.07
Lysine	3.80±0.33 ^b	5.96±0.75 ^a
Methionine	1.91±0.54	3.52±0.51
Histidine	0.93±0.22 ^b	2.17±0.26 ^a
Phenylalanine	4.75±0.67 ^b	7.46±0.75 ^a
Arginine	5.43±0.86 ^b	9.74±0.66 ^a
Tyrosine	4.56±0.81	6.59±0.70
Asparagine	0.53±0.08 ^b	1.00±0.10 ^a
Glutamine	14.15±3.39	10.17±2.05
Tryptophan	0.25±0.16 ^b	0.90±0.15 ^a
<i>Nucleotides (µM/g)</i>		
Hypoxanthine	1.99±0.14	2.14±0.12
Uridine	0.58±0.04	0.65±0.07
Guanosine monophosphate (GMP)	0.09±0.04	0.07±0.02
Inosine-5'-phosphate (IMP)	2.77±0.07	2.47±0.23

618 Means within a same row with different superscripts (a,b) differ significantly p<0.05.

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Table 4. Concentration (mmol/kg meat) of identified metabolites in 1⁺ grade LT muscles from Hanwoo cows and steers

Item	Steer	Cow
AMP	0.94±0.07	0.98±0.06
Acetate	0.38±0.03 ^b	0.54±0.03 ^a
Alanine	2.87±0.16	2.79±0.13
Betaine	1.49±0.22	1.40±0.06
Carnosine	7.45±0.54	8.33±0.73
Choline	1.03±0.20	0.90±0.11
Creatine	23.73±0.76 ^b	26.32±0.65 ^a
Creatinine	0.84±0.10 ^b	1.11±0.05 ^a
Fumarate	0.29±0.06	0.24±0.03
Glucose	6.24±0.11	6.89±0.15
Glutamine	3.39±0.61	2.80±0.38
Glutathione	0.51±0.04	0.42±0.03
Glycerol	4.53±0.40	4.31±0.22
Glycine	1.22±0.04 ^b	1.39±0.05 ^a
Inosine	0.62±0.03 ^b	0.75±0.02 ^a
Isoleucine	0.64±0.12	0.87±0.14
Lactate	32.14±2.01	26.89±1.37
Leucine	0.99±0.19	1.04±0.10
Methionine	0.79±0.03	0.87±0.03
N,N-Dimethylglycine	0.08±0.01	0.08±0.00
N-Nitrosodimethylamine	0.41±0.03	0.37±0.01
O-Acetylcarnitine	0.61±0.06	0.65±0.06
Taurine	2.65±0.28	2.61±0.14
Trimethylamine N-oxide	3.79±0.33 ^b	4.95±0.32 ^a
Tyrosine	0.27±0.02 ^b	0.34±0.03 ^a
Valine	0.63±0.04	0.70±0.08
sn-Glycero-3-Phosphocholine	1.49±0.26	1.44±0.03

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

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Table 5. Relative percentage (%) of fatty acids in 1⁺ grade LT muscles from Hanwoo cows and steers

Item	Steer	Cow
C14:0	2.80±0.30	2.69±0.29
C16:0	31.05±0.88 ^b	36.50±0.89 ^a
C16:1n7	3.60±0.32	5.01±0.56
C18:0	12.17±1.14	11.19±1.86
C18:1n7	0.35±0.05 ^b	0.61±0.06 ^a
C18:1n9	47.86±1.56 ^a	41.48±1.87 ^b
C18:2n6	1.86±0.21	2.00±0.26
C18:3n3	0.07±0.01	0.08±0.01
C18:3n6	0.05±0.00	0.05±0.00
C20:1n9	0.11±0.02	0.29±0.09
C20:4n6	0.05±0.01	0.05±0.01
C20:5n3	0.00±0.00	0.00±0.00
C22:4n6	0.04±0.00	0.04±0.01
C22:6n3	0.00±0.00	0.00±0.00
Saturated fatty acids (SFA)	46.02±1.70	50.38±1.79
Unsaturated fatty acids (UFA)	53.98±1.70	49.62±1.79
Monounsaturated fatty acids (MUFA)	51.92±1.72	47.40±2.01
Polyunsaturated fatty acids (PUFA)	2.06±0.21	2.22±0.27
n3 fatty acids	0.07±0.01	0.08±0.01
n6 fatty acids	2.00±0.20	2.14±0.27
n6/n3 fatty acids	30.31±1.52	27.89±2.95

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

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634 **Table 6.** Mean scores (6-points scale) for sensory traits of 1⁺grade LT muscles from Hanwoo
 635 cows and steers

Item	Steer	Cow
Tenderness	4.22±0.15	4.36±0.25
Juiciness	3.78±0.12	3.93±0.17
Flavor	3.91±0.12	3.98±0.24
Roasty flavor	4.27±0.67	4.20±0.66
Taste	3.56±0.14	3.62±0.30
Overall acceptability	4.02±0.08	4.06±0.22

636 Score distribution: Juiciness: (1) very dry to extremely juicy (6); Taste: intensity of taste, (1)
 637 very weak to very strong (6); Tenderness: (1) very tough to very tender (6); Beef flavor:
 638 intensity of beefy flavor, (1) very weak to very strong (6); Roasty flavor: intensity of roasty
 639 flavor, (1) very weak to very strong (6) Overall acceptability: (1) extremely dislike to extremely
 640 like (6).

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