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Author	Soyoung Jang ^{1,*} , Youngho Lim ^{1,*} , Sanghun Park ¹ , Gyutae Park ¹ , Nayoung Choi ¹ , Sehyuk Oh ¹ , Chaeri Kim ¹ , Jimin An ¹ , Yeongi Hong ¹ , Sol-Hee Lee ¹ , Sung-Sil Moon ^{2,†} , Jungseok Choi ^{1,†}
Affiliation	¹ Department of Animal Science, Chungbuk National University, 28644, Republic of Korea ² Sunjin Co., Seoul, 05372, Korea
Special remarks – if authors have additional information to inform the editorial office	* These authors contributed equally as first author to this work. † Corresponding author.
ORCID (All authors must have ORCID) https://orcid.org	Soyoung Jang (https://orcid.org/0009-0001-1146-2695) Youngho Lim (https://orcid.org/0000-0002-0238-4736) Sanghun Park (https://orcid.org/0000-0003-4804-0848) Gyutae Park (https://orcid.org/0000-0003-1614-1097) Nayoung Choi (https://orcid.org/0000-0002-4782-1098) Sehyuk Oh (https://orcid.org/0000-0003-4105-2512) Chaeri Kim (https://orcid.org/0009-0004-5493-3401) Jimin An (https://orcid.org/0009-0006-9164-1955) Yeongi Hong (https://orcid.org/0009-0007-8524-933x) Sol-Hee Lee (https://orcid.org/0000-0003-1124-7095) Sung-Sil Moon (https://orcid.org/0000-0003-2734-8931) Jungseok Choi (https://orcid.org/0000-0001-8033-0410)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jungseok Choi
Email address – this is where your proofs will be sent	jchoi@chungbuk.ac.kr

Secondary Email address	
Postal address	Department of Animal Science, Chungbuk National University, Cheongju, Chungcheongbuk-do Korea, 28644
Cell phone number	+82-01-3235-2127
Office phone number	+82-43-261-2551
Fax number	+82-43-261-2773

7

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Sung-Sil Moon
Email address – this is where your proofs will be sent	ssmun@sj.co.kr
Secondary Email address	
Postal address	Sunjin Co., Seoul, 05372, Korea
Cell phone number	+82-010-2560-0960
Office phone number	+82-02-2225-0271
Fax number	+82-02-471-9388

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Quality and storage characteristics of Hanwoo Pemmican by replacing canola oil

Abstract (within 250 words)

This study investigated the effects of replacing beef tallow with canola oil (CA) on the quality characteristics and storage stability of pemmican. Six treatment groups were established: CON (beef tallow), CA1 (2.5% CA), CA2 (5.0% CA), CA3 (7.5% CA), CA4 (10% CA), and CA5 (12.5% CA). Samples were stored at 4°C for 2 weeks. Fatty acid composition, proximate composition, color, water activity (a_w), and texture profile analysis (TPA) were assessed. Additionally, pH, volatile basic nitrogen (VBN) and 2-thiobarbituric acid reactive substances (TBARS) were measured at 0, 7, and 14 days. The levels of saturated fatty acids decreased with higher CA content, while oleic acid and linoleic acid increased. Results showed that a_w decreased with increasing CA levels ($P < 0.05$). TPA indicated decreasing trends in hardness, chewiness and gumminess as CA levels increased. During the first week of storage, pH decreased for CON and CA1 but increased for CA2-5 ($P < 0.05$). CA5 exhibited the highest TBARS throughout storage period, suggesting increased lipid oxidation. VBN showed a decreasing trend with higher CA levels, indicating improved protein stability. Therefore, replacing beef tallow with CA can improve the composition of fatty acid and storage characteristics. Based on the findings, CA4 emerged as the most suitable option. These findings provide valuable insights into optimizing pemmican formulation for improved quality and shelf life.

Keywords: Pemmican, Canola oil, Vegetable oil, Meat product, Beef tallow

37

38 Meat products are valuable sources of protein, essential amino acids, crude fat and
39 various nutrients such as minerals. And recently there has been a rapid increase in
40 consumer demand for meat worldwide (Ursachi et al., 2020). Dried meat food products
41 have the advantage of being conveniently consumed due to their small size compared to
42 their rich protein content, and the salting and drying processes greatly extend the shelf
43 life of products by inhibiting the growth of microorganisms and other bacteria thanks to
44 their low water activity (Mediani et al., 2022). Additionally, dried meat products are
45 manufactured by seasoning prepared meat with spices and additives, then preserving it
46 through low temperature drying and smoking, which makes the production process
47 relatively simple (Konieczny et al., 2007). Therefore, the consumption of these dried
48 meat products has gained popularity due to the ever-changing preferences of consumers,
49 their growing interest in high-protein foods, and the desire for convenience (Aykın,
50 2023).

51 Among these options, 'Pemmican' is a dried meat product made by combining dried
52 meat with animal fat (Ngapo et al., 2021). Throughout history, pemmican has been a
53 popular choice for providing a convenient source of nutrition during arduous travels or
54 extended periods of labor in harsh climates (Kark et al., 1945). It offers a concentrated
55 dose of energy, thanks to its high fat and protein content, and can be further enriched
56 with vitamins and minerals by incorporating berries (Merriam, 1955). Additionally,
57 pemmican is easy to carry and has excellent storage stability at room temperature.

58 Compared to dried meat products obtained from other meat sources, products derived
59 from beef are highly popular due to their rich flavor and versatility (Aung et al., 2023).
60 In this study, Hanwoo, a premium Korean beef, was selected as the meat for making
61 pemmican. Renowned for its excellent marbling, tenderness, and rich flavor, Hanwoo

62 significantly enhances the quality of the final meat product (Joo et al., 2017). Its high-
63 quality protein and favorable fat composition make it an ideal choice for meat products,
64 contributing to both nutritional value and taste. As a result, many studies are being
65 conducted to incorporate it into meat products.

66 However, excessive intake of saturated fats found in animal fats can increase low-
67 density lipoprotein (LDL) cholesterol levels, leading to obesity, diabetes, hypertension,
68 and various cardiovascular diseases (Maki et al., 2021). Therefore, the WHO and FDA
69 recommend reducing the content of saturated fatty acids by including unsaturated plant
70 oils to prevent chronic diseases (Vogli et al., 2014). Additionally, incorporating
71 vegetable oils into meat products has been identified as an effective approach for
72 lowering cholesterol and saturated fatty acid content, while also enhancing the levels of
73 natural antioxidants such as tocopherols, β -carotene, and various phenolic compounds
74 (Rodríguez et al., 2012). This, in turn, improves the nutritional value of these products.
75 As a result, the food industry has been actively researching the substitution of animal
76 fats with vegetable oils.

77 Canola oil (CA) is a vegetable oil derived from the genus *Brassica* in the Cruciferae
78 family (Chew, 2020). It is known for having the lowest saturated fatty acid content
79 among commercially available edible oils, with 5-8% saturated fatty acids (SFA), 30-
80 35% polyunsaturated fatty acids (PUFA) and 60-65% monounsaturated fatty acids
81 (MUFA) (Goyal et al., 2021). The high concentration of unsaturated fatty acids in CA,
82 such as oleic acid and linoleic acid, has been shown to reduce levels of LDL cholesterol,
83 thus contributing to a lower risk of diseases like heart disease and diabetes (Okuyama et
84 al., 2016). In addition to its appropriate fatty acid composition, CA also contains natural
85 antioxidants such as various phenolic compounds and tocopherols, specifically the γ -
86 isomer. These antioxidants have the ability to inhibit spoilage (Przybylski, 2005).

87 However, effectively incorporating plant-based oils with a high proportion of PUFA,
88 such as CA, into products presents challenges. These challenges include the oxidative
89 instability caused by their high unsaturation and the fluidity of vegetable oils causes
90 physical instability (Jiang and Xiong, 2015). As a result, researchers face the significant
91 challenge of providing oxidative stability while utilizing vegetable fats that contain a
92 high amount of unsaturated fatty acids. Many studies are being conducted to propose
93 the most suitable fatty acid composition and ratio from both health and quality
94 perspectives.

95 The objective of this study is to enhance the fatty acid composition of pemmican by
96 substituting beef tallow with CA. Furthermore, we aim to determine the optimal
97 concentration of CA that maintains the physicochemical properties and storage stability
98 of the product. The findings of this research will serve as valuable foundational data for
99 future studies on pemmican.

100

101

Materials and Methods

102

Materials

103

Hanwoo top round meat and beef tallow were obtained from a butcher shop located in

104

Chungcheongbuk-do. Raisins (Raisin, Nutree Co., Paju, Korea), dried blueberries

105

(Songrim Food Co., Kimpo, Republic of Korea), dried cranberries (Dried Cranberries,

106

Nuts Farm Co., Gwangju, Republic of Korea), and canola oil (CJ Co., Seoul, Republic

107

of Korea) were used.

108

109

Preparation of Pemmican

110

The process of manufacturing pemmican is shown in figure 1. First, the Hanwoo top

111

round meat was thinly sliced $20 \times 0.5 \times 20$ cm (L \times W \times H) and then cut into wide

112 slices before being dried in a 77°C dry oven (SH-DO-360 FH, Samheung, Seoul,
113 Republic of Korea) for 17 h. Next, the dried meat was freeze-dried using a freeze dryer
114 (FDU-2100, EYELA, Japan). The meat was dried twice to prevent ice crystal formation
115 and to improve drying efficiency during freeze-drying. The dried meat, raisins, dried
116 blueberries, and dried cranberries were ground using a blender (HMF-4010SS, Hanil
117 Electric, Seoul, Republic of Korea) and mixed together. Beef fat was rendered at 120°C
118 for about 30 min to produce beef tallow, which was then double-strained through two
119 stain-resistant sieves to remove impurities. Afterwards, the mixture and the beef tallow
120 in liquid form, along with CA added according to the blending ratios shown in table 1,
121 were mixed and shaped before being frozen overnight at -20°C. Finally, the frozen
122 mixture was cut into samples measuring 2 × 2 × 4 cm (L × W × H) for the experiment.
123 A total of six treatment groups (CON, Beef tallow 25%; CA1, Beef tallow 22.5% + CA
124 2.5%; CA2, Beef tallow 20% + CA 5%, CA3, Beef tallow 17.5% + CA 7.5%; CA4,
125 Beef tallow 15% + CA 10%; CA5, Beef tallow 12.5% + CA 12.5%) were
126 manufactured.

127

128 **Fatty acid composition analysis**

129 The method described by Lepage and Roy (1986) was used to methylate the samples at
130 100°C for 1 h. Hexane was added after cooling to separate the fatty acid methyl esters.
131 The upper layer of the sample was collected. A gas chromatograph with a capillary
132 column (100 m × 0.25 mm i.d. × 0.20 µm film thickness) was utilized to quantify the
133 fatty acid methyl esters. The carrier gas used was nitrogen. The initial oven temperature
134 was maintained at 180°C, and the final temperature was maintained at 240°C (2°C per
135 min). The temperatures of both the injector and detector were kept at 250°C.

136

137 **Proximate composition**

138 The contents of moisture, crude fat, crude protein, crude ash and carbohydrates were
139 measured using AOAC (2012). The 105°C air oven drying method was used to
140 determine the moisture content, crude protein content was analyzed using the Kjeldahl
141 method, crude ash content was determined using the dry ashing method at 550°C and
142 crude fat content was determined using the Folch method, and the carbohydrate content
143 was determined by subtracting the moisture, crude ash, crude fat, and crude protein
144 from the sample, as outlined in the method by Hussain et al. (2009).

145

146 **Color**

147 The color of the inner surface of the pemmican was measured using a standardized
148 Spectro colorimeter (CM-26d, Konica, Tokyo, Japan) against a white plate (L^* , 89.39;
149 a^* , 0.13; b^* , -0.51). The CIE L^* (lightness), CIE a^* (redness), and CIE b^* (yellowness)
150 values were obtained, and used a D65 illuminant.

151

152 **Water activity (a_w)**

153 Samples were placed in moisture activity sample cups, sealed, and equilibrated at room
154 temperature for 12 h to ensure consistent experimental conditions. Water activity was
155 then measured using an AquaLab 4TE (METER group, Pullman, USA).

156

157 **Texture profile analysis (TPA)**

158 Pemmican cubes, measuring $1.00 \times 1.00 \times 1.00$ cm ($L \times W \times H$), were analyzed using a
159 rheometer (Model Compac-100, Sun Scientific Co., LTD, Tokyo, Japan). The probe
160 utilized had an area of 3.14 cm^2 , with a load cell weight of 10 kg and a cross-head speed

161 of 200 mm/min. To determine the hardness, springiness, cohesiveness, chewiness, and
162 gumminess, the calculations followed the methodology outlined by Bourne (1978).

163

164 **pH**

165 To measure the pH value of pemmican, 6 g of sample was mixed with 54 mL of
166 distilled water, homogenized at 10,000 rpm for 60 s using a Bihon Seiki Ace
167 homogenizer (Osaka, Japan), and subsequently measured using a pH meter (Orion
168 Star™ A211, Thermo Scientific, Waltham, MA, USA).

169

170 **2-thiobarbituric acid reactive substance (TBARS)**

171 TBARS were measured using the method described by Witte et al. (1970). A 10g
172 sample was homogenized with 70% perchloric acid (Samchun Chemicals, Pyeongtaek,
173 Korea) diluted to make 10% perchloric acid 15 mL, and then 20 mL of distilled water,
174 at 10,000 rpm for 30 s. The homogenate was then filtered through Whatman No.2 filter
175 paper to obtain the filtrate. Next, 5 mL of the filtrate was mixed with 5 mL of 2-
176 thiobarbituric acid (Sigma Aldrich, Darmstadt, Germany) and left to stand in the dark
177 for 16 h. After 16 h, absorbance was measured at 529nm using a Spectrophotometer.
178 (mobi, MicroDigital Co., Ltd., Seongnam, Korea). The standard curve for
179 malondialdehyde used in the experiment was calculated with $x=0.0011$ ($r=0.999$),
180 $y=0.1975$, where x =TBARS value and y =absorbance.

181

182 **Volatile basic nitrogen (VBN)**

183 Pearson's method (1968) was used for measuring VBN levels. Initially, a 3 g sample
184 was homogenized with 45 mL of distilled water at 10,000 rpm for 60 s. The resulting
185 mixture was then filtered through Whatman No. 2 filter paper. Subsequently, 3 mL of

186 the filtrate was transferred to the outer chamber of a conway unit. In the inner chamber,
187 1 mL of 0.01 M appropriate reagent (Sigma Aldrich, Darmstadt, Germany) and 4 drops
188 of indicator solution (0.066% methyl red + 0.066% bromocresol green) were added.
189 Additionally, 1 mL of 50% K₂CO₃ (Samchun Chemicals, Pyeongtaek, Korea) was
190 added the outer chamber. The mixture was allowed to culture at 37°C for 120 min.
191 Following culturing, the solution in the inner chamber was titrated with 0.01 M sulfuric
192 acid. Ultimately, VBN was quantified as mg per 100 g of sample (mg%).

$$193 \text{ VBN (mg/100g)} = ((A-B) \times F \times 28.014 \times 100) / (\text{amount of sample})$$

194 A: the amount of sulfuric acid injected (mL)

195 B: the amount of H₂SO₄ injected into the blank (mL)

196 F: 0.02 N H₂SO₄ standardized index

197 28.014: amount of N required to titrate 1 mL of 0.02 N H₂SO₄

198

199 **Statistical Analysis**

200 The experiment results were analyzed with three or more repetitions, and all statistical
201 analyses were conducted using SPSS (26.0). To compare the significance of treatment
202 groups and storage periods, One-way ANOVA analysis was performed, followed by
203 One-way Analysis of Variance and Duncan's multiple range test ($P < 0.05$) for mean
204 and standard deviation.

205

206 **Results & Discussion**

207 **Fatty acids composition**

208 The fatty acids composition of pemmican, in which beef tallow was replaced with CA is
209 shown in table 2. As the level of CA increased, there was a significant decrease in the
210 content of SFA, while the content of MUFA and PUFA significantly increased ($P <$

211 0.05). The main SFA in pemmican were stearic acid (C18:0) and palmitic acid (C16:0),
212 while the major unsaturated fatty acids (UFA) were oleic acid (C18:1n9) and linoleic
213 acid (C18:2n6). These findings align with the results reported by Lee (2010), which
214 indicated a similar fatty acid composition in Hanwoo beef fat and pemmican. CA is
215 known to primarily contain UFA, with oleic acid at 62.41% and linoleic acid at 20.12%
216 (Zambiasi et al., 2007). The increase in the content of these fatty acids in the CA
217 treatment groups can be attributed to the high ratio of oleic acid and linoleic acid in CA.
218 This corresponds to the results reported by Koo et al. (2009), which demonstrated an
219 increase in UFA, such as oleic acid, in hamburger patties produced with CA, and the
220 results reported by Moon et al. (2021), which showed an increase in MUFA with
221 increasing CA content in emulsified sausages produced with varying ratios of horse fat
222 and CA. However, it should be noted that UFA are relatively susceptible to oxidation
223 compared to SFA, which poses a risk of reducing fat hardness and deteriorating fat
224 color during storage (DeLany et al., 2000). Therefore, it is necessary to determine an
225 appropriate ratio of mixed oils to ensure oxidative stability.

226

227 **Proximate composition**

228 The quality characteristics of pemmican, in which beef tallow was replaced with CA is
229 shown in table 3. Among all the treatment groups, there were no significant differences
230 observed in moisture, crude fat, crude protein, crude ash and carbohydrate content ($P >$
231 0.05). These findings are in line with previous studies that found no significant
232 differences in the proximate composition of pork patties when 50% of animal fat was
233 replaced with plant-based oil, compared to the control group (Lu et al., 2017).
234 Furthermore, when animal fat was replaced with CA in hamburger patties, there were
235 no significant differences observed in the proximate composition compared to the

236 control group (Koo et al., 2009). Additionally, the fat and protein content in beef
237 burgers with added CA did not differ significantly from the control group (Onopiuk et
238 al., 2022). Therefore, it can be concluded that replacing up to 12.5% of animal fat with
239 CA does not affect the proximate composition of pemmican.

240

241 **Color**

242 In terms of color, no significant differences were observed in lightness and redness
243 among the treatment groups ($P > 0.05$). Pemmican inherently exhibits a very dark color.
244 Therefore, the addition of CA does not seem to significantly affect the lightness and
245 redness. The lowest yellowness value was observed in the CON group, and there were
246 no significant differences in the CA 1-5 groups ($P > 0.05$). The yellowness increased as
247 the level of CA increased, suggesting that the yellow hue of CA itself may have
248 influenced the color. CA's yellowness hue is known to be caused by natural pigments
249 like carotenoids and chlorophylls found in oil (Przybylski, 2005), and these compounds
250 have been reported to impact the yellowness of meat products (Bolognesi and Garcia,
251 2018). However, the color of meat products is primarily affected by variations in raw
252 materials rather than changes in color due to the type of animal fat used. Therefore, the
253 color changes in pemmican are considered minimal.

254

255 **a_w**

256 Water activity is a critical parameter in food that affects stability, microbial reactions,
257 and the types of microorganisms present (Tapia et al., 2020). Dried meat products need
258 to maintain a stable a_w to prevent quality changes during storage (Sun et al., 2002). In
259 all treatment groups, the a_w values of pemmican were consistently low, at 0.40 or below.
260 Furthermore, there was a decreasing trend in a_w with increasing levels of CA addition,

261 with CA5 showing significantly the lowest value ($P < 0.05$). Animal fat is retained more
262 efficiently within the protein matrix, and its particles act as a barrier against water,
263 allowing the meat to retain moisture better (Kumar, 2021). Therefore, it is determined
264 that as the level of beef tallow decreases, a_w decreases. Low moisture activity foods are
265 often lightweight and stable at room temperature, making them convenient for
266 consumers as they can be easily carried and stored at ambient temperature, such as
267 snacks, dried fruits, and jerky. The results of this study suggest that as the level of CA
268 addition increases, there is a decrease in a_w , indicating better inhibition of microbial
269 growth and quality changes. This implies that the addition of CA contributes to
270 enhancing storage safety, extending shelf life, and preserving product quality.

271

272 **TPA**

273 Hardness, springiness, chewiness, and gumminess showed a decreasing trend as the
274 level of CA increased. However, cohesiveness did not exhibit any significant
275 differences ($P > 0.05$). These findings are consistent with Park et al. (2005), who
276 observed that replacing animal fat with vegetable oil reduced the hardness of pork
277 patties. Wood et al. (2004) also reported that the hardness of adipose tissue is greatly
278 influenced by the physical properties of fat, which are determined by fatty acids, and
279 this can impact the meat quality. The major fatty acids composing pemmican have
280 specific melting points: palmitic acid (16:0) at 62°C, stearic acid (18:0) at 70°C, oleic
281 acid (18:1) at 13°C, and linolenic acid (18:3) at -11°C (Knothe and Dunn, 2009). It is
282 inferred that the decrease in hardness is due to the inability of fat to retain moisture
283 because of the low melting point of unsaturated fatty acids. Furthermore, it has been
284 reported that hardness decreases as the ratio of unsaturated fatty acids, which have weak
285 intermolecular forces due to their molecular structure, increases compared to saturated

286 fatty acids (Hur et al., 2005) and it has been reported that adding vegetable oil to meat
287 products can soften the protein matrix structure, resulting in a smoother texture (Cho et
288 al., 2023). Therefore, the results of this study suggest that pemmican, in which animal
289 fat was replaced with CA containing high levels of unsaturated fatty acids, will exhibit a
290 softer texture compared to the CON.

291

292 **pH**

293 The pH of pemmican, in which beef tallow was replaced with CA, during the 14 days of
294 storage is shown in figure 2. The pH of meat products is a significant factor in
295 evaluating freshness, color, and shelf life, serving as an important indicator for
296 assessing the freshness of meat products (Tamkutė et al., 2021). The treatment groups
297 with added CA exhibited significantly lower pH values compared to the CON group at
298 day 0 ($P < 0.05$). This finding aligns with the results of a study by Lee et al. (2015),
299 which observed a decrease in pH of sausage emulsion with 16% CA addition, replacing
300 animal fat with vegetable oil. This decrease in pH enhances the safety of meat products
301 by deactivating pathogens and inhibiting quality changes caused by spoilage
302 microorganisms, thereby improving product stability and extending shelf life (Ammor
303 and Mayo, 2007). Significant increases in pH were observed in the CA2, CA3, CA4,
304 and CA5 treatment groups until day 7 ($P < 0.05$), followed by a decreasing trend at day
305 14. Hydrolysis, a chemical reaction that breaks the ester bonds of triglycerides in
306 vegetable oils, results in the formation of free fatty acids and glycerol, which can
307 subsequently lower the pH of the product. (Fakhri and Qadir, 2011). Therefore, it can be
308 predicted that triglyceride hydrolysis of CA occurred from day 7 onwards. This is
309 consistent with reported results showing that the pH increased until the 7th day and then
310 decreased in press ham with added vegetable oil (Dzudie et al., 2004), as well as in beef

311 patties with animal fat replaced by brown rice oil and olive oil, where the pH increased
312 until the 7th day of storage (Seo et al., 2011).

313

314 **TBARS**

315 The TBARS values of pemmican, in which beef tallow was replaced with CA, during
316 the 14 days of storage is shown in figure 3. A major cause of quality deterioration in
317 meat products is lipid oxidation, which leads to undesirable changes in nutritional value,
318 taste, appearance, and texture, and can potentially generate toxic substances (Sun et al.,
319 2011). In the case of TBARS in pemmican, there was a decreasing trend in TBARS
320 values as the level of CA increased at day 0, and no significant increase in TBARS
321 values was observed as the storage period elapsed in the other treatment groups, except
322 for CA5 ($P > 0.05$). CA contains a significant amount of tocopherol, also known as
323 vitamin E (Matthaus et al., 2016). α -tocopherol primarily protects unsaturated fatty
324 acids from lipid radicals (Monahan et al., 1992). Therefore, higher levels of α -
325 tocopherol in meat products indicate better antioxidant activity, enhancing oxidative
326 stability. Carotenoids present in CA also scavenge peroxy radicals, protecting PUFA
327 from oxidation and stabilizing carbon-centered radicals by resonance (Domínguez et al.,
328 2019). However, CA5 showed significantly the highest values at both day 7 and day 14
329 ($P < 0.05$). Unsaturated fatty acids are more susceptible to lipid oxidation compared to
330 saturated fatty acids (Rael et al., 2004). The oxidation of PUFA deteriorates the color,
331 flavor, and quality of meat (Adeyemi and Olorunsanya, 2012). This aligns with the
332 reported decrease in oxidative stability when using vegetable fats in meat products
333 (Kılıç and Özer, 2019). The lipid peroxidation inhibition provided by phenolic
334 compounds can help reduce oxidative stress at low replacement ratios. However, as the
335 amount of oil and unsaturated fatty acids increases, so does oxidative sensitivity, which

336 can diminish this benefit, and finding the optimal ratio is crucial (Xu et al., 2015).

337 Therefore, it can be inferred that lipid oxidation occurred due to the susceptibility of

338 unsaturated fatty acids to spoilage when CA was replaced at levels above 10%.

339

340 **VBN**

341 The VBN values of pemmican, in which beef tallow was replaced with CA, during the 2

342 weeks of storage is shown in figure 4. VBN is a numerical indicator that measures the

343 presence of volatile amines like ammonia nitrogen and trimethylamine. It reflects the

344 freshness of meat during refrigerated storage, and in South Korea, the permissible limit

345 for VBN in meat products is regulated to be 20 mg% (Jeon and Choi, 2012). For

346 pemmican, there was a decrease in VBN as the proportion of CA increased, both at 0

347 and 14 days. This can be attributed to the antimicrobial and antioxidant effects of

348 phenolic compounds and tocopherol present in CA, which inhibit protein degradation

349 (Li et al., 2021). Also, the aldehydes and ketones generated from the oxidation of fatty

350 acids affect the quality and shelf life of meat, and these compounds can influence VBN

351 levels (Geng et al., 2024). These results suggest that the decrease in TBARS also likely

352 contributed to the reduction in VBN levels. These findings are consistent with studies

353 that found low VBN values in ground pork with added vegetable fats such as pork fat,

354 olive oil, and soybean oil (Youn et al., 2007). In conclusion, replacing animal fat with

355 CA in pemmican appears to reduce the VBN content, thereby improving the product's

356 shelf life.

357

358 **Conclusion**

359 This study aimed to improve the fatty acid composition of a dried meat product called

360 pemmican by replacing beef tallow with canola oil (CA). The study also examined the

361 quality characteristics and storage stability of pemmican based on the level of CA
362 substitution.

363 The proximate composition of pemmican with CA replacing animal fat did not vary
364 significantly across all treatment groups. However, as the proportion of CA increased,
365 the pH and water activity (a_w) decreased. The addition of CA did not impact the
366 lightness of the product but did slightly increase its yellowness. Furthermore, as the
367 level of CA increased, the hardness, springiness, chewiness, and gumminess of the
368 pemmican decreased, resulting in a softer texture. The substitution of animal fat with
369 CA led to an increase in monounsaturated fatty acids and polyunsaturated fatty acids
370 content and a decrease in saturated fatty acids content and Notably, there was a
371 significant increase in oleic acid and linoleic acid content. Storage evaluation conducted
372 at 4°C on days 0, 7, and 14 showed no significant differences in 2-thiobarbituric acid
373 reactive substances (TBARS), except for the CA5 treatment. In terms of volatile basic
374 nitrogen (VBN), a decreasing trend was observed with increasing levels of CA addition.

375 In conclusion, replacing animal fat with CA in the production of pemmican improves
376 the fatty acid composition and enhances stability against microbial growth, thanks to the
377 decreased pH and a_w . Additionally, it inhibits protein degradation and lipid oxidation,
378 although an increase in TBARS was observed in the CA5 treatment, indicating lipid
379 deterioration. Overall, substituting animal fat with CA in pemmican increases the
380 content of unsaturated fatty acids, suggesting superior nutritional quality. The CA4
381 treatment at a concentration of 10% is considered the most optimal.

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Tables and Figures

546 Table 1. The formulation of Pemmican with different ratio of beef tallow and canola oil

Ingredients (%)		CON	CA1	CA2	CA3	CA4	CA5
Main	Dried meat	60.0	60.0	60.0	60.0	60.0	60.0
	Dried berry ¹	15.0	15.0	15.0	15.0	15.0	15.0
	Beef tallow	25.0	22.5	20.0	17.5	15.0	12.5
	Canola oil	0	2.5	5.0	7.5	10.0	12.5
Additive	Salt	1.2	1.2	1.2	1.2	1.2	1.2

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548¹⁾ Dry berries are a 1:1:1 ratio of dried cranberries, dried raspberries, and raisin

Table 2. Fatty acids composition of Pemmican with different ratio of beef tallow and canola oil

Traits (%)	CON	CA1	CA2	CA3	CA4	CA5
Myristic acid (C14:0)	2.26±0.03 ^{ab}	2.52±0.56 ^a	2.00±0.06 ^{abc}	1.73±0.02 ^{bc}	1.63±0.01 ^c	1.48±0.02 ^c
Palmitic acid (C16:0)	25.51±0.10 ^a	25.84±2.27 ^a	22.90±0.11 ^b	21.00±0.04 ^{bc}	19.85±0.03 ^{cd}	18.45±0.09 ^d
Palmitoleic acid (C16:1n7)	3.96±0.03 ^{ab}	4.21±0.69 ^a	3.47±0.06 ^{bc}	3.09±0.01 ^{cd}	2.91±0.00 ^{cd}	2.68±0.02 ^d
Stearic acid (C18:0)	12.04±0.12 ^a	10.25±1.56 ^b	10.59±0.18 ^{ab}	10.01±0.05 ^{bc}	9.22±0.02 ^{bc}	8.50±0.07 ^d
Oleic acid (C18:1n9)	52.77±0.03 ^f	53.47±0.34 ^e	54.69±0.09 ^d	56.01±0.05 ^c	56.79±0.04 ^b	57.73±0.09 ^a
Linoleic acid (C18:2n6)	2.42±0.00 ^d	2.60±1.22 ^d	4.55±0.04 ^c	5.87±0.01 ^b	6.91±0.00 ^{ab}	8.05±0.02 ^a
γ-Linoleic acid (C18:3n6)	0.05±0.00 ^a	0.04±0.01 ^{ab}	0.04±0.00 ^{ab}	0.04±0.00 ^b	0.04±0.00 ^b	0.03±0.00 ^b
Linolenic acid (C18:3n3)	0.10±0.00 ^e	0.25±0.28 ^e	0.84±0.03 ^d	1.25±0.02 ^c	1.62±0.00 ^b	2.00±0.02 ^a
Eicosenoic acid (C20:1n9)	0.67±0.00 ^{cd}	0.64±0.07 ^d	0.72±0.02 ^{bc}	0.79±0.00 ^{ab}	0.82±0.01 ^a	0.86±0.01 ^a
Arachidonic acid (C20:4n6)	0.23±0.00	0.19±0.05	0.21±0.00	0.21±0.00	0.21±0.00	0.21±0.00
Total			100			
Saturated fatty acids (SFA)	39.81±0.01 ^a	38.60±1.27 ^a	35.49±0.02 ^b	32.74±0.01 ^c	30.70±0.02 ^d	28.43±0.03 ^e
Unsaturated fatty acid (UFA)	60.19±0.01 ^e	61.40±1.27 ^e	64.51±0.02 ^d	67.26±0.01 ^c	69.30±0.02 ^b	71.57±0.03 ^a
Monounsaturated fatty acid (MUFA)	57.39±0.00 ^f	58.32±0.29 ^e	58.87±0.05 ^d	59.89±0.04 ^c	60.53±0.02 ^b	61.27±0.08 ^a
Polyunsaturated fatty Acid (PUFA)	2.80±0.01 ^d	3.07±1.55 ^d	5.64±0.07 ^c	7.37±0.04 ^b	8.77±0.00 ^{ab}	10.29±0.05 ^a

SFA/UFA	0.66	0.63	0.55	0.49	0.44	0.40
PUFA/SFA	0.07	0.08	0.16	0.23	0.29	0.36

CON = Beef tallow 25%, CA1 = Beef tallow 22.5% + canola oil 2.5%, CA2 = Beef tallow 20% + canola oil 5%, CA3 = Beef tallow 17.5% + canola oil 7.5%, CA4 = Beef tallow 15% + canola oil 10%, CA5 = Beef tallow 12.5% + canola oil 12.5%. ^{a-f} Different letters within each row indicate significant differences determined by mean \pm standard deviation ($P < 0.05$).

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Table 3. Quality characteristics of Pemmican with different ratio of beef tallow and canola oil

Traits (%)	CON	CA1	CA2	CA3	CA4	CA5
Moisture	6.79±0.42	6.43±0.29	6.65±0.54	6.77±0.57	6.77±0.49	6.22±0.26
Protein	45.18±2.55	44.34±1.64	46.20±0.99	45.57±2.51	45.90±2.99	45.35±3.40
Fat	38.44±1.03	38.02±2.76	38.13±1.59	36.03±2.31	37.30±2.28	39.00±2.08
Ash	0.55±0.13	0.53±0.16	0.74±0.04	0.71±0.19	0.74±0.11	0.72±0.04
Carbohydrate	9.04±1.08	10.58±1.78	8.28±1.47	10.92±4.79	9.29±5.13	8.76±3.61
CIE L*	25.16±0.47	25.68±0.87	25.18±0.88	25.22±0.72	25.56±0.74	25.78±0.73
CIE a*	4.37±0.41	4.43±0.54	4.31±0.49	4.87±0.25	4.23±0.96	4.90±0.37
CIE b*	4.60±0.64 ^b	4.88±0.56 ^{ab}	5.32±0.71 ^{ab}	5.00±1.05 ^{ab}	5.80±0.62 ^a	5.42±0.73 ^{ab}
Water activity (a _w)	0.38±0.01 ^a	0.36±0.00 ^{bc}	0.37±0.01 ^{ab}	0.34±0.01 ^d	0.35±0.01 ^{cd}	0.32±0.01 ^e
Hardness (kg)	0.42±0.02 ^a	0.27±0.05 ^b	0.26±0.05 ^b	0.25±0.06 ^b	0.19±0.04 ^b	0.18±0.02 ^b
Springiness (%)	15.79±2.19 ^{ab}	17.88±1.01 ^{ab}	19.76±5.30 ^a	15.16±3.24 ^{ab}	12.00±1.11 ^b	12.43±3.56 ^{ab}
Cohesiveness (%)	57.74±6.91	51.45±1.02	42.04±5.62	43.43±9.29	50.88±1.24	41.67±11.79
Chewiness (kg)	0.04±0.00 ^a	0.02±0.00 ^b	0.02±0.01 ^b	0.02±0.00 ^b	0.01±0.00 ^b	0.01±0.00 ^b
Gumminess (kg)	0.24±0.02 ^a	0.14±0.02 ^b	0.11±0.04 ^b	0.10±0.05 ^b	0.10±0.02 ^b	0.08±0.07 ^b

CON = Beef tallow 25%, CA1 = Beef tallow 22.5% + canola oil 2.5%, CA2 = Beef tallow 20% + canola oil 5%, CA3 = Beef tallow 17.5% + canola oil 7.5%, CA4 = Beef tallow 15% + canola oil 10%, CA5 = Beef tallow 12.5% + canola oil 12.5%. The carbohydrate value is calculated by subtracting the average of moisture, protein, fat, and ash values from 100.

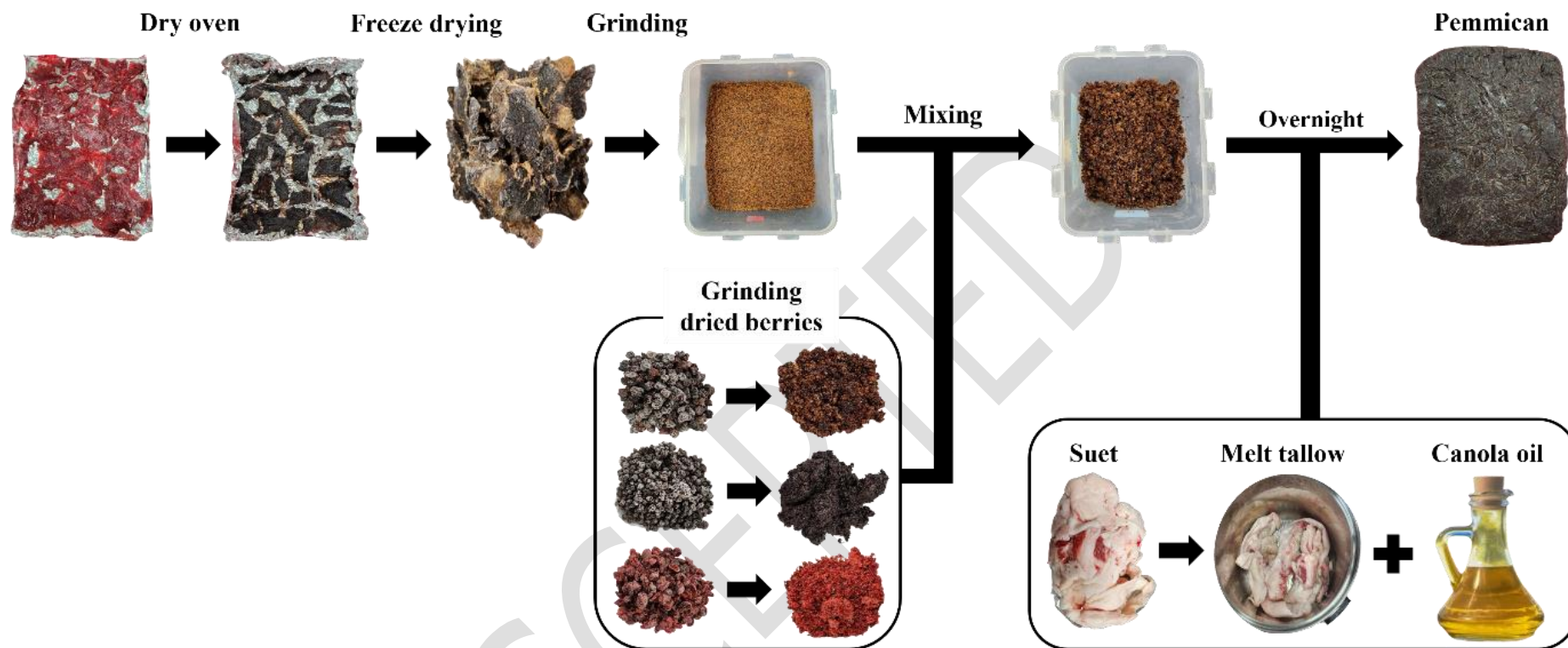


Figure 1. Manufacturing process of Pemmican with different ratio of beef tallow to canola oil.

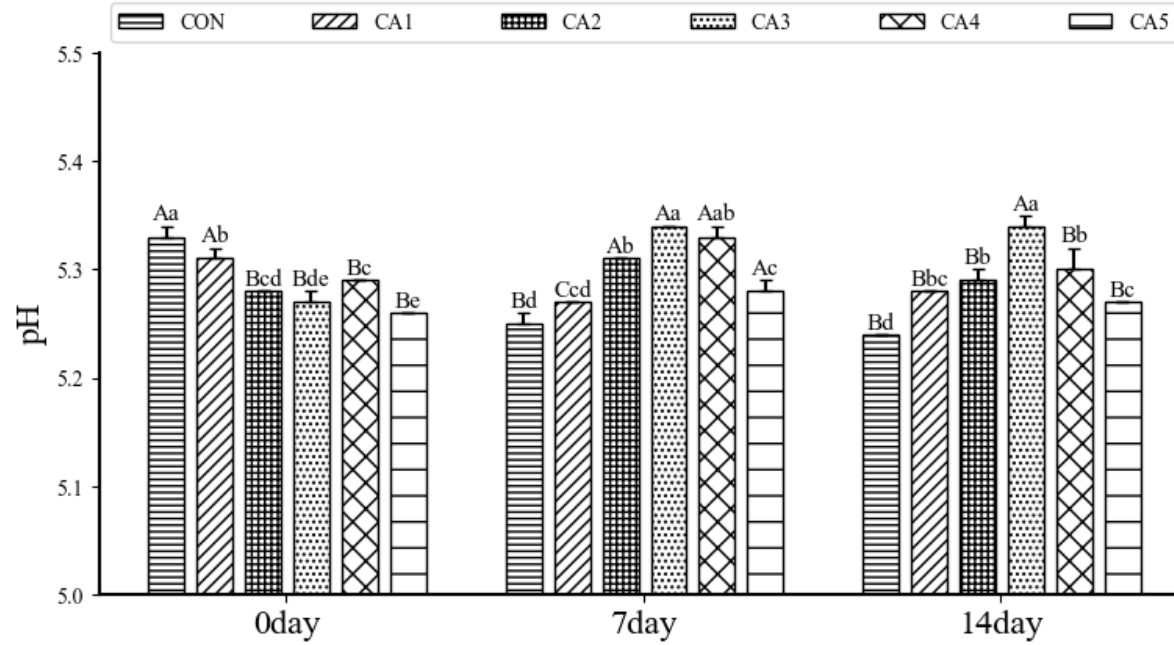


Figure 2. pH of Pemmican with different ratio of beef tallow and canola oil.

CON = Beef tallow 25%, CA1 = Beef tallow 22.5% + canola oil 2.5%, CA2 = Beef tallow 20% + canola oil 5%, CA3 = Beef tallow 17.5% + canola oil 7.5%, CA4 = Beef tallow 15% + canola oil 10%, CA5 = Beef tallow 12.5% + canola oil 12.5%. ^{A-B} Different letters within each treatment indicate significant differences determined by mean \pm standard deviation ($P < 0.05$). ^{a-c} Different letters within each day indicate significant differences determined by mean \pm standard deviation ($P < 0.05$).

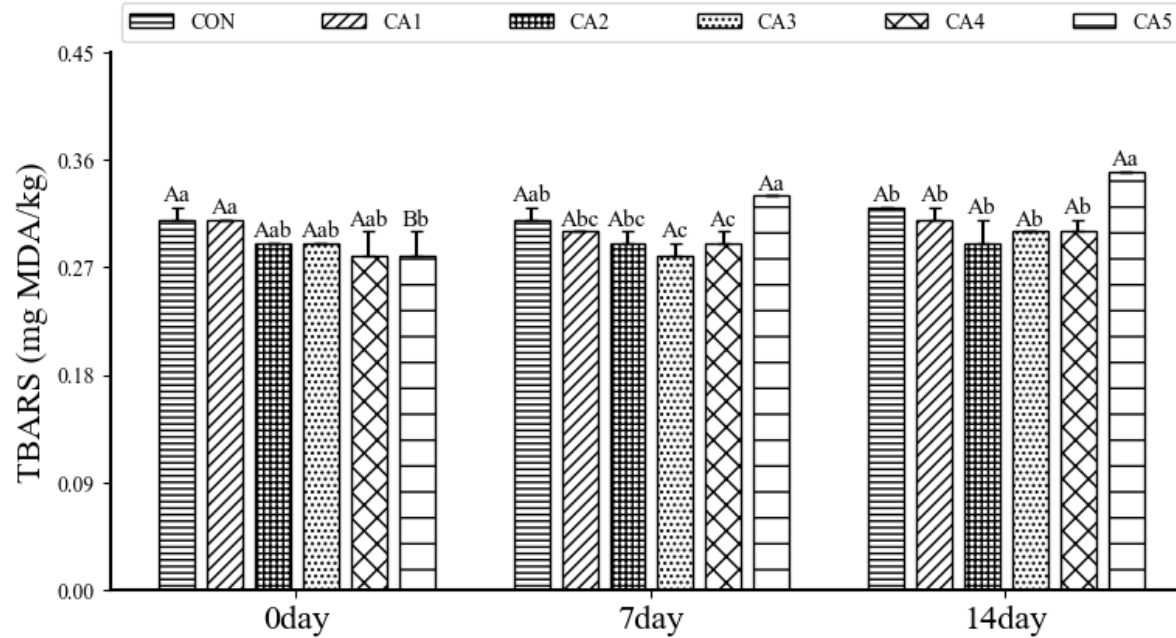


Figure 3. 2-Thiobarbituric acid reactive substance (TBARS) of Pemmican with different ratio of beef tallow and canola oil.

CON = Beef tallow 25%, CA1 = Beef tallow 22.5% + canola oil 2.5%, CA2 = Beef tallow 20% + canola oil 5%, CA3 = Beef tallow 17.5% + canola oil 7.5%, CA4 = Beef tallow 15% + canola oil 10%, CA5 = Beef tallow 12.5% + canola oil 12.5%. ^{A-B} Different letters within each treatment indicate significant differences determined by mean \pm standard deviation ($P < 0.05$). ^{a-c} Different letters within each day indicate significant differences determined by mean \pm standard deviation ($P < 0.05$).

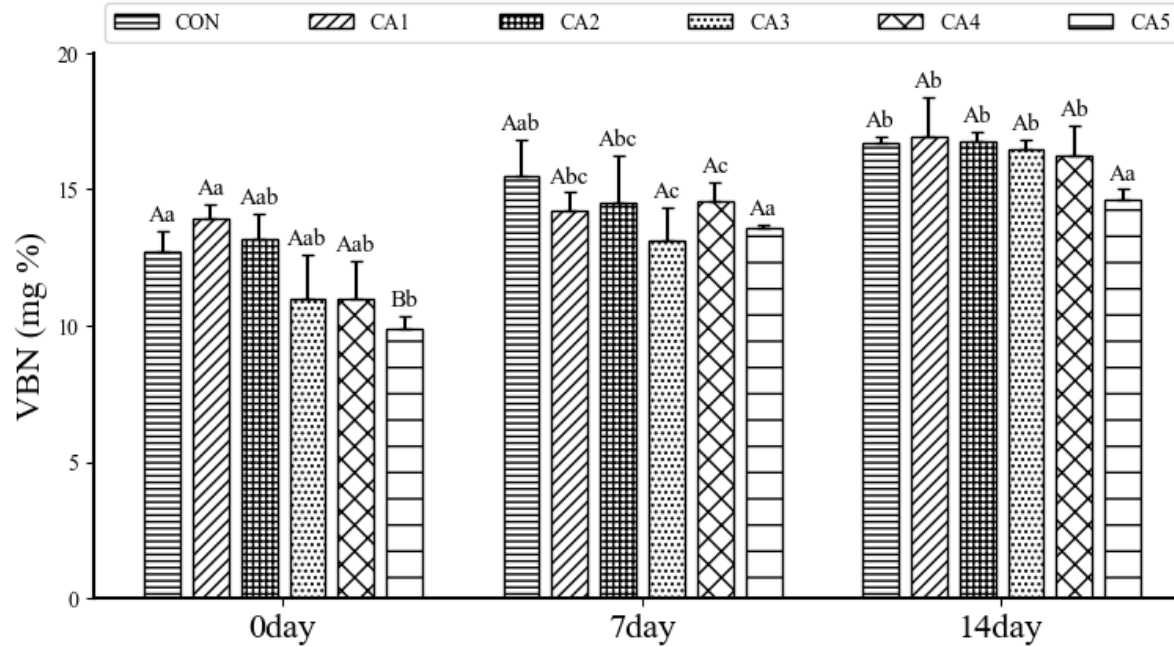


Figure 4. Volatile basic nitrogen (VBN) of Pemmican with different ratio of beef tallow and canola oil.

CON = Beef tallow 25%, CA1 = Beef tallow 22.5% + canola oil 2.5%, CA2 = Beef tallow 20% + canola oil 5%, CA3 = Beef tallow 17.5% + canola oil 7.5%, CA4 = Beef tallow 15% + canola oil 10%, CA5 = Beef tallow 12.5% + canola oil 12.5%. ^{A-B} Different letters within each treatment indicate significant differences determined by mean \pm standard deviation ($P < 0.05$). ^{a-c} Different letters within each day indicate significant differences determined by mean \pm standard deviation ($P < 0.05$).