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1 **Peptide from RuBisCO protein hydrolysate and its application in beef meat**
2 **preservation**

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25 **Abstract** – The objective of this work was to purify a small (705 Da) and hydrophilic
26 antimicrobial peptide (Arg-Asp-Arg-Phe-Leu) from RuBisCO protein hydrolysate and to
27 evaluate its effect on the microbiological and oxidative stability of minced beef meat during
28 refrigeration. The TBARS values were significantly affected by the storage period and the
29 concentration of bioactive peptide. The antioxidant effect was similar to that of the BHT used
30 as control. The counts of all determined microbiological indicators were significantly affected
31 by the addition of the Arg-Asp-Arg-Phe-Leu. Arg-Asp-Arg-Phe-Leu can be effectively
32 utilized as food ingredients in meat for enhancing their shelf life and oxidative stability.

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34 **Index Terms:** Antioxidant; antimicrobial; bioactive peptide, beef meat.

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Introduction

50
51 Fresh products as minced meat contains adequate amount of water and abundance of
52 proteins and essential nutrients with favorable pH for supporting microbial growth. Meat is
53 highly perishable products and requires appropriate preservation (Domínguez et al. 2018).
54 Therefore, several synthetic additives have long been directly applied as food additives or
55 used as processing aids with primary intention of prolonging shelf life and preserving quality
56 of meat and meat products (Munekata et al., 2020). The application of chemical preservatives
57 such as butylated hydroxytoluene (BHT) and propyl gallate (PG) has been questioned because
58 of the potential toxic and carcinogenic effects (Hashemi et al. 2017). Not only pressure of the
59 consumer health concerns, but also a trend towards natural food additives so called “clean-
60 labeling” has driven exploring of natural antimicrobial compounds as an alternative to
61 synthetic food additives (Domínguez et al. 2019; Lorenzo et al. 2018). Advancement in
62 bioactive peptide research has showed that the peptides derived from food proteins could be
63 natural potential candidates to replace the synthetic compounds (Chakrabarti et al., 2018;
64 Wang et al., 2015). Indeed, antimicrobial peptides of industrial waste could be used as
65 preservatives (Lafarga & Hayes, 2014). To date, several animal peptides such as lysozyme,
66 lactoferrin, and nisin are approved for application in meat and meat products (Silveira et al.,
67 2021).

68 Recently, the food industries have taken interest to peptides derived from agro-
69 industrial waste. Green juice derived from bio-refinery of Alfalfa (*Medicago sativa*), is often
70 considered as a source of pollution, but it is an excellent protein-rich source and has become
71 an alternative for peptides generation with great interest for food formulation (Hadidi et al.,
72 2019). Ribulose-1,5-bisphosphate carboxylase oxygenase, most commonly known by the
73 shorter name RuBisCO. It's the most abundant plant protein and possesses unique
74 characteristics, a favorable nutritional profile, beneficial bioactivities, and desirable physical

75 attributes, making it a promising candidate for use in food applications (Grácio et al., 2023).
76 Several studies showed that RuBisCO have been reported to contain several bioactive
77 peptides compared to commonly consumed food proteins, except milk proteins (Corrêa et al.,
78 2023). In recent previous studies antibacterial and antioxidant peptides derived from the
79 enzymatic hydrolysis of Alfalfa RuBisCO were isolated and identified (Kobbi et al., 2015;
80 Kobbi et al., 2017). The antibacterial peptide (Arg-Asp-Arg-Phe-Leu) was previously
81 identified from protein hydrolysates of Alfalfa RuBisCO prepared with pepsin® (Kobbi et al.,
82 2015). Arg-Asp-Arg-Phe-Leu a small (705 Da) peptide with one positive charges at pH 7
83 belongs to the great subunit of the RuBisCO, which has a large antibacterial spectrum as
84 against Gram positive and Gram-negative bacteria (Kobbi et al., 2015). This bioactive peptide
85 has several attractive properties and attributes that make it a potential promising candidate for
86 application as preservative in the storage and distribution of various meat products. The
87 objective of this work was to purify a small (705 Da) and hydrophilic antimicrobial peptide
88 (Arg-Asp-Arg-Phe-Leu) from RuBisCO protein hydrolysate and to evaluate its effect on the
89 microbiological and oxidative stability of minced beef meat during refrigeration.

90 **Materials and methods**

91 All common chemicals and solvents were of analytical grade from commercial
92 sources: Sigma-Aldrich (Saint-Quentin Fallavier, France) or Flandres Chimie (Villeneuve
93 d'Ascq, France). Synthetic peptide was obtained from GeneCust Europe (Biotechnology
94 Laboratory of Luxembourg S.A). The beef meat was obtained from a local purveyor.

95 The purification of RuBisCO was realized by a simple method based on pH and
96 solvent effect, according to the method described by Kobbi et al., (2017). Primary purification
97 of an interest protein from a crude medium is specifically to eliminate impurities depending
98 on the solubility and chemical properties of RuBisCO, as its overall charge, solubility or pHi.
99 In order to isolate a soluble fraction, 100 g of green juice was solubilized in 1 L of distilled

100 water or 1 L of ammonia (NH₄OH) at 0.1 M and pH 11. Suspensions were centrifuged at
101 10,000 g for 20 min at 4°C to eliminate the green protein and all debris. The RuBisCO
102 containing base 1 at pH 10 was repurified to retrieve the second supernatant 2. Base 2 was
103 repurified again to recover supernatant 3. After three successive centrifugations at pH 10, all
104 insoluble fractions were removed, and, hence, three supernatants were summed up to recover
105 all RuBisCO. Proteins were then precipitated by the acidification of the medium at pH 3 using
106 10 mM of formic acid. This was followed by a last centrifugation step at 10,000 g at 4 °C for
107 20 min. The precipitate was washed by ultrapure water and RuBisCO was then lyophilized.
108 A 10% (w/v) aqueous solution of the lyophilized RuBisCO was adjusted to pH 10 and then
109 centrifuged at 10,000 g for 5 min. The supernatant was adjusted to optimal pH (pH 3.0) and
110 temperature (37°C) for pepsin[®]. The hydrolysis reaction was started by the addition of the
111 enzyme at a 1:100 (U/mg) enzyme/ protein ratio. The enzymatic reaction was conducted at 37
112 °C and pH 3 for 24 h. The hydrolysis reactions are stopped after 24 h by increasing the pH to
113 10 with 1 M NaOH and then stored at -20 °C for further use. The proportion of none
114 hydrolyzed RuBisCO and then hydrolyzed RuBisCO was deduced from the peak areas by
115 HPLC.

116 The degree of hydrolysis (DH) is defined as the ratio of the number of peptide bonds cleaved
117 to the total number of peptide bonds of protein substrate. DH is determinate by the OPA
118 (o-phthaldialdehyde) method using leucine as standard (Nielsen et al., 2001). A fresh OPA
119 solution was prepared extemporaneously with 25 mL of 100 mM sodium tetrahydroborate, 5
120 mL of 10% SDS solution (w/v), 80 mg of OPA (dissolved in 1 mL of methanol) and 0.01 mL
121 of β-mercaptoethanol were adjusted to a final volume of 50 ml with distilled water. To assay
122 proteolysis with RuBisCO protein as substrates, an aliquot (usually 0.01–0.05 ml containing
123 5–100 mg proteins) was added to 1.0 ml of OPA reagent in a 1.5 ml quartz cuvette. The

124 solutions were mixed briefly and incubated for 2 min at room temperature, and then the
125 absorbance at 340 nm was measured.

126 The RuBisCO hydrolysis was analyzed by RP-HPLC on a C4 column according to the
127 process previously described with slight modifications ([Trovalset et al., 2007](#)). The liquid
128 chromatographic system consisted of a Waters 600E automated gradient controller pump
129 module, a Waters Wisp 717 automatic sampling device and a Waters 996 photodiode array
130 detector. Spectral and chromatographic data were stored on a NEC Image 466 Image 466
131 computer. Millennium software was used to plot, acquire, and analyze chromatographic data.
132 Chromatographic processes were performed with a VydacC4 column (250 mm×10 mm). The
133 mobile phase composed of water/trifluoroacetic acid (1000:1, v/v) was used as eluent A,
134 while acetonitrile/trifluoroacetic acid (1000:1, v/v) was used as eluent B. The flow rate was
135 0,6 ml/min. Samples were filtered through 0.22mm filters and then injected. The gradient
136 applied was 0-10 min, 100% A; 25-95min, 21- 81% B; 96-110min, 100- 0% B; and the HPLC
137 system were equilibrated for 10 min with 100% A. On-line UV absorbance scans were
138 performed between 215 and 325 nm at a rate of one spectrum per second with a resolution of
139 1.2 nm. Chromatographic analyzes were completed with Millennium software.

140 The RuBisCO hydrolysate was adjusted to pH 7.5 and NaCl was added to a fixed volume to
141 reach a final concentration of 2.0 M of salt ([Adoui et al., 2013](#)). The solution was stirred for
142 30 min at room temperature before being centrifuged at 10,000 g for 10 min at 4°C. The pellet
143 was washed twice by centrifugation with phosphate buffer (5 mM, pH 7.5) to remove soluble
144 proteins, and then resuspended in phosphate buffer (5 mM, pH 7.5, 100 mM NaCl) to 20
145 times the original hydrolysate volume. The final pH of the peptide hydrolysate was adjusted
146 between 1.5 to 2. After one hour stirring at 7 °C, the pellet was centrifuged (10,000 g, 10
147 min), and the supernatant was recovered. The solution was stored at -20°C until performing
148 the antibacterial assay and analysis by RP-HPLC.

149 The molecular mass and peptide sequencing were done in positive ion mode using
150 Electrospray ionisation-mass spectrometry (ESI-MS) and the tandem mass spectrometry
151 (MS/MS), respectively. ESI mass spectrometry was performed using a triple quadrupole
152 instrument Applied Biosystems API 3000 (PE Sciex, Toronto, Canada) equipped with an
153 electrospray ion source. The system is controlled by the Analyst Software 1.4, allowing the
154 control of the spectrometer, the analysis and the processing data. Interpretations of spectra
155 MS-MS were made with the Bioanalyst software. The freeze-dried samples from RP-HPLC
156 were dissolved in acetonitrile/water (20/80; v/v) containing 0.1% formic acid for the positive
157 mode. The solution was injected (nebulised) uninterrupted, by a pump (Model 22, Harvard
158 Apparatus, South Natick, USA) with a flow rate of 5 μ l/min. The potential of ionisation was
159 of 5000 V in positive mode. At the time of the recording of the spectrum, 30 scans on average
160 were added (MCA mode) for each spectrum. The gases used (nitrogen and air) were pure (up
161 to 99%) and produced by a compressor Jun-Air 4000-40M and a nitrogen generator Whatman
162 model 75-72 (Whatman Inc, Haverhill, MA the USA). The polypropylene glycol (PPG) was
163 used for the calibration and the optimisation of the machine. The peptide sequence was
164 determined from the CID spectrum of the protonated analyse $[M+H]^+$ by MS/MS
165 experiments. Peptide sequences were done using the bioanalyst software (Applied
166 Biosystems, USA).

167 The antimicrobial peptide (Arg-Asp-Arg-Phe-Leu) was chemically synthesized by the
168 conventional method: Fmoc solid phase synthesis, according to the method described by
169 [Fields and Noble \(1990\)](#). Arg-Asp-Arg-Phe-Leu peptide was synthesized on a Fmoc-Arg
170 (Pbf)-WangTentagel® S resin (0.25 mmol/g from Iris Biotech, 50 μ mol scale) using an
171 Intavis peptide synthesizer (Proteigene) and standard Fmoc/tert-butyl. Activation of the amino
172 acids (10 eq) was performed separately prior to the coupling step using a mixture of
173 TBTU/HOBt/DIEA: 10 eq/10 eq/30 eq in DMF. A capping step was performed after each

174 coupling with a mixture of Ac₂O/NMM/DMF: 5/10/75 v/v/v. At the end of the synthesis, the
175 Fmoc protecting group was removed using 20% piperidine in DMF. The resin was washed
176 with DMF (4 × 2 min) and CH₂Cl₂ (4 × 2 min) and dried in vacuo. Deprotection and
177 cleavage of the peptide from the resin was performed with a mixture of TFA/TIS/H₂O: at
178 room temperature. The mixture was precipitated in cold diethyl ether. The peptide was
179 collected by centrifugation, dissolved in deionized water and lyophilized. Arg-Asp-Arg-Phe-
180 Leu was purified by RP-HPLC.

181 The hemolytic activity of the Arg-Asp-Arg-Phe-Leu peptide was determined by methods of
182 [Dathe et al. \(1996\)](#), slightly modified. Five milliliters of bovine blood were centrifuged at
183 3500 rpm for 10 min to isolate erythrocytes, which were then washed three times with 10mM
184 sodium phosphate, pH 7.5, containing NaCl 9 g/L (NaCl/Pi). The cell concentration stock
185 suspension was adjusted to 10⁹ cells /mL. The cell suspension (12 mL) along with varying
186 amounts of peptide stock solution and the buffer were pipetted into Eppendorf tubes to give a
187 final volume of 50 mL. The Eppendorf tubes with 2.5 × 10⁸ cells/mL were then incubated at 37
188 °C during 40 min. After centrifugation (5000 rpm, 5 min), 30 mL of supernatant was diluted
189 in 500 mL water. The absorbance of the diluted solution was measured at 420 nm. The
190 absorbance obtained after treating erythrocytes with only NaCl/Pi and SDS (0.2%) was taken
191 as 0 and 100%, respectively. The test was carried out in triplicate.

192 The beef patties were composed with commercial beef containing 0.28% (w/w) of salt, 14%
193 (w/w) of fat and about 18% of proteins. Salt (70% NaCl and 30% KCl) was added at a rate of
194 1.5% to the minced meat. The sample was subdivided into four treatments: a negative control,
195 the meat without additive, a positive control, the meat with two different BHT concentrations
196 (0.1 and 0.5% (w/w)) and the meat with two different Arg-Asp-Arg-Phe-Leu peptide
197 concentrations (0.1 % and 0.5% (w/w)). The meat sample preparation was realized under
198 strong aseptic conditions to avoid contaminations. Patties of 10 g were placed in plastic foam

199 meat trays, wrapped with film polyethylene and kept at 4 °C for fourteen days. Four samples
200 were taken from each treatment at fixed day in order to evaluate their oxidation and the
201 potential microbial contamination. A strict sanitation procedure was followed during the
202 preparation of meat samples to avoid microbial contamination.

203 Lipid oxidation was evaluated using the thiobarbituric acid-reactive substance (TBARS) as
204 previously described by [Witte et al., \(1970\)](#). For each prepared formula five grams of minced
205 meat were added to 12.5 mL of solution containing trichloroacetic acid (20% w/v) and
206 phosphoric acid (2 M). The mixture was then adjusted to 25 mL of ultrapure water, shaken
207 and filtered using Buchner funnel through Whatman paper. After that, 5 mL of the filtrate was
208 then transferred into a glass test tube and an equal volume of 2-thiobarbituric acid (5 mM)
209 was added. The test tube was then well shaken and incubated at room temperature for 17 h.
210 The absorbance of the mixture was measured at 520 nm and the results were expressed as
211 malondialdehyde equivalents per meat kilogram (mg MDA/kg meat).

212 Microbial count was carried out at days 0, 1, 3, 6, 9, and 11 of storage at 4°C according to the
213 following procedure. 45 mL sterilized peptone solution (0.1% w/v) was added to each five
214 grams of beef patty in a sterile stomacher bag, then homogenized. Decimal dilutions up to 10⁵
215 prepared from the initial concentration (100 mg/mL) and 100 µL of the appropriate dilutions
216 were spread out on different selective media. Total viable counts were determined using plate
217 count agar (PCA), potato dextrose agar (PDA) for yeasts and molds counts, Man, Rogosa and
218 Sharpe (MRS) agar for lactic acid bacteria and violet, red bile lactose (VRBL) agar for
219 coliform counts were used after incubation at 37 °C for 24h. Microbiological count was
220 expressed as the log₁₀ of colony-forming units per gram of patty (log₁₀ CFU/g).

221 All data were submitted to Analysis of Variance (ANOVA) and differences between means
222 were evaluated by Duncan's Multiple Range Test. The data were analyzed using the

223 Statistical Package for the Social Sciences (SPSS) version 10.0 (Chicago, Illinois, USA).
224 Differences were considered significant at $p < 0.05$.

225 **Results and Discussion**

226 Figure 1 shows the RP-HPLC chromatogram from the peptic RuBisCO proteins purified from
227 green juice of Alfalfa. Generated hydrolysate (DH = 18%) was mainly intermediate peptides
228 (produced from the beginning of hydrolysis and disappearing gradually as hydrolysis
229 advance) (Abou-Diab et al., 2021). This standard condition following hydrolysis process led
230 to a complex hydrolysate containing many peptides, intermediate or final, with various
231 characteristics (Kleekayai & FitzGerald, 2022). The chromatographic profile (Figure 2A)
232 showed the peptide population with retention times between 10 and 50 min which
233 corresponding to antimicrobial peptides. According to the mass spectrometry analyses (Figure
234 2B), one antimicrobial pentapeptide was identified: Arg-Asp-Arg-Phe-Leu. The MIC of the
235 purified peptide varied between 2.17 ± 0.1 mM (for *Listeria innocua* and *Bacillus subtilis*)
236 and 2.83 ± 0.2 mM (for *Escherichia coli* and *Micrococcus luteus*). Peptides showed an
237 outstanding antibacterial activity against both Gram positive and Gram-negative bacteria by
238 damaging the cell membrane integrity (Bi et al., 2020; Tu et al., 2019; Medeiros et al., 2018).
239 Arg-Asp-Arg-Phe-Leu peptide did not cause hemolysis. The hemolytic activity of the peptide
240 against human erythrocytes was determined as an indication of its toxicity towards
241 mammalian cells. The highest concentration tested corresponding to this concentration of
242 peptide. No hemolysis was observed up to a peptide concentration corresponding to five times
243 the MIC. The positively charged residues can significantly reduce the toxicity of the peptides
244 towards the mammalian cells, so with the increasing positive charges, the hemolytic activity
245 of the peptides reduced reasonably (Lohan et al., 2023; Sengkhui et al., 2023).
246 Significant changes in TBARS occurred over the sampling period when Arg-Asp-Arg-Phe-
247 Leu peptide was added to chilled minced beef meat. Analysis of variance of the TBARS

248 values was determined between the different samples. The result showed that the TBARS
249 values increased throughout the eleven days experiment. This fact was clearly marked for the
250 control sample (without additives) which highest TBARS values value than all tested
251 conditions by the end of storage (It goes from value 0.56 ± 0.07 mg MDA for day 1 to a value
252 of 2.31 ± 0.18 mg MDA for day 11). While the TBARS values of the meat supplemented with
253 either 0.5% or 0.1% (w/w) Arg-Asp-Arg-Phe-Leu peptide, were significantly lower ($p <$
254 0.05) than the meat without additives, used as control. Moreover, the efficiency in ground
255 meat preservation increased proportionally with peptide concentration. But this difference was
256 clear from day 6 (Figure 3) because no significant effect between the four treated groups has
257 been observed during the first three days of the experiment, but their TBARS values were
258 significantly lower than the control (Figure 3). From day 6, the power antioxidant of Arg-
259 Asp-Arg-Phe-Leu at 0.5% (w/w) was higher (0.83 ± 0.04 mg MDA/kg) than the other two
260 treated groups (peptide at 0.1% and BHT at 0.1%) with an average value of 1.75 ± 0.31 mg
261 MDA and 1.25 ± 0.13 mg MDA respectively. Thus, the peptide at 0.5% (w/v) can protect beef
262 meat against lipid oxidation and extend their storage time. The TBARS values of the meat
263 supplemented with either peptide 0.5% or BHT 0.5% (w/v), used as reference, have no
264 significant difference and were similar after 9 days of storage at 4 °C of an average value
265 TBARS of 0.91 ± 0.21 mg MDA that is two times less than the control (2.145 ± 0.36 mg MDA)
266 (Figure 3). Consequently, the BHT at 0.5% (w/v) and Arg-Asp-Arg-Phe-Leu at 0.5% (w/w)
267 reduced the lipid oxidation on meat of 60% (Figure 3) compared to the control. This tendency
268 continued until 11 days. At the end of the experiment, the BHT at 0.5 (w/w) and Arg-Asp-
269 Arg-Phe-Leu 0.5% (w/w) had no significant difference to protect against the phenomenon of
270 lipid oxidation with a TBARS-value about of 0.96 mg MDA/kg meat. The results of the
271 present study show that adding the Arg-Asp-Arg-Phe-Leu' peptide derived from protein
272 hydrolysates of Alfalfa RuBisCO at 0.5% (w/w) protects beef patties against lipid oxidation.

273 The work confirms the results obtained by [Kobbi et al. \(2017\)](#) which revealed that the Alfalfa
274 RuBisCO hydrolysate (DH = 18.8 %) exhibited strong antioxidant abilities, including the
275 prevention of linoleic acid oxidation, reduction of ferric ion, and stability of ABTS*.
276 Therefore, green juice derived from bio-refinery of Alfalfa offers a promising source of
277 antioxidant peptides of interest for various functional food applications. The antioxidant
278 activity of this peptide was probably due to its low molecular weight (705 Da) and the
279 presence of aromatic residue Phenylalanine (Phe) which can quench free radicals by direct
280 electron transfer to act as an antioxidant ([Geng et al., 2023](#); [Jeampakdee et al., 2020](#); [Chi et
281 al., 2015](#)). Peptides with highly potent inhibitory activity have hydrophobic amino acids as
282 leucine (Leu), at the N-terminal position in the sequences ([Shi et al., 2022](#); [Nwachukwu et al.,
283 2019](#)). The microbial count increased proportionally with the storage time and reached the
284 highest values at the end of chill period. Among the experimental groups, the negative control
285 group showed the most rapid increase in the number of total microorganisms (from $4.29 \pm$
286 0.04 to 8.14 ± 0.31 log CFU/g), followed by samples treated with Arg-Asp-Arg-Phe-Leu
287 peptide and BHT ([Figure 4a](#)). Arg-Asp-Arg-Phe-Leu peptide efficiency on bacterial growth
288 inhibition was tested using 0.1%, and 0.5 % concentrations. In general, the microbial growth
289 decreased significantly with the addition of Arg-Asp-Arg-Phe-Leu peptide and with the
290 highest concentrations of this compound during the storage period ([Figure 4a](#)). Nevertheless,
291 the group with a 0.5% (w/w) of Arg-Asp-Arg-Phe-Leu peptide showed the slowest increase in
292 the number of total viable colonies until the eleventh day (7.12 ± 0.12 log CFU/g). The
293 coliform count on a VRBL medium shows that no significant difference ($p > 0.05$) has been
294 observed between the four treated groups with additives during the first three days ([Figure
295 4b](#)). But beyond this day, the group with Arg-Asp-Arg-Phe-Leu peptide at 0.5% (w/w)
296 showed efficiency more important and comparable to that of BHT 0.5% (w/w) with the
297 coliform counts of 4.73 ± 0.04 log CFU/g, at day 11. Therefore, this peptide can reduce by

298 32% the number of total coliforms in meat compared to the control. For the yeasts and molds,
299 the most effective additives were the Arg-Asp-Arg-Phe-Leu at 0.5% (w/w) and BHT 0.5%
300 (w/w), with the yeasts and molds counts of 5.64 ± 0.25 and 5.63 ± 0.06 log CFU/g,
301 respectively, at day 11 (Figure 4c). These effects were similar to BHT 0.5% (w/w) (no
302 significant difference). On the other hand, the groups treated with BHT 0.1% (w/w) and Arg-
303 Asp-Arg-Phe-Leu at 0.1% (w/w) are less effective and they were presented no significant
304 difference with the yeasts and molds counts 6.28 ± 0.25 and 6.19 ± 0.25 log CFU/g
305 respectively, at day 11, compared to group without additive with 7.11 ± 0.62 log CFU/g. At
306 last, for the growth of lactic acid bacteria (Figure 4d), during the first three days no bacterial
307 proliferation has been observed for the four treated groups with additives (from 6.01 ± 0.42 to
308 6.35 ± 0.12 log CFU/g), except for the untreated group, the number of lactic acid bacteria was
309 increased from 2.23 ± 0.81 log CFU/g at day 0 to 3.53 ± 0.12 log CFU/g at day 3. After 6
310 days, three treated groups (with peptide 0.5%, BHT 0.1% and BHT 0.5%) showed a
311 significant difference with the negative control group (Figure 4d). Once again, the most
312 effective group were Arg-Asp-Arg-Phe-Leu at 0.5% (w/w) and BHT 0.5% (w/w). At this
313 time, the microbial counts were of 4.67 ± 0.83 log CFU/g for the untreated group and $3.24 \pm$
314 0.18 log CFU/g for the group treated with the Arg-Asp-Arg-Phe-Leu peptide at 0.5% (w/w).
315 Many studies have reported the impact of antimicrobial peptides on meat and its products
316 (Zagorec & Champomier-Verges, 2017). Antimicrobial peptide can potentially be used to
317 extend the quality of highly perishable foods during storage (Gogliettino et al., 2020).
318 Antimicrobial peptides have unique structural characteristics. The length of amino acids and
319 the composition of antimicrobial peptides that have already been studied are extremely
320 diverse. Their tendency to adapt to various structural changes when they come into contact
321 with membranes is exclusive (Torres et al., 2019). The cationic peptides exert antibacterial
322 activity by interacting with negatively charged bacterial membrane to increase membrane

323 permeability and lead to cell membrane lysis and cell content release. Upon approaching the
324 cytoplasmic membrane through electrostatic interaction with the microbial membrane,
325 antimicrobial peptides bind to the microbial membrane and interact with the anionic
326 components of the plasma membrane (Rajagopal & Walker, 2017; Baek et al., 2016).

327 **Conclusion**

328

- 329 1. A small antibacterial peptide from RuBisCO was isolated and purified.
- 330 2. Improvement of oxidative stability of minced beef meat during refrigeration.
- 331 3. The peptide reduced the lipid oxidation about 60% to delay meat rancidity.
- 332 4. Inhibition of the microbial growths under refrigeration during 14 days.
- 333 5. The peptide can be effectively utilized as food ingredients in meat products.

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340

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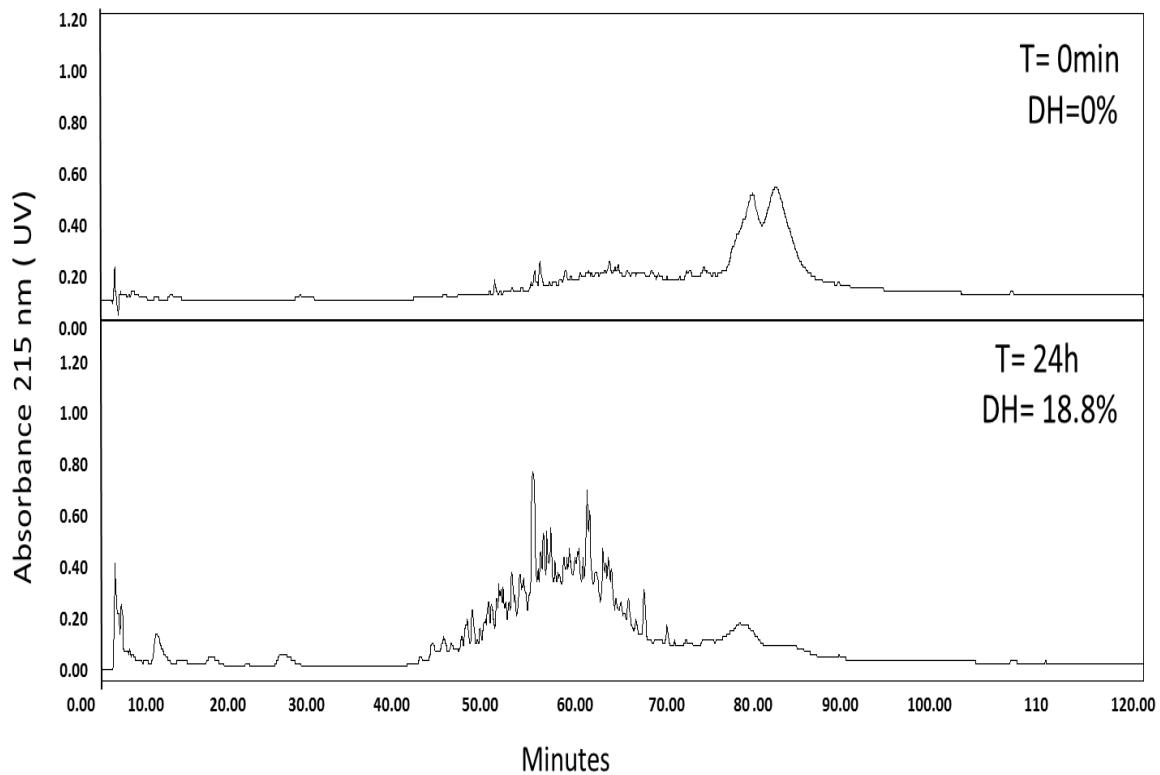
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508 **Figure 1.** Chromatographic profiles of RuBisCO without hydrolysis (DH=0%) and RuBisCO

509 hydrolysis at 215 nm by RP-HPLC with a C4 column at 18.8% hydrolysis degree for 24 h.

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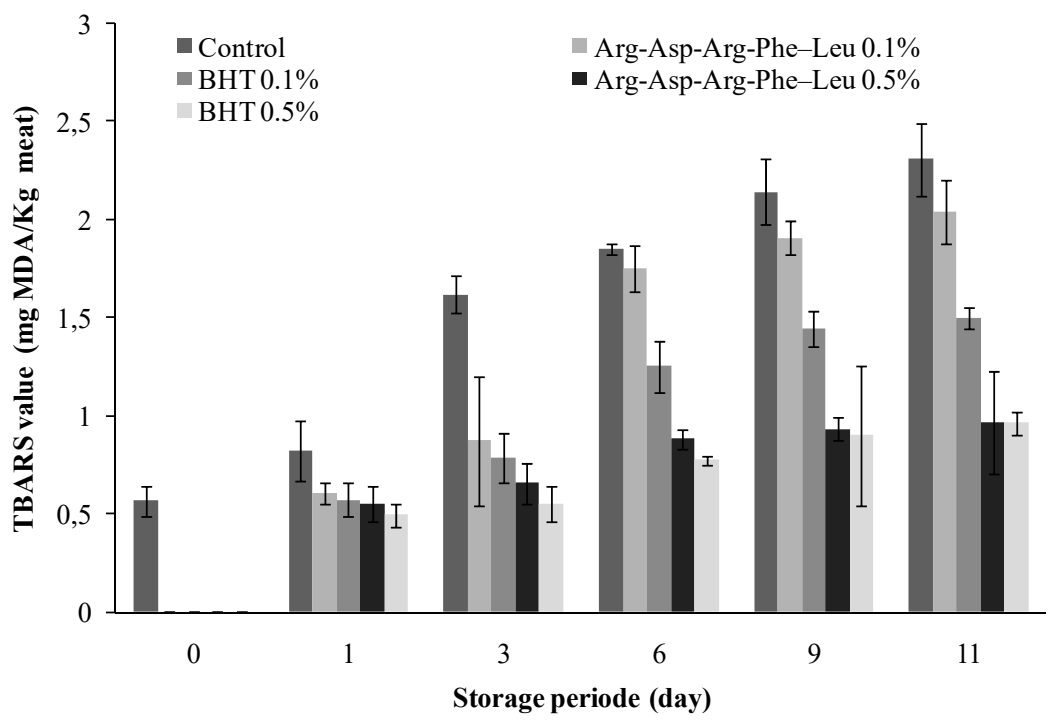
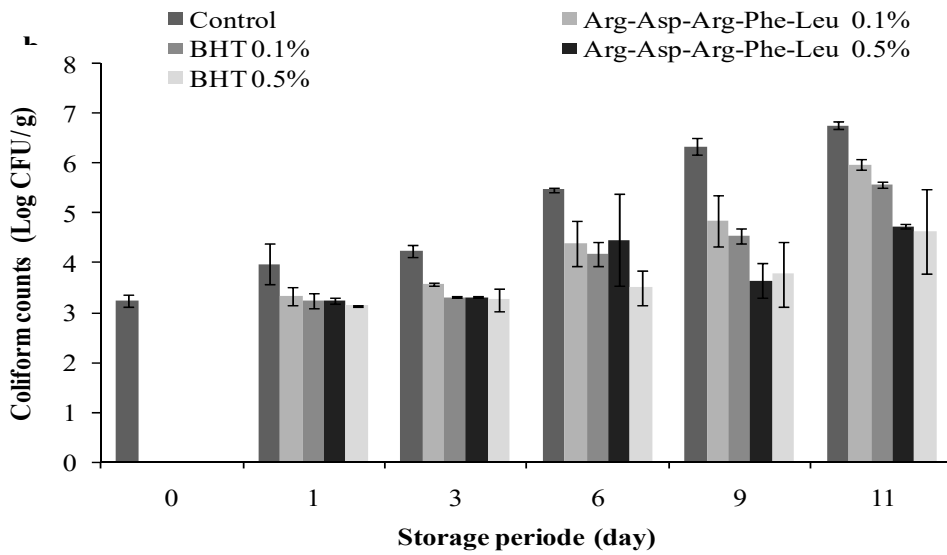
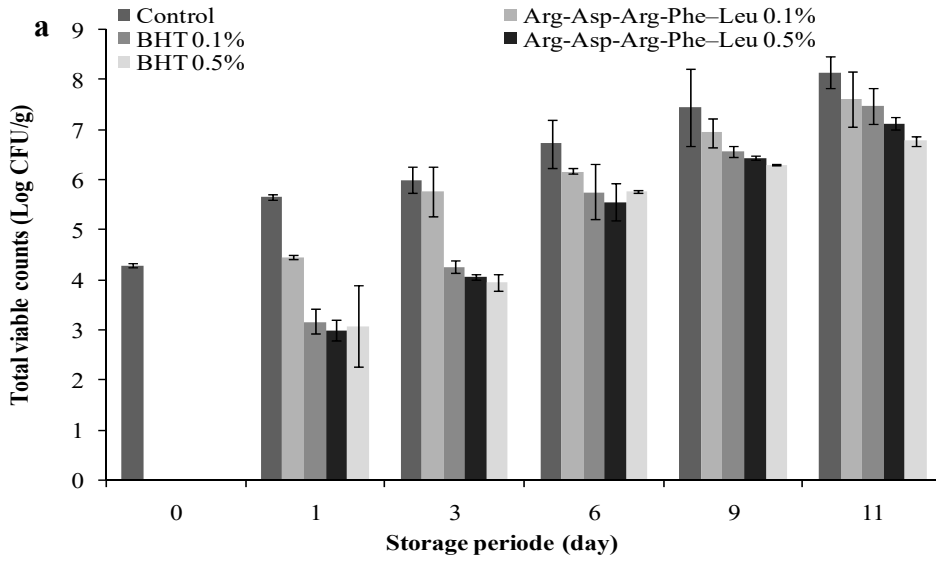


Figure 3. Lipid oxidation evaluation of the meat treated with BHT and Arg-Asp-Arg-Phe-Leu peptide at concentrations of 0.1 and 0.5% (w/w) during storage under refrigeration.

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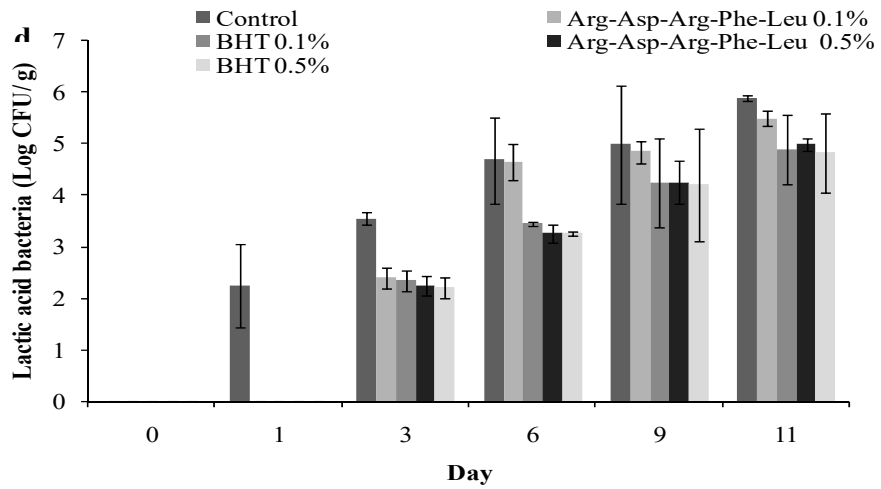
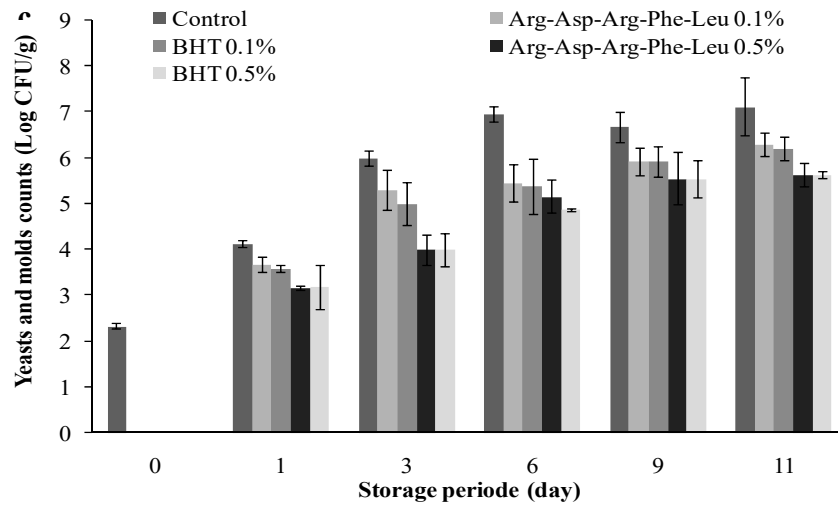
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614 **Figure 4.** Microbial changes in meat treated with BHT and Arg-Asp-Arg-Phe-Leu peptide at

615 concentrations of 0.1 and 0.5% (w/w) during storage under refrigeration. **(a)** Total viable

616 counts, **(b)** Coliform counts, **(c)** Yeasts and molds counts **(d)** Lactic acid bacteria counts.