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Evaluation of Gamma Irradiation and Moringa Leaf Powder on Quality Characteristics of Meat Balls under Different Packaging Materials

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36 **Abstract**

37 This study was carried out to investigate the effects of irradiation doses (0, 1.5 and 3kGy) on the
38 physicochemical and microbial qualities of meat balls with or without moringa leaf powder
39 (MLP) and 14 days under refrigerated storage. The results indicated that irradiation and storage
40 caused significant changes on physicochemical attributes of meatballs and the decreasing trend
41 in stability was observed in meatballs stored under aerobic packaging. Highest pH and TVBN
42 were observed in samples treated with 3kGy at day 14 in aerobic packaging without MLP. The
43 addition of MLP and higher doses of irradiation reduced the total aerobic bacteria and coliforms
44 counts. Different treatments did not affect the sensory quality of chicken samples. Hence, it was
45 concluded that irradiation dose at 1.5kGy with the addition of MLP and vacuum packaging may
46 enhanced the safety, quality as well as stability of chicken meat during storage intervals.

47 **Keywords.** *Aerobic and vacuum packaging, gamma irradiation, moringa leaf powder, quality*
48 *attributes, sensory evaluation*

49 **Practical application**

50 The health benefits of meat and its products make them popular among the consumers. Hence,
51 this study indicates the significance of irradiation (at low dose) in preserving the meat for long
52 time without causing immense deteriorative changes in its quality. Moreover, the addition of
53 moringa leaf powder (a natural antioxidant) and type of packaging (vacuum packaging) plays a
54 vital role in maintaining the shelf life and stabilizing the safety of meat without causing major
55 changes in its physicochemical, functional and sensory characteristics.

56 **Introduction**

57 Meat consumption is considered as highly esteem diet in most places around the world due to its
58 high nutritional value. Meat is regarded as a rich source of zinc, essential amino acids, heme iron
59 and bioavailable B vitamins (Pereira & Vicente, 2013). It is the first-choice source of animal
60 protein for many people all over the world (Ekmekcioglu et al., 2018).

61 The need for ensuring meat products of high quality, consumer attractiveness and cost
62 effectiveness has made the industrialists, meat processors, distributors and retailers to confirm
63 the availability of these required features (Valdramidis & Koutsoumanis, 2016). It is not easy for
64 meat-based products to make stable its shelf life in dynamic market due to its high perishability
65 character without employing some appropriate preservation techniques. To enhance the shelf life
66 of meat and its products various traditional preservation approaches have been utilized such as
67 dehydration; heat processing, low temperature preservation, smoking and curing, but these
68 conventional preservation methods have certain restrictions such as average shelf life, expansive
69 and less stability. Moreover, numerous environmental and safety issues are also linked with the
70 application of these methods that may develop serious environmental and health issues.
71 Therefore, for safety enhancement of meat innovative techniques must be used in spite of these
72 traditional methods that do not cause adverse effects on health (Troy et al., 2016). Thus, a wide
73 range of novel processing, packaging and preservation methods has been emerging to increase
74 the shelf stability and quality of meat products. These innovative techniques preserve the meat
75 without disturbing the quality and sensory characteristics of muscle food (Feng et al., 2018).

76 Researchers have investigated a novel method that is the use of radiation for the decontamination
77 of meat from different food and meat borne pathogens. It is quite efficient method as it has less
78 residual effect on meat products in comparison to the use of different chemicals for
79 decontamination (FAO, 2016). Radiation was first time applied in food materials at the institute
80 of technology in Massachusetts. It was documented that irradiation can enhance the shelf life and
81 eradicate the microorganisms of food product (Fadhel et al., 2016).

82 Irradiation preserves food products with minimal harm to the nutritional, functional and sensory
83 attributes and has recently become one of the effective techniques for the preservation of food
84 products. Irradiation is considered as preservation technology and gaining importance in muscle
85 food worldwide (Bhat et al., 2016). All types of pathogenic microorganism in meat can be
86 decontaminated by irradiation application but it also has negative effect as it reduces the quality
87 due to change in oxidation reduction environment (Ahn et al., 2013). The gamma irradiation can
88 also be applied on different fruits processing for enhancing the safety and shelf life (Panou et al.,
89 2020).

90 Another major issue in maintaining the quality of meat is lipid oxidation (Feng & Ahn, 2016).
91 Anti-oxidant addition can resolve the meat oxidation issue. The use of natural preservative is a
92 promising technology to enhance the shelf-life of meat and meat products. Natural preservatives
93 includes plants, herbs, fruits and vegetables powders or extracts have antimicrobial and
94 antioxidant properties. *Moringa oleifera* is one of the naturally founded antioxidant which is also
95 called as drumstick or horse radish tree is native to Southeast Asia, Arabia, Pakistan,
96 Bangladesh, India, Africa and South America (Falowo et al., 2017). *Moringa oleifera* L. leaf
97 extracts have been reported in literature as edible and promising sources of natural antioxidants
98 (Bartolome et al., 2013). The leaves have unique concern in the preservation of food as they are
99 highly enriched with proteins, essential amino acids such as cystine, tryptophan, methionine and
100 lysine, minerals (particularly iron) and provitamin A, vitamins B and C (Oyeyinka & Oyeyinka,
101 2018) and also because of presence of bioactive substances such as, flavonoids, ascorbic acid,
102 carotenoids and phenolics. It plays a significant role as a natural anti-oxidant and shelf life
103 enhancer (Al-Juhaimi et al., 2016). The combination of gamma irradiation and moringa leaf
104 powder has synergistic effect on the safety of meat products (Nisar et al., 2019).

105 Application of a novel and advance techniques are useless without applying proper packaging, it
106 has an active role in, safety enhancement, preserving quality and sensory characteristics of the

107 product. Plastics are usually used for meat as a packaging material, more specifically, laminates,
108 in which good humidity barrier performance and polymer layers with oxygen-barrier properties
109 like polyamide or polyethylene tetrathalate. Packaging materials with good oxygen barrier
110 attributes can be employed for low oxygen vacuum packaging. An air-permeable packaging is
111 generally characterized as aerobic packaging, whereas vacuum packaging involves placing a
112 product in a pack possessing low-oxygen permeability.

113 Considering the facts discussed above, the objective of this study was to determine the anti-
114 microbial effect of irradiation with addition of moringa leaf powder as an antioxidant for safety
115 enhancement of meat and meat products, to improve the shelf life of meat with gamma
116 irradiation and to examine the stability of gamma treated meat under different storage conditions
117 (Aerobic and Vacuum) and time intervals.

118 **Material and methods**

119 **Procurement of raw material**

120 This research study was conducted at Nuclear Institute for Food and Agriculture, Peshawar
121 (NIFA) and Institute of Home and Food Sciences, Government College University, Faisalabad,
122 Pakistan. The moringa leaf powder (MLP) and chicken meat were collected from the local
123 market. Chicken was grinded and half of the meat was mixed with 2% MLP. Chicken samples
124 were then placed in polythene bags and were transferred to NIFA for gamma irradiation. All
125 reagents and chemicals used in this study were procured from Sigma Aldrich (Tokyo, Japan).

126 **Irradiation dose**

127 The chicken meat samples were placed in 12 packets (6 each for aerobic and vacuum packaging).
128 The samples were than subjected to different irradiation doses (0, 1.5 and 3 kGy). After
129 treatment the samples were placed in refrigerator storage at 4 °C up to 14 days. The
130 physicochemical and functional analysis of treated samples was performed at 0, 7 and 14 days of
131 interval.

132 **Physicochemical analysis**

133 **pH**

134 The pH was determined in a homogenate of the sample with distilled water (1→10) using a
135 digital pH meter (Model 520A, Orion Research inc., Boston, USA) that was calibrated with

136 standard pH buffers of 4.01, 7.00 and 10.01 at 25 °C. Three replicate measures were taken, and
137 the mean value derived.

138 **Total volatile basic nitrogen value**

139 The TVBN value was determined as described by Qiao et al., 2017. Meat sample (5 g) was
140 homogenized (Heiddph: 595-08000-00-2, Germany) with 45 mL of DW for 30 s; 5 mL of the
141 homogenate was mixed with an equal volume of 10% trichloroacetic acid (TCA; w/v in distilled
142 water). Thus, obtained TCA extract was used to determine the value of TVBN. One milliliter of
143 TVBN reagent was added into the inner well of the Conway unit, and 1 mL TCA extract was
144 added into the outer well, followed by the addition of 1 mL saturated potassium carbonate
145 (K₂CO₃). The Conway unit was immediately sealed with an airtight ground glass plate, rotated
146 clockwise and counter-clockwise, and incubated at room temperature for 3 h. The TVBN reagent
147 in the inner well was back-titrated with 0.02 N sulfuric acid (H₂SO₄) until the blue coloring
148 changed to pink. The TVBN value was calculated using the following equation

$$149 \quad \text{“N” mg/mL of extract} = 14 \times a \times b$$

$$150 \quad \text{TVBN value (mg/100mL)} = 100 \times N$$

151 $14 =$ molecular weight of nitrogen, $a =$ normality of H₂SO₄,

152

153 $b =$ volume of H₂SO₄, (titration value)

154

155 **Hunter color**

156 Hunter colorimeter was used to determine the surface color values of the chicken samples, white
157 calibration plate ($L = 89.2$, $a = 0.921$, and $b = 0.783$) is used as standardized measurements. An
158 average of 3 random readings of color values (lightness (L), redness (a), and yellowness (b))
159 were obtained of each sample surface.

160 **Heme pigment**

161 The extraction of heme pigments from chicken meat samples were extracted following
162 the protocol of Warriss (1979). Chicken sample (4 g) was homogenized (Heiddph: 595-08000-
163 00-2, Germany) for 10 s at 13000 rpm with cold phosphate buffer (20 mL, 40 mM, pH 6.8). The
164 resultant homogenate was stored for 1 hour at 4°C. The extract was then subjected to

165 centrifugation for 30 min at 5000 rpm, the mixture was then filtered through Whatman No.1
166 filter paper. Ultraviolet–visible (UV/VIS) spectrophotometer (U-1800, Japan) was used to
167 measure the absorbance of sample at different wavelength (525, 545, 565, and 572 nm). The
168 method of Krzywicki et al, (1982) was followed to determine the relative concentration of heme
169 pigments, such as myoglobin, oxymyoglobin and metmyoglobin. The relative concentrations (%)
170 of Mb, MbO₂, and MMb were calculated using the following equations:

171 $[Mb] = (0.369R_1 + 1.140R_2 - 0.941R_3 + 0.015) \times 100$

172 $[OxyMb] = (0.882R_1 - 1.267R_2 + 0.809R_3 - 0.361) \times 100$

173 $[MetMb] = (-2.541R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100$

174 Where R₁, R₂, R₃ are absorbance ratios A⁵⁷²/A⁵²⁵, A⁵⁶⁵/A⁵²⁵, A⁵⁴⁵/A⁵²⁵, respectively.

175 **Microbial analysis**

176 The determination of microbes (total aerobic bacteria and coliforms) in chicken samples were
177 done according to the methodology described by Helrich, (1990). Total aerobic bacteria were
178 spread on Plate Count Agar (Merck, Darmstadt, Germany) in petri dishes, which were then
179 incubated at different time and temperature (35 °C for 24–48 hour and 25 °C for 3–5 days).
180 Enumeration of coliform was performed with solid medium method by using double layer
181 VRBA (Violet Red Bile Glucose Agar, Oxoid) and VRBA (Oxoid) with 4-methylumbelliferyl-b-
182 D-glucuronide (MUG). The plates were then incubated for 18-24 hour at 35 °C. The total
183 coliforms were counted by the formation of dark red, lactase positive and 0.5–2 mm diameter
184 colonies surrounded by a reddish zone.

185 **Storage Study**

186 The irradiated meat samples were kept in storage for 7 and 14 days at 4°C to study the changes
187 in its physicochemical and functional analysis throughout the storage interval (Walkling-Ribeiro
188 et al., 2009).

189 **Sensory evaluation**

190 The sensory evaluation of gamma and moringa leaf powder treated chicken meatballs sample
191 was conducted at storage interval of 0, 7, and 14 by trained panelists using a 9-point hedonic
192 scale where “0” being the lowest and “9” being the highest score (Meilgaard et al., 2007). The

193 chicken meatballs were analyzed for different quality attributes (appearance, taste, texture,
194 flavor, and overall acceptability).

195 **Statistical analysis**

196 All experiments were conducted in triplicate (n=3) and an ANOVA test using SPSS version 11.5
197 was used to compare the mean values of each treatment. Significant differences between the
198 means of parameters were determined by using the Tukey test ($p < 0.05$) (Steel & Torrie, 2012).

199 **Results and Discussion**

200 **Physicochemical parameters**

201 The effect of different irradiation doses on the physicochemical parameters of meat samples
202 including pH, hunter color and heme pigment of meat samples incorporated with or without
203 moringa leaf powder were examined. Chemical composition and nutritional quality of meat and
204 meat products are affected by several factors including processing and storage and are also
205 influenced by species. Results for changes in physicochemical parameters (pH, hunter color and
206 heme pigment) of untreated and processed meat samples kept at refrigerator in aerobic and
207 vacuum packaging for a maximum period of 14 days are presented and described.

208 **pH**

209 pH is one of the important factor as it is closely related with the stability of the bioactive
210 compounds in meat and meat products (Sánchez-Moreno et al., 2006). The pH value of meat
211 samples with or without moringa leaf powder showed significant decrement ($p \leq 0.05$) with the
212 increment of irradiation doses and storage days. The mean values of pH irradiated with different
213 doses of gamma radiations, stored in aerobic and vacuum packaging are given in (table 1). The
214 pH value in aerobic and vacuum packaging on 0 day after different treatments ranged from
215 5.61 ± 0.01 to 5.76 ± 0.01 respectively, whereas, the pH values in aerobic packaging during storage
216 ranged from 5.70 ± 0.01 to 6.02 ± 0.01 and in vacuum packaging ranged from 5.71 ± 0.02 to
217 6.06 ± 0.01 respectively. The Higher pH (more acidic) was found with 1.5 kGy with the addition
218 of 2% MLF on storage day 0 (5.61 ± 0.01) in both aerobic and vacuum packaging; lower pH
219 (6.06 ± 0.01) was found with 1.5 kGy without MLF on storage day 14. This result is similar with
220 the findings of Chouliara et al. (2008), who concluded that pH of irradiated meat samples kept in
221 aerobic and modified atmospheric packaging tends to reduce slightly throughout the storage

222 period of 25 days. Pelicia et al. (2014) also determined that pH tends to decrease in meat with the
223 high dose of gamma irradiation kept in both aerobic and vacuum packaging.

224 The increment in pH level by irradiation processing may be because of the breakdown of lactic
225 acid production and due to hydrolysis of glycogen which accumulates in meat (Kumar et al.,
226 2017). The stability of pH level in irradiated samples with moringa leaf powder is because the
227 radiations and antioxidants inhibit the growth of lactic acid bacteria which causes the rise in pH
228 in meat samples (Miyagusku et al., 2008).

229 **Total volatile basic nitrogen (TVBN) value**

230 The TVBN value of meat samples with or without moringa leaf powder showed significant
231 increment ($p \leq 0.05$) with rise of irradiation doses and storage period. The mean TVBN value of
232 meat samples on storage day 0 varied from 2.25 ± 0.01 to 4.15 ± 0.02 mg/100mL among different
233 applied treatments, whereas the TVBN value during storage in aerobic packaging ranged from
234 2.43 ± 0.02 to 4.51 ± 0.01 mg/100mL, while in vacuum packaging ranges from 2.37 ± 0.01 to
235 4.42 ± 0.01 mg/100mL respectively (Table 1). Higher TVBN value (4.51 ± 0.01 mg/100mL) were
236 found in meat samples (without MLF) treated with 3 kGy irradiation dose and stored in aerobic
237 packaging for 14 days. The similar effect of high dose irradiation causing the increment in
238 TVBN value of chicken kababs was examined by Al-Bachir et al. (2010). In another study by
239 Kwon et al. (2011), it was determined that the TVBN level increases in raw and cooked chicken
240 meat when exposed to gamma radiation dose of 0-5kGy.

241 However, it was also demonstrated that the increment in TVBN values were suppressed by
242 irradiation during storage as compared to control and it was also observed that addition of
243 moringa leaf powder also suppressed the production of volatiles in meat samples during storage.
244 The amount of TVBN components also vary in type of packaging, meat samples stored in
245 vacuum packaging showed less value of TVBN throughout the storage as compared to meat
246 samples in aerobic packaging. This result is similar with the findings of Yun et al. (2014), who
247 reported that volatile compounds were enhanced by irradiation process in ready to eat chicken
248 breast, but they tend to increase slowly as compared to un-irradiated meat samples during the
249 storage time period. Furthermore, Li et al. (2017) reported that the volatiles substances found to
250 be increased in high dose irradiated pork samples as compared to low dose irradiated samples
251 during storage. The role of antioxidants in suppressing the volatiles production induced by

252 irradiation process during storage was demonstrated by Hwang et al. (2015). According to the
253 author the mugwort extracts and ascorbic acids when added into meat samples inhibits the
254 increment of volatile substances during storage.

255 The increment in TVBN level by irradiation processing may be because of the breakdown of
256 nitrogenous compounds present in meat samples (Ahn et al, 2004). The stability of TVBN level
257 in irradiated samples and samples with moringa leaf powder during storage might be due to the
258 fact that irradiation and antioxidants both helps in controlling the production of spoilage bacteria
259 which may causes high amount of TBVN value in meat samples (An et al., 2017). The results are
260 in agreement with the findings of Arshad et al. (2019); Arshad et al. (2020).

261 **Hunter color**

262 Color is one of the most important sensory attribute of any product. The color value of meat
263 samples with or without moringa leaf powder showed significant changes ($p \leq 0.05$) in all
264 treatments and during storage. The lightness L^* value of meat samples increased till 1.5 kGy
265 dose, whereas it showed decrement with the rise of irradiation dose. On the other hand, the a^*
266 and b^* values increased significantly with the increment of irradiation dose (Table 2). According
267 to the obtained data the higher L^* , a^* and b^* value was observed at 1.5 kGy with 2% MLF
268 throughout storage in vacuum packaging that ranged from 70.00 ± 1.00 to 78.66 ± 0.57 , 4.04 ± 0.05
269 to 7.48 ± 0.01 and 15.55 ± 0.09 to 13.00 ± 0.100 respectively, while minimum L^* , a^* and b^* values
270 were observed in control at 0 day.

271 According to our result the storage days do not show any variation in L^* , a^* and b^* color values,
272 but different irradiation treatments significantly affect the color values of meat samples.
273 Moreover, we concluded that meat samples stored in vacuum packaging show minimally
274 changes in color values as compared to meat samples stored in aerobic packaging. The
275 incorporation of natural antioxidant (moringa leaf powder) resulted in the variation in L^* , a^* and
276 b^* color values of irradiated and non-irradiated samples throughout the storage period in both
277 types of packaging. The similar effect of storage was determined in the study of Li et al. (2017),
278 who examined that L^* , a^* and b^* values of color parameters remain unchanged during storage
279 for 14 days. However, the rise in irradiation doses enhanced the color values. Present results are
280 in agreement with Zhao et al. (2017) on effect of irradiation on quality of vacuum packed
281 chopped beef samples, also agrees with our finding. The authors concluded that color of meat

282 samples changes with the increment of irradiation dose level irrespective of storage time and
283 packaging. The change in color as observed in irradiated meat samples incorporated with
284 moringa leaf powder is in favor of previous study by Liz and Getty, (2013), who demonstrated
285 that antioxidants when mixed with meat samples transfers their natural color into the meat thus
286 causing some variation in meat color.

287 Irradiation degrades the water molecules in meat that generates oxidizing and reducing
288 compounds such as hydroxyl radicals, hydrogen atoms, etc. and lead to the changes in meat color
289 (Thakur and Singh, 1994). The oxidizing compound can convert myoglobin to metmyoglobin
290 and can also lead to lipid oxidation by removing ferric ion from heme, whereas the oxidation-
291 reduction potential is lowered by reducing compounds in meat (Min et al., 2010).

292 **Heme Pigment**

293 The assessment of heme pigment especially myoglobin is used to observe the quality parameters
294 of meat like color, microbial growth, shelf life and rancidity (Chaijan et al., 2007). The heme
295 pigment value of irradiated meat samples with or without moringa leaf powder in aerobic and
296 vacuum packaging showed significant changes ($p \leq 0.05$) among all treatments and storage days.
297 The mean values of myoglobin, oxymyoglobin and metmyoglobin are shown in Table 3. The
298 amount of myoglobin varied from 28.98 ± 0.86 to 34.08 ± 0.06 %, oxymyoglobin ranged from
299 8.98 ± 0.15 to 11.77 ± 0.06 % and metmyoglobin ranged from 42.12 ± 0.31 to 45.05 ± 0.04 %
300 respectively, on storage day 0. The higher value of myoglobin was observed in 3 kGy samples
301 with MLF on day 0 (35.65 ± 0.03 %), higher value of oxymyoglobin was 20.61 ± 0.12 %, observed
302 at 3 kGy with MLF on day 14, while the highest value of metmyoglobin (65.90 ± 0.07 %) was
303 observed on day 14 at 3 kGy without MLF in vacuum packaging.

304 According to present results, the myoglobin pigment decreased, whereas the oxymyoglobin and
305 metmyoglobin pigments increased with the rise in irradiation dose level and storage days in both
306 aerobic and vacuum packaging. This result correlates with the findings of An et al. (2017) who
307 reported that heme pigments of irradiated smoked duck meat increased during storage except
308 myoglobin which tends to decrease with the increase in storage days. Reddy et al. (2015), also
309 reported that myoglobin pigment decreased during storage, whereas, oxymyoglobin and
310 metmyoglobin increased as the storage day increases in irradiated samples of mutton kheema. In
311 our study, we also found that the heme pigments level in the meat samples incorporated with

312 moringa leaf powder though exhibited the same trend as meat samples without the antioxidant,
313 but the level of heme pigments is greater in those samples as compared to ones without
314 antioxidants. Cunha et al. (2018), also examined that addition of antioxidants in meat samples
315 slightly inhibits the degradation of myoglobin during storage, thus provided better contents of
316 heme pigments in meat samples.

317 The irradiation process through many different mechanisms causes the catabolism of myoglobin
318 pigment into oxymyoglobin and metmyoglobin pigments thus resulting in decrement of
319 myoglobin level and increment in other two pigment compounds. Myoglobin possess prooxidant
320 capacities by many ways as it could act as a free radical by decomposing hydroperoxide. free
321 radicals helps in lipid oxidation and produces hydroxyl radicals from water that converts
322 myoglobin into metmyoglobin due to ionizing radiation (Aliakbarlu et al., 2015).

323 **Microbial analysis**

324 The effect of different irradiation doses on the microbes of irradiated and non-irradiated meat
325 samples with or without moringa leaf powder were examined before and after storage under
326 vacuum and aerobic packaging. Total aerobic bacteria and coliform values of irradiated meat
327 samples with or without moringa leaf powder showed significant changes ($p \leq 0.05$) in all
328 treatment and storage time interval (Table 4). The results of total bacterial count in meat samples
329 after being exposed to irradiation processing, demonstrate that irradiation have the great potential
330 in minimizing and eliminating the microbes from the meat and meat products. The highest value
331 of TAB was found in control (9.81 ± 0.04 log CFU/g) on day 14 in aerobic packaging, whereas
332 the lowest TAB value was observed in 3 kGy treated sample w/ MLF (4.17 ± 0.02 log CFU.g) on
333 day 0. Coliforms were only discovered in control samples with or without MLF, whereas, they
334 were absent in all irradiated meat samples. The similar effect of gamma irradiation eradicating
335 the microbes from fried chicken dices was observed by Chen et al. (2016). Kanatt et al. (2010),
336 also examined the effect of irradiation in controlling the growth of microorganism in different
337 meat products. The gamma radiation eradicating the no of coliforms in smoked duck meat
338 samples was also reported by An et al. (2017). Moreover, according to our result, the
339 incorporation of Moringa leaf powder in meat samples act as a resistant against microbial
340 growth, therefore, the no of bacterial count become more less in antioxidant meat samples after
341 irradiation as compare to non-antioxidant irradiated meat samples. This examinations corelates

342 with the observation of Cunha et al. (2018), who demonstrate that antioxidants play a vital role in
343 preserving the different meat products.

344 Though irradiation reduced the amount of TAB but during storage their number tends to increase
345 slightly. On the other hand, irradiation eradicates the coliforms from the meat samples which do
346 not appear in storage days as well. These findings agree with the observations of Fallah et al.
347 (2010) who examined that gamma radiation inactivates pathogenic bacteria from chicken meat
348 samples, moreover very less amount of bacteria were found in irradiated samples when kept in
349 storage for 25 days. In another study, Mantilla et al. (2011) reported that the shelf life of chicken
350 breast fillets improves with the irradiation treatment and when kept in storage under vacuum
351 packaging as compared to unirradiated samples in aerobic packaging. Henriques et al. (2013) and
352 Arzina et al. (2012) also reported that irradiation dosage of 1.5-2.5kGy is enough to eradicate the
353 pathogenic bacteria in refrigerated stored meat samples. The growth of microorganism is
354 controlled by irradiation process as it kills them with its high doses of radiation (Montiel et al.,
355 2013).

356 **Sensory evaluation**

357 Sensory assessment of a product correlates with consumer approach, believes and awareness.
358 Product development requires an efficient hedonic evaluation for documentation and
359 interpretation of sensory results perceived by the trained panelists. The mean sensory score of
360 appearance, flavor, texture, taste and overall acceptability of formulated chicken meatballs in
361 aerobic and vacuum packaging are shown in Tables 5 and 6, respectively. According to present
362 results, different treatments did not affect the sensory result, but it showed variation with storage
363 days and type of packaging. the highest value of appearance (8.25 ± 0.70), flavor (7.5 ± 1.35),
364 texture (7.1 ± 0.77), taste (7.3 ± 1.03), overall acceptability (7.5 ± 0.81) was awarded to control (0
365 kGy) on day 0 in both aerobic and vacuum packaged meatballs samples. Whereas the lowest
366 value of appearance (6.1 ± 1.43 in aerobic packaging), flavor (6.1 ± 0.90 in vacuum packaging),
367 texture (5.7 ± 1.20 in aerobic packaging), taste (6.0 ± 0.90 in vacuum packaging) and overall
368 acceptability (6.2 ± 0.90 in vacuum packaging) is also at 0 kGy but on storage day 14.

369 The sensory attributes showed decrement in their value throughout the storage time. These
370 findings are in accordance with Fallah et al. (2010) who concluded that appearance score of both
371 irradiated and non-irradiated samples were good at day 1, but after storage day 15 the non-

372 irradiated barbecued chicken exhibit less sensory score as compared to irradiated one. Al-Bachir
373 et al. (2010) also reported that the flavor of prepared chicken kababs was well accepted by the
374 panelist without irradiation treatment on day 1, all irradiated chicken kababs were also had good
375 sensory score on day 1 and throughout the storage period, but non-irradiated samples start losing
376 their acceptability of flavor as the storage days increases. Regarding texture, Benerjee et al.
377 (2016), reported that the hardness, chewiness and gumminess in mutton patties were lower in
378 control on day 0, as compared to irradiated mutton patties. Furthermore, Gertzou et al. (2017)
379 reported that sensory attributes are better with combination of vacuum packaging and ozonation.
380 In another study by Kanatt et al. (2005), it was observed that un-irradiated meat products
381 exhibited good taste on day 1 but when kept in storage their taste become less accepted due to
382 bacteria which altered the taste as compared to irradiated meat products which inhibited the
383 growth of pathogenic bacteria. Irradiation facilitated the lipid oxidation which cause changes in
384 sensory attributes and made the product less acceptable, but overall acceptability was in the
385 range of acceptance.

386 **Conclusion**

387 This study indicated that irradiation preserves food products with minimal harm to the
388 nutritional, functional and sensory attributes at lower doses. Phytochemicals originating from
389 moringa leaf powder play an important role in stabilizing and protecting the fat, amino acid and
390 other physicochemical and functional components in meat and meat products. The type of
391 packaging helped in extending the shelf life of meat and meat products. The irradiation doses
392 minimize the total aerobic bacteria and eliminates the coliform from the meat samples during
393 storage. Different treatments did not affect the sensory quality of chicken meatballs, but the
394 variation was observed with the storage period.

395 **Authors' contributions**

396 The contribution of the each author for this manuscript was as follows, MSA and MY designed
397 the experimental plan and MFS conducted the analysis and drafted the manuscript. MKK and
398 MA helped in execution of the project. MARS helped in revising the manuscript and SS helped
399 in data analysis. It is also confirmed that all the authors read and approved the final manuscript.

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Table 1. pH and TVBN in aerobic and vacuum packaged meat samples during storage

		pH		TVBN (mg/100mL)	
		Aerobic	Vacuum	Aerobic	Vacuum
0kGy	0	5.76±0.01 ^f	5.76±0.01 ^g	2.76±0.02 ^a	2.76±0.02 ^a
	7	5.88±0.01 ^{cd}	5.82±0.01 ^e	3.20±0.01 ^b	3.01±0.02 ^b
	14	5.97±0.01 ^b	5.93±0.01 ^{bc}	3.54±0.02 ^c	3.27±0.02 ^b
1.5kGy	0	5.70±0.01 ^g	5.70±0.01 ^{ef}	3.70±0.02 ^c	3.70±0.02 ^c
	7	5.82±0.01 ^e	5.97±0.01 ^b	3.89±0.02 ^d	3.82±0.02 ^d
	14	5.95±0.01 ^b	6.06±0.01 ^a	3.97±0.01 ^e	3.78±0.01 ^c
3kGy	0	5.77±0.01 ^f	5.77±0.01 ^{hi}	4.15±0.02 ^e	4.15±0.02 ^b
	7	5.90±0.01 ^c	5.81±0.04 ^{ef}	4.44±0.01 ^f	4.36±0.01 ^e
	14	6.02±0.01 ^a	5.91±0.01 ^{cd}	4.51±0.01 ^e	4.42±0.01 ^{ef}
0kGy + 2% MLF	0	5.67±0.01 ^{gh}	5.67±0.01 ⁱ	2.25±0.01 ^a	2.25±0.01 ^a
	7	5.74±0.01 ^f	5.76±0.03 ^{fg}	2.43±0.02 ^a	2.37±0.01 ^a
	14	5.82±0.01 ^e	5.86±0.01 ^{de}	2.51±0.02 ^a	2.45±0.01 ^a
1.5kGy + 2% MLF	0	5.61±0.01 ⁱ	5.61±0.01 ^{gh}	3.36±0.01 ^c	3.36±0.01 ^c
	7	5.70±0.01 ^g	5.89±0.01 ^{cd}	3.58±0.02 ^{bc}	3.50±0.02 ^{bd}
	14	5.86±0.01 ^d	5.99±0.01 ^b	3.71±0.01 ^c	3.59±0.02 ^c
	0	5.67±0.01 ^h	5.67±0.01 ^j	3.67±0.01 ^c	3.67±0.01 ^c

3kGy + 2% MLF	7	5.77±0.01 ^f	5.71±0.02 ^{gh}	3.78±0.01 ^d	3.70±0.01 ^d
	14	5.88±0.01 ^{cd}	5.86±0.02 ^{de}	3.89±0.01 ^d	3.77±0.01 ^d

Results are means of triplicates (±SD). Values with different letters in columns are significantly different (Tukey's test, $p \leq 0.05$). MLF; Moringa

Leaf Powder

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Table 2. Hunter color in aerobic and vacuum packaged meat samples during storage

Results are means of triplicates (\pm SD). Values with different letters in columns are significantly different (Tukey's test, $p \leq 0.05$). MLF; Moringa Leaf Powder

Treatments	Storage Days	Aerobic			Vacuum		
		L*	a*	b*	L*	a*	b*
0kGy	0	54.00±1.00 ^{def}	4.04±0.06 ^h	13.00±0.100 ⁱ	54.00±1.00 ^{ef}	4.04±0.05 ^f	13.00±0.100 ^g
	7	53.00±1.00 ^{ef}	4.05±0.01 ^h	12.96±0.01 ⁱ	58.00±1.00 ^f	4.24±0.01 ^f	13.38±0.03 ^g
	14	51.33±1.52 ^f	4.08±0.01 ^h	12.85±0.05 ⁱ	56.66±0.57 ^f	4.29±0.01 ^f	13.77±0.02 ^{fg}
1.5kGy	0	61.00±1.00 ^b	5.57±0.04 ^f	14.23±0.15 ^{de}	61.00±1.00 ^b	5.57±0.04 ^d	14.23±0.15 ^c
	7	62.66±0.57 ^b	5.56±0.01 ^f	14.18±0.03 ^{def}	71.33±0.57 ^b	5.90±0.01 ^d	14.83±0.05 ^c
	14	62.00±1.00 ^b	5.60±0.01 ^f	14.35±0.02 ^d	73.00±1.00 ^b	5.97±0.01 ^d	14.94±0.02 ^{bc}
3kGy	0	53.00±1.00 ^{ef}	5.09±0.07 ^g	14.00±0.10 ^{efg}	53.00±1.00 ^c	5.09±0.07 ^c	14.00±0.10 ^c
	7	52.00±1.00 ^{ef}	5.11±0.02 ^g	13.96±0.01 ^{fgh}	66.66±0.57 ^c	5.57±0.01 ^c	14.63±0.06 ^{cd}
	14	54.33±0.57 ^{de}	5.08±0.02 ^g	13.98±0.07 ^{efgh}	66.00±1.00 ^c	5.62±0.01 ^c	14.73±0.02 ^{cd}
0kGy + 2% MLF	0	57.66±0.57 ^c	6.72±0.07 ^e	13.73±0.05 ^h	57.66±0.57 ^d	6.72±0.07 ^e	13.73±0.05 ^{ef}
	7	56.66±0.57 ^{cd}	6.79±0.02 ^{de}	13.76±0.01 ^{gh}	61.33±0.57 ^d	6.85±0.01 ^e	13.99±0.05 ^{ef}
	14	56.66±0.57 ^{cd}	6.71±0.03 ^e	13.79±0.01 ^{gh}	62.66±0.57 ^{de}	6.90±0.01 ^e	14.29±0.44 ^{de}
1.5kGy + 2% MLF	0	70.00±1.00 ^a	7.15±0.04 ^b	15.13±0.20 ^{bc}	70.00±1.00 ^a	7.15±0.04 ^a	15.13±0.20 ^a
	7	69.33±0.57 ^a	7.53±0.15 ^a	15.26±0.05 ^{ab}	77.66±0.57 ^a	7.43±0.01 ^a	15.44±0.05 ^a
	14	68.00±1.00 ^a	7.12±0.01 ^{bc}	15.40±0.01 ^a	78.66±0.57 ^a	7.48±0.01 ^a	15.55±0.09 ^a
3kGy + 2% MLF	0	63.00±1.00 ^b	6.94±0.08 ^d	14.93±0.15 ^c	63.00±1.00 ^b	6.94±0.08 ^b	14.93±0.15 ^{ab}
	7	63.66±0.57 ^b	6.89±0.01 ^d	14.88±0.03 ^c	73.33±0.57 ^b	7.22±0.01 ^b	15.31±0.05 ^{ab}

14

63.00±1.00^b

6.95±0.01^{cd}

14.96±0.01^c

73.33±1.52^b

7.28±0.01^b

15.26±0.04^{ab}

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Table 3. Heme pigment in aerobic and vacuum packaged meat samples during storage

Results are means of triplicates (\pm SD). Values with different letters in columns are significantly different (Tukey's test, $p \leq 0.05$). MLF; Moringa Leaf Powder

Treatments	Storage Days	Aerobic			Vacuum		
		Mb (%)	OxyMb (%)	MetMb (%)	Mb (%)	OxyMb (%)	MetMb (%)
0kGy	0	33.36 \pm 0.43 ^a	8.98 \pm 0.15 ^l	42.12 \pm 0.31 ^l	34.44 \pm 0.14 ^c	9.12 \pm 0.06 ^l	42.86 \pm 0.07 ^k
	7	26.30 \pm 0.13 ^e	10.86 \pm 0.04 ^{jk}	51.51 \pm 0.49 ^g	26.26 \pm 0.32 ^h	13.75 \pm 0.19 ^g	53.71 \pm 0.22 ^g
	14	18.13 \pm 0.09 ^j	13.78 \pm 0.14 ^g	57.28 \pm 0.25 ^d	19.58 \pm 0.34 ^m	15.59 \pm 0.05 ^e	61.79 \pm 0.13 ^d
1.5kGy	0	30.73 \pm 1.18 ^b	10.96 \pm 0.01 ^{jk}	44.64 \pm 0.14 ⁱ	33.93 \pm 0.06 ^c	11.05 \pm 0.01 ^j	44.89 \pm 0.02 ^j
	7	22.63 \pm 0.20 ^{gh}	12.84 \pm 0.16 ^h	54.56 \pm 0.19 ^e	26.71 \pm 0.19 ^h	14.45 \pm 0.08 ^f	52.80 \pm 0.13 ^h
	14	16.71 \pm 0.21 ^k	15.64 \pm 0.25 ^e	59.45 \pm 0.16 ^b	18.50 \pm 0.04 ⁿ	17.51 \pm 0.20 ^c	63.84 \pm 0.14 ^c
3kGy	0	28.98 \pm 0.86 ^c	10.47 \pm 0.35 ^k	43.90 \pm 0.07 ^j	32.38 \pm 0.20 ^d	10.99 \pm 0.01 ^j	44.63 \pm 0.05 ^j
	7	19.43 \pm 0.06 ⁱ	14.55 \pm 0.19 ^f	53.60 \pm 0.16 ^f	24.57 \pm 0.06 ⁱ	13.58 \pm 0.14 ^g	55.80 \pm 0.10 ^f
	14	15.84 \pm 0.03 ^k	17.63 \pm 0.09 ^c	57.30 \pm 0.10 ^d	17.64 \pm 0.24 ^o	16.35 \pm 0.11 ^d	65.90 \pm 0.07 ^a
0kGy + 2%	0	34.08 \pm 0.06 ^a	9.07 \pm 0.05 ^l	42.96 \pm 0.02 ^k	34.08 \pm 0.06 ^b	9.07 \pm 0.05 ^k	42.96 \pm 0.02 ^k
MLF	7	28.68 \pm 0.24 ^{cd}	11.75 \pm 0.25 ⁱ	50.83 \pm 0.01 ^h	28.68 \pm 0.24 ^f	12.62 \pm 0.05 ^h	51.79 \pm 0.14 ⁱ
	14	23.81 \pm 0.16 ^f	16.67 \pm 0.29 ^d	56.92 \pm 0.01 ^d	22.65 \pm 0.16 ^k	15.59 \pm 0.14 ^e	60.73 \pm 0.13 ^e
1.5kGy + 2%	0	33.17 \pm 0.08 ^a	11.77 \pm 0.06 ⁱ	45.05 \pm 0.04 ⁱ	36.34 \pm 0.07 ^a	11.96 \pm 0.03 ⁱ	44.99 \pm 0.01 ^j
MLF	7	27.50 \pm 0.04 ^d	15.70 \pm 0.38 ^e	54.70 \pm 0.30 ^e	29.57 \pm 0.10 ^e	15.48 \pm 0.11 ^e	53.67 \pm 0.30 ^g

	14	21.52±0.19 ^h	18.58±0.13 ^b	61.62±0.31 ^a	23.54±0.26 ^j	18.50±0.14 ^b	64.63±0.31 ^b
3kGy + 2%	0	31.26±0.08 ^b	11.22±0.06 ^{ij}	44.97±0.01 ⁱ	35.65±0.03 ^b	11.72±0.03 ⁱ	44.79±0.03 ^j
MLF	7	22.72±0.22 ^{fg}	16.89±0.07 ^d	50.76±0.12 ^h	27.67±0.16 ^g	16.48±0.10 ^d	59.90±0.07 ^f
	14	15.71±0.13 ^k	19.48±0.07 ^a	58.74±0.33 ^c	20.59±0.15 ^l	20.61±0.12 ^a	64.81±0.09 ^b

Table 4. Mean values of microbial load in aerobic and vacuum packaged meat samples during storage

Treatments	Storage Days	Aerobic		Vacuum	
		TAB (log CFU/g)	Coliform (log CFU/g)	TAB (log CFU/g)	Coliform (log CFU/g)
0kGy	0	7.03±0.01 ^d	5.23±0.02 ^d	7.03±0.01 ^e	5.23±0.02 ^b
	7	8.53±0.01 ^b	6.35±0.01 ^b	7.62±0.01 ^b	5.71±0.28 ^a
	14	9.81±0.04 ^a	7.12±0.01 ^a	8.15±0.01 ^a	5.98±0.10 ^a
1.5kGy	0	5.15±0.02 ^h	ND	5.15±0.02 ^j	ND
	7	5.36±0.02 ^g	ND	5.68±0.01 ^h	ND
	14	5.98±0.02 ^f	ND	5.98±0.01 ^g	ND
3kGy	0	4.23±0.02 ^m	ND	4.23±0.02 ⁿ	ND
	7	4.52±0.01 ^l	ND	4.65±0.01 ^l	ND
	14	4.67±0.01 ^k	ND	4.77±0.02 ^k	ND
0kGy + 2% MLF	0	6.74±0.02 ^e	4.86±0.04 ^f	6.74±0.02 ^f	4.86±0.04 ^c
	7	7.02±0.01 ^d	5.17±0.01 ^e	7.03±0.01 ^d	4.90±0.01 ^b
	14	7.24±0.02 ^c	5.35±0.01 ^c	7.31±0.09 ^c	4.97±0.01 ^b
1.5kGy + 2% MLF	0	5.04±0.01 ⁱ	ND	5.04±0.01 ^k	ND
	7	5.12±0.01 ^h	ND	5.03±0.01 ^j	ND
	14	5.38±0.01 ^g	ND	5.20±0.01 ⁱ	ND
3kGy + 2% MLF	0	4.17±0.02 ⁿ	ND	4.17±0.02 ^o	ND

7	4.46±0.03 ^l	ND	4.24±0.02 ⁿ	ND
14	4.74±0.01 ^j	ND	4.31±0.04 ^m	ND

Results are means of triplicates (±SD). Values with different letters in columns are significantly different (Tukey's test, $p \leq 0.05$). MLF; Moringa Leaf Powder

Table 5. Sensory scores of meatballs stored in aerobic packaging

Treatments	Storage Days	Appearance	Flavor	Texture	Taste	OA
0kGy	0	8.25±0.70 ^a	7.5±1.35 ^a	7.1±0.77 ^{ab}	7.3±1.03 ^a	7.5±0.81 ^{ab}
	7	7.15±1.30 ^b	6.7±1.03 ^b	6.8±0.95 ^b	6.9±1.16 ^b	6.8±0.97 ^b
	14	6.1±1.43 ^c	6.2±0.91 ^c	5.7±1.20 ^c	6.1±1.42 ^c	6.3±1.10 ^c
1.5kGy	0	7.86±0.71 ^{ab}	7.2±1.30 ^b	6.9±0.80 ^b	7.2±0.90 ^a	7.2±0.67 ^c
	7	7.1±0.71 ^b	6.5±1.10 ^b	6.2±0.90 ^b	6.5±1.20 ^b	6.4±0.82 ^b
	14	6.8±0.74 ^c	5.9±0.80 ^c	5.8±1.40 ^c	6.3±1.25 ^c	6.1±1.02 ^c
3kGy	0	7.5±0.53 ^{ab}	7.3±1.15 ^a	7.0±0.90 ^a	7.1±1.01 ^a	7.3±0.77 ^{ab}
	7	7.2±1.30 ^b	6.8±0.95 ^b	6.7±1.52 ^b	6.9±1.19 ^b	6.9±0.77 ^b
	14	6.5±1.52 ^c	6.5±0.90 ^c	6.1±1.20 ^c	6.5±1.04 ^c	6.4±1.03 ^c
0kGy + 2% MLF	0	8±0.65 ^a	7.4±1.32 ^a	7.0±1.10 ^{ab}	7.2±0.98 ^a	7.4±0.74 ^a
	7	7.5±0.70 ^b	6.9±1.14 ^b	6.7±0.99 ^b	6.7±1.06 ^b	6.9±0.77 ^b
	14	7.1±1.20 ^c	6.4±1.01 ^c	6.2±1.30 ^c	6.4±1.10 ^c	6.5±1.32 ^c
1.5kGy + 2% MLF	0	7.8±0.77 ^{ab}	7.3±1.16 ^{ab}	7.0±0.87 ^a	7.3±1.15 ^a	7.4±0.93 ^{abc}
	7	7.2±0.82 ^b	6.6±0.70 ^b	6.7±1.03 ^b	6.4±1.07 ^b	6.7±0.85 ^b

3 kGy + 2% MLF	14	6.9±0.90 ^c	6.4±0.74 ^c	6.3±0.93 ^c	6.3±1.03 ^c	6.4±1.05 ^c
	0	7.6±0.80 ^b	7.4±1.43 ^{ab}	7.0±0.90 ^{ab}	7.1±0.88 ^a	7.3±1.01 ^{bc}
	7	6.8±1.30 ^b	6.5±1.33 ^b	6.6±0.90 ^b	6.2±0.98 ^b	6.5±0.98 ^b
	14	6.76±1.42 ^c	6.3±1.21 ^c	6±1.10 ^c	6.2±1.07 ^c	6.1±1.10 ^c

The values are mean ± SD of ten independent determinations. The means carrying different letters in a column differed significantly. OA: Overall Acceptability; MLF: Moringa Leaf Powder

Table 6. Sensory scores of meatballs stored in vacuum packaging

Treatments	Storage Days	Appearance	Flavor	Texture	Taste	OA
0kGy	0	8.25±0.70 ^{ab}	7.5±1.35 ^b	7.1±0.77 ^{ab}	7.3±1.03 ^a	7.5±0.81 ^a
	7	7.3±0.72 ^b	6.4±1.21 ^b	6.9±1.18 ^b	6.8±1.16 ^b	6.8±0.88 ^b
	14	6.4±0.80 ^c	6.1±0.90 ^c	6.0±1.36 ^c	6.0±0.90 ^c	6.2±0.90 ^c
1.5kGy	0	7.86±0.71 ^d	7.2±1.30 ^{ab}	6.9±0.80 ^{ab}	7.2±0.90 ^a	7.2±0.67 ^a
	7	6.9±0.71 ^b	6.7±1.21 ^b	6.8±1.10 ^b	6.4±1.13 ^b	6.7±0.80 ^b
	14	6.8±0.82 ^c	6.4±1.02 ^c	6.3±1.32 ^c	6.2±1.43 ^c	6.3±0.77 ^c
3kGy	0	7.5±0.53 ^b	7.3±1.15 ^b	7.0±0.90 ^a	7.1±1.01 ^a	7.3±0.77 ^a
	7	7.3±0.77 ^b	6.2±1.22 ^b	6.9±0.98 ^b	6.8±1.07 ^b	6.8±0.84 ^b
	14	6.8±0.85 ^c	6.3±1.10 ^c	6.2±1.08 ^c	6.4±1.41 ^c	6.3±0.77 ^c
0kGy + 2% MLF	0	8±0.65 ^a	7.4±1.32 ^a	7.0±1.10 ^b	7.2±0.98 ^a	7.4±0.74 ^a
	7	7.5±0.99 ^b	6.9±0.94 ^b	6.5±0.90 ^b	6.6±1.33 ^b	6.8±0.91 ^b

	14	7±1.01 ^c	6.4±0.74 ^c	6.1±1.41 ^c	6.3±1.21 ^c	6.1±1.01 ^c
1.5kGy + 2% MLF	0	7.8±0.77 ^{bc}	7.3±1.16 ^a	6.9±0.87 ^{ab}	7.2±1.15 ^a	7.4±0.93 ^a
	7	7.2±1.10 ^b	6.9±1.30 ^b	6.8±1.17 ^b	6.3±1.10 ^b	6.8±0.85 ^b
	14	6.7±0.77 ^c	6.4±1.04 ^c	6.4±1.10 ^c	6.2±1.15 ^c	6.3±0.74 ^c
3 kGy + 2% MLF	0	7.6±0.80 ^{cd}	7.4±1.43 ^b	6.8±0.90 ^{ab}	7.1±0.88 ^a	7.3±1.01 ^a
	7	7.1±0.71 ^b	6.3±1.25 ^b	6.7±1.18 ^b	6.3±1.23 ^b	6.6±0.84 ^b
	14	6.6±0.74 ^c	6.2±1.17 ^c	6.1±1.30 ^c	6.1±1.20 ^c	6.2±0.91 ^c

The values are mean ± SD of ten independent determinations. The means carrying different letters in a column differed significantly. OA: Overall Acceptability; MLF: Moringa Leaf Powder



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