Benedito Fort, JJ.; Cambrero, MI.; Ortuño Cases, C.; Cabeza, MC.; Ordoñez, JA.; De La Hoz, L. (2011). Modeling and optimization of sensory changes and shelf-life in vacuum-packaged cooked ham treated by E-beam irradiation. Radiation Physics and Chemistry. 80(3):505-513. doi:10.1016/j.radphyschem.2010.11.001.

Modeling and optimization of sensory changes and shelf-life in vacuum packaged cooked ham treated by E-beam irradiation

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ABSTRACT

The E-beam irradiation of vacuum-packaged RTE cooked ham was carried out to establish the dose required to achieve the food safety objective (FSO) and to minimize changes in selected sensory attributes. Cooked ham was irradiated with doses ranging from 1 to 4 kGy. After the treatment the microbial inactivation of *Listeria monocytogenes,* the shelf-life of the product and some sensory attributes (appearance, odor and flavor) were determined. The inactivation of *Listeria monocytogenes* was satisfactorily described by a first-order kinetics equation (R^2=0.99). The influence of the irradiation dose on appearance, odor and flavor was modeled through the Gompertz (R^2=0.99, for appearance) and activation/inactivation (R^2=0.99, for odor and flavor) equations. A model was also developed to determine the shelf-life of irradiated cooked ham depending on the irradiation dose (R^2>0.91). The dose that maximized the scores of the sensory attributes was 0.96 kGy resulting in an acceptable sensory quality for 80 days. It is possible to apply up to 2 kGy to ensure microbial safety while provoking no significant changes in the above mentioned sensory attributes.

Key words: microbial safety, modeling, quality attributes, shelf-life, E-beam irradiation.

1. Introduction

Nowadays, people's dietary habits are undergoing a transformation. Traditional food is being replaced by ready-to-eat (RTE) products (IAEA, 2003; Jacxsens et al., 2002; Hoz et al., 2006). Although consumers are demanding foods with short preparation times, there is also great concern about the need for a healthy diet which drives consumers to demand more and more natural foods, free of chemical additives (Jacxsens et al.,2002). On the other hand, the meat industry is focused on manufacturing long shelf-life RTE products in domestic portions from processed blocks (Cabeza et al., 2009; Gil-Díaz et al., 2009). The rising number and severity of food poisoning outbreaks world-wide has increased public awareness of the microbial safety of foods, including meats (Maurice, 1994). Therefore, it is necessary to apply some preservation technique to these products to both reduce the spoilage microorganisms and to guarantee the microbial safety (Zhu et al., 2005).

Although thermal treatments have been the most commonly used technique for reducing the microbial load of foods, they can destroy heat-sensitive nutrients and affect properties such as flavor, odor, appearance or texture. Non-thermal methods allow foods to be processed at lower temperatures than when they are pasteurized, and, therefore, flavors, essential nutrients, and vitamins undergo minimal or no changes. Foods can be non-thermally processed by irradiation (Patterson et al., 1993; Zhu et al., 2005), high hydrostatic pressure (Lakshmanan and Dalgaard, 2004), the use of antimicrobials (Vogel et al., 2006), ultrasound (Knorr et al., 2004), filtration, and electrical methods such as pulsed electric fields (Barbosa-cánovas et al., 1998a), light pulses (Wang et al., 2005), and oscillating magnetic fields (Barbosa-Cánovas et al., 1998b). Due to recent technological developments over the last decade, E-beam irradiation processing has been receiving special attention. Additionally, it is a very useful method for sanitizing RTE foods, since it is not feasible to apply the conventional technologies to these products with that goal in mind (Hoz et al., 2006; Sommers et al.,

2003; Thayer et al., 1990; Zhu et al., 2005).

Irradiation is an effective way to eliminate pathogens present in foods, including *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica*, *Escherichia coli* O157:H7, and others (Burgess et al., 2010; Cabeza et al., 2009; Schilling et al., 2009). However, some reports (Arthur et al., 2005; Lee and Ahn, 2005; Rababah et al., 2010) indicate that its application to meat is limited since irradiation can produce changes in the aroma, color and flavor that significantly affect consumer acceptance. The odor of irradiated meat has been described as being like rotten egg, sweet, cooked meat, barbecued corn, burnt, sulphur, metallic, alcohol, acetic acid, liver-like serumy, and bloody (Brewer, 2009; Hampson et al.,1996; Lee et al., 1996). It is, therefore, critical to carefully adjust the irradiation doses to achieve an adequate level of microbial inactivation to produce only negligible changes in sensory properties, thereby avoiding consumer rejection of the irradiated product.

Since food quality and food safety normally require opposing process conditions, the modeling and optimization of food preservation processes can lead to an equilibrium between both factors allowing the optimal process conditions to be found. In this regard, several studies have been carried out to optimize the processing and storage of different meat products in order to prevent oxidative damage, the growth of pathogens or the loss of antioxidant components (Álvarez et al., 2007; Marselles-Fontanet and Martin-Belloso, 2007; Shi and Le Maguer, 2000). To formulate an optimization problem, mathematical models that describe the effect of the process variables not only on the inactivation of microorganisms, but also on the quality attributes and the shelf-life of the products, must be obtained.

Examples of models to describe the inactivation of microorganisms or other compounds (quality factors, enzymes, etc.) are the Gompertz function (Ding et al., 2010), the Weibull distribution (Bermúdez-Aguirre et al., 2009), the Fermi Distribution

(Elez-Martinez et al., 2006) or the activation/inactivation model (Soysal, 2008).

On the other hand, models for describing the microbial growth include the modified Gompertz equation (Huang, 2010) or the Hill's Model (Wang, 2010).

Due to the great potential of E-beam irradiation as a method of sanitizing RTE meat products (Cabeza et al., 2007; Hoz et al., 2008), it is necessary to develop mathematical models which describe the response of microorganisms and the quality factors of meat products treated using this technology. Moreover, it is very interesting to develop optimization procedures to find the optimum process conditions. The main objective of this work was to optimize the irradiation treatment of vacuum packaged cooked ham.

2. Materials and methods

2.1. Estimation of the food safety objective (FSO)

Assuming a contamination of 10 cells/g, the performance criterion values (Gorris, 2005) of 1.7D and 5.09D reductions of the load of *L. monocytogenes*, for EC and USDA statements respectively, have been previously determined (Cabeza et al., 2007) in order to reach the FSO in cooked ham.

2.2. Organism

The *L. monocytogenes* Scott A (CIP 103575, serotype 4b) strain was used. The strain was maintained by freezing (−40 °C) in trypticase soy broth (TSB; Difco, BD, Sparks, MD) adding 10% glycerol as the cryogenic agent. Fresh culture was prepared for each experiment by removing a piece of frozen culture from vials and inoculating it into 9 ml of TSB, then incubating at 32 °C for 24 h. The culture was then centrifuged (at 4 °C) and the pellet suspended in a beaker with 50 ml sterile saline solution, which yielded a bacterial load of approximately 10^8 cells/ml. The slices were contaminated by

133 immersion in the beaker for a few seconds. In experiments, a large number of cells
134 were used to calculate the radioresistant parameters precisely.

135

136 **2.3. Sample preparation and irradiation treatment**

137 Heat processed cooked ham blocks (1 kg weight) packed into cylindrical (diameter 7
138 cm, length 25 cm) thermoplastic bags were purchased in a local supermarket. Slices (2
139 mm thick) were cut in an electric machine, whose knife and contact surfaces were
140 previously thoroughly cleaned and then washed in sterile distilled water. For
141 microbiological purposes, slices were contaminated as described above. The
142 contaminated (for microbial analysis) and uncontaminated (for shelf-life determination)
143 slices (1 for microbial and 3–6 per bag for shelf-life analysis) were vacuum packaged to
144 reach about 20 kPa in 10×10 cm laminated film bags of low gas permeability (diffusion
145 coefficient of 35 cm^3/24 h m^2 bar to oxygen and 150 cm^3/24 h m^2 bar to carbon
146 dioxide). The samples were transferred (less than 1 h) in insulated polystyrene boxes
147 to the irradiation plant (IONISIOS sterilization SA, Tarancón, Cuenca, Spain) and
148 irradiated under an electron beam radiation source, which operates at 10 MeV. The
149 radiation doses employed were between 1 and 4 kGy and the dose absorbed by the
150 samples was checked by determining the absorbance of cellulose triacetate dosimeters
151 (ASTM, 2000) simultaneously irradiated with samples. The experiments were carried
152 out in triplicate and at room temperature (18 – 20 °C). During treatment, the product
153 temperature went up by less than 2 °C. After E-beam treatment, samples were
154 transferred to the laboratory and stored at 4 ºC until use.

155 **2.4. Microbial analyses**

156 To count the *L. monocytogenes* survivors, about 10 g of the material was weighed and
157 homogenized with 90 ml of a sterile saline solution in a Stomacher bag. Counts were
158 determined on the surface of plates with trypticase soy agar (Difco) and the use of a

159 spiral plate system (model Eddy Jet, IUL Instrument, Barcelona, Spain). Plates were
160 incubated at 32 °C for 24 h. Colonies were enumerated with an automatic counter
161 (Countermat Flash model, IUL Instrument, Barcelona, Spain).

162 **2.5. Shelf-life determination**

163 The shelf-life of irradiated RTE cooked ham slices was determined by periodically
164 counting the bacterial number and by means of a sensory analysis (odor and visual
165 appearance) of samples stored at 4 °C. Non-irradiated vacuum-packaged slices were
166 used as controls. From a bacterial point of view, the shelf-life was considered to end
167 when the bacterial count was higher than 10^7 cfu./g.

168 **2.6. Sensory analysis**

169 To determine the possible sensory differences between the non-treated (0 kGy) and
170 irradiated samples (1, 2, 3 and 4 kGy) stored at 4 ºC, a triangular test, a rank order
171 test, and a descriptive test were performed. Samples were evaluated by a panel of
172 twenty tasters (ten females and ten males) selected from among the members of the
173 “Departamento de Nutrición, Bromatología y Tecnología de los Alimentos”. The
174 panelists were previously trained in the sensory assessment of meat products. In this
175 training, several models (“fresh cooked ham”, “concentrated meat broth”, “hot culture
176 medium”, “burnt beef broth”, “scalded feather”, “burnt feather”, “pungent pepper”,
177 “cooked cabbage”, “spoiled milk” and “spoiled vacuum cooked ham”) were prepared as
178 reference in order to familiarize the testers with the flavors expected to be produced by
179 the E-beam treatment. The analyses were performed as described by Cabeza et al.
180 (2008). The evaluation was carried out between meals (after breakfast but before the
181 midday meal). The samples were allowed to sit for 20 min to reach room temperature.
182 The evaluations were performed in individual booths built according to the International
183 Standards Organization DP 66.58 criteria (ISO, 1981). The tasters received unsalted
184 crackers and water at room temperature to cleanse the palate between samples. Three

185 independent tests were performed to evaluate appearance, odor, and flavor. White
186 fluorescent light was used during appearance analysis. The odor and flavor of samples
187 were evaluated under red light conditions just after opening the bags.

188 The forced-choice option of the triangle test (ISO, 1981) was chosen, in which the
189 tasters must select the sample that, in their opinion, is different. All the possible
190 combinations of untreated and irradiated samples were tested. These sensory
191 analyses were carried out on the 0 and the 1st day after treatment and on the 16 and
192 17th days of storage at 4 ºC. To complement the triangle test, tasters were asked to
193 indicate their reasons for selecting one particular sample of the three used in the
194 analysis.

195 For the rank order test, the tasters were instructed to rank samples in order of
196 preference, according to the proximity of the sensory characteristic (appearance, odor,
197 or flavor) of the sample analyzed to the optimal sensory quality of the cooked ham
198 (appearance: bright red color, high marbling degree; odor: richness and intensity,
199 absence of off-odors; flavor: typical cooked ham flavor intensity, richness of taste
200 notes, cured and rancid intensity, absence of off-flavors, and intensity of aftertaste). For
201 this test, a 5-point scale (1, lowest preference; 5, highest preference) was used. No
202 repetitions were allowed. Results of the rank order test were used to obtain the sum of
203 ranks, which corresponds to the sum of scores of cooked ham preference for a specific
204 sensory characteristic (calculating the sum of the products of values given to each
205 sample on a 5-point scale multiplied by the number of times that each sample was
206 allocated to a specific score).The significance level of data obtained in these tests was
207 determined by Friedman's rank addition according to the model proposed by Joanes
208 (1985) and the tables for multiple comparison procedures for the analysis of ranked
209 data (Christensen et al., 2006). The sum of the ranks, as quantitative values of the
210 sensory evaluation, was used in the modeling, statistical analysis and optimization of

211 the irradiation process. The rank order test was performed on the 2nd and 18th days,
212 after sample treatment and storage at 4 ºC.

213 Panelists were also asked to provide information about the cooked ham characteristics
214 (appearance, odor, and flavor and any off-sensory aspect) following a profile
215 descriptive analysis. This procedure was carried out on the 3rd and 19th days, after
216 irradiation treatment and storage at 4 ºC.

217 **2.7. Modeling inactivation of Listeria monocytogenes Scott A**

218 The kinetic data of the organism inactivation was analyzed using first-order kinetics
219 (Eagerman, 1976), with the variable time replaced by the treatment dose (d_{irr}) (Eq. 1
220 and 2). $N(d_{irr})$ and $N(d_0)$ are the number of microorganisms after an irradiation dose
221 (d_{irr}) and without treatment (d_0), respectively and k is the rate constant (kGy^{-1}) for
222 given treatment conditions. k values were obtained from the linear regression analysis
223 of log $[N(d_{irr}) / N(d_0)]$ versus dose.

224 $$\log\left[N\left(d_{irr}\right)/N\left(d_0\right)\right] = -k\ d_{irr} \quad (1)$$

225 **2.8. Modeling of quality factors**

226 Appearance, odor and flavor were described using adapted inactivation models, such
227 as the Gompertz Function (Eq. 2), the Weibull Distribution (Eq. 3) and the
228 Activation/Inactivation model (Eq. 4). Normally, these equations relate the response
229 factor to the variable time, however, in this work time was replaced by the irradiation
230 dose.

231 $$\log\left[Q\left(d_{irr}\right)/Q\left(d_0\right)\right] = C\ \exp\left[-\exp\left(A + B\ d_{irr}\right)\right] - C\ \exp\left[-\exp(A)\right] \quad (2)$$

232 $$\log\left[Q(d_{irr})/Q(d_0)\right] = -b\ d_{irr}^{\ n} \quad (3)$$

233 $$\log\left[Q(d_{irr})/Q(d_0)\right] = \left[d_{irr}/\left(k_1 + k_2\ d_{irr}\right)\right]\ \left[1 - \log(1 + \exp(k_3\ d_{irr}))^m\right] \quad (4)$$

Where $Q(d_{irr})$ and $Q(d_0)$ are the scores assigned by tasters for the quality attributes (appearance, odor or flavor) after 2 and 18 days of different irradiation treatments (d_{irr}) and without treatment (d_0), respectively. A, B and C; b and n; m and k_i (i=1, 2 and 3), are the kinetic constants for the Gompertz Function, the Weibull Distribution and the Activation/Inactivation model, respectively. The models were fitted to the scores obtained after 2 and 18 days of treatment.

2.9. Modeling of the bacterial growth. Shelf-life of cooked ham.

In the present study, the growth of the surviving spoilage microorganisms during the storage period was fitted using Hills's model (Eq. 5). This model was developed by Hills and coworkers and it is based on two concepts: synthesis and cell division and biomass availability (Hills and Mackey, 1995; Hills and Wright, 1994).

$$N(t) = N_0 - [[\, k - \exp(\, Pt + P\exp(\, kt\,)\,)\,]/[P + k]] \quad (5)$$

Where N(t) and N_0 is the microbial population after time t and the initial time, respectively, after a specific irradiation dose. This model includes two different kinetic parameters, P and k, which depend on the environmental factors. The shelf-life was obtained from Eq. 5, taking the limit of cfu/g to be 10^7. Eq. 5 was adapted to incorporate the irradiation dose and to be able to describe the microorganism growth during the storage time after different doses of treatment.

2.10. Statistical analysis and optimization of the irradiation process

The kinetic constants of the models were calculated by minimizing the sum of the square differences between experimental and predicted data using the Excel Solver tool. The root mean square error (RMSE, Eq. 6) and the coefficient of determination (R^2, Eq. 7) were used to evaluate the goodness of the fit and the accuracy of the estimation. RMSE is a measure of the standard error in the estimation, whereas R^2 is a

statistical parameter that defines the variability explained by the model (Schemper, 2003).

$$RMSE = \sqrt{\frac{\sum_{k=1}^{n} (y_k - y_k^*)^2}{N}} \quad (6)$$

$$R^2 = 1 - \left(S_{yx}^2 / S_y^2\right) \quad (7)$$

Where y is the experimental data; y* is the estimated value; N is the number of experimental values and S_y and S_{yx} are the total standard deviation and the standard deviation of the estimation, respectively.

The main objective of this work was to optimize the irradiation process, which involves determining the dose that best preserves the quality characteristics (defined by the objective function) of the product while ensuring the food safety and a minimum shelf-life (restraints).

In the optimization problem, the models for the microbial inactivation and the changes in the sensory attributes were used. In the case of the sensory properties, the models selected were those describing food quality (odor, flavor and appearance) after 18 days of treatment. This was because interest was focused on a shelf-life of over 2 days and 18 days was a more realistic storage period.

The optimization was based on a mathematical problem that contained the objective function, the restraints and the decision variable. The objective function (to be maximized) was defined as the sum of the scores for odor, flavor and appearance. The prior modeling of experimental scores was necessary and this was done by using different models (previously described) and selecting the best model for each attribute according to the defined statistical parameters. Therefore, the sum of the selected models for odor, flavor and appearance represented the objective function.

281 Two restraints were considered, one related to food safety, regarding the number of log
282 reductions that must be reached according to the European Commission's or United
283 States Department of Agriculture's safety criterion, which was calculated through Eq. 1.
284 On the other hand, the second restraint was related with the shelf-life of the product,
285 given by Eq. 5, with the minimum period before the count of 10^7 cfu/g was reached
286 being 60 days

287 Finally, the decision variable was the irradiation dose, which must be sought to
288 maximize the objective function while coping with the restraints. The Solver tool
289 (Microsoft Excel ™) was used to solve this optimization problem.

290

291 **3. Results and discussion**

292 Mathematical models were used to describe the effect of the irradiation dose on the
293 microbial inactivation and sensory characteristics of vacuum packaged cooked ham
294 slices. These equations were used in the optimization of the process and the dose
295 which minimized the effect on the quality properties (objective function) thereby
296 ensuring the safety of the product and the required shelf-life was chosen (restraints).
297 Finally, once the optimum irradiation dose was calculated for each FSO (EC, USDA),
298 the shelf-life of the product was determined through the corresponding model.

299 **3.1. Modeling inactivation of Listeria monocytogenes Scott A**

300 Treatments at 1, 2, 3 and 4 kGy were considered to obtain the survival curve and the
301 D-value. The experimental data obtained for the inactivation of *L. monocytogenes* in
302 cooked ham slices after E-beam irradiation was satisfactorily described by Eq. 8
303 (R^2=0.997, RMSE=0.133). The inactivation kinetics of this strain of *L. Monocytogenes*
304 is extensively reported previously (Cabeza et al., 2007).

305 $$\log N(d_{irr}) = -2.7802\, d_{irr} + 7.5882 \quad (8)$$

306 The response of *L. monocytogenes* to the irradiation treatment fits first-order
307 inactivation kinetics, showing about 2.78 log reductions as the dose increases by 1
308 kGy. The strain of *L. monocytogenes* used here presented the lowest D-value (0.36
309 kGy), compared with other listeria strains (Cabeza et al, 2007). This means that, to
310 avoid the risk that comes from the routine use of *L. monocytogenes* Scott A in
311 experiments, it is possible to work normally with more resistant strains (Dion et al.,
312 1994; Grant and Patterson, 1992; Mendonca et al., 2004). Other authors have used
313 non-linear models (Gompertz) to describe the inactivation kinetics of *L. monocytogenes*
314 (Linton et al., 1995).

315 **3.2. Modeling of quality factors**

316 The panel of tasters assessed cooked ham slices after E-beam irradiation in order to
317 evaluate the sensory properties of the samples after both a short storage period (2
318 days) and also one of 18 days. The data obtained for the quality properties (odor, flavor
319 and appearance) after irradiation was fitted to three mathematical models (the
320 Gompertz Function, Weibull's Distribution and the Activation/Inactivation model). For
321 each quality property, a model was selected according to the statistical parameters, R^2
322 and RMSE, and used for the optimization procedure. Table 1 shows the mean scores
323 obtained via the rank order test for the three attributes assessed, including the
324 significant differences between the doses applied for each attribute.

325 **3.2.1. Appearance**

326 The intensity of the treatment had a similar effect on the samples analyzed after both 2
327 and 18 days of storage (Figure 1). Non-treated samples and samples treated with 1
328 kGy obtained a similar preference, which indicates that this dose is not high enough to
329 affect this feature significantly. When analyzing samples stored for both 2 and 18 days,
330 it was possible to observe a clear decrease in consumer acceptance for doses of over
331 2 kGy, which shows that once this threshold is exceeded, the irradiation negatively

affects the appearance. In the triangular analysis, only significant differences ($p < 0.05$) between non-irradiated and irradiated samples at doses of over 2 kGy were found. In the color descriptive analysis, samples with 3 and 4 kGy were judged to be darker and brown-gray. These sensory results coincide completely with those obtained by means of the instrumental color analysis of several irradiated meat products, since a progressive decrease in redness (*a** values of tristimulus colorimeter) is observed as the irradiation treatment becomes more intense (Cabeza et al., 2007; Nam and Ahn, 2003).

The fact that, after 18 days, the preference of non-treated samples obtained the highest score indicates that the growth of the surviving spoilage microorganism did not significantly affect the appearance of cooked ham. Therefore, this attribute seems to be mainly affected by the intensity of the irradiation treatment.

Table 2 shows the values for the different parameters obtained when the appearance was fitted to the three previously described models. As regards how the dose affects the appearance, the calculated statistical parameters show that, although all the models provided a good fit, the Gompertz Function offered the best result ($R^2 = 0.99$; RMSE = 0.001 and 0.002 for 2 and 18 days, respectively). The goodness of the model fitting can also be observed in Figure 1.

3.2.2. Odor and flavor

Flavor is a sensory property which is defined as the combined chemical sensations of taste and smell. Consequently, odor and flavor behaved in a similar way (Table 1, Figure 2), after both 2 and 18 days of storage. After 2 days of irradiation, non-treated samples were better accepted than treated ones. When 1 kGy was applied, a decrease in the score was observed, although this dose was not high enough to provoke any significant differences ($p>0.05$) in odor and flavor, compared to non-treated samples. A significant ($p<0.05$) decrease in the acceptance of odor and flavor was observed for a dose of 2 kGy, compared to non-treated samples, although the difference was not

359 significantly different ($p>0.05$) from 1 kGy treated samples. Overall, the samples were
360 poorly rated as the dose increased. In the triangular analysis, significant differences
361 ($p<0.05$) were found at doses ≥ 3 kGy as compared with 0 and 1 kGy. In the irradiated
362 samples, doses ≥ 2 kGy gave rise to weak off-odors and off-flavors defined as “hot
363 culture medium”, “burnt beef broth”, “scalded feather” and a negligible, astringent-feel
364 aftertaste. The higher the irradiation dose, the more intense the odor. These changes
365 have been observed by other authors and they have been associated with the
366 irradiation treatment (Brewer, 2009; Hampson et al., 1996; Jo et al., 1999; Nam and
367 Ahn, 2003). Brewer (2009) reported that irradiation can induce the formation of iso-
368 octane-soluble carbonyl compounds in the lipid fraction and low molecular weight, acid-
369 soluble carbonyls in the protein fraction of meat. Raising the irradiation dose increases
370 these compounds.

371 As for odor, the most widely accepted samples after 18 days of storage were those
372 treated with 1 kGy (Figure 2). The high score reached by these, compared to non-
373 treated ones, could be due to the growth of spoilage organisms which generated off-
374 odors and off-flavors, reducing the cooked ham preference. In samples treated with
375 doses of over 1 kGy, the preference began to decrease (Table 1, Figure 2). This fact
376 was not attributed to the spoilage microorganisms, which were more affected than
377 when using 1 kGy, but rather, as it happened after 2 storage days, was a consequence
378 of the dose. In the descriptive analysis, the odor of the non-irradiated samples was
379 defined as moderately lactic acid, and sour. A similar slight off-flavor was detected in
380 these samples, but not in irradiated samples. These changes may be explained by the
381 fact that the presence of a high number of spoilage organisms, presumably lactic acid
382 bacteria (De Pablo et al., 1989). In irradiated samples, however, the original spoilage
383 microbiota was effectively reduced by the E-beam in such a way that the off-odor and
384 off-flavor in the samples treated at doses ≥ 3kGy were associated to the irradiation

385 treatment. However, in the samples irradiated at 2 kGy, the formerly perceived off-odor
386 and off-flavor were minimized during the storage time.

387 Several authors (Houser et al., 2005; Zhu et al., 2003) have reported that irradiation
388 has negative effects on the odor of some RTE meat products, such as ham and pork
389 frankfurters, while in others, like irradiated turkey ham, it increases dimethyl disulfide
390 concentration and sulphur odor and flavor as the dose rises from 0 to 2 kGy. It has also
391 been shown to induce the production of hydrocarbons, 1,7-hexadecadiene, 1,7,10-
392 hexadecatriene, and 6,9-heptade-cadiene in hams and sausages (Kwon et al., 2007).

393 Table 3 shows the values of the parameters obtained when the odor and flavor were
394 fitted to the three models (the Gompertz Function, Weibull's Distribution and the
395 Activation/Inactivation model). After 2 days of treatment, the three models satisfactorily
396 fitted the acceptance of odor and flavor, the Gompertz Function being the one that
397 provided the best result for odor (Figure 2; R^2 = 0.998; RMSE = 0.008) and the Weibull
398 Distribution for flavor (R^2 = 0.998; RMSE = 0.007). After 18 days of treatment, the
399 Activation/Inactivation model satisfactorily described odor (Figure 2) and flavor (R^2 =
400 0.999; RMSE = 0.007 and 0.003 for odor and flavor, respectively).

401 **3.3. Modeling of the bacterial growth. Shelf-life of cooked ham.**

402 The growth of surviving spoilage microorganisms in the E-beam treated vacuum
403 packaged cooked ham was progressively slower and the lag-phase longer as the dose
404 of irradiation increased (Figure 3). The growing behavior was properly described using
405 the Hills model (Eq. 5). Table 4 shows the values for the kinetic constants of the model
406 and the statistics of the fit. As can be observed, the high values of the explained
407 variance and the values of RMSE demonstrate how accurately the model fits the
408 experimental data.

409 Table 4 shows that similar values of P parameter were obtained, while the values of
410 the k parameter decreased as the dose increased. To obtain a single equation that

predicted the growth of spoilage microorganisms, for any irradiation dose, P was assigned a constant value of 0.21, and a relationship between k and the dose was determined using the Weibull model (Eq. 9).

$$k\,(d_{irr}) = k\,(d_0)\cdot 10^{\left[\frac{1}{2.303}(d/\alpha)^{\beta}\right]} \quad (9)$$

Where $k(d_{irr})$ and $k(d_0)$ represent the value for a dose with treatment (d_{irr}) and without (d_0); α and β are the model constants (α=0.037; β=0.38;R^2=0.955; RMSE=0.257). By substituting Eq. 9 in Eq. 5, Eq. 10 was obtained, which satisfactorily described the growth of spoilage microorganisms in the vacuum packaged cooked ham for the different irradiation doses applied. Although the fit for the different doses was slightly poorer (Table 4), the model (Eq. 10) permitted a good estimation of the population growth (Figure 3) and, therefore, it can be used to interpolate the growth for doses in the range of 0 to 4 kGy. Equation 10 will be used to calculate the shelf-life in the optimization procedure considering the limit to be 10^7 cfu/g.

$$N\,(d_{irr},t) = N_0 - \left[\frac{\left[\left[\left[k(d_0)\cdot 10^{\left[\frac{1}{2.303}\left[\frac{d_{irr}}{\alpha}\right]^{\beta}\right]}\right] - \exp\left[P\cdot t + P\exp\left(t\cdot k(d_0)\cdot 10^{\left[\frac{1}{2.303}\left[\frac{d_{irr}}{\alpha}\right]^{\beta}\right]}\right)\right]\right]\right]}{P + k(d_0)\cdot 10^{\left[\frac{1}{2.303}\left[\frac{d_{irr}}{\alpha}\right]^{\beta}\right]}}\right] \quad (10)$$

3.4. Optimization of the irradiation process

The optimization strategy was based on the maximization of the objective function, defined as the sum of the scores of odor, flavor and appearance. The restraints were related to the safety conditions (Eq. 1) and the shelf-life of the product (Eq. 10).

Initially, it was necessary to find equations which included the objective function and the restraints. These equations have been previously described and selected and they depend on the dose of treatment, which will be the decision variable.

433 The preference of the appearance and odor after 18 days was described by means of
434 the Gompertz Function, using the parameters from Tables 2 and 3. The acceptance of
435 flavor after 18 days was described through the Activation/Inactivation model, using
436 parameters from Table 3. The restraint of the minimum shelf-life (60 days) was
437 described using Eq. 10 and the safety restraint of the number of log reductions for the
438 *L. monocytogenes* population was described using Eq. 8 and it was different for EC
439 compared to USDA. Therefore, two different criteria will be considered in the
440 optimization.

441 Using the Solver tool, the dose was sought which maximized the sum of odor, flavor
442 and appearance scores (objective function) and allowed the specific number of log
443 reductions in the microbial population (safety criterion) and a minimum shelf-life of 60
444 days to be achieved.

445

446 **3.4.1. European Commission's safety criterion**

447 The safety condition established by EC required reductions of the initial microbial
448 concentration of 1.7 log cfu/g. According to the inactivation kinetics of *L.*
449 *monocytogenes*, the minimum dose of irradiation required was 0.81 kGy (Figure 4,
450 Limit EC). The optimum dose will be the one that maximizes the sum of the
451 appearance, odor and flavor scores. The best appearance score was in the range of 0
452 kGy up to 1.8 kGy (in the irradiation range where the appearance is not affected).
453 However, it can be observed that the flavor (log $[F(d_{irr})/F(d_0)]$ =0.1178) and odor (log
454 $[O(d_{irr})/O(d_0)]$ =0.1798) values calculated at 0.81 kGy are close but not at the maximum
455 of their curves. The intensity of 0.81 kGy reduced the initial microbial concentration by
456 1.7 log cfu/g and allowed a shelf-life of 78.5 days to be obtained (Figure 5, calculated
457 from Eq. 10).

When the optimization procedure was applied using the tool Solver, the optimum dose was identified as 0.96 kGy. Moreover, this dose improved the safety restraint obtaining 2.02 log cfu/g reductions. Therefore, the EC safety criterion was met and the shelf-life was extended to 79.46 days (Figure 5), longer than the imposed limit of 60 days.

3.4.2. United States Department of Agriculture's safety criterion

The safety criterion established by USDA required reductions of the initial microbial concentration of 5.2 log cfu/g. This restraint imposes the dose be higher than 2.49 kGy. The appearance, odor and flavor (log $[A(d_{irr})/A(d_0)]$=-0.0656, log $[O(d_{irr})/O(d_0)]$ =-0.0342 and log $[F(d_{irr})/F(d_0)]$ =-0.0900) values calculated (Figure 4), are not close to the maximum of the curves and if the dose increases, all the sensory attributes will decrease (Figure 4). This dose allowed a shelf-life of 86.23 days to be obtained.

According to the safety levels required by USDA (5.2 log cfu/g reductions), using a dose of 2.49 kGy leads to slight changes being observed in the sensory quality after 18 days, compared to non-treated samples. Nevertheless, once the threshold of 2.49 kGy is exceeded, the irradiation will rapidly affect the ham quality, diminishing the acceptability of this product. According to the safety levels required by USDA (5.2 log cfu/g reductions) the optimum dose calculated through Solver tool is coincident with the limit of 2.49 kGy, necessary to meet the safety restraint.

4. Conclusions

Models were formulated to describe the effect of the irradiation dose on the quality attributes (odor, flavor and appearance) of vacuum packaged RTE cooked ham. The spoilage microorganisms affected the odor and flavor of non-treated samples leaving the appearance unaffected. In treated samples, doses of up to 2 kGy did not affect

484 appearance, while doses of around 1 kGy gave the highest scores for odor and flavor
485 after 18 storage days. The EC and USDA safety criterion was followed and the
486 optimum irradiation dose was calculated, reaching an equilibrium between the safety
487 and the quality of the meat products. Mathematical modeling and the use of
488 optimization procedures can lead to a better understanding of the irradiation process
489 and they allow the optimum operational conditions to be determined.

490

491 **5. Acknowledgments**

492 The authors acknowledge the financial support from the project CSD2007-00016
493 (CONSOLIDER-INGENIO 2010) funded by the Spanish Ministry of Science and
494 Innovation.

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FIGURE CAPTIONS

Figure 1. Effect of E-beam irradiation on the appearance of vacuum packaged RTE cooked ham after 2 and 18 storage days. Continuous line: Gompertz Function.

Figure 2. Effect of E-beam irradiation on the odor of vacuum packaged RTE cooked ham after 2 and 18 days. Continuous line: Gompertz function; dashed line: Activation/Inactivation model.

Figure 3. Growth of spoilage microorganisms on vacuum packaged RTE cooked ham after different doses of E-beam irradiation. Continuous line: modified Hill's model.

Figure 4. Effect of E-beam irradiation on the sensory properties (Q: appearance, odor or flavor; A: appearance; F: flavor; O: odor). A, F and O → modeled curves. EC limit: minimum dose of 0.81 kGy to reach reductions of the initial microbial concentration of 1.7 log cfu/g; Optimum dose for EC criterion: 0.96 kGy; Limit and optimum dose for USDA criterion: 2.49 kGy to reach reductions of initial microbial concentration of 5.2 log cfu/g.

Figure 5. Modeling of the spoilage microorganism's growth after 0.814 kGy (dotted line), 0.96 kGy (continuous line) and 2.49 kGy (dashed line) of E-beam irradiation treatment.

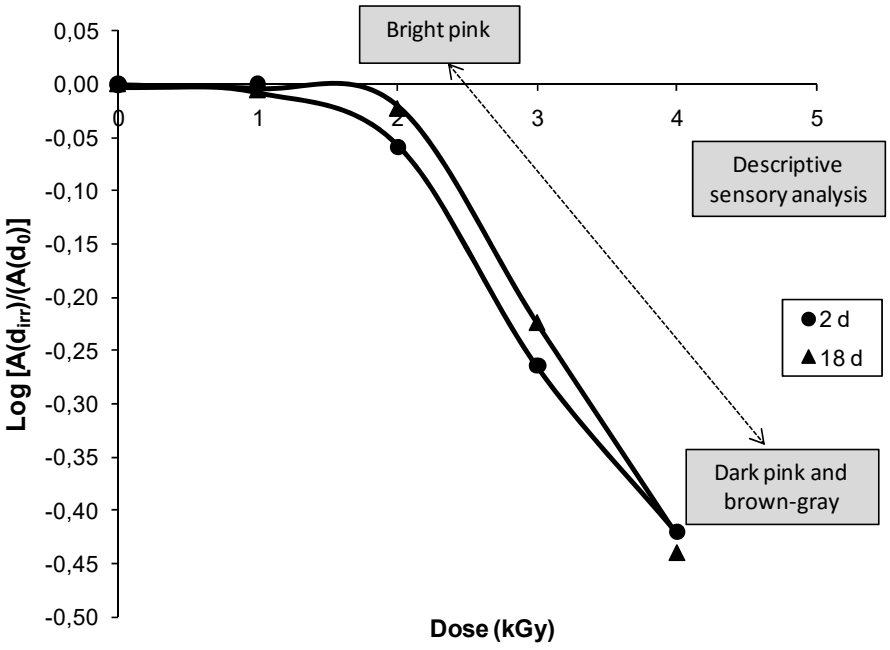
Bright pink
Descriptive sensory analysis
Dark pink and brown-gray
2 d
18 d
Log [A(d_irr)/(A(d_0)]
Dose (kGy)
0,05
0,00
-0,05
-0,10
-0,15
-0,20
-0,25
-0,30
-0,35
-0,40
-0,45
-0,50
0
1
2
3
4
5

Figure 1.

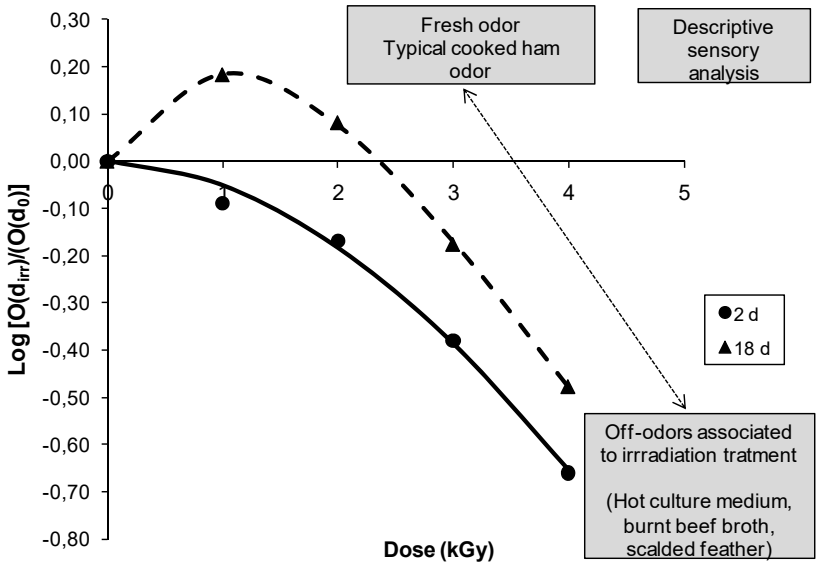
Fresh odor
Typical cooked ham odor
Descriptive sensory analysis
0,30
0,20
0,10
0,00
-0,10
-0,20
-0,30
-0,40
-0,50
-0,60
-0,70
-0,80
0
1
2
3
4
5
Log [O(d_irr)/(O(d_0)]
Dose (kGy)
2 d
18 d
Off-odors associated to irrradiation tratment
(Hot culture medium, burnt beef broth, scalded feather)

Figure 2.

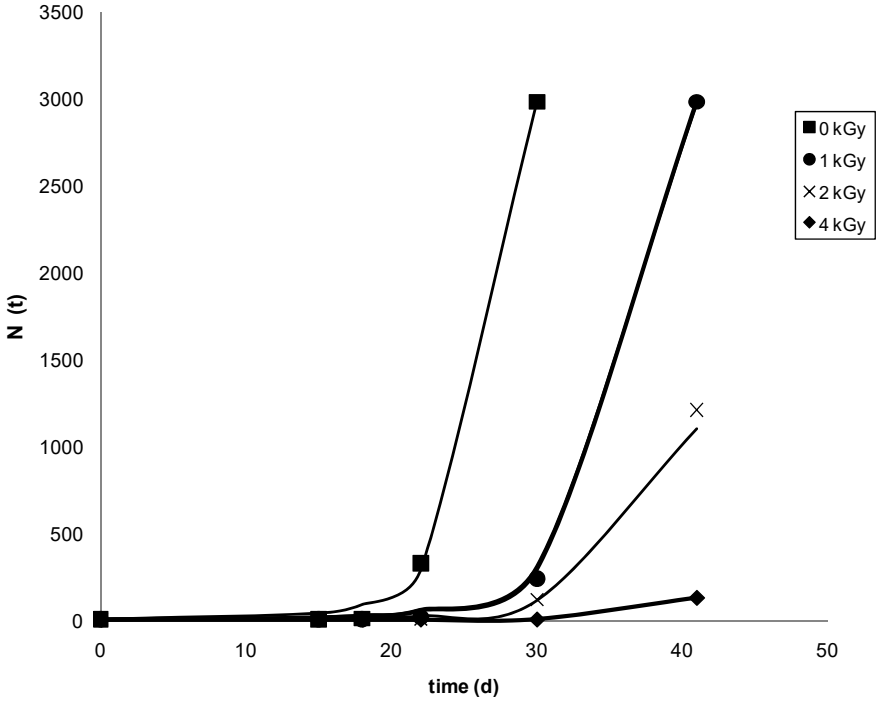
3500
3000
2500
2000
1500
1000
500
0
N (t)
0
10
20
30
40
50
time (d)
0 kGy
1 kGy
2 kGy
4 kGy

Figure 3.

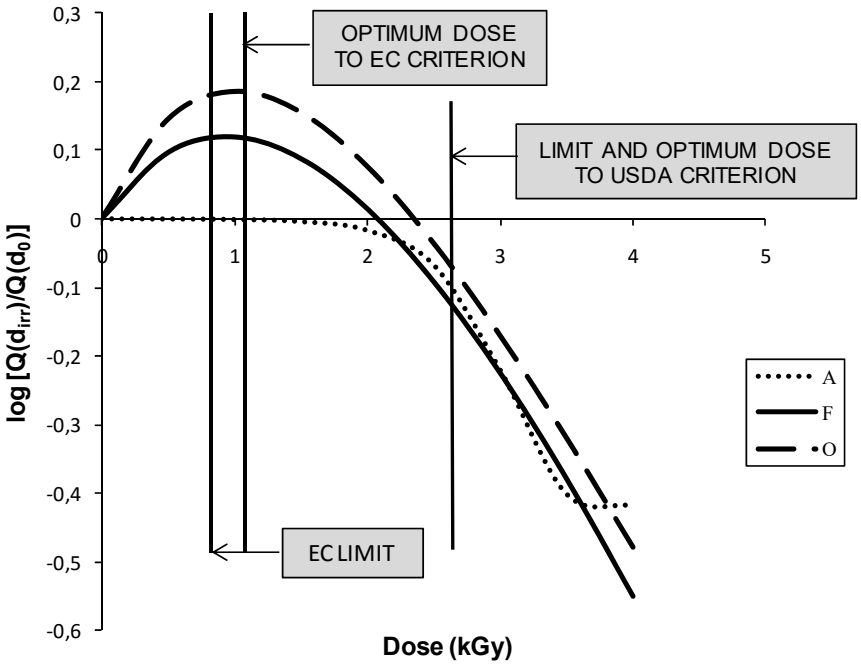
OPTIMUM DOSE
TO EC CRITERION
LIMIT AND OPTIMUM DOSE
TO USDA CRITERION
EC LIMIT
A
F
O
0,3
0,2
0,1
0
-0,1
-0,2
-0,3
-0,4
-0,5
-0,6
0
1
2
3
4
5
log [Q(d_irr)/Q(d_0)]
Dose (kGy)

Figure 4.

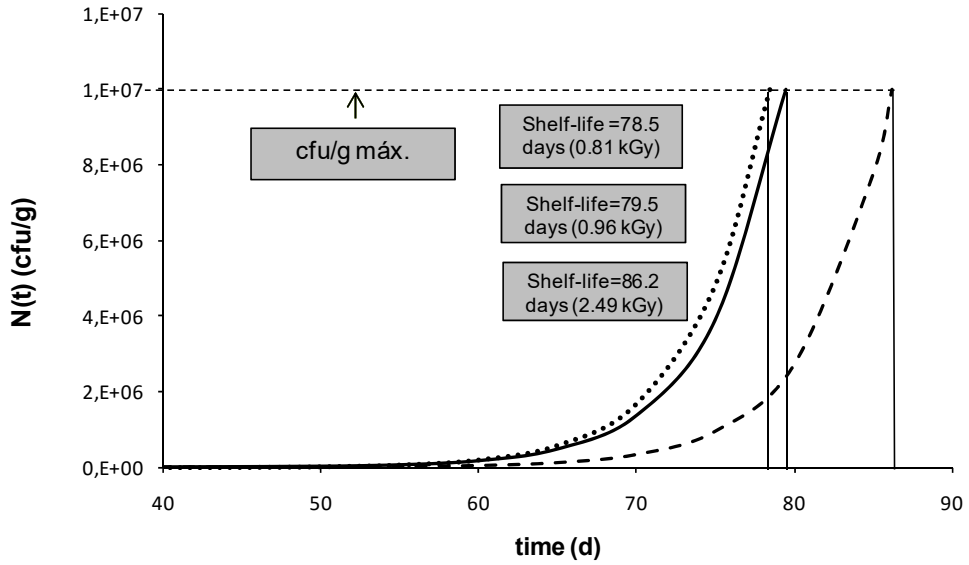
1,E+07
1,E+07
8,E+06
6,E+06
4,E+06
2,E+06
0,E+00
N(t) (cfu/g)
40
50
60
70
80
90
time (d)
cfu/g máx.
Shelf-life =78.5 days (0.81 kGy)
Shelf-life=79.5 days (0.96 kGy)
Shelf-life=86.2 days (2.49 kGy)

Figure 5.

Table 1. Sensory evaluation by rank order test of vacuum packaged RTE cooked ham after 2 and 18 days of irradiation treatment.

Irradiation treatment (kGy)	Appearance		Odor		Flavor	
	2 days	18 days	2 days	18 days	2 days	18 days
0	79_{a}	77_{a}	96_{a}	$63_{b,c}$	91_{a}	71_{a}
1	79_{a}	76_{a}	$78_{a,b}$	96_{a}	$81_{a,b}$	93_{a}
2	$69_{a,b}$	$73_{a,b}$	$65_{b,c}$	$76_{a,b}$	$60_{b,c}$	74_{a}
3	$43_{b,c}$	$46_{b,c}$	$40_{c,d}$	42_{c}	$41_{c,d}$	42_{b}
4	30_{c}	28_{c}	21_{d}	21_{c}	27_{d}	20_{b}

Final score= (N1 X 1) + (N2 X 2) + (N3 X 3) + (N4 X 4) + (N5 X 5), where N1, N2, N3, N4, and N5 are the number of panellists that ranked the sample in position 1 (minimal preference), 2, 3, 4, or 5 (maximum preference) in the rank order test. Within a column, values with different letters are significantly different ($P < 0.05$).

Table 2. Estimated model parameters for modeling the effect of the irradiation dose on the appearance of vacuum packaged RTE cooked ham after 2 and 18 storage days.

	Storage time	Parameters		R^2	RMSE
Gompertz Function	2 days	A	-5.725	0.999	0.001
		B	1.906		
		C	0.422		
	18 days	A	-7.972	0.999	0.002
		B	2.544		
		C	0.440		
Weibull Distribution	2 days	b	0.019	0.973	0.030
		n	2.259		
	18 days	b	0.008	0.979	0.027
		n	2.880		
Inactivation / Activation model	2 days	k_1	4.201	0.932	0.048
		k_2	99.852		
		k_3	27.758		
		m	1.594		
	18 days	k_1	2.500	0.949	0.043
		k_2	99.888		
		k_3	27.743		
		m	2.179		

Table 3. Estimated model parameters for modeling the effect of the irradiation dose on the odor and flavor of vacuum packaged RTE cooked ham after 2 and 18 storage days.

		ODOR				FLAVOR			
	Storage time	Parameters		R^2	RMSE	Parameters		R^2	RMSE
Gompertz Function	2 days	A	-3.426	0.998	0.008	A	-2.477	0.995	0.019
		B	0.522			B	0.804		
		C	3.329			C	0.662		
	18 days	A	-52.403	0.701	0.142	A	7.382	0.901	0.083
		B	17.209			B	-2.474		
		C	0.477			C	-0.596		
Weibull Distribution	2 days	b	0.052	0.992	0.024	b	0.061	0.998	0.007
		n	1.825			n	1.564		
	18 days	b	0.001	0.770	0.124	b	0.003	0.908	0.080
		n	4.549			n	3.779		
Inactivation / Activation model	2 days	k_1	52.564	0.996	0.013	k_1	52.624	0.996	0.013
		k_2	37.859			k_2	38.085		
		k_3	-3.574			k_3	-0.769		
		m	0.352			m	0.383		
	18 days	k_1	0.935	0.999	0.007	k_1	1.054	0.999	0.003
		k_2	0.942			k_2	1.249		
		k_3	0.225			k_3	0.081		
		m	0.405			m	0.322		

Table 4. Estimated kinetic constants and statistical parameters (R^2; RMSE) obtained from the fit of Hill's model to the growth of remaining spoilage microorganisms in irradiated vacuum packaged RTE cooked ham.

	Hill's model				**Hill's model (k depending on dose)**		
Dose (kGy)	P	k	R^2	RMSE (cfu/g)	k	R^2	RMSE (cfu/g)
0	0.205	0.940	0.998	54	0.554	0.978	189
1	0.178	0.063	0.998	41	0.016	0.999	36
2	0.218	0.004	0.999	13	0.005	0.999	12
4	0.222	0.0004	0.998	2	0.001	0.914	116