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Modeling and optimization of the E-beam treatment of chicken steaks and hamburgers, considering food safety, shelf-life, and sensory quality.

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ABSTRACT

The present work was carried out to model the effect of E-beam treatment on the safety, shelf-life and sensory attributes of two poultry products, steaks and hamburgers, and to optimize the radiation treatment. The inactivation of *Salmonella* spp. by means of different radiation doses was modeled using a first order kinetics. The shelf-life was studied by periodically counting the bacterial number in samples. For the modelling of experimental data, only the exponential phase of growth was taking into account. The effect of the radiation dose on the sensory attributes (appearance, odor and flavor) and instrumental color (*L**, *a** and *b** parameters) was modeled using the Gompertz function and the Activation-Inactivation or linear models. The optimization of the radiation dose was carried out by maximizing the sensory scores of samples and minimizing the instrumental color changes. The safety and the shelf-life of samples were assured by introducing constraints in the optimization problem. In the case of hamburgers, the optimum calculated dose was 2.04 kGy, which guarantees the safety of the product and provides the best combination of sensory and instrumental attributes. As regards the steaks, the optimum assessed dose was 1.11 kGy, significantly lower than for hamburgers.

KEYWORDS: radiation, *Salmonella*, appearance, color, objective function, constraint,

1. INTRODUCTION

Nowadays, the sale of food products in individual or domestic portions is very common. In the meat industry, it is possible to differentiate two categories of these presentations: that corresponding with convenience, ready-to-eat (RTE) foods, including different kinds of cooked or dry-cured meats and dry fermented sausages, and small pieces of ready-to-cook (RTC) meat (steaks, hamburgers, etc.). This last category is one of the most common products in the poultry meat industry. To prepare these products, it is necessary to carry out several operations, such as cutting, mixing or shaping, which increase the risk of cross-contamination, the most important contaminant organisms in this case being *Salmonella* spp. and *Campylobacter jejuni* (Nachamkin, 2007; Cutter et al., 2012). Despite efforts by the industry to remove *Salmonella* from meat and poultry carcass, it is not possible to avoid the meat contamination from both the animals during slaughtering and the industrial equipment, which are the main sources of *Salmonella* contamination, not only in the case of the carcasses but also the small meat pieces (Lillard, 1990; Domingues et al., 2002; Ruban et al., 2010; Panisello et al., 2000; Cutter et al., 2012). Consequently, there is a need to improve microbial control so as to minimize the contamination of the final product, in order both to reduce the incidence of foodborne pathogens and to extend the shelf-life of the products.

Heat treatment is the technique most commonly used to reduce the microbial load of many foods. However, this technique cannot be applied to fresh meat due to the associated changes in the product characteristics (flavor, odor, color, appearance or texture). Therefore, to reduce the bacterial number in meat products, including pathogens, different non-thermal sanitizing treatments have been proposed. Of them, high hydrostatic pressure (Lakshmanan and Dalgaard, 2004), oscillating magnetic fields (Barbosa-Cánovas et al. 1998), light pulses (Hierro et al., 2012) or E-beam radiation (Cabeza et al., 2007; Garcia-Márquez et al., 2012) are sound alternatives.

Irradiation is known as an effective way to eliminate foodborne pathogens, such as *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica* or *Escherichia coli* O157:H7 (Cabeza et al., 2007, 2009; Schilling et al., 2009). However, it has been reported that this technology has limited application for meat, since an excessive level of irradiation can produce changes in the sensory properties of treated products, which could significantly affect the consumer acceptance (Arthur et al., 2005; Lee and Ahn, 2005). In this regard, the odor of excessively irradiated meat has been described as being like rotten egg, cooked meat, hot culture medium, sulphur, alcohol, acetic acid, liver-like serumy, and bloody (Brewer, 2009; Cabeza et al., 2007). Therefore, the adjustment of the radiation dose is a critical point; it must be set to a

level that allows an adequate level of microbial inactivation to be achieved (food safety), while at the same time minimizing the changes in treated meat to avoid consumer rejection and, an additional, valuable feature, to extend the shelf-life.

The mathematical modelling of the influence of the radiation dose on the microbial load reduction and the sensory properties retention of E-beam treated products, permits the quantification of effects and, in a later step, the optimization of treatments (Benedito et al., 2011). For that purpose, it is necessary to consider models that define the inactivation of microorganisms, the change in the sensory properties and the growth of spoilage microorganisms after treatments in order to estimate the product shelf-life. The model proposed by Bigelow (1921) is probably one of the most commonly used to describe microbial thermal inactivation. This traditional first-order model was developed as a way of quantifying the effect of the duration of the heat treatment on the reduction of the viable organisms. A similar model was used, replacing the time variable by the radiation dose, to assess the influence of this factor on the inactivation of *L. monocytogenes* in vacuum-packaged cooked ham (Benedito et al., 2011). Other models that have also been used, not only to define microorganism or enzyme inactivation, but also the kinetic changes of quality attributes, are the Gomperzt function (Ding et al. 2010) or the Activation-Inactivation model (Soysal, 2008).

The shelf-life of products treated by means of any preservation technology is a factor which it is very important to take into account in order to achieve process optimization and, in the case of meat, it is linked to the growth of microorganisms that produce the spoilage of food. In this regard, of the models used to define the microbial growth, Hill's model or the modified Gompertz equation (Huang, 2010) are worth mentioning. However, the classical linear models have also been used because of their simplicity and the acceptable way they fit to numerous different cases (McKellar and Lu, 2004).

Then, the optimization of the E-beam treatment using the modeling of the radiation effects on the microbial and quality factors of the treated products may be an interesting tool for process design. Although this type of optimization has barely been reported, it has been developed for vacuum-packaged cooked ham (Benedito et al., 2011). However, as far as the authors know, this strategy has not been applied to raw poultry meat. Therefore, the goal of this work was to model the effect of E-beam on the safety, shelf-life and sensory attributes of two chicken products (steaks and hamburgers) and to optimize the radiation treatment, maximizing the product quality while guaranteeing its microbial safety and a reasonable shelf-life.

2. MATERIALS AND METHODS

2.1.Microorganisms

The *Campylobacter jejuni* is a very sensitive microorganism to ionizing radiation being the reported decimal reductions (D-value) lower than 0.2 kGy (Clavero et al., 1994; Verde et al., 2004). For this reason, the target organism selected for this study was the other main pathogenic bacteria present in poultry meat, i.e. Salmonella spp., namely *Salmonella enterica* serovar Enteritidis (CECT4300) and *Salmonella enterica* serovar Typhimurium (CECT 443). The strains were maintained by freezing (-40 ºC) in trypticase soy broth (TSB; Difco), adding 10 % glycerol as the cryogenic agent. Fresh cultures were prepared for each experiment by removing a piece of frozen culture from vials and inoculating it into 9 mL of TSB, then incubating it at 32 ºC for 24h. The cultures were then centrifuged (at 4 ºC) and the pellet suspended in beakers with 50 mL sterile saline solution, which yielded a bacterial load of approximately 10^8 cells/mL that were used to contaminate meat samples.

2.2. Sample preparation and radiation treatment

Chicken breasts, immediately separated from the carcass, were obtained in a local market and transported to the laboratory at 4 ºC. Several breasts were cut into steaks (8-12 g, 3 mm thickness). To establish the death kinetics of Salmonellae, a batch of these steaks was contaminated by immersion for one minute in the bacterial suspension obtained as previously described. The contaminated (kinetics studies) and uncontaminated (for the determination of color, sensory characteristics and shelf-life) samples were packed in a plastic bag of low gas permeability (diffusion coefficient of 35 cm^3 24 $h^{-1}m^{-2}$ bar to oxygen and 150 cm^3 24 $h^{-1}m^{-2}$ bar to carbon dioxide) and thermo-sealed. Other set of breasts was chopped (hamburger samples) using a domestic mincing machine and divided in two batches. In order to mimic the deep cross contamination produced in the industry during chopping operation, a set was contaminated with a bacterial suspension (bs) at the ratio 1ml bs/20 g meat previously to be minced. Then, aliquots of 20 g (contaminated and no contaminated batches) were placed into Petri dishes (0.5 cm diameter and 0.5 cm height). Once hamburgers were ready, they were handled as described for steaks. The samples were transported (less than 1 h) in insulated polystyrene boxes (<5ºC) to the irradiation plant (IOSNISIOS sterilization S.A., Tarancón, Cuenca, Spain) and irradiated under an electron beam radiation source, which operates at 10 MeV. The radiation doses employed were 0, 1, 2, 3, and 4 kGy. The actual dose absorbed by the samples was determined by assessing the absorbance of cellulose triacetate dosimeters (STM, American Society of Testing and Materials, 2000) simultaneously irradiated with the samples. The experiments were carried out in triplicate at room temperature (18 - 20 ºC) and,

after treatment, the samples were transferred to the laboratory and stored at 4 ºC until use. During treatment, the product temperature increased by less than 2 ºC.

2.3. Microbial analyses

To count the number of total viable organisms in hamburgers and steaks, 10 g of sample were homogenized with 90 mL of a sterile saline solution in a Stomacher bag for two minutes. Counts were determined on the surface of plates with TSB and using a spiral plate system (model Eddy Jet, IUL Instrument, Barcelona, Spain). Since the fresh poultry only presents a very complex microbiota dominated by the indigenous microbiota, a selective medium VRBG (violet red bile glucose agar, Oxoid) was used for *Salmonellae* enumeration in order to avoid the count of background microbiota. Previously, it was observed that this strategy did not affect the growth of the survival cells (Cambero et al., 2011). In every case, plates were incubated at 32 ºC for 24-36 h. Colonies were enumerated with an automatic counter (Countermat Flash model, IUL Instrument, Barcelona, Spain).

2.4. Sensory analyses

Independent triangular, rank order and descriptive test trials were carried out on three sensory characteristics of meat samples, appearance, odor and flavor, in order to determine the possible influence of the radiation dose (0, 1, 2, 3 and 4kGy) on meat quality. The samples were evaluated by a panel of twenty tasters (ten women and ten men). The panelists were previously trained and familiarized with the terms necessary to describe the sensory characteristics of the chicken steaks and hamburgers, such as general appearance (color and brightness), odor (richness and intensity), off-odor (absence), taste (richness of taste notes, off-taste absence, tenderness, juiciness and after-taste intensity) as well as the expected off-sensory features resulting from the E-beam treatment (off-odors and taste, such as hot culture medium, sulfuric, metallic, scalded feather, burnt feather, pungent pepper). The evaluations were performed in individual booths built according to the International Standards Organization DP66.58 criteria (ISO, 1981a). The evaluation was carried out between meals, after breakfast, and before the midday meal. To reduce fatigue, panel members performed three sessions per day with a minimum break of 1 h between sessions. Three independent tests were performed to evaluate appearance, odor and flavor.

Prior to sensory analysis, the samples were removed from the refrigerator and maintained in a temperature-controlled room at 10 ºC for no more than 1 h. For the appearance test, closed thermo-sealed bags with the steak or hamburger samples were supplied to the panelist, thus avoiding direct contact with the sample. For the odor test, the bags with samples were opened

just before the test. To evaluate the flavor, samples of around 5 g were cooked in a domestic microwave (800 W) at 30 % of the total power for 60 s. Afterwards, they were maintained (2-5 min) at 75 ºC in an infrared oven until the test. The tasters received unsalted crackers and water at room temperature to cleanse the palate between samples. White fluorescent light was used during appearance analysis. The odor and flavor of the samples were evaluated under red light conditions.

The sensory analyses were carried out just after treatment and after 5 days of storage at 4 ºC. The triangle test (ISO, 1981b) was performed by the forced choice option, in which the tasters must choose the sample that, in their opinion, is different. All the possible combinations of untreated and irradiated samples were tested.

For the rank order test, five samples (untreated and treated) were supplied for each test and the panelists were instructed to rank samples in order of preference, according to the proximity of the sensory characteristic (appearance, odor or flavor) of the analyzed sample to the fresh product. For this, a 5-point preference scale (in which 1 corresponded to the lowest and 5 to the highest) was used. No repetitions were allowed. Results of the rank order test were used to obtain the sum of ranks, which corresponds to the sum of the scores of sample preference (the sum of the products of the value given for each sample on a 5-point scale multiplied by the number of times that each sample was allocated this specific score) for a specific sensory characteristic. The significance level of data was determined by the Friedman rank addition following the model proposed by Joanes (1985) and the tables for multiple comparison procedures for the analysis of ranked data (Christensen et al., 2006). The sum of the ranks, as quantitative values of the sensory evaluation, was used in the modeling, statistical analysis, and optimization of the irradiation process.

Panelists were also asked to provide information regarding specific characteristics (color, brightness, odor, taste, texture, any off-sensory feature) of the chicken samples by following a profile descriptive analysis (ISO, 2003). They were also asked to qualify the intensity of these sensations with the following terms: negligible or very slight, slight, moderate and strong.

2.5. Instrumental color determination

The color of the sample surface was measured using a tristimulus colorimeter (Minolta Chroma Meter CR300, Minolta Corporation, NJ) provided with a 10º standard observer and a D65 standard illuminant. In each sample, the L, a and b coordinates (CIE-Lab) were measured in quadruplicate. The measurements were taken the same day of the irradiation treatment and

after 5 days of storage at 4 ºC and in both cases after 4-5 minutes after opening the bags containing the samples.

2.6. Shelf-life determination

To assess the shelf-life, irradiated and non-irradiated samples were removed from the bags and the total viable counts were determined. From a microbiological point of view, the end of shelf-life was established when this exceeded the value of 5×10^7 cfu/g. Analyses were performed just after E-beam treatment (0 days) and at various times during storage (2, 4, 6, 8 and 11 days) until the end of the shelf-life. The shelf-life was also assessed by means of sensory analysis (odor and visual appearance).

2.7. Estimation of the food safety objective (FSO)

For Salmonellae, the "zero tolerance" (absence in 25 g of product) is a universal criterion and, therefore, it has been adopted in most microbiological regulations. This level may be considered as equivalent to a FSO of 4 cells/100 g (log10 = -1.39). Previously, it has been reported that, according to the zero tolerance criterion, a treatment of 1.5 kGy warrants safe ready-to-eat intermediate moisture foods (dry-cured ham, beef or smoked tuna), which accounted for a Salmonellae reduction of 2.3 D. The water activity (a_w) of these products is lower than 0.88 (Cambero et al., 2012) and, therefore, *Salmonellae* are unable to grow. However, since the value of a_w is higher (about 0.985) in fresh poultry, the growth of *Salmonellae* is possible, particularly if a temperature abuse (for example, a temperature increase up to 8 - 10 ºC) is produced during distribution or even at home. This fact and the reasoning used previously for sanitizing mayonnaise potato salad (Cambero et al. 2012), since the egg is also a product in which the *Salmonellae* acquire importance, was followed in this case to increase the degree of decimal reduction to 2.7 D to achieve the FSO.

2.8. Modeling inactivation of *Salmonella* spp. by E-beam radiation

To describe the influence of the radiation dose on the inactivation of *S*. Enteritidis and *S*. Typhimurium, a first order kinetics model was used, replacing the time variable by the treatment dose (d_{irr}) (**Equation 1**), according to Benedito et al. (2011).

$$Log\left(N_{d_{irr}}\right) = Log\left(N_{d_0}\right) - k \cdot d_{irr} \quad (1)$$

Where $log(N_{d_{irr}})$ and $log(N_{d_0})$ are the decimal logarithm of the number of microorganisms (cfu/g) after a radiation dose of d_{irr} and without treatment, d_0, respectively, and *k* is the rate constant (kGy^{-1}).

2.9. Modeling the E-beam radiation effect on sensory properties

To describe the effect of radiation on the sensory attributes measured by panelists, several inactivation models were assessed. However, only those which most accurately fitted the data are included. Specifically, the Gompertz Function (**Equation 2**) and the Activation-Inactivation model (**Equation 3**) (Mckellar and Lu, 2004) were adapted, replacing the time of treatment by the irradiation dose:

$$Log\left(\frac{S_{d_{irr}}}{S_{d_0}}\right) = C \cdot exp\big(-exp(A + B \cdot d_{irr})\big) - C \cdot exp\big(-exp(A)\big) \quad (2)$$

$$Log\left(\frac{S_{d_{irr}}}{S_{d_0}}\right) = \left[\frac{d_{irr}}{k_1 + k_2 \cdot d_{irr}}\Big(1 - log\big(1 + exp(k_3 \cdot d_{irr})\big)^m\Big)\right] \quad (3)$$

where $S_{d_{irr}}$ and S_{d_0} are the scores assigned by panelists for the appearance, odor or flavor of samples irradiated using different doses (d_{irr}) or without irradiation (d_0). The *A*, *B* and *C* variables correspond with the model parameters of Gompertz Function and the k_1, k_2, k_3 and *m* correspond with those of the Activation-Inactivation model. The models were fitted to the data of sensory parameters corresponding to the same day that irradiation took place (0 days) and after 5 days of storage at 4 ºC.

2.10. Modeling the influence of E-beam radiation on instrumental color.

From the experimentally measured CIE-Lab coordinates (a^* and b^*), the chroma was calculated (**Equation 4**).

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

The influence of the radiation dose on chroma was described by a linear relationship (**Equation 5**).

$$Chroma = p + q \cdot d_{irr} \quad (5)$$

2.11. Modeling the E-beam radiation effect on the product shelf-life.

Throughout storage, the changes in the total microorganism count in hamburgers and steaks, radiated or not, were modeled through a first order kinetics model. The meat could be considered as a biological reactor where organisms are in the exponential growth phase. Under these conditions, the number of microorganisms during storage could be estimated from a balance of biomass (**Equation 6**).

$$\frac{d(M_S X)}{dt} = \mu M_s \quad (6)$$

where

M_s represents the mass of meat sample (g), X the number of microorganisms per mass unit (ufc/g), t the storage time (days) and μ the rate of microorganism growth. Considering only the exponential growth phase, μ is the constant maximum growth rate. **Equation 7** is obtained by integrating **Equation 6**.

$$Ln\left(\frac{X_t}{X_0}\right) = \mu \cdot t \quad (7)$$

where X_0 and X_t stand for the number of microorganisms at the beginning of storage and after time t, respectively.

2.12. Model fitting and statistical analyses of experimental data

The parameters of the models included in **Equations 1**, **5** and **7** were identified by linear regression using the Microsoft Excel 2007™ software. In the case of **Equations 2** and **3**, the model parameters were identified by minimizing the sum of the square differences between the experimental and calculated data of sensory properties using Solver tool from Microsoft Excel™.

In order to assess the ability of these models to fit the experimental data, the percentage of explained variance (*%var*, **Equation 8**) and the mean relative error (*MRE*, **Equation 9**) were computed (Lipson and Sheth, 1973).

$$\%\ var = \left(1 - \frac{S_{se}^2}{S_s^2}\right) \quad (8)$$

$$MRE = \frac{100}{N}\sum_{i=1}^{N}\frac{|v_{ei} - v_{ci}|}{v_{ei}} \quad (9)$$

where S^2_s and S^2_{se} are the variance of the sample and the estimation, respectively, N the number of experimental data and v_{ei} and v_{ci} the values of the experimental and calculated variables, respectively.

On the other hand, the multifactor ANOVA and the LSD (Least Significant Difference) intervals (Statgraphics Plus 5.1; Statistical Graphics Corp.) were calculated to evaluate the significance of the differences between the experimental measurements carried out on samples treated with different radiation doses.

2.13. Optimization of the radiation process

The main objective of the study was to optimize the radiation dose in order to achieve the inactivation of Salmonellae, and then to reach a safe level, with minimum changes in the quality factors of poultry. Then, after modeling the influence of irradiation on both microbiological and quality factors, a mathematical optimization approach was performed. For that, an objective function was defined, the decision variables selected and some restraints were considered. The highest quality of meat was linked with the highest sensory property scores (appearance, odor and flavor) and the smallest color changes (instrumentally measured). Therefore, the objective function chosen was the ratio between the sum of scores for the sensory parameters divided by the color changes and the optimization algorithm was set to maximize this function. The restraints considered were those related with both the food safety and the shelf-life of the product. For food safety, it was considered that the irradiation treatment must produce a 2.7D reduction of *Salmonella* spp., according to the FSO defined in **Section 2.8**. On the other hand, the shelf-life was considered to be expired when the bacterial counts reached the level 5 x 10^7cfu/g. The decision variable was the radiation dose. The optimization problem was solved using the Generalized Reduced Gradient method available in the Solver tool of Microsoft Excel™.

3. RESULTS AND DISCUSSION

3.1. Influence of radiation on the inactivation of *Salmonella* spp.

As expected, the radiation of chicken samples led to a reduction in the load of Salmonellae proportional to the intensity of the treatment. As can be observed in **Table 1**, the first order kinetics model proposed in **Equation 1** was adequate for describing the effect of the radiation dose on the lethal power of E-beam. Thus, the percentage of explained variance (*%var*) of the model was above 98% for both *S.* Enteritidis and *S*. Typhimurium. For the latter, the inverse of the identified *k* (**Table 1**), the decimal reduction dose (*D*), was 0.41 kGy in steaks and 0.52 kGy in hamburgers, and this difference was statistically significant ($p<0.05$). These data coincide with what is reported in literature being then the microbial inactivation rate dependent on the way the meat has been processed (Radomyski et al., 1994). In any event, the *D* values obtained are in the reported range. Thus, Thayer et al. (1990) reported a value of 0.53 kGy for mechanically deboned poultry, Grant and Patterson (1991) observed values from 0.40 to 0.44 kGy for pork and more recently, a figure of 0.53 kGy has been reported for both dry fermented sausages (Cabeza et al., 2009) and dry cured ham (Cambero et al., 2012).

The *D*-values observed for *S*. Enteritidis, 0.37 kGy and 0.39 kGy in steaks and hamburgers respectively, were significantly lower ($p<0.05$) than those found for *S*. Typhimurium. Moreover,

317 the differences between steaks and hamburgers were not significant (**Table 1**) for this serovar.
318 In this case, the values found in the literature were more variable, ranging from 0.77 kGy in
319 mechanically deboned chicken to 0.17 kGy in growth media (Thayer et al., 1990), although
320 similar values to those obtained in the present study have also been reported, e.g. 0.37 kGy in
321 poultry meat (Mulder et al., 1977) or 0.41 kGy in dry fermented sausages (Cabeza et al., 2009).

322 From these results, it may be concluded that *S*. Enteritidis was significantly more sensitive to
323 radiation than *S*. Typhimurium and, therefore, the latter may be considered as the target
324 microorganism in poultry steaks and hamburgers in order to ensure the product safety.

325 **3.2. Effect of irradiation on meat quality**

326 **3.2.1. Sensory attributes**

327 In the triangular analysis performed just after radiation treatment, no significant differences
328 ($p > 0.05$) were detected in the sensory attributes of hamburgers when samples of 0 and 1 kGy
329 were compared (**Table 2**). All the other combinations of sample treatments (both for chicken
330 steaks and hamburgers) exhibited differences in, at least, one sensory characteristic. Many
331 combinations showed differences in two or three sensory attributes (**Table 2**). In general,
332 significant differences ($p < 0.05$) in appearance and odor were found between the untreated
333 and radiated samples. No significant differences ($p > 0.05$) were detected between samples
334 treated at 3 and 4 kGy (Table 2). The results of the microbiological analysis indicated that the
335 untreated chicken products were not suitable for consumption after 5 days of refrigerated
336 storage ($> 5 \times 10^7$ cfu/g), which agrees fully with the flavor analysis performed. Significant
337 differences ($p < 0.05$) in flavor were detected when 1 kGy treated samples were compared
338 with those subjected to doses of over 2 kGy.

339 The results of the sensory analysis obtained by means of the rank order test on the chicken
340 products are shown in **Table 3**. As in the case of the triangular test, the panelists' scores
341 showed that there were significant differences ($p<0.05$) in appearance, odor and flavor
342 between samples irradiated with different doses, not only for hamburger samples but also for
343 steaks (**Table 3**). Storage for 5 days at 4 ºC also affected the sensory attributes. Thus, in the
344 case of their appearance, the hamburger samples treated with doses above 2 kGy showed
345 higher scores than non-radiated ones **(Table 3)**, even after 5 days of storage. The appearance
346 descriptive analysis of samples showed that the yellowish aspect of fresh hamburgers became
347 pink-purple; the higher the radiation dose, the more intense the pink. In this sense, the
348 samples irradiated at 3 or 4 kGy showed a similar pattern to that described for pork or turkey
349 hamburgers. Nam and Ahn (2002) attributed the increase in the pink and red color of

350 irradiated meat to the formation of a carbon monoxide-myoglobin complex induced by the
351 production of carbon monoxide and reducing conditions during radiation treatment. Samples
352 treated at 2 kGy retained the appearance during storage but the non-radiated samples (0 kGy)
353 and those treated with 1 kGy became pale and grayish.

354 On the contrary, for the steak samples (**Table 3**), the higher score obtained for samples
355 irradiated above 2 kGy became, after storage, lower than that of non-radiated steaks. The 2
356 kGy treated samples exhibited a pink color and a high degree of brightness that, despite not
357 being the conventional color of chicken steaks, was associated with a higher quality fresh
358 meat. However, after 5 days of storage, the color of the samples treated above 3 kGy became
359 pale, yellowish and grayish.

360 The dose applied also affected the odor just after radiation (0 days), reducing the scores for
361 both steaks and hamburgers as the radiation dose increased (**Table 3**). However, after 5 days
362 of storage, samples radiated at doses over 2 kGy scored better than non-radiated ones, and
363 the odor differences between radiation doses decreased. The odor of samples radiated at 1
364 and 2 kGy was described as “scalded feather”, while at 3 and 4 kGy it was defined as
365 "irradiated" and "sulfured", which could make the commercialization of this product difficult.
366 However, the intensity of this odor decreased after the storage time, achieving a final value
367 which is adequate for consumption. Similar findings concerning the elimination of radiation
368 off-odors during storage have been reported (Nam and Ahn, 2003; Brewer, 2009; García-
369 Márquez et al., 2012), which has been explained by the dissipation of some volatiles (e.g.,
370 dimethyl disulfide, dimethyl trisulfide, S-methyl ester) aroused during radiation (Du et al.,
371 2002).

372 As regards the flavor, the results after radiation (0 days) were similar to those found for odor
373 (**Table 3**). Just after treatment, steaks and hamburgers treated at doses higher than 2 kGy
374 showed lower scores than non-treated samples. In the descriptive analyses, irradiated samples
375 (particularly those treated at doses over 2 kGy) were judged less juicy and they presented a
376 very slight taint of ‘‘burnt’’ or ‘‘hot culture medium’’ and a slightly astringent aftertaste,
377 although they were considered acceptable for sale. It should be emphasized that the samples
378 were cooked before carrying out the test, which can favor the dissipation of off-odors during
379 cooking (Hashim et al., 1995). In general, after storage, panelists’ scores of the samples treated
380 with 1, 2, 3 or 4 kGy only showed non-significant differences (**Table 3**). Several authors have
381 reported (Du and Ahn, 2002; Du et al., 2002) that post-radiation storage could allow the flavor
382 to return to levels close to those of the untreated products as the volatile compounds are lost.

383 The adapted Gompertz Function (**Equation 2**) and the Activation-Inactivation model (**Equation**
384 **3**) were fitted to the scores attributed by panelists to the appearance, odor and flavor
385 characteristics of chicken-breast steaks and hamburgers. **Table 4** shows the identified model
386 parameters, only including the model that best fitted each sensory attribute. Thus, the
387 Gompertz function was the model that best fitted the appearance data (**Table 4, Figure 1**). In
388 the case of odor and flavor, the Activation-Inactivation model achieved the highest values of
389 percentage of explained variance (**Table 4**). Flavor is defined as the combined chemical
390 sensations of taste and smell. Consequently, odor and flavor behaved in a similar way just after
391 irradiation (0 days) and after 5 days of storage at 4 ºC. The model was able to describe the
392 influence of the applied irradiation dose on the odor of meat samples (**Figure 2**). The data
393 concerning flavor was found to follow a similar tendency to that shown in **Figure 2**.

394 The fact that the radiation dose just after treatment had a different effect from the one after
395 5, days of storage should be highlighted. After treatment, the tendency exhibited by the model
396 between the doses applied and the odor or flavor scores was negative (**Figure 2**); the lowest
397 scores were achieved as the applied radiation dose rose. This pattern changed for the samples
398 stored for 5 days. In this case, the increase in the radiation dose led to an increase in the
399 scores, but once a maximum was reached, the increase in the radiation dose brought about a
400 decrease in the scores. Therefore, this behavior showed the existence of an optimum radiation
401 dose, which provided the best flavor and odor after 5 days of storage.

402 **3.2.2. Instrumentally determined color**

403 The experimental results obtained from the CIE Lab analysis are shown in **Table 5**. The steak
404 samples had lower a^*, b^* and L^* values than the hamburger samples, probably due to the fact
405 that the surface directly exposed to the air is much higher for the latter meat product. No clear
406 trend was identified between the radiation dose or storage time and the L^* coordinate in
407 hamburger samples. In steak samples, the radiation produced a decrease in the L^* value
408 compared to the non-radiated samples, but no relationship was identified between L^* and the
409 radiation dose applied. The L^* values of the untreated steaks after 5 days of storage at 4 ºC
410 were lower than those obtained just after the E-beam treatment, which could be related to
411 the loss of the surface water. However, the brightness of the radiated samples was not
412 significantly (p<0.05) affected by storage.

413 With regard to the a^* and b^* parameters, the radiation affected both coordinates. Then, the
414 values measured just after radiation rose as a larger radiation dose was applied (**Table 5**),
415 which means that both the hamburger and the steak samples became pink in colour. Thus, the

instrumental measurements of color were consistent with the results obtained in the sensory analyses (**Tables 2 and 3**) and the color changes observed by other authors (Nam and Ahn, 2002) in meat and meat products after radiation. Some authors have speculated that when the myoglobin is primarily in the $MbFe^{3+}$ form, the radiation produces an increase in both the *a** (Giroux et al., 2001; Satterlee et al., 1972) and *b** (Brewer, 2009) values in all meat species.

After 5 days of storage, the values of *b** for steaks were lower than those observed just after radiation, but the relationship between *b** and the radiation dose was maintained. Similar behavior has been observed in fresh pork loin (Garcia-Marquez et al., 2012). However, in the case of the hamburgers, no significant differences were detected in the *b** values associated with storage.

Therefore, it being difficult to identify a clear influence on the *L** coordinate, the *a** and *b**parameters were the main ones affected by the E-beam treatment. For this reason, the evaluation of the global changes in sample color induced by radiation was carried out by chroma (**Equation 4**). As can be observed in **Figures 3A** and **3B**, the relationship between radiation dose and chroma followed a linear trend. **Equation 5** was an adequate means of describing this relationship (**Table 6**), as can be observed in **Figures 3A** and **3B** and from the mean relative error value obtained, in all cases lower than 5.26 %. The low value of the percentage of explained variance obtained for the samples stored for 5 days can be attributed to the wide experimental variability of the data.

As may be seen in **Table 6**, the y-intercept, *p*, was higher in hamburgers than in steaks according to the higher values of *a** and *b** measured in the former product. The slope of the linear relationship, *q*, of the just radiated hamburgers was almost twice as high as that obtained for steaks, which indicates that the color of hamburger was more sensitive than that of steaks to the increase in radiation. However after 5 days of storage, the identified slopes for hamburgers and steaks were quite similar.

3.3. Modeling of microorganism growth in E-beam radiated samples.

Compared with the non-radiated samples, radiation slowed down the growth of the surviving microorganisms (**Figure 4**). From **Table 7** (columns μ regardless of dose), it may be observed that the proposed model (**Equation 7**) was found to be an adequate method of describing microbial growth, the percentage of explained variance being higher than 97 % and the mean relative error lower than 7.5%.

The higher the dose applied, the lower the kinetic parameters of the model (μ regardless of dose), which shows how the microorganisms' growth rate was affected by radiation. Radiation

was found not only to have an effect on the initial inactivation of bacteria as shown in **Section 3.1,** but also on their survival and multiplication rate, leading to a delay in meat spoilage. This relationship, between μ and the radiation dose, was also linear for both the hamburger (**Equation 10**) and steak (**Equation 11**) samples.

$$\mu = -0.196 \cdot d_{irr} + 1.716 \; ; \quad r^2 = 0.96 \tag{10}$$

$$\mu = -0.276 \cdot d_{irr} + 1.529 \; ; \quad r^2 = 0.93 \tag{11}$$

Therefore, from **Equations 10** and **11** it was possible to estimate the μ parameter by only taking the applied radiation dose into account. While the y-intercept was quite similar for both products (similar growth when no irradiation was applied), the negative slope for steak samples was 40 % higher than those of the hamburger samples. This indicates that the increase in the irradiation dose was more effective at slowing down the microorganisms' growth in steaks than in hamburgers.

For the purposes of obtaining a single equation that predicted the growth of microorganisms at any irradiation dose, **Equations 10** and **11** were combined with **Equation 7**, yielding **Equation 12** for hamburgers and **Equation 13** for steaks.

$$Ln\left(\frac{X_t}{X_0}\right) = (-0.196 \cdot d_{irr} + 1.716) \cdot t \tag{12}$$

$$Ln\left(\frac{X_t}{X_0}\right) = (-0.276 \cdot d_{irr} + 1.529) \cdot t \tag{13}$$

As can be observed from the *%var* and *MRE* (%) (**Table 7,** columns μ dependent on dose), the fit of the model proposed in **Equations 12** and **13** is slightly poorer than the fit provided by **Equation 7,** where μ was fitted for each radiation considered. However, **Equations 12** and **13** allowed the post-radiation microorganism growth to be predicted in the interval from 0 to 4 kGy for both steaks and hamburgers, only considering the radiation dose as a factor. Accordingly, this model was used to estimate the shelf-life of meat samples in the optimization procedure.

3.4. Process optimization.

The aim of the defined optimization problem was to find the radiation dose that provided the best panelists' scores for appearance and odor and reduced the instrumentally measured color change in samples after 5 days of storage. The radiation effect on the appearance scores was described using the Gompertz function, the influence it had on the flavor scores by means of the Activation-Inactivation model (parameters shown in **Table 4**) and how it affected color

changes using chroma (**Table 7**). Therefore, the objective function to be maximized is shown in **Equation 14**.

$$OF = \frac{\left[C \cdot exp\left(-exp(A + B \cdot d_{irr})\right) - C \cdot exp\left(-exp(A)\right)\right] + \left[\left[\frac{d_{irr}}{k_1 + k_2 \cdot d_{irr}}\left(1 - log\left(1 + exp(k_3 \cdot d_{irr})\right)^m\right)\right]\right]}{p + q \cdot d_{irr}} \quad (14)$$

Two constraints to the objective function were considered: first, the radiation dose applied must produce a log reduction of Salmonellae of 2.7 *D* (**Section 2.8**) and, secondly, during the 5-day shelf-life of both products, steaks and hamburgers, the bacterial count must be under 5 x 10^7cfu/g. The effect of the irradiation dose on Salmonellae was modeled through **Equation 1** using the estimated parameters shown in **Table 1** and the influence on the bacterial growth by **Equations 12 and 13**, for hamburgers and steaks, respectively and the estimated parameters in **Table 7**.

The optimization results obtained for hamburger samples showed that the optimum radiation dose was 2.04 kGy. This value was the result of combining the changes in the appearance, odor and chroma with the increase in radiation dose (**Figure 5A**). The appearance factor exhibited an increasing trend while odor showed a maximum value at a dose of around 1.1 kGy, subsequently decreasing the more dose was applied. The chroma value also increased linearly with the radiation dose applied, which means that the color difference between radiated and non-radiated samples increased in line with the radiation dose. For that reason, this factor was placed in the denominator in the objective function. As a result, the objective function showed a maximum value, representing the optimum irradiation dose which provides the best combination of sensory and instrumental attributes as considered by **Equation 14**. This estimated optimum dose of 2.04 kGy was enough to ensure that the microorganism will grow less (1.2 x 10^6cfu/g) than the limit considered (5 x 10^7cfu/g) throughout the shelf-life period in question (5 days). Furthermore, it was also enough to exceed the FSO for salmonella (2.7*D*), since the treatment will produce a reduction of 3.95*D* (**Figure 5A**).

As for the steaks, the evolution of appearance score (decreased as the irradiation dose increased) differ from those found in the case of hamburgers, which affected the evolution of the objective function (**Figure 5B**). Thus, the maximum value of the objective function was reached at a lower radiation dose, i.e., 0.95 kGy than the obtained for hamburgers. This means that, above this dose, the sensory features and instrumental color parameters of radiated samples were worse than the optimum and this fact would negatively affect the consumer acceptance. This level of dose guarantees that the 5-day shelf-life constraint is achieved, since

511 the number of microorganisms predicted by the model will be $8.4 \cdot 10^5$cfu/g, almost 2 log units
512 lower than 5 x 10^7cfu/g. However, the Salmonellae number will be reduced by 2.3*D*, a value
513 lower than the constraint (2.7*D*), which, in turn, means that it will not be possible to reach the
514 FSO. As a consequence, it will be necessary to increase the radiation dose in order to achieve
515 the safety goal. The application of the model provided an optimum radiation dose of 1.11 kGy,
516 which, as can be observed in **Figure 5B**, was the minimum value necessary to achieve the FSO
517 for Salmonellae. Therefore, the proposed optimization methodology allowed us to find the
518 radiation dose that provided the best values of the selected quality factors while, at the same
519 time, achieving the necessary microbial safety and stability of the product.

520 **4. CONCLUSIONS**

521 The models proposed were an adequate means of describing the effect of a radiation dose on
522 both the sensory (appearance, odor and flavor) and instrumental (color) attributes of the two
523 raw chicken products (steaks and hamburgers). In the same way, the influence of radiation on
524 the inactivation of *Salmonella* spp. and the shelf-life of radiated samples was properly
525 predicted. From this mathematical modeling and by applying an optimization procedure to the
526 defined objective function, it was possible to identify an optimum radiation dose that provided
527 the best quality attribute values but also guaranteed the food's safety and stability
528 requirements. This procedure can help the process management by permitting objective
529 decision-making to be adopted.

530

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Table 1. First order kinetics modeling (Equation 1) of the *Salmonella* spp. inactivation in chicken meat as a function of the radiation dose

Product	Microorganisms	*Log* (Nd_0)	*k* (kGy^{-1})	*% var*	*MRE* (%)
Steaks	***S.* Enteritidis**	8.23±0.28	2.67±0.15	98.26	12.14
	***S.* Typhimurium**	8.28±0.16	2.43±0.09	99.25	2.99
Hamburgers	***S.* Enteritidis**	9.06±0.24	2.60±0.14	98.55	9.66
	***S.* Typhimurium**	8.79±0.14	1.94±0.07	99.15	4.34

$log(N_{d_0})$ decimal logarithm of the number of microorganisms (cfu/g) before treatment; *k* is the rate constant; %var is the percentage of explained variance by the model; MRE(%) is the mean relative error of the model

Table 2. Sensory characteristics of untreated and E-beam radiated chicken breast products (hamburgers and steaks) showing significant differences (P < 0.05) in the triangular test just after treatment (0) and after 5 days of storage at 4 ºC.

Dose (kGy)	0[a]	1	2	3	4	
0[a]		O_5	A_0, O_0, F_0, A_5, O_5,	A_0, O_0, F_0, A_5, O_5	A_0, O_0, F_0, A_5, O_5	**Hamburgers**
1	A_0, O_0, O_5			A_0, O_0, F_0, A_5, O_5	A_0, O_0, F_0, A_5, O_5, F_5	
2	A_0, O_0, O_5	O_0		O_5	A_0, O_0, F_0, O_5	
3	A_0, O_0, F_0, O_5	A_0, O_0, F_0	O_0, F_0			
4	A_0, O_0, F_0, O_5	A_0, O_0, F_0, O_5	O_0, F_0, O_5			
	Steaks					

A_0, O_0, F_0, A_5, O_5, F_5: Significant differences ($p < 0.05$) in appearance (A), odor (O) and flavor (F) after 0 ($_0$) or 5 ($_5$) days of storage at 4 ºC

[a] In non-radiated samples, the flavor test was not performed since they were spoiled after 5 days of storage.

Table 3. Sensory evaluation by means of the rank order test of untreated and E-beam radiated chicken breast products (hamburgers and steaks) just after treatment (0) and after 5 days of storage at 4 ºC.

	Hamburgers						Steaks					
	Appearance		Odor		Flavor		Appearance		Odor		Flavor	
Dose	Storage days											
(kGy)	0	5	0	5	0	5	0	5	0	5	0	5
0	22cα	31bα	92aα	20bβ	92a	NP	34cβ	69aα	98aα	20cβ	88a	NP
1	43bcα	48bα	82aα	87aα	74aα	89aα	32bβ	65aα	78abα	80aα	82aα	74aα
2	62abα	57abα	53bβ	76aα	66aα	76abα	68aα	58aα	60bα	79aα	67aα	65aα
3	84aα	84aα	44bβ	74aα	38bβ	66bα	88aα	57aβ	32cβ	70abα	35bβ	66aα
4	89aα	80aα	29bα	43bα	30bα	49bα	78aα	51aβ	32cα	51bα	28bβ	75aα

Different Latin characters in the same column (a,b,c) or Greek characters in the same row (α, β) for each sensory feature of each chicken product indicates significant differences ($p < 0.05$).

Final scoring = $(N_1 \times 1)+(N_2 \times 2)+(N_3 \times 3)+(N_4 \times 4)+(N_5 \times 5)$, where N_1, N_2, N_3, N_4 and N_5 are the number of panelists that ranked the sample in position 1 (the lowest), 2, 3, 4 or 5 (the highest) in the rank order test.

NP, Flavor test was not performed in non-radiated samples since they were spoiled after 5 days of storage. In this case, only the flavor of the irradiated samples was evaluated [Final scoring = $(N_1 \times 1)+(N_2 \times 2)+(N_3 \times 3)+(N_4 \times 4)$].

Table 4. Parameters of the models used for describing the effect of radiation on the appearance, odor and flavor of chicken breast products (hamburgers and steaks) after 0 and 5 days of storage at 4 ºC.

			Hamburgers		Steaks	
Attribute	**Model**	**Parameters**	**0 days**	**5 days**	**0 days**	**5 days**
Appearance	**Gomperzt**	**A**	0.23	0.29	27.01	0.34
		B	-0.89	-0.75	-13.14	-0.24
		C	0.90	0.63	0.39	-0.38
	%var		0.99	0.93	0.98	0.93
Odor	**Activation-Inactivation**	**k_1**	22.33	1.66	55.26	0.32
		k_2	55.82	-4.80	44.18	0.31
		k_3	47.98	3.47	48.31	0.67
		m	0.94	-2.72	0.77	0.91
	%var		0.97	0.88	0.88	0.99
Flavor	**Activation-Inactivation**	**k_1**	47.98	0.31	11.57	$1 \cdot 10^{-10}$
		k_2	52.79	0.09	81.60	$1 \cdot 10^{-6}$
		k_3	5.55	0.91	29.85	1.63
		m	0.53	1.06	1.10	1.80
	%var		0.94	0.99	0.92	0.97

A, *B* and *C* and k_1, k_2, k_3 and m are the parameters of the models relating the effect of the radiation dose on the sensory scores (appearance, odor and flavor). Equations 2 and 3.

Table 5. CIE-Lab analysis of untreated and E-beam radiated chicken breast products (hamburgers and steaks) just after treatment (0) and after 5 days of storage at 4 ºC.

Chicken breast product	Dose (kGy)	L^*		a^*		b^*	
		Days of storage					
		0	5	0	5	0	5
Hamburger	**0**	62.5±0.6 a,α	62.0±0.8 a,α	13.6±0.8 a,α	14.7±0.5 a,α	8.9±0.7 a,α	8.9±0.5 a,α
	1	62.2±0.6 a,α	62.4±0.6 a,α	15.4±0.7 a,α	15.5±0.6 a,α	9.3±0.7 a,α	9.6±0.6 a,α
	2	60.7±0.7 a,α	62.4±0.9 a,α	16.3±0.8 ab,α	15.1±0.9 a,α	10.2±0.8 ab,α	9.0±0.9 a,α
	3	63.3±0.7 a,α	61.0±0.9 a,β	17.3±0.7 b,α	15.9±0.7 ab,β	12.0±0.8 b,α	9.6±0.4 a,β
	4	61.6±0.7 a,α	62.0±0.8 a,α	18.3±0.8 b,α	17.4±0.8 b,α	12.2±0.8 b,α	11.7±0.6 b,α
Steak	**0**	59.2±0.8 a,α	57.6±0.6 a,β	3.2±0.3 a,α	2.7±0.5 a,α	3.0±0.3 a,α	2.3±0.3 a,β
	1	55.6±0.9 b,α	56.4±0.7 ab,α	4.0±0.4 a,α	3.3±0.4 ab,α	3.7±0.3 a,α	3.0±0.4 b,β
	2	53.5±0.8 b,α	55.0±0.9 b,α	4.3±0.3 ab,α	3.9±0.2 b,α	4.0±0.6 ab,α	3.5±0.5 b,β
	3	53.5±0.9 b,α	55.0±0.9 b,α	5.3±0.3 b,α	3.7±0.4 b,β	4.4±0.3 b,α	3.6±0.4 b,β
	4	54.0±0.7 b,α	55.0±0.9 b,α	5.2±0.3 b,α	5.4±0.6 c,α	5.1±0.4 b,α	4.9±0.3 c,α

*L**: lightness, *a**: redness, *b**: yellowness.

Different Latin characters in the same column (a,b,c) or Greek characters in the same row (α, β) for each parameter of each chicken product indicates significant differences ($p < 0.05$).

Table 6. Linear relationships between the radiation dose and chroma in samples of chicken breast products (hamburgers and steaks).

Meat sample	Days after radiation	p	q	% var	MRE (%)
Hamburger	0	16.393	1.457	99.14	0.74
	5	16.935	0.787	71.26	3.25
Steak	0	4.508	0.738	97.37	2.02
	5	3.506	0.823	89.09	5.26

p and *q* are the parameters of the model relating the effect of the radiation dose on chroma (Equation 5); %var is the percentage of explained variance by the model; MRE(%) is the mean relative error of the model

Table 7. Parameters of first order kinetics model for microorganism growth in samples of chicken breast products (hamburgers and steaks), treated with different E-beam doses.

Dose (kGy)	Hamburgers						Steaks					
	μ regardless of dose			μ dependent on dose			μ regardless of dose			μ dependent on dose		
	μ	% var	MRE (%)	μ	% var	MRE (%)	μ	% var	MRE (%)	μ	% var	MRE (%)
0	1.81	99.01	2.81	1.72	98.13	3.20	1.57	99.09	2.31	1.57	98.85	2.53
1	1.45	97.78	4.53	1.50	97.31	4.42	1.08	97.59	4.59	1.22	92.06	7.79
2	1.35	94.63	7.39	1.31	94.41	7.63	1.01	97.01	3.81	0.96	96.39	3.85
3	1.12	98.37	2.87	1.12	98.37	2.87	0.84	98.47	2.20	0.69	87.39	5.63
4	1.01	97.96	3.44	0.92	95.49	4.94	0.31	97.22	1.05	0.41	63.30	5.23

μ the rate of microorganism growth; %var is the percentage of explained variance by the model; MRE(%) is the mean relative error of the model