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Modelling the effect of gamma irradiation on the inactivation and growth kinetics of psychrotrophic bacteria in squid rings during refrigerated storage. Shelf-life predictions

Alejandra Tomac [a,*], Rodolfo Horacio Mascheroni [b,c,d], María Isabel Yeannes [a,c]

[a] Grupo de Investigación Preservación y Calidad de Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Juan B. Justo 4302, Mar del Plata B7608FDQ, Argentina
[b] CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos), CONICET La Plata – UNLP, Calle 47 y 116, La Plata 1900, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, 5° piso, (1033), CABA, Argentina
[d] MODIAL, Facultad de Ingeniería, UNLP, Argentina

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ABSTRACT

Psychrotrophic bacteria behaviour, when gamma irradiation is applied with shelf-life extension purposes to a fresh squid product, was modelled. In this regard, the effect of gamma irradiation at 0, 1.8, 3.3 and 5.8 kGy on psychrotrophic microorganisms in vacuum-packed squid (*Illex argentinus*) rings was analysed during storage at 4–5 °C. First-order kinetics satisfactorily described the radio-induced inactivation of the initial psychrotrophs population. The growth of surviving bacteria during storage was fitted to two empirical models: modified Gompertz model and a polynomial expression dependent on irradiation dose and storage time. In turn, the influence of irradiation dose on kinetic parameters of Gompertz model was described by second order polynomials. Both proposed models satisfactorily described the behaviour of psychrotrophs as affected by gamma irradiation, allowing accurate shelf-life predictions for doses up to 5.3 kGy. Considering the predictions accuracy, complete Gompertz model was preferred and model validation was done for irradiation at 4.8 kGy.

1. Introduction

Irradiation processing of food has demonstrated to be a safe and effective method for reducing or eliminating biological hazards in foods (WHO, 1994). After more than 50 years of safety studies, irradiation of one or more foods has been approved in more than 60 countries (Sommers and Fan, 2006). When food is exposed to ionising radiations, the critical target is the microorganism cell DNA (Moseley, 1989). In turn, some chemical changes occur in food main constituents, but nutritional adequacy is not compromised (ICGFI, 1999; Josephson et al., 1978). Gamma irradiation has been used as an effective method to extend the shelf-life of fish products by reducing qualitatively and quantitatively the microbial population (Arvanitoyannis and Stratakos, 2010). The microbial resistance to the irradiation treatment is affected by different factors such as irradiation process parameters, microorganisms characteristics, and product parameters. According to Mañas and Pagán (2005) Gram negative bacteria, the typical spoiling flora of fishery products, are among the least irradiation resistant microorganisms. Microorganisms response to irradiation treatment has been described to fit first-order inactivation kinetics (Mañas and Pagán, 2005) from which the D_{10}-value can be estimated. The D_{10}-value is the irradiation dose necessary to reduce microbial counts of a particular microorganism or group of microorganisms in one logarithmic cycle.

Quality, shelf-life and safety of foods are determined mainly by the presence and growth of spoilage and pathogenic microorganisms. The feasibility of developing safe foods is based on the adequate understanding of microorganisms behaviour towards inactivation agents. Furthermore, the knowledge of the relations between intrinsic (pH, water activity, etc.) and extrinsic (temperature, atmosphere, etc.) factors and responses of spoilage and pathogenic microorganisms in foods is essential to assess and manage their safety and shelf-life (McMeekin et al., 1993). Quantification and description of these relations through mathematical models represent a great benefit for food technology since they allow making predictions of microorganisms growth or decline (Coll Cárdenas et al., 2001; McMeekin et al., 1993; Zwietering et al., 1990). Primary models describe the response of microorganisms with time for given conditions. Gompertz equation and modified Gompertz equation (Zwietering et al., 1990) are among the most widely used primary models (Gibson and Roberts, 1989). Mathematical modelling of microbial growth also represents great advantages for food industry in terms of costs and time saving. In particular, finding a

* Corresponding author. Tel.: +54 223 481 6600x286.
E-mail addresses: atomac@fi.mdp.edu.ar, alejandratomac@hotmail.com (A. Tomac).

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mathematical expression that predicts microbial behaviour as affected by gamma irradiation would imply the reduction of time and costs involved with preliminary irradiation tests. Furthermore, these models would allow shelf-life predictions when food is treated with gamma irradiation with shelf-life extension purposes.

Illex argentinus is the most abundant squid species of the Southwest Atlantic Ocean region and the second fishery in volume for Argentina, representing about 27% of total marine catches (MINAGRI, 2007). Fresh and frozen squid products (mantle, fins, tentacles and rings) supply mainly domestic and international markets, respectively. After squid is caught, it spoils due to microbial and enzymatic reactions. According to Huss (1995), the initial as well as the spoiling flora of fish is often dominated by Gram negative psychrotrophic bacteria. Lapa-Guimarães et al. (2005) observed psychrotrophic bacterial increases of 4 logarithmic cycles in whole *Loligo plei*, during iced storage.

Tomac and Yeannes (2012) worked for the first time preserving a slightly processed product of *I. argentinus* by gamma irradiation, improving its quality and shelf-life.

Considering the aforementioned, the objectives of this work were:

- To experimentally determine psychrotrophic bacteria counts in squid rings irradiated at 0, 1.8, 3.3 and 5.8 kGy, during storage at 4–5 °C.
- To describe mathematically the inactivation of psychrotrophic bacteria by gamma irradiation and to estimate the D_{10}-value.
- To predict psychrotrophic bacteria growth in irradiated vacuum-packed squid rings during refrigerated storage by fitting:
- Experimental growth curves of psychrotrophs to a primary empirical growth model (modified Gompertz) and finding mathematical expressions that describe Gompertz parameters dependence on irradiation dose (secondary models).
- Experimental data to an empirical polynomial model which describes psychrotrophic bacteria growth dependence on irradiation dose and storage time.
- To find an expression that predicts shelf-life of vacuum-packed squid rings for different radiation doses.

2. Materials and methods

2.1. Samples source, irradiation treatment and storage conditions

Peeled squid (*I. argentinus*) mantle rings (1.2 ± 0.3 cm wide), pre-treated with a sodium polyphosphate solution, were acquired in Mar del Plata (Argentina). Samples of 110 ± 2 g (20 rings approx.) were vacuum packed in our laboratory using bags of LDPE and PA (Cryovac®, 125 μm) and a Minimax 430 M machine (Sevivac, Argentina). Samples were transported (4 ± 3 °C) to the semi-industrial irradiation facility of the Ezeiza Atomic Centre (National Atomic Energy Commission of Argentina, activity: 600,000 Ci) and gamma irradiated with a Cobalt-60 source at 1.8, 3.3 and 5.8 kGy (minimum absorbed doses). Doses were determined with Amber Perspex dosimeters. Irradiated and non-irradiated samples (control, 0 kGy) were stored at 4–5 °C during 29 days. Samples were analysed before irradiation (day 0) and days 1, 5, 8, 12, 15, 19, 22, 26 and 29. There were 3 samples for each analysis day and for each dose.

2.2. Microbiological analysis

Ten grams of sample in saline solution (0.85%) with 0.1% w/w peptone (ICMSF, 1983) made to 100 ml were macerated in a Stomacher 400 Homogenizer. Counts of psychrotrophic bacteria were performed on Plate Count Agar, incubating at 7 ± 0.5 °C for 10 days (ICMSF, 1983). Analyses were done in triplicate.

2.3. Modelling psychrotrophic bacteria inactivation induced by gamma irradiation. Determination of D_{10}-value

First-order kinetics was used to model psychrotrophs inactivation due to gamma irradiation, by replacing the variable time by irradiation dose (d) in Eq. (1), where $N(d_0)$ and $N(d)$ are the number of psychrotrophic microorganisms before irradiation treatment and after an irradiation dose, respectively. The rate constant value, k (kGy^{-1}) for given treatment conditions were obtained from the linear regression analysis of log $[N(d)/N(d_0)]$ versus irradiation dose. The value of D_{10} for psychrotrophic microorganisms was determined by calculating the reciprocal of the slope ($1/k$).

$$\log [N(d)/N(d_0)] = -k * d \tag{1}$$

2.4. Modelling psychrotrophic bacteria growth during refrigerated storage. Shelf-life predictions and model validation

To predict shelf-life extension gained by gamma irradiation, two different approaches based on empirical modelling were used to model the growth curves of the surviving psychrotrophs in irradiated vacuum-packed squid rings during storage at 4–5 °C:

2.4.1. Modified Gompertz model

Experimental data of psychrotrophic growth curves for each irradiation dose applied (0, 1.8, 3.3 and 5.8 kGy) were fitted to a primary growth model, modified Gompertz equation (Zwietering et al., 1990):

$$\log N = \log N_0 + A \exp\left[-\exp\left(\frac{\mu e}{A}(L-t)\right) + 1\right] \tag{2}$$

where $\log N$ is the decimal logarithm of the number of psychrotrophic bacteria counts at time t and $\log N_0$ the psychrotrophic counts the day after irradiation treatment ($\log$CFU/g), μ is the specific growth rate ($\log$CFU/g day^{-1}), L is the lag phase duration (days), A is the logarithmic population increase (difference between the upper asymptote ($\log N$ when time t tends to infinity) and the initial counts) and e is the Euler number (2.7182 approx.). Data was fitted to modified Gompertz equation by nonlinear regression (using Marquardt algorithm) with OriginPro® version 8.0 software (OriginLab Corporation, Northampton, MA). The fitting and the accuracy of the estimations for experimental data obtained from each radiation dose was evaluated by the Residual Sum of Squares (RSSs) and the determination coefficient (R^2). Considering that RSS is a measure of the discrepancy between the data and the estimation model, a small RSS would indicate a tight fit of the model.

In order to predict psychrotrophic bacterial counts for different radiation doses it was necessary to find a relationship between Gompertz kinetic parameters (μ, L and A) and the irradiation dose. To find these secondary models, data were fitted to second order polynomial equations: $\mu = \mu_1 + \mu_2 d + \mu_3 d^2$; $L = L_1 + L_2 d + L_3 d^2$ and $A = A_1 + A_2 d + A_3 d^2$. Afterwards, a full model (the complete modified Gompertz model, **CMGM**) was developed by introducing the inactivation equation (Eq. (1)) and the secondary models into the modified Gompertz equation (Eq. (2)).

2.4.2. Polynomial model

Polynomial regression was used to fit experimental data to an empirical expression in order to describe the dependence of surviving psychrotrophs growth on irradiation dose and storage time. A polynomial model was proposed:

$$y = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \beta_3\chi_1^2 + \beta_4\chi_2^2 + \beta_5\chi_1\chi_2 \quad (3)$$

where y is the decimal logarithmic count of psychrotrophic bacteria (log CFU/g), χ_1 and χ_2 are the independent variables irradiation dose (kGy) and storage time (days), respectively, β_0 is the independent coefficient, β_1 and β_2 are the linear terms, β_3 and β_4 are the quadratic terms and β_5 is the interaction coefficient.

The adequacy of the fitting model and the significance of linear, quadratic and interaction terms in the global model were studied through analyses of variance (ANOVA) of the polynomial models using R (R Development Core Team, 2008).

The polynomial model and the CMGM fit to experimental data were compared by the percentage average relative error (PEr), calculated considering the number of data analysed (n) by:

$$\mathrm{PEr} = 100\frac{1}{n}\sum_{i=1}^{n}\left|\frac{\text{Experimental value} - \text{Calculated value}}{\text{Experimental value}}\right|$$

A different set of experimental data was used to validate the CMGM and to evaluate the accuracy of the shelf-life predictions. Psychrotrophic bacterial counts of squid (*I. argentinus*) rings treated with gamma irradiation at 4.8 kGy and stored at 4–5 °C during 77 days (Tomac et al., 2012) were used for CMG model validation. Psychrotrophs counts were determined on days 1, 5, 8, 13, 16, 19, 22, 26, 33, 40, 47, 54, 68 and 77. The CMGM predictions of psychrotrophic counts (log N) were plotted against the experimental values in order to analyse their correlation. Also, the estimation of the CMGM shelf-life was compared with the experimental value.

2.5. Statistical analysis

A two-ways ANOVA test ($p < 0.05$) was used to analyse the significance of irradiation dose (0, 1.8, 3.3 and 5.8 kGy), days of storage (0, 1, 5, 8, 12, 15, 19, 22, 26 and 29 d) and interaction between them. Tukey test was used to compare means ($p < 0.05$). The R software (R Development Core Team, 2008) was used.

3. Results and discussion

3.1. Microbiological results

Before irradiation treatment, initial psychrotrophic bacterial counts in vacuum-packed squid rings were 4.45 ± 0.01 log CFU/g. Microbiological analysis carried out the day after irradiation indicated that gamma irradiation at 1.8, 3.3 and 5.8 kGy significantly ($p < 0.05$) reduced initial counts to 3.50, 2.66 and <1 log CFU/g, respectively. Meanwhile, the day after irradiation was applied, psychrotrophic bacterial counts increased in nonirradiated samples up to 4.51 log CFU/g.

During refrigerated storage, psychrotrophs counts in nonirradiated sample significantly ($p < 0.05$) increased until the end of the storage period. In turn, the growth of surviving bacteria in irradiated samples was retarded by gamma irradiation in a dose dependent way. Experimental growth curves for each irradiation treatment are shown in Fig. 2.

The bactericidal effect of irradiation has been described by many authors in fishery products (Arvanitoyannis and Stratakos, 2010) such as in irradiated whole anchovies (Lakshmanan et al., 1999) and *Merluccius hubbsi* (Lescano et al., 1990), among others. When exposed to irradiation, the main occurrence of damage in the microbial cell is in the DNA (Mañas and Pagán, 2005). Moseley (1989) explained that the hydroxyl radical ($OH^{\cdot}$) is able to react with the sugar-phosphate backbone in the DNA chain causing the appearance of single and double strand breaks.

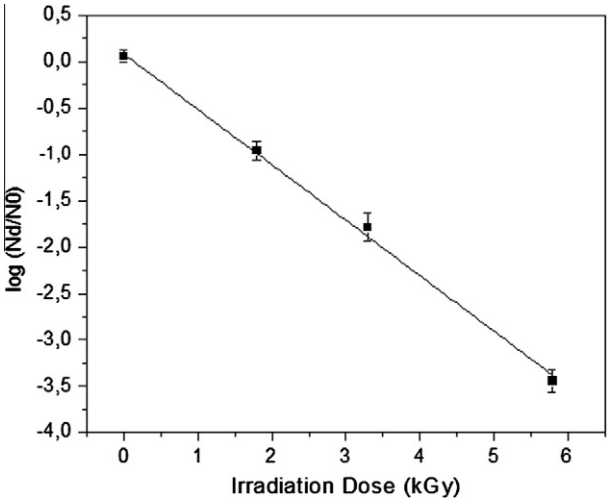

Fig. 1. Survival curve of psychrotrophic bacteria in squid rings treated with gamma irradiation (mean ± standard error, $n = 3$).

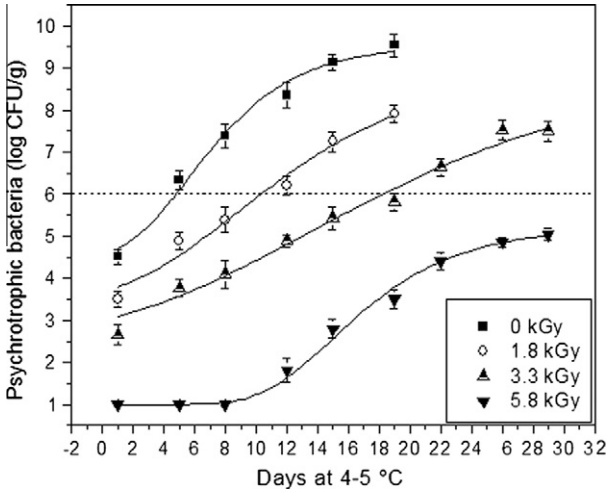

Fig. 2. Psychrotrophic bacteria counts (log CFU/g) evolution during storage of squid rings treated with gamma irradiation (mean ± standard error, $n = 3$). Continuous lines represent the fitting of the modified Gompertz equation.

3.2. Modelling psychrotrophic bacteria inactivation

Psychrotrophic bacteria inactivation induced by gamma irradiation is described in the survival curve that appears in Fig. 1. From the linear regression analysis it was derived Eq. (4), which satisfactorily described ($R^2 = 0.998$) the inactivation of psychrotrophic bacteria caused by gamma irradiation. For this product, when the irradiation dose increases 1 kGy, a 0.606 log reduction in psychrotrophic counts is accomplished. From this relation the D_{10}-value for psychrotrophs was obtained as the reciprocal of the slope, in accordance with various authors (Bassen et al., 1989; Wang et al., 2010), indicating that 1 logarithmic cycle reduction is achieved with 1.65 kGy. As mentioned in the introduction, several factors (food composition, temperature, packaging atmosphere, etc.) influence on the resistance to microbial inactivation by irradiation, that is the reason why different D_{10}-values for the same microorganism can be found in literature. Though in this case no reports of D_{10}-values for psychrotrophic bacteria in irradiated squid were found, Prachasitthisakdi et al. (1984) worked with frozen shrimp and from the psychrotrophic bacteria inactivation that they

informed D_{10}-value for psychrotrophs was estimated to be 1.176. Some authors have reported shoulders in the typical survival curves for bacterial inactivation by irradiation. They attributed it to repairable cellular damage when low doses are applied (Moseley, 1989). In this work no shoulders were detected (Fig. 1).

Eq. (4) is useful to predict microbial inactivation caused by gamma irradiation between 0 and 5.8 kGy, provided the initial bacterial count is known.

$$\log N(d) = -0.606 * d + 4.572 \quad (4)$$

3.3. Modelling psychrotrophic bacteria growth during refrigerated storage

3.3.1. Empirical growth model

Modified Gompertz equation is an empirical model that has been largely used to model microbial growth (Gibson and Roberts, 1989). Various works indicate that experimental data were better modelled with Gompertz compared to other models.

Fig. 2 shows experimental psychrotrophic populations (dots) for squid rings irradiated at 0, 1.8, 3.3 and 5.8 kGy fitted to modified Gompertz equation (solid black line). It can be seen that experimental data presented a sigmoid pattern. Gompertz parameters (μ, L, A), determination coefficient (R^2) and Residual Sum of Squares (RSSs) of the adjusted modified Gompertz model for different irradiation doses are shown in Table 1. All four experimental sets of data were well fitted to Gompertz, as indicated by the high determination coefficients (R^2 = 0.964–0.994) and a low RSS (<0.5). Other authors have satisfactorily fitted experimental microbial growth in food with Gompertz model (Gianuzzi et al., 1998; Huang, 2010; Lebert et al., 2000). In particular, Zhang et al. (2006) modeled microbial growth kinetics in gamma irradiated fresh-cut lettuce using modified Gompertz equation.

Results obtained from fitting Gompertz equation to experimental data indicated that specific growth rate (μ) tended to decrease with increasing irradiation dose with respect to nonirradiated sample. However, $\mu_{5.8kGy}$ was higher than $\mu_{3.3kGy}$. This phenomenon could be explained by the flora selection caused by irradiation at a higher dose (5.8 kGy). The initial inactivation of the predominant flora caused by irradiation would have allowed the subsequent growth of more radio resistant flora, initially inhibited by the predominant species. This behaviour was also explained by Kodo (1990) based on results found in irradiated scomber fillets. The effect of irradiation dose on lag phase (L) was evident for the higher dose applied in this work, since irradiation at 5.8 kGy significantly increased L in about 8 units, meanwhile irradiation at 1.8 and 3.3 kGy did not cause a considerable extension of the lag phase. The length of the lag phase depends on a wide variety of factors including the bacterial population and the time required to recover from physical damage induced by irradiation. The effect of irradiation is proportional to the dose intensity, indicating that higher doses would then cause microbial death and cell damage to a greater extent, so bacterial reductions of the initial flora would be larger, as observed previously. Samples irradiated at higher doses will have smaller bacterial counts, and this population will be seriously injured. Cells that are damaged may require more time to synthesise macromolecules and repair damage before they can divide. During the lag phase, there is no increase in cell number, but there are significant metabolic activities inside the cells, such as repair mechanisms. For these reasons the lag phase in the samples irradiated at higher dose intensity would be longer, since counts would be reduced and the inactivated (but not death) surviving flora would be repairing the damage induced by irradiation until they are capable of growing.

The relationship between the parameters μ, L and A, and the irradiation dose was determined by fitting data to second order polynomial equations. The best fitting polynomial expression for each parameter was:

$$\mu = 0.4329 - 0.1232d + 0.0169d^2 \ (R^2 = 0.990)$$

$$L = 1.4963 - 1.3812d + 0.4670d^2 \ (R^2 = 0.976)$$

$$A = 5.0590 + 0.8392d - 0.1626d^2 \ (R^2 = 0.865) \quad (5)$$

Galati et al. (2011) satisfactorily described the influence of temperature on Gompertz parameters (μ, L and A) with second order polynomials when modelling the effect of temperature and water activity on the growth of *Aspergillus parasiticus* in maize.

By introducing secondary polynomial models (Eqs. (5)) into Gompertz equation (Eq. (2)), as well as the inactivation model derived from experimental data (Eq. (4)) a full model was developed, the complete modified Gompertz model:

$$\log N = -0.606d + 4.572 + \left(5.059 + 0.839d - 0.163d^2\right) \exp E \quad (6)$$

where

$$E = \left[-\exp\left(\frac{\left(0.433 - 0.123d + 0.017d^2\right) e}{\left(5.059 + 0.839d - 0.163d^2\right)} \times \left(1.496 - 1.381d + 0.467d^2 - t\right)\right) + 1 \right]$$

d is the irradiation dose (kGy), N is the psychrotrophic bacteria count for an irradiation dose (log CFU/g), e is the Euler number and t the storage time in days. This complete model is useful to predict psychrotrophic populations for a determined irradiation dose applied, provided the initial microbial count is given, for an irradiation dose range of 0–5.8 kGy. Microbial counts predictions of this model presented 4.5% PEr.

3.3.2. Polynomial model

A polynomial model was proposed to analyse the fitting of the data to a simpler mathematical expression that describes the dependence of psychrotrophic bacteria growth on irradiation dose and storage time. Statistical analyses of the complete model (Eq. (2)) indicated that the quadratic term of the variable irradiation dose β_3 was not significant ($p > 0.05$). The model that best

Table 1
Gompertz parameters (μ, L, A), determination coefficient (R^2) and Residual Sum of Squares (RSSs) of the adjusted modified Gompertz model to psychrotrophic bacteria growth curves of squid rings irradiated at 0, 1.8, 3.3 and 5.8 kGy.

Radiation dose (kGy)	M (log CFU/g days^{-1})[a]	L (days)	A (log CFU/g)	R^2	RSS
0	0.429 ± 0.066	1.251 ± 0.865	5.181 ± 0.361	0.98	0.193
1.8	0.278 ± 0.044	1.206 ± 1.265	5.653 ± 1.391	0.96	0.279
3.3	0.200 ± 0.020	1.341 ± 1.250	6.398 ± 1.061	0.98	0.427
5.8	0.290 ± 0.024	9.342 ± 0.561	4.385 ± 0.215	0.99	0.099

[a] Mean value ± standard error.

described the experimental data ($R^2 = 0.98$) was $y = 4.9330 - 0.8169\chi_1 + 0.2772\chi_2 - 0.0018\chi_2^2 - 0.0140\chi_1\chi_2$ (Eq. (7)) being y the psychrotrophic counts in log(CFU/g) and χ_1 and χ_2 the independent variables dose and storage time, respectively. Table 2 depicts the results of the ANOVA test for the significant model ($p < 0.0001$). The prediction surface given by this model showed the different rates of psychrotrophs growth during storage and the higher initial counts reductions reached by higher doses, as described by the inactivation model (figure not shown). This model allows estimating the psychrotrophic counts for a given radiation dose with 11.9% of PEr.

3.3.3. Shelf-life prediction and model validation

The usefulness of these models lies in the feasibility to predict shelf-life of a slightly processed squid product, when treated with gamma irradiation. For this, shelf-life was estimated as the amount of days until psychrotrophic counts reached 1×10^6 CFU/g (horizontal dotted line in Fig. 2), which is considered the maximum recommended value for marine species (Lapa-Guimarães et al., 2002; Özden et al., 2007), and was also found to coincide with evident signs of spoilage in *I. argentinus* rings (Tomac et al., 2012). In order to find the expressions that predict shelf-life with both models, the variable time (t) in the CMGM (Eq. (6)) and the polynomial model (Eq. (7)) was expressed as function of irradiation dose. In turn, $\log N$ was replaced by $\log(1 \times 10^6$ CFU/g) and different storage times were calculated for irradiation doses between 0 and 5.8 kGy. Both models predictions, together with experimental results are shown in Fig. 3, where it was plotted predicted shelf-life in days at 4–5 °C against irradiation dose. Both models could give accurate predictions for irradiation at 0, 1.8 and 3.3 kGy. Shelf-life calculated with complete modified Gompertz model and the polynomial model for these doses had 5.0% and 6.5% percentage average relative error, respectively. It was not possible to make accurate predictions of the shelf-life with the proposed models for irradiation doses above 5.5 kGy, for which the curve in Fig. 3 becomes asymptotic to the y-axis, indicating that shelf-life predictions would lack of accuracy for irradiation above 5.3 kGy. This is in agreement with experimental results since the psychrotrophs growth curve for samples irradiated at 5.8 kGy tends to become flat towards the end of storage period indicating that psychrotrophic counts would not reach the recommended maximum (Fig. 2). This would indicate that when irradiating at doses of 5.8 kGy psychrotrophs are injured in such way that even if they were able to repair the damage and grow, microbial populations would not be able to reach counts above the recommended limit value. This behaviour was also observed in later works regarding squid rings irradiation, where psychrotrophic bacteria initially inhibited by gamma irradiation was not able to exceed 1×10^7 CFU/g in samples irradiated at 4.8 kGy, and did not growth in samples irradiated at 8.4 kGy, during 77 days at refrigerated storage (Tomac et al., 2012). According to Mañas and Pagán (2005) ionising radiation can cause sub-lethal injury to microorganisms, indicating that they would be able to repair the damage and outgrow only if experimental conditions are suitable. However, the stress caused by ionising radiation affects the microorganisms homeostasis and they might completely use of their energy when trying to repair mechanisms to recover it. As a consequence, they might become metabolically exhausted and die (Leistner, 2000).

Table 2
ANOVA results for the polynomial model.

Coefficients	Estimate	Standard error	p Value
β_0	4.9330	0.1541	<0.00001
β_1	−0.8169	0.0402	<0.00001
β_2	0.2772	0.0189	<0.00001
β_4	−0.0018	0.0007	<0.1
β_5	−0.0140	0.0029	<0.00001

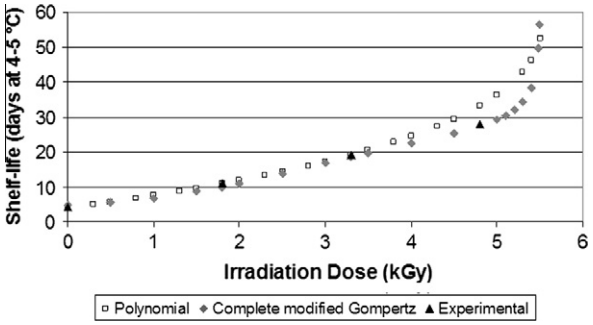

Fig. 3. Predicted and experimental shelf-life for squid rings during storage at 4–5 °C.

3.3.3.1. Complete modified Gompertz model validation. The CMGM was validated using a new set of independent data, which was obtained by irradiating squid rings at a different irradiation dose (4.8 kGy) than the ones used to adjust the model (1.8, 3.3 and 5.8 kGy). The correlation between the CMGM predictions and the experimental values of psychrotrophic bacterial counts ($\log N$) for squid rings irradiated at 4.8 kGy during storage was high ($R^2 = 0.969$). This would indicate that the performance of the CMGM with the independent set of data analysed was good. Furthermore, the shelf-life (time to reach 1×10^6 CFU/g) was experimentally determined in 28 days and the CMGM prediction was 27.5 days. So, the CMGM proposed in this work was useful to predict the shelf-life of squid rings.

4. Conclusions

Inactivation of psychrotrophic bacteria by gamma irradiation was accurately described by first-order kinetics for a dose range of 0–5.8 kGy, which in turn allowed the estimation of the D_{10}-value for these microorganisms. Psychrotrophic growth curves for irradiated squid rings during storage at 4–5 °C were fitted to two different empirical models, polynomial and modified Gompertz with replacement of its parameters by dose-dependent polynomial expressions. Both proposed models allowed accurate predictions of psychrotrophic bacteria behaviour in vacuum-packed squid rings during refrigerated storage as affected by gamma irradiation (for irradiation doses ranging between 0 and 5.8 kGy). Taking into account the mathematical simplicity of the expressions, polynomial model could be considered being a better option. However, the percentage average relative error for the polynomial model was higher than the one for the CMGM (11.9% against 4.5%). Also, considering the availability of developed computing power, mathematical simplicity of polynomial model seems to loose relevance in the present. The CMGM model was preferred, and it was satisfactorily validated using independent data of squid rings irradiated at 4.8 kGy. The CMGM cannot give accurate predictions of the shelf-life for dose above 5.3 kGy, since bacterial counts of rings irradiated above this dose would not reach the maximum recommended value that was used to estimate the shelf-life. In this context, the CMGM proposed in this work could be recommended as a useful tool to make predictions of shelf-life extension achieved by gamma irradiation up to 5.3 kGy of vacuum-packed squid rings stored at 4–5 °C.

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