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Research note

# Modeling total volatile basic nitrogen production as a dose function in gamma irradiated refrigerated squid rings

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The behavior of a chemical spoilage index for marine products (total volatile basic nitrogen – TVBN) was modeled as a function of the irradiation dose in *Illex argentinus* rings stored in refrigeration. The effect of gamma irradiation at 0, 1.8, 3.3 and 5.8 kGy on TVBN was analyzed in vacuum-packed squid rings during storage at 4 ± 1 °C. The modified Gompertz model satisfactorily described TVBN behavior for each irradiation dose ($R^2 > 0.980$; RMSE < 5.7). Gompertz model parameters ($\mu$, $A$, $L$) were modeled with dose-dependent second order polynomials, for doses ranging between 0 and 5.8 kGy, in order to develop a complete model that is useful for predicting TVBN as a function of the dose in squid rings. Model validation was carried out using independent data of TVBN in squid rings irradiated at 4.8 kGy. The developed model can be used to predict TVBN production in gamma irradiated squid meat.


## 1. Introduction

Squid *Illex argentinus* is the most abundant squid species of the South West Atlantic Ocean (Brunetti, Ivanovic, & Sakai, 1999), with average annual catch in Argentina exceeding 166,000 tons (2000–2012 period, MINAGRI, 2013). Squid is highly perishable because of its particular composition, being mainly deteriorated by the action of gram-negative bacteria (Huss, 1999). One of the main chemical changes during storage of marine product used to assess fish and seafood quality is the gradual accumulation of certain volatile amines in the flesh, which include trimethylamine (TMA), dimethylamine (DMA) and ammonia (Huss, 1999). Marine species contain trimethylamine-N-oxide (TMAO), a body fluid freezing-point depressor involved in fish osmotic regulation. TMAO is reduced to TMA by spoilage bacteria and therefore it is used as a spoilage indicator, being responsible of the typical fishy odor developed during spoilage (Huss, 1999; Pedrosa-Menabrito & Regenstein, 1990). DMA is produced from TMAO by intrinsic enzyme activity during frozen and cold storage (Huss, 1999), being produced in equimolar quantities with formaldehyde. It can be considered an effective marker of fish freshness of many white fish species (Connell, 1975; Huss, 1999; Pedrosa-Menabrito & Regenstein, 1990). One of the most widely used parameters to evaluate fish quality is Total Volatile Basic Nitrogen (TVBN), which includes the measurement of TMA, DMA and ammonia (produced by the deamination of amino-acids and nucleotide catabolites) (Huss, 1999). TVBN is considered particularly useful to assess quality of squid (LeBlanc & Gill, 1984; Woyewoda & Ke, 1980). Gamma irradiation is a safe technology for preserving different foodstuffs by means of the inactivation of spoiling and pathogenic bacteria (Josephson, 1983; Urbain, 1986; WHO, 1994, 1999). There have been numerous studies on the shelf-life extension of fish products treated with ionizing radiation (IAEA, 1969; ICGFI, 1991; Kilgen, 2001; Lescano, Kairiyama, Narvaiz, & Kaupert, 1990; Narvaiz, Lescano, Kairiyama, & Kaupert, 1989; Nickerson, Licciardello, & Ronsivalli, 1983), including the inactivation of spoiling bacteria by gamma irradiation in squid (*Illex argentinus*) rings (Tomac et al., 2012, Tomac, Mascheroni, & Yeannes, 2013; Tomac & Yeannes, 2012). The mathematical modeling of TVBN spoilage index behavior with respect to gamma irradiation is important to assess and manage food safety and shelf-life, and is convenient for the food industry since it avoids costs and time related to preliminary irradiation experiences. Modified Gompertz equation (Zwietering, Jongenburger,

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Rombouts, & Van Riet, 1990) is among the most widely used microbial growth primary models (Gibson & Roberts, 1989) and it has also been used to model other biological phenomena, like the growth of blue shrimp (*Penaeus stylirostris*) (Sepúlveda Medina, 1996). The objective of this work was to model the effect of different gamma irradiation doses on the TVBN production in squid rings, during refrigerated storage.

## 2. Materials and methods

### 2.1. Raw material source, treatment and storage

Peeled squid (*Illex argentinus*) mantle rings (1.2 ± 0.3 cm wide), pre-treated with a sodium polyphosphate solution (at 1 ± 1 °C) were acquired in Mar del Plata (Argentina). Immediately after capture and before processing, whole squids were maintained at 0–2 °C with ice flakes. Rings samples of 110 ± 2 g (20 rings approx.) were vacuum-packed in heat-sealed bags of LDPE/PA (Cryovac®, 125 μm) with a Minimax 430M machine (Sevivac, Argentina). Samples were transported in polystyrene boxes with cooling gel ice packs to the semi-industrial irradiation facility of the Ezeiza Atomic Centre (National Atomic Energy Commission, Argentina, activity: $2.22 \times 10^{16}$ Bq). Samples were divided into four lots consisting of 40 samples each, that were gamma irradiated with a Cobalt-60 source at 0, 1.8, 3.3 and 5.8 kGy, respectively (minimum absorbed doses; dose rate: 10.94 kGy/h). Doses were determined with Amber Perspex dosimeters. Irradiated and non-irradiated samples (control, 0 kGy) were stored at 4 ± 1 °C and analyzed before irradiation (day 0) and at 1, 5, 8, 12, 15, 19, 22, 26 and 29 days after irradiation.

### 2.2. Total volatile basic nitrogen (TVBN) determination

TVBN was determined by the commercial method adapted from the direct distillation method (Giannini, Davidovich, & Lupín, 1979). Results were expressed as mgN 100 $g^{-1}$ of wet sample. Determinations were done in triplicate.

### 2.3. Modeling TVBN production during refrigerated storage

Experimental data of TVBN curves for each irradiation dose (0, 1.8, 3.3 and 5.8 kGy) were fitted to a primary growth model, the modified Gompertz equation (Zwietering et al., 1990), in which bacterial counts were replaced by TVBN, as indicated in Eq. (1):

$$\mathrm{TVBN} = \mathrm{TVBN}_0 + A\exp\left[-\exp\left(\frac{\mu e}{A}(L-t)\right)+1\right] \quad (1)$$

where TVBN is the value of TVBN at time $t$ and $\mathrm{TVBN}_0$ the value observed the day after irradiation (mgN 100 $g^{-1}$), $\mu$ is the TVBN production rate (mgN 100 $g^{-1}$ $day^{-1}$), $L$ is the initial phase during which there are no changes in TVBN (days), A is the complete TVBN change over the entire period and $e$ is the Euler number (2.7182). Data was fitted to modified Gompertz equation by non linear 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 Root Mean Square Error (RMSE) and the determination coefficient ($R^2$). Considering that RMSE is a measure of the discrepancy between the data and the model estimations, a small value would indicate a tight fit of the model. RMSE was calculated using Eq. (2), where $X_{ip}$ is the predicted value, $X_i$ is the experimental value and $n$ is the number of data pairs:

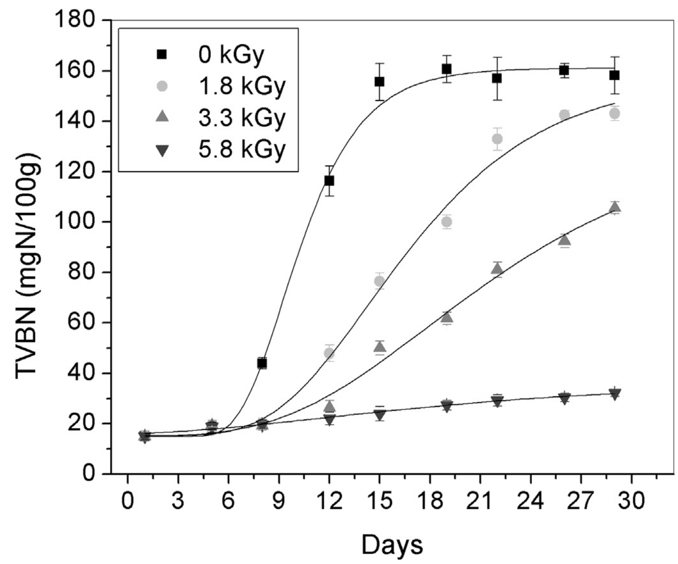


**Fig. 1.** TVBN (mgN 100 $g^{-1}$) evolution during storage (4 ± 1 °C) of irradiated and control squid rings (mean ± standard error, $n = 2$). Solid lines represent the modified Gompertz model adjustment.

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(X_{ip}-X_i)^2} \quad (2)$$

In order to predict TVBN for different radiation doses it was necessary to find a relationship between Gompertz kinetic parameters ($\mu$, $L$ and $A$) and the irradiation dose. To develop 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 $A = A_1 + A_2 d + A_3 d^2$ (where $d$ is the radiation dose). Afterwards, a full model (the complete Gompertz model, **CGM**) was developed by introducing the secondary models into the modified Gompertz equation (Eq. (1)).

The **CGM** fit to experimental data was analyzed by the percentage average relative error (PEr), calculated considering the number of data analyzed ($n$) by:

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

### 2.4. Model validation

The complete model was validated using an independent set of experimental data: TVBN results of squid (*Illex argentinus*) rings treated with gamma irradiation at 4.8 kGy during 77 days of storage at 4 ± 1 °C (Tomac et al., 2012). TVBN values were determined on days 0, 1, 5, 8, 13, 16, 19, 22, 26, 33, 40, 47, 54, 68 and 77. Complete model TVBN predictions were plotted against experimental values in order to analyze the goodness of the predictions.

### 2.5. Statistical analyses

A two-ways ANOVA test ($p < 0.05$) was used to analyze 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-project software (R Development Core Team, 2008) was used.

## 3. Results and discussion

### 3.1. Modeling TVBN evolution in irradiated rings

Fig. 1 shows TVBN evolution in squid rings during refrigeration for the tested irradiation doses.

Initial TVBN value was 15.2 ± 0.2 mgN/100 g, similar to the findings of Melaj, Sánchez-Pascua, Casales, and Yeannes (1997) in *Illex argentinus* and of Sungsri-in, Benjakul, and Kijroongrojana (2011) in *Loligo formosana*. TVBN depends on various factors such as the TMAO muscle content, which depends in turn of the specimen size, the feeding state, the fishing zone, the season, and also on the samples microbial load (Huss, 1999). TVBN significantly increased ($p < 0.05$) during storage in control and irradiated samples, but at different production rates depending on the irradiation dose. TVBN increase during squid storage has also been observed by other authors (Lapa-Guimaraes et al., 2005; Sungsri-in et al., 2011). The effect of the dose was significant ($p < 0.05$) on the TVBN production, with lower TVBN values found for higher doses, for a given storage time. After a constant behavior period, TVBN increased in all samples, being significantly higher ($p < 0.05$) in samples irradiated at 0, 1.8, 3.3 and 5.8 kGy, respectively. These results can be related to the bacterial inactivation observed in squid rings due to gamma irradiation (Tomac et al., 2012, 2013; Tomac & Yeannes, 2012), since TMA is produced by bacterial activity, contributing to TVBN value. Other authors have informed lower TVBN values as a consequence of gamma irradiation in various fish products (Badr, 2012; Jeevanandam, Kakatkar, Doke, Bongirwar, & Venugopal, 2001; Lakshmanan, Venugopal, Venketashvaran, & Bongirwar, 1999; Narvaiz et al., 1989).

From Fig. 1 it can be observed that TVBN experimental curves for unirradiated and 1.8 kGy irradiated rings presented a sigmoidal behavior, suggesting that experimental results could be properly adjusted with a growth model, such as Gompertz model modified by Zwietering et al. (1990). The adjustment of the four experimental data sets (TVBN of rings irradiated at 0, 1.8, 3.3 and 5.8 kGy) to the modified Gompertz equation is represented by solid lines in Fig. 1. The four data sets were accurately modeled by Gompertz model, as indicated by the high determination coefficients values obtained ($R^2 > 0.980$), the low RMSE (<5.7) and the high significance of each dose adjustment ($p < 0.00001$). In Table 1 are shown these values together with the Gompertz model parameters estimations ($\mu$, $L$, $A$).

The TVBN production rate ($\mu$) significantly decreased ($p < 0.05$) with higher irradiation dose intensity. Doses of 5.8, 3.3 and 1.8 kGy caused a reduction in $\mu$ of about 19, 15 and 11 units (mgN 100 g$^{-1}$ days$^{-1}$), respectively, in comparison with the production rate observed in non irradiated rings. In a same way, the highest TVBN value reached during storage ($A$) was also lower at higher doses, being 125 units lower in rings irradiated at 5.8 kGy compared to control. The $L$ parameter (time during which there were no changes in the TVBN value) showed an increasing tendency with increasing doses, except for 5.8 kGy, that it was 4 days lower with respect to $L$ of control rings. However, the production rate of samples irradiated at 5.8 kGy was much lower (0.73 mgN 100 g$^{-1}$ days$^{-1}$) than the one observed in non irradiated rings. These results are in agreement with the microbial inactivation observed in the samples, which was also dose-dependent (Tomac et al., 2013). Other authors have modeled TVBN production during storage of osmotically dehydrated sea bream using first order equations (Tsironi, Salapa, & Taoukis, 2009).

**Table 1**
Gompertz model parameters ($\mu$, $L$, $A$), determination coefficient ($R^2$) of the modified Gompertz model adjusted to TVBN production experimental curves.

| Dose (kGy) | $\mu$ (mgN 100 g$^{-1}$ days$^{-1}$) | $L$ (days) | $A$ (mgN 100 g$^{-1}$) | $R^2$ | RMSE |
|---|---|---|---|---|---|
| 0 | 20.85 ± 1.70 | 6.64 ± 0.17 | 146.16 ± 2.06 | 0.998 | 3.95 |
| 1.8 | 9.19 ± 0.77 | 8.66 ± 0.57 | 142.54 ± 6.52 | 0.996 | 4.57 |
| 3.3 | 5.04 ± 0.36 | 9.22 ± 0.69 | 117.99 ± 14.75 | 0.991 | 5.68 |
| 5.8 | 0.73 ± 0.07 | 1.54 ± 0.97 | 20.52 ± 2.55 | 0.983 | 1.75 |

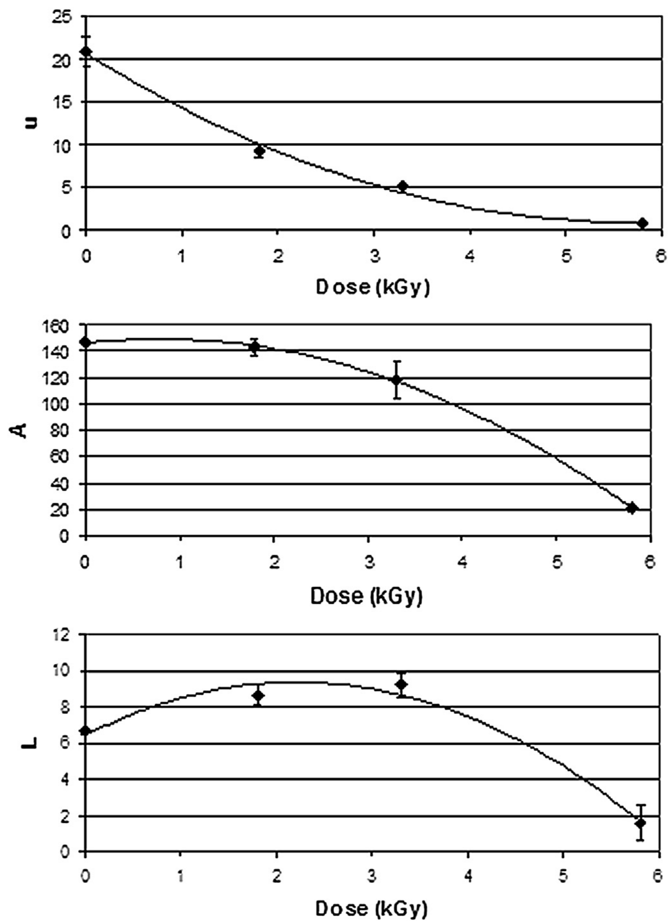

**Fig. 2.** Polynomial adjustment of $\mu$, $L$ and $A$ parameters depending on the irradiation dose.

Gompertz model parameters were adjusted to second order polynomial equations, in order to find secondary models depending on the irradiation dose. In Fig. 2 it is shown this adjustment for $A$, $\mu$ and $L$ parameters, which polynomial expressions and determination coefficients were:

$$\begin{aligned} \mu &= 20.585 - 6.9653d + 0.6153d^2 \, (R^2 = 0.994) \\ A &= 145.76 + 8.1416d - 5.1194d^2 \, (R^2 = 0.999) \\ L &= 6.4451 + 2.6597d - 0.6009d^2 \, (R^2 = 0.979) \end{aligned} \quad (3)$$

By introducing Equation (3) in Equation (1) the complete Gompertz model (**CGM**) was developed, which expression is represented by Equation (4) as follows:

$$\text{NBVT} = \text{NBVT}_0 + \left(145.76 + 8.1416d - 5.1194d^2\right) \exp E \quad (4)$$

where

$$E = \left[ -\exp\left( \frac{(20.585 - 6.9653d + 0.6153d^2)e}{(145.76 + 8.1416d - 5.1194d^2)} \times \left(6.4451 + 2.6597d - 0.6009d^2 - t\right)\right) + 1 \right]$$

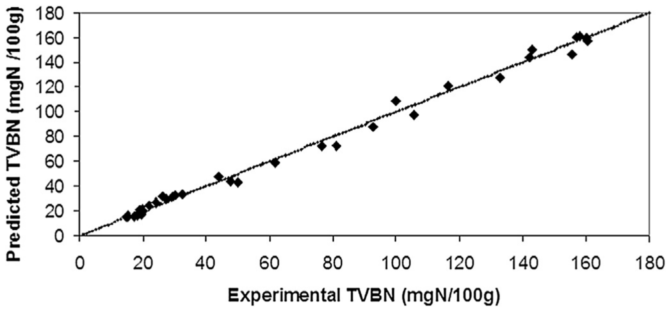


**Fig. 3.** Predicted and experimental TVBN values in squid rings.

This proposed model allowed accurate TVBN predictions in squid rings with 6.4% EPr, for doses ranging between 0 and 5.8 kGy, provided the initial TVBN value is known.

Fig. 3 shows predicted versus experimental TVBN values, indicating prediction goodness by the proximity of the points to the bisector line.

### 3.2. Complete model validation

Independent data of another squid rings irradiation experience at 4.8 kGy, during 77 days of refrigerated storage (Tomac et al., 2012) were used to validate the developed complete model. The complete Gompertz accurately estimated TVBN values for rings irradiated at 4.8 kGy with 7.0% EPr (RMSE $< 6.3$) indicating that the proposed model is useful to predict with precision the TVBN behavior in irradiated squid rings during refrigerated storage.

These results indicate that the developed model allows estimations of a chemical spoilage index, such as TVBN, when gamma irradiation is applied to a minimally processed squid product with shelf-life extension purposes.

## 4. Conclusions

A strong dependence of TVBN production rate with irradiation dose was observed, finding lower rates at higher doses. It was feasible to find a mathematical model that accurately describes TVBN production during refrigerated storage of gamma irradiated squid rings, by means of a microbial growth mathematical equation, which parameters were modeled as a function of the irradiation dose using second order polynomials. In this way, a complete model was developed that allows TVBN predictions with 6.4% percentage average relative error for squid rings irradiated between 0 and 5.8 kGy. Also, the model was satisfactorily validated with independent data from an *Illex argentinus* rings irradiation at 4.8 kGy. The developed model is useful to predict the effect of different irradiation doses on a spoilage parameter related to the commercialization time (TVBN), with a simple and fast method.

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