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# Development of a predictive model for the growth kinetics of aerobic microbial population on pomegranate marinated chicken breast fillets under isothermal and dynamic temperature conditions

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ABSTRACT

The aim of this study was the development of a model to describe the growth kinetics of aerobic microbial population of chicken breast fillets marinated in pomegranate juice under isothermal and dynamic temperature conditions. Moreover, the effect of pomegranate juice on the extension of the shelf life of the product was investigated. Samples (10 g) of chicken breast fillets were immersed in marinades containing pomegranate juice for 3 h at 4 °C following storage under aerobic conditions at 4, 10, and 15 °C for 10 days. Total Viable Counts (TVC), *Pseudomonas* spp and lactic acid bacteria (LAB) were enumerated, in parallel with sensory assessment (odor and overall appearance) of marinated and non-marinated samples. The Baranyi model was fitted to the growth data of TVC to calculate the maximum specific growth rate ($\mu_{max}$) that was further modeled as a function of temperature using a square root–type model. The validation of the model was conducted under dynamic temperature conditions based on two fluctuating temperature scenarios with periodic changes from 6 to 13 °C. The shelf life was determined both mathematically and with sensory assessment and its temperature dependence was modeled by an Arrhenius type equation. Results showed that the $\mu_{max}$ of TVC of marinated samples was significantly lower compared to control samples regardless temperature, while under dynamic temperature conditions the model satisfactorily predicted the growth of TVC in both control and marinated samples. The shelf-life of marinated samples was significantly extended compared to the control (5 days extension at 4 °C). The calculated activation energies ($E_a$), 82 and 52 kJ/mol for control and marinated samples, respectively, indicated higher temperature dependence of the shelf life of control samples compared to marinated ones. The present results indicated that pomegranate juice could be used as an alternative ingredient in marinades to prolong the shelf life of chicken.



## 1. Introduction

It is well established that the short shelf life of meat, including poultry is due to microbial activity that can be manifested as visible growth (e.g., slime, colonies), textural changes, off-odors and off-flavors (Nychas et al., 2008). This situation leads to significant economic losses for the meat industry which is looking for effective, natural preservation methods that provide poultry and poultry products with extensive shelf life and at the same time meet consumers' demands for high quality, convenience and improved flavor.

Marination is the process of immersing muscle origin foods, e.g. meat and/or fish in a usually acidic solution supplemented with spices or herbs in order to (i) add flavor as well as enhance the sensory quality in a natural way, and (ii) tenderize it (Lemos et al., 1999; Bjorkroth, 2005; Pathania et al., 2010). Previous studies have shown that the nature of some marination ingredients such as alcoholic beverages, fruit juices or essential oils may lead to safer products with prolonged shelf life (Friedman et al., 2007; Cadun, 2008; Pathania et al., 2010; Kargiotou et al., 2011; Nisiotou et al., 2013; Thanissery and Smith, 2014). The most common ingredients traditionally employed in marination include lemon, vinegar and wine. However, gastronomes and researchers have turned their attention to novel constituents that will combine more effectively the improvement of sensory characteristics with the

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extension of the shelf life. Pomegranate and its products (juice, sauces) could be used for this reason (Gokoglu et al., 2009; Keşkekoğlu and Üren, 2014).

Pomegranate (*Punica granatum*) is a well-known crop since the ancient times, but nowadays it is becoming more and more popular due to its undeniable benefits. The considerable content of the edible part of the fruit in acids, vitamins, polysaccharides, polyphenols and minerals (Lansky and Newman, 2007; Tzulker et al., 2007; Cassano et al., 2011; Bagci Onsekizoglu, 2014) renders the pomegranate a very healthful fruit with antioxidant (Gil et al., 2005; Naveena et al., 2008; Turkyilmaz et al., 2013) and antimicrobial properties (Braga et al., 2005; Al-Zoreky, 2009; Vaithiyanathan et al., 2011; Turkyilmaz et al., 2013; Kapetanakou et al., 2015).

The use of pomegranate as an ingredient in marination has not received proper attention by the scientific community and there is little information on the effect of pomegranate marinades on the population dynamics of the indigenous microbiota and the extension of shelf life of chicken during aerobic storage (Bazargani-Gilani et al., 2015).

Thus, the objectives of this study were: (i) the development of a new product (marinated chicken breast fillet) using pomegranate juice as a novel marinade ingredient, (ii) the determination of the shelf life of marinated chicken breast fillets, and (iii) the development of a product-specific model based on the growth of aerobic microbiota, and the validation of the developed model under dynamic temperature conditions.

## 2. Materials and methods

### 2.1. Marination and storage conditions of chicken breast fillets

Fresh chicken breast fillets were purchased from a local meat market in Athens and immediately transported to the laboratory under temperature-controlled conditions. On arrival, chicken blocks were aseptically cut into pieces (10 g) of similar size (3 × 2 × 1 cm) using a sterile knife. For the marination of the samples, a marinade prepared at home consisting of: 70 mL commercially available 100% natural pomegranate juice (Rodion, Elliniki Agora A.E., Chalkidiki, Greece), 30 mL olive oil (Altis, ELAIS/UNILEVER, Athens (50% refined−50% virgin olive oil)), 0.1 g dried thyme (AB Vassilopoulos, Athens) and 2 g honey (Attiki-Pittas, Kryoneri Attikis). The ingredients of the marinade mixed with intense agitation, exactly as the household practice for the preparation of marinades. Marination was carried out in metal trays by immersing fillets in the marinade solution for 3 h at 4 °C. The ratio quantity of chicken meat/volume of marinade was 200 g of chicken meat/300 mL of marinade. After marination, samples were removed from the trays, allowed to drain off for a few seconds and then placed into sterile Petri dishes that were wrapped with oxygen-permeable plastic film and stored at 4, 10, and 15 °C for 9 days in high precision (±0.5 °C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan). Untreated (without marination) chicken samples were also prepared and used as control. Two independent experiments were conducted with two samples analyzed each time ($n = 4$) for each treatment and each temperature condition.

### 2.2. Microbiological analysis

Microbiological analysis was undertaken for the enumeration of chicken breast fillets indigenous microbiota at the beginning (time 0) and every 24 h until the end of storage. Chicken samples (10 g) were added aseptically to 90 mL of sterile quarter-strength Ringer's solution (LAB 100Z, LAB M, Bury, UK) and homogenized in a stomacher (Lab Blender, Seward Medical, London, UK) for 60 s at room temperature. Serial decimal dilutions in the same medium were prepared and 0.1 or 1 mL aliquots of the appropriate dilutions were spread or poured in duplicate on the following agar media: Tryptic Glucose Yeast Agar (402145, Biolife, Milan, Italy) for total viable counts (TVC), incubated at 30 °C for 48 h, Pseudomonas agar base (LAB 108, supplemented with CFC (Cephalothin, Fucidin, Cetrimide) selective supplement with product code X108, LAB M) for pseudomonads, incubated at 25 °C for 48 h, de Man-Rogosa-Sharpe agar (401728, Biolife) (pH adjusted to 5.7) for lactic acid bacteria (LAB), overlaid with the same medium and incubated at 30 °C for 72 h. After incubation, typical colonies for each microbial group were enumerated and colony counts were logarithmically transformed (log cfu/g). Results are presented as average values (±standard deviation) of duplicate samples analyzed at each sampling point. In addition, the pH of both chicken samples and marinades was recorded by a pH meter (Metrohm 691 pH meter, Ion Analysis, Herisau, Switzerland) after the end of microbiological analysis with direct immersion of the glass electrode in the marinade solution or in the sample homogenate.

### 2.3. Sensory analysis

Sensory evaluation was conducted by a panel of 10 in-house trained assessors at the end of storage. The panelists were all staff of the laboratory experienced in sensory evaluation of meat. Marinated and non-marinated samples were evaluated on the basis of odor and overall appearance using a 3-point hedonic scale (1-desirable; 2-acceptable; 3-unacceptable) in which scores > 2 indicated the end of shelf life. A score of 2 characterized the semi-fresh samples, namely the samples that changed from typical fresh to deteriorated but still considered acceptable by the panelists. This score (2) was set as the threshold for the end of the shelf life even if samples were assessed with this score for only one of the sensory attributes assayed.

### 2.4. Model development and validation

Total Viable Counts (TVC) growth data of untreated and marinated samples stored under isothermal conditions (4, 10, and 15 °C) were fitted to the primary model of Baranyi and Roberts (1994) (available at www.combase.cc) to obtain the kinetic parameters of maximum specific growth rate ($\mu_{max}$ [$h^{-1}$]) and lag time duration ($\lambda$ [h]). The values of $\mu_{max}$ were further modeled as a function of storage temperature using a square root type model (eq. (1)) (Ratkowsky et al., 1983).

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad (1)$$

where b is a constant, T is the storage temperature (°C), and $T_{min}$ is the theoretical minimum temperature for growth. The parameters of the model were calculated using the IPMP (Integrated Pathogen Modeling Program) software (Huang, 2014).

The model developed under isothermal conditions was validated against observed growth of the TVC under non-isothermal conditions using two different scenarios of periodic temperature changes. The prediction of the growth profile of TVC under non-isothermal conditions was based on the combination of the primary model, the square root model and the time/temperature profile of the samples which were numerically integrated with respect to time (eq. (2) and (3)) (Gougouli et al., 2008).

$$\frac{dy}{dt} = \{b[T(t) - T_{min}]\}^2 \left(\frac{q}{q+1}\right)\left(1 - \frac{y}{y_{max}}\right)y \quad (2)$$