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Meta-analysis of D-values of proteolytic *Clostridium botulinum* and its surrogate strain *Clostridium sporogenes* PA 3679

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ABSTRACT

Foodborne botulism is a serious disease resulting from ingestion of preformed *Clostridium botulinum* neurotoxin in foodstuff. Since the 19th century, the heat resistance of this spore forming bacteria has been extensively studied in order to guarantee the public health associated with low acidic, ambient stable products. The most largely used heat resistance parameters in thermal settings of such products are the $D_{121.1\ °C}$ values (time required to have a 10-fold decrease of the spore count, at 121.1 °C) and the z-values (temperature increase to have a 10-fold decrease of D-values).

To determine $D_{121.1\ °C}$ and z-values of proteolytic *C. botulinum* and its nontoxigenic surrogate strain *C. sporogenes* PA3679, a dataset of 911 D-values was collected from 38 scientific studies. Within a meta-analysis framework, a mixed-effect linear model was developed with the log D-value (min) as response and the heat treatment temperature as explicative variable. The studies (38), the *C. botulinum* strains (11), and the heat treatment media (liquid media and various food matrices, split into nine categories in total) were considered as co-variables having a random effect. The species (*C. botulinum* and *C. sporogenes*) and the pH (five categories) were considered as co-variables having a fixed effect.

Overall, the model gave satisfactory results with a residual standard deviation of 0.22. The heat resistance of proteolytic *C. botulinum* was found significantly lower than the *C. sporogenes* PA 3679 one: the mean D-values at the reference temperature of 121.1 °C, in liquid media and pH neutral, were estimated to 0.19 and 1.28 min for *C. botulinum* and *C. sporogenes*, respectively. On the other hand, the mean z-values of the two species were similar: 11.3 and 11.1 °C for *C. botulinum* and *C. sporogenes*, respectively. These results will be applied to thermal settings of low-acid ambient stable products.

1. Introduction

Foodborne botulism is a serious disease resulting from ingestion of preformed *Clostridium botulinum* neurotoxin in foodstuff. This disease is particularly associated with proteolytic *Clostridium botulinum*, which is a mesophilic anaerobic spore forming bacteria, with a minimum growth temperature of 10 °C–12 °C and a maximum of 45 °C–50 °C. Spores of proteolytic *C. botulinum* are also known to be the most heat-resistant form of pathogenic organisms in low-acid, ambient-stable canned products (Frazier, 1967; Stumbo, 1973).

The first objective of this study was to quantify the heat resistance of proteolytic *C. botulinum* and particularly to estimate the parameters $D_{121.1\ °C}$ (time required to have a 10-fold decrease of the spore count at 121.1 °C (Katzin et al., 1943)) and z-values (temperature increase to have a 10-fold decrease of D-values), widely used in thermal treatment settings. There are many scientific publications reporting studies in which these two key parameters have been determined. In the original article concerning the proteolytic *C. botulinum* in canned food (Esty and Meyer (1922)), the highest heat resistance at 121.1 °C was reported to be 0.21 min. This value is still used nowadays as a reference in the canning industry (Rosnes et al., 2012). Likewise, a z-value of 10 °C is generally applied when calculating equivalence in thermal processes of ambient stable products (Holdsworth, 1985; Lund and Peck, 2000; Pflug and Odlaug, 1978). Using the existing literature, it is now possible to include in the analysis of the log D-values the heterogeneity due to the studies (which might be associated with different experimental protocols carried out over the time), the media in which the heat resistance experiments was performed (which might explain differences between results obtained in liquid laboratory media and in food matrices), the strains used in the experiments,…etc.

The second objective of this study was to compare the heat resistance of proteolytic *C. botulinum* and of *C. sporogenes* PA 3679. This latter is a nontoxigenic surrogate strain of *C. botulinum*, often used in challenge-test study performed in low-acid shelf-stable foods. It is

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generally admitted that the heat resistance of these two bacteria are similar, although the use of *C. sporogenes* PA 3679 might be seen as a fail-safe thermal processing surrogate (Brown et al., 2012).

Despite an abundant literature on both proteolytic *C. botulinum* and *C. sporogenes* PA 3679, there is no meta-analysis of all the D-values collected over the time with the objective of quantifying the effect of high temperature (≥100 °C) on the heat resistance and subsequently recalculating z-values and $D_{121.1\ °C}$ values of spores of both bacteria. Nonetheless, there are recent studies compiling information on these bacteria, for instance based on a set of 176 log D-values extracted from three different reviews, van Asselt and Zwietering (2006) estimated that the z-values of proteolytic *C. botulinum* was 10.2 °C and the $D_{121.1\ °C}$ value 0.21 min. Likewise, Brown et al. (2012) compiled a substantial amount, although limited to 121 °C, of D-values of *C. botulinum* and *C. sporogenes* PA 3679 (210 and 70 data, respectively) and made a comparison of heat resistance between these two species at 121 °C.

Meta-analysis is a statistical technique for combining estimated treatment effects from independent comparable studies, it is widely used in medical research and it is gaining interest in food safety (Gonzales-Barron and Butler, 2011). Among others, studies of *Salmonella* prevalence on pig carcasses (Gonzales Barron et al., 2008), growth rates of *Escherichia coli* O157 on ground beef (Vialette et al., 2005) and consumer behavior and practices (Patil et al., 2005) have been reported. Meta-analysis technique has been recently applied to heat resistance characterization. For instance, Jaloustre et al. (2012) have studied the inactivation of *Clostridium perfringens* vegetative cell in beef-in-sauce; Rigaux et al. (2013) have studied the heat resistance of *Geobacillus stearothermophilus*. In both cases, a mixed-effect model has been developed, with the logarithm of D-values as the statistical response and the temperature as the explicative variable. To explicitly account for the heterogeneity of the different studies (different articles from which the D-values were collected) the statistical analysis included this factor as a random-effect co-variable.

Here, a meta-analysis was carried out on 911 D-values collected from 38 scientific studies, of which 394 D-values on proteolytic *C. botulinum* (23 studies) and 517 on *C. sporogenes* PA 3679 (20 studies). The temperature range varied between 100 and 143 °C. Beside the study, there were two random effect co-variables incorporated in the analysis: the *C. botulinum* strains and the heat treatment media, and one fixed-effect co-variable: the pH during heat treatment (separated into five categories).

2. Material and methods

2.1. Data collected

The D-values of proteolytic *C. botulinum* (whatever the strain) and *C. sporogenes* PA 3679 were collected as most widely as possible. The collect of data was based on a literature search, carried out through various scientific platforms. Articles published from 1922 up to 2013, were gathered. The pieces of information extracted from the articles were log D values, temperature, media, species and strain (for *C. botulinum*). Beside these key information, when mentioned, pH and water activity were recorded.

Initially, 73 articles dealing with heat resistance of *C. botulinum* or *C. sporogenes* PA 3679 were collected. However, 23 publications did not contain D-values. Moreover, in the relevant publications some data were discarded from the meta-analysis due to the following reasons: i) presence of an extra factor such as high pressure or addition of a chemical compound to accelerate the thermal inactivation, ii) a study in which there was only one D-value collected (singleton study).

Next, when checking the range of temperature at which the D-values were collected, it was noticed that only few data were collected at temperature below 100 °C and 110 °C for *C. botulinum* and *C. sporogenes*, respectively. To avoid an extrapolation to these low temperatures, it was then decided to discard these data (3 for *C. botulinum* and 11 for *C. sporogenes*, extracted from 4 different studies) from the meta-analysis. Nevertheless, these data were kept in the study for validation. Likewise, the singleton study (8 data coming from 8 different studies) were kept for validation.

Finally, a total of 911 D-values, collected from 38 studies were used in the meta-analysis. The temperature range varied between 100 and 140 °C for *C. botulinum* and between 110 and 143 °C for *C. sporogenes*. In Table 1, the number of data collected per study, species and heat treatment media is provided.

2.2. Meta-analysis — mixed effect linear model

The prediction of the survival of the micro-organisms in food requires certain parameters such as the intrinsic resistance of the micro-organism, the effects of the change of the chemical or physical environment characteristics. The most widely used parameter in the thermal treatments is the time of decimal reductions (D-values). In our study, the decimal logarithm of the D-values was analyzed in a mixed effect linear model. The logarithm transformation is very common in microbiology and particularly when assessing the temperature effect on the inactivation rate (Jaloustre et al., 2012; Rigaux et al., 2013; van Asselt and Zwietering, 2006).

The log D-values were analyzed as a function of:

- the temperature applied during the heat treatment with a fixed effect.
- the sources from which the data were collected. The factor "studies" was analyzed as a co-variable having a random effect.
- the medium in which the heat treatment was applied. Initially, from the sources used, more than 100 different media were recorded. The media were then grouped into 9 categories: liquid media (laboratory medium and food-based preparation with water), milk and dairy products, meat and meat products, fish and seafood products, vegetable, canned vegetable, frozen vegetable, oil and cream, and finally, starch based products (e.g. rice, pasta). The factor "media" was analyzed as a co-variable having a random effect.
- the species. When the data were collected, only *C. botulinum* and *C. sporogenes* species were deliberately retrieved as the objective of this study was to quantify the effect of temperature on *C. botulinum* and its surrogate strain. Consequently, the factor "species" was analyzed as a co-variable having a fixed effect.
- the strain. For *C. botulinum*, all data were collected whatever the strain was. When there were two or more experimental data with the same strain, this strain was kept in the analysis as such. A total of 10 different strains were then analyzed. An 11th strain category was created artificially, it corresponded to the experimental study for which either the strain was not mentioned, or, there was only one datum for one given strain. The factor *C. botulinum* strain was analyzed as a co-variable having a random effect. On the other hand, for *C. sporogenes*, only data on the surrogate strain PA 3679 were collected, the model did not include a *C. sporogenes* strain effect.
- the pH of heat treatment. Beside the media, some authors have either recorded the pH of the media in which the heat treatment was applied, or, studied the effect of the pH itself. Unfortunately, the pH value was not systematically mentioned in the articles (561 pH values on 911 data). Then, it was decided to range the studies into five pH categories: i) $pH < 4.5$; ii) $4.5 \le pH < 5.0$; iii) $5.0 \le pH < 5.5$; iv) $5.5 \le pH < 6.0$ and v) $pH \ge 6.0$ or not mentioned. The "not mentioned" pH category was merged with the "$pH \ge 6.0$" category as generally the heat resistance studies in which the pH is not mentioned are carried out in laboratory medium at near neutral pH. The factor pH was analyzed as a co-variable having a fixed effect.