International Journal of Food Microbiology 338 (2021) 108985

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Using extended Bigelow meta-regressions for modelling the effects of temperature, pH, °Brix on the inactivation of heat resistant moulds

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ARTICLE INFO

Keywords:
Meta-analysis
D-value
z-Value
Neosartorya
Byssochlamys
Talaromyces
Juice
Pasteurization

ABSTRACT

The management of Heat Resistant Moulds (HRMs) is considered a great challenge for the juice fruit industry. *Neosartorya*, *Byssochlamys* and *Talaromyces* are three out of the main genera isolated from fruit juices that show great resistance to heat treatments. Several inactivation parameters can be found in the literature, however all of them were carried out in specific food matrices and using diverse inactivation methods. Thus, this meta-analysis study synthesizes the thermal resistance parameters of the three HRMs by adjusting extended Bigelow-based meta-regression models to data on inactivation experiments conducted in different liquid media. The meta-analytical data, extracted from publications between 1969 and 2017, was composed of decimal reduction time (D), inactivation method, temperature of inactivation, pH, °Brix, age of spores, and type of medium (model, juice, concentrates). Pooled D* values (D at 90 °C, pH 3.5 and 12° Brix) were estimated for *B. fulva* (1.95 min; 95% CI: 1.21–3.11 min), *Talaromyces* (4.03 min; 95% CI: 3.43–4.74 min), *Neosartorya* (0.5.35 min; 95% CI: 4.10–7.08 min), and *B. nivea* (10.32 min; 95% CI: 5.81–18.4 min). It was found that increasing the soluble solids in concentrates tends to cause a lower decrease in the heat resistance of *Neosartorya* and *Talaromyces* than increasing the soluble solids in model liquid or juices ($p = 0.001$; 0.012). In general, the screw-capped tubes and three neck round inactivation methods render higher D* values ($p < 0.05$) than the thermal death tubes, the polyethylene bag and the capillary methods. Spores of *Talaromyces* (overall $z_{pH} = 7.56$; 95% CI: 5.13–13.5) and *Neosartorya* (overall $z_{pH} = 7.07$; 95% CI: 5.04–10.8) appear to be more thermal sensitive to a decrease in medium pH than spores of *Byssochlamys* (overall $z_{pH} = 4.34$; 95% CI: 3.20–6.73). The meta-regression models presented in this study can be valuable for estimating pooled inactivation kinetic parameters to be used by the fruit juice industry in the management of thermal processes and in the determination of shelf-life.

1. Introduction

The fruit juice industry faces a great challenge in managing the spoilage of their products by heat resistant moulds (HRMs) and associated economic losses (Salomão, 2018; Snyder and Worobo, 2018; Tournas, 1994a, 1994b). The main HRM genera isolated from fruit juices are *Neosartorya*, *Byssochlamys*, *Talaromyces* and *Eupenicillium* (Massaguer et al., 2014; Salomão, 2018; Sant'Ana et al., 2009a, 2009b; Tournas, 1994a, 1994b). The contact with the soil may be considered the main source of fruit contamination by these HRMs. Because of this, fruits such as strawberry, pineapple, peach, apples and berry are highly susceptible to contamination and spoilage by HRMs (Berni et al., 2017a, 2017b; Evelyn and Silva, 2017; Rico-Munoz et al., 2019; Sant'Ana et al., 2009a, 2009b; Santos et al., 2018; Tournas, 1994a, 1994b).

Fruit products are prone to the spoilage by HRMs because these fungi can survive thermal processing and further grow under low oxygen tension, low pH (under 4.5) and can synthesize enzymes that affect juice stability (Sant'Ana et al., 2009a, 2009b; Santos et al., 2019; Tournas, 1994a, 1994b). HRMs can withstand thermal processes applied to fruit products because of their ability to form ascospores during their sexual phase (Massaguer et al., 2014; Tournas, 1994a, 1994b), which is associated with their high thermal tolerance (Sant'Ana et al., 2009a, 2009b; Tournas, 1994a, 1994b; Tremarin et al., 2017). Even though strategies have been employed in the field to reduce the initial contamination of

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https://doi.org/10.1016/j.ijfoodmicro.2020.108985
Received 27 December 2019; Received in revised form 12 August 2020; Accepted 14 November 2020
Available online 21 November 2020
0168-1605/

fruits by HRMs, the contamination of fruit products by these microorganisms remains a challenge and counts of 1–9 ascospores per 100 g or mL have been reported (Massaguer et al., 2014; Sant'Ana et al., 2008; Santos et al., 2018; Tournas, 1994a, 1994b). Given their resistance, even with such a low concentration of ascospores, some HRMs have been found to be able to survive pasteurization processes of fruit juices (Sant'Ana et al., 2009a, 2009b). In fact, in these cases, the heat treatments applied by the industry may induce the germination of dormant ascospores and the further growth of HRM, leading to fruit products spoilage (Sant'Ana et al., 2010; Santos et al., 2019) and production of mycotoxins (Oteiza et al., 2017; Sant'Ana et al., 2008; Sant'Ana et al., 2009a, 2009b), which may represent health risk for consumers.

Because of their high thermal tolerance, several studies have been done towards assessing strategies towards the inactivation of HRM in fruit products (Amaeze, 2013; Berni et al., 2017a, 2017b; Beuchat, 1986; King et al., 1979; Sant'Ana et al., 2009a, 2009b; Souza et al., 2017; Splittstoesser and Splittstoesser, 1977). Nevertheless, these studies have been conducted for specific and individualized HRM, in specific model foods or beverages, and using diverse methods to obtain the inactivation kinetic parameters. However, it is known that the characteristics of the food matrix, the strain and the inactivation method employed will influence the obtained inactivation kinetic parameters of HRMs (Basaran-Akgul, 2013; Delgado et al., 2012; Fujikawa et al., 2001). If not accurately estimated, the inactivation kinetic parameters may result in undesired sensory changes if thermal resistance was overestimated (Petruzzi et al., 2017) or may impact on the production of shelf-stable fruit products if thermal resistance was underestimated (Sant'Ana et al., 2008).

Given the above, for realistic design of thermal processes applied to fruit products, the knowledge of the inherent variability regarding the food matrix, microorganisms and method employed for estimation of inactivation kinetics must be known. A proper way to unveil the variability in the inactivation kinetic parameters of HRMs and further allow their easy use for the design of thermal processes, comprise their integration into global models capable of extracting pooled or overall heat resistant kinetic parameters. Meta-analysis comprises powerful approaches for synthesizing and merging numerous outcomes from related experimentations carried out even with dissimilar methods (Gonzales-Barron et al., 2019). Thus, this work aimed to combine results from thermal inactivation experiments of HRMs in fruit juices, by developing meta-regression models based on an extended Bigelow equation. With this approach, values of decimal reduction time (D) taken from the literature can be described as a function of temperature of inactivation, pH and Brix, as well as by study characteristics codified a posteriori such as inactivation method, type of medium, fruit matrix and age of spores. It is expected that global inactivation parameters of HRMs, as summarized from the meta-regression models, can be helpful to the fruit juice industry in the delineation of heat treatment processes and determination of shelf-life.

2. Material and methods

2.1. Literature search and data extraction

Literature search was carried out using the bibliographic databases Pubmed, Scopus and Web of Science to find primary sources on challenge studies aiming to characterise the thermal inactivation kinetics of spores of the genera *Neosartorya*, *Byssochlamys* and *Talaromyces* in liquid media. The electronic search was performed systematically and aimed to find quality studies validated by the scientific community such as original articles and theses. Articles were searched, with no year restriction, with appropriate key terms in English, Spanish and Portuguese, using a formula that combined terms regarding the heat resistant mold (*Neosartorya*, *Byssochlamys*, *Talaromyces*), the thermal treatment (pasteurization, sterilization, "heat resistance", "heat treatment", "thermal resistance", "thermal treatment") and the lethality parameters (D, z, "D value", "z value"), to identify studies that published D-values obtained from conventional heat treatment experiments. The articles retrieved from the three bibliographic databases were combined in the JabRef software version 4.3.1. After elimination of duplicate sources, abstracts were first screened for adequacy, and afterwards full texts were checked for completeness of the data required for this meta-analysis. Primary sources were considered as eligible if they reported the type and characteristics of liquid medium, including pH and °Brix, the inactivation method, and the D value, or alternatively the data from where D value could be estimated. Thus, a total of 25 studies were regarded as appropriate for inclusion in the meta-analysis, since they contained required data on inactivation of *Byssochlamys*, *Neosartorya* and *Talaromyces* in liquid media such as buffer, glycose solution and fruit juices, concentrates, pulp or puree. These primary studies, and the number of D values thereof extracted, were: Amaeze (2013) [230 D values], Bayne and Michener (1979) [16], Berni et al. (2017a, 2017b) [24], Beuchat (1986) [75], Conner and Beuchat (1987) [12], Engel and Teuber (1991) [21], Evelyn et al. (2016) [2], Hoffman (2004) [16], King et al. (1969) [7], King et al. (1979) [4], King and Halbrook (1987) [11], King and Whitehand (1990) [26], Marcolino (2003) [6], Rajashekhara et al. (1996) [14], Rajashekhara et al. (1998) [12], Rajashekhara et al. (2000) [12], Salomão et al. (2007) [9], Sant'Ana et al. (2009a, 2009b) [4], Slongo et al. (2005) [33], Slongo and de Aragão (2007) [6], Splittstoesser and Splittstoesser (1977) [16], Splittstoesser and Churey (1991) [22], Tournas (1994a, 1994b) [12], Tranquillini et al. (2017) [6], and Zimmermann (2012) [10].

From each of them, the following study characteristics were obtained: D-value (***D***, min); inactivation temperature (***T***, °C); pH (***pH***); °Brix (°***Brix***); type of medium (***Type t*** = {"model", "juice", "concentrate"}); fruit (**f**, a series of fruits, but also including "water", "glycose solution", "ringer solution" and "buffer"); use of preservative (***Preserv p*** = {"yes", "no"}); inactivation method (***Inac m*** = {capillary tubes – "CAP", screw tubes – "SCW", three-neck round – "TNR", thermal death tubes – "TDT", polythene bags – "PTB", Erlenmeyer – "Erlen"}), age of spores (***Age***, days) and study (***j*** = {"1", "2", … "25"}). It is worth mentioning that the variable ***Type*** intended to differentiate between model menstruum and actual liquid food; and within liquid food, to further differentiate according to consistency. Thus, ***Type*** was a moderator purposely coded, directly from information provided in the primary study, to group buffer, glycose solution, ringer solution and water into the class "model", food of lower consistency such as fruit juices in the class "juice", and thicker food such as fruit concentrates, puree and pulp into the class "concentrate".

2.2. Meta-regression

A Bigelow-type secondary model was used to describe the heat resistance of *Byssochlamys*, *Neosartorya* and *Talaromyces* in liquid matrices, as affected by inactivation temperature, and matrix pH and °Brix. A °Brix term was used instead of the water activity term of the original Bigelow equation, since water activity information was seldom provided in the primary studies.

$$logD = logD^* - \left(\frac{1}{z_T}\right)(T - T^*) - \left(\frac{1}{z_{pH}^2}\right)(pH - pH^*)^2 - \left(\frac{1}{z_{^\circ Brix}}\right)(^\circ Brix - ^\circ Brix^*) \quad (1)$$

In Eq. (1), D is the decimal reduction time at the constant temperature T and at the pH and °Brix of the food/model matrix; T^* is the reference temperature (set at 90 °C); pH* and °Brix* are the reference pH and °Brix (chosen to be 3.5 and 12°, respectively, to correspond to the common pH and total soluble solids of fruit juices); z_T is the conventional thermal z-value; z_{pH} is the distance of pH from pH* which leads to a ten-fold change in decimal reduction time; $z_{^\circ Brix}$ is the distance of °Brix from °Brix* which leads to a ten-fold change in decimal reduction time; and D^* is the decimal reduction time at T^*, pH* and

Brix*.

As mentioned earlier, other study characteristics or moderators (namely, fruit, type of beverage (i.e., model, juice, concentrate), addition of preservative (yes/no), age of spores (day), inactivation method (SCW, PTB, CAP, TDT, Erlen, TNR), culture medium and species) were extracted from the primary studies in an attempt to investigate how they affect the decimal reduction time D and, consequently, the thermal parameters D^*, z_T, z_{pH} and $z_{^\circ Brix}$. To this effect, the basis Bigelow model (Eq. (1)) was transformed into an overarching linear mixed-effects meta-regression model to describe each of the three fungi data sets. Since such transformations are data-driven, the meta-regression architecture chosen for each fungi data set had to meet two requirements: (i) to be parsimonious and provide good representation of the data, and (ii) to have a fully interpretable arrangement of variables. Model development was carried out in a systematic manner that involved: appraisal of data structure, confounding and correlation; definition of potential clustering variables and their appropriate location for random effects as well as variance structure; choice of significant and meaningful fixed effects; and assessment of goodness-of-fit through validations graphs (i.e., residuals versus fitted, histogram of residuals, residuals versus explanatory variables, and influential observations). With a reduced number of candidate models, comparison was undertaken through log likelihood comparison and information criteria using the maximum likelihood estimator. Thus, for each of the three fungi, several meta-regression models were tested, but only the best ones are presented here.

To describe the combined effects of heating temperature and pH and °Brix of the liquid medium on the thermal resistance of fungal spores, the following Bigelow-based meta-regression architectures provided the best representation of the data for *Neosartorya* (Eq. (2)), *Byssochlamys* (Eq. (3)) and *Talaromyces* (Eq. (4)).

$$logD^*_{jfm} = (\alpha_0 + \alpha_{1m} Inac_m + \alpha_2 log(Age)) + u_{jf} = logD^*_{mean\ m} + u_{jf}$$

$$\frac{1}{z_{T\,jfp}} = \beta_1 + \beta_2 Preserv_p + v_{jf}$$

$$\frac{1}{z^2_{pH}} = \delta_1$$

$$\frac{1}{z_{^\circ Brix\,t}} = \gamma_1 + \gamma_{2t} Type_t \tag{2}$$

$$logD^*_{jmst} = (\alpha_0 + \alpha_{1m} Inac_m + \alpha_{3s}\text{Species}_s + \alpha_{4t} Type_t) + u_j = logD^*_{mean\ mst} + u_j$$

$$\frac{1}{z_{T\,jt}} = \beta_1 + \beta_{3t} Type_t + v_j$$

$$\frac{1}{z^2_{pH}} = \delta_1$$

$$\frac{1}{z_{^\circ Brix\,t}} = \gamma_1 + \gamma_{2t} Type_t \tag{3}$$

$$logD^*_{jfm} = (\alpha_0 + \alpha_{1m} Inac_m) + u_{jf} = logD^*_{mean\ m} + u_{jf}$$

$$\frac{1}{z_{T\,p}} = \beta_1 + \beta_2 Preserv_p$$

$$\frac{1}{z^2_{pH}} = \delta_1$$

$$\frac{1}{z_{^\circ Brix}} = \gamma_1 \tag{4}$$

where α_0 is an intercept, α_{1m} is the set of fixed effects of the m methods of inactivation (a class variable consisting of the levels SCW, PTB, CAP, TDT, Erlen or TNR), α_2 is the effect of a one-log increment in the spores age (day), α_{3s} is the set of fixed effects of the s species of fungus, and α_{4t} is the set of fixed effects of the t types of media (a class variable consisting of model, juice and concentrate). The value of log D^*_{mean} represents the mean of the log-transformed decimal reduction time at the reference T^*, pH* and *Brix applicable to the entire population of liquid media, yet it is an intercept allowed to take up different independent values arising from the variability either between primary studies j or between the interaction levels study j × fruit f. The intercept random effects due to primary study (u_j) or due to the interaction study × fruit (u_{jf}) are assumed to have a normal distribution with mean zero and variance s^2_u.

The coefficient β_1 is the mean effect of a 1-°C increment in temperature difference (T-T^*) for the entire population of liquid media; β_2 is the coefficient of the interaction term between addition/non-addition of a preservative (p) and temperature. Such interaction term allows for a shift in the temperature difference slope if any preservative is added to the liquid medium. The set of parameters β_{3t} represents the interaction between type of medium (t) and temperature slope, which allowed for z_T values to be different between juices and concentrates. Furthermore, the remaining unexplained variability in the temperature slope linear predictor ($1/z_T$) was extracted by placing random-effects v_j/v_{jf} with subject of variation primary study j or the interaction study × fruit jf. These random effects were assumed to have a normal distribution with mean zero and variance s^2_v.

The coefficient δ_1 represents the effect of a one-unit increment in pH difference (pH-pH*) while γ_1 the effect of a one-unit increment in °Brix difference (°Brix-°Brix*) on the logarithm of the thermal death time D. Since γ_1 was found to be affected by the type of liquid medium, the coefficients γ_{2t} of the interaction term between Type (t levels) and the °Brix difference slope were calculated. Such interaction enables each type of medium (i.e., model, juice and concentrate) to have its own $z_{^\circ Brix}$.

The meta-regressions explained above (Eqs. (2), (3) and (4)) were fitted as mixed-effects linear models (Eqs. (5), (6) and (7), respectively),

$$\begin{aligned} logD_{ijfmpt} = {} & (\alpha_0 + \alpha_{1m} Inac_m + \alpha_2 log(Age) + u_{jf}) \\ & + (\beta_1 + \beta_2 Preserv_p + v_{jf})(T - T^*) \\ & + \delta_1 (pH - pH^*)^2 \\ & + (\gamma_1 + \gamma_{2t} Type_t)(^\circ Brix - {}^\circ Brix^*) + \varepsilon_{ijfmpt} \end{aligned} \tag{5}$$

$$\begin{aligned} logD_{ijmst} = {} & (\alpha_0 + \alpha_{1m} Inac_m + \alpha_{3s}\text{Species}_s + \alpha_{4t} Type_t + u_j) \\ & + (\beta_1 + \beta_{3t} Type_t + v_j)(T - T^*) \\ & + \delta_1 (pH - pH^*)^2 + (\gamma_1 + \gamma_{2t} Type_t)(^\circ Brix - {}^\circ Brix^*) + \varepsilon_{ijmst} \end{aligned} \tag{6}$$

$$\begin{aligned} logD_{ijfmp} = {} & (\alpha_0 + \alpha_{1m} Inac_m + u_{jf}) + (\beta_1 + \beta_2 Preserv_p)(T - T^*) \\ & + \delta_1 (pH - pH^*)^2 + \gamma_1 (^\circ Brix - {}^\circ Brix^*) + \varepsilon_{ijfmp} \end{aligned} \tag{7}$$

As all variances can be thought of as realisations of a *primary study* (Eq. (6)) or *study* × *fruit* (Eqs. (5) and (7)), the presence of heterogeneity was assessed by the Wald's test of significance of each of the variance, s^2_u and s^2_v parameters. The Wald Z statistic is a common likelihood-based statistic computed as the parameter estimate divided by its asymptotic standard error, and it is frequently used in meta-analysis to assess the significance of the covariance parameters in heterogeneity analysis (Whitehead et al., 2002).

Hence, if s^2_u and s^2_v were statistically significant, the between-study variability τ^2 was approximated by $s^2_u + s^2_v$, and the I^2 statistics or intra-class correlation, estimating the proportion of between-study variability from the total variance, can be approached as $(s^2_u + s^2_v)/(s^2_u + s^2_v + s^2)$, where s^2 is the variance of the normally-distributed residual random errors $\varepsilon_{ijfmpt}/\varepsilon_{ijmst}/\varepsilon_{ijfmp}$. In order to estimate the fraction of between-study variance explained by the moderators (R^2), a null model (i.e., the simple Bigelow model, Eq. (1), without moderators) was also fitted, and its between-study variability (τ^2_{null}) extracted. The value of R^2 was finally estimated as $[(\tau^2_{null} - \tau^2)/\tau^2_{null}]$. In addition, estimates of log D^*, z_T,

z_{pH} and $z_{°Brix}$ as affected by fungus species, type of medium, inactivation method, fruit and addition of preservative, were computed. The computation of log D^* values and their confidence intervals was carried out by the method of estimable linear combinations from the fitted meta-regression parameters (McLean et al., 1991); this is, by solving $(\alpha_0 + \alpha_{1m}Inac_m + \alpha_2 \log(Age) + u_{jf})$, $(\alpha_0 + \alpha_{1m}Inac_m + \alpha_{3s}\text{Species}_s + \alpha_{4t}Type_t)$ and $(\alpha_0 + \alpha_{1m}Inac_m + u_{jf})$ for *Neosartorya*, *Byssochlamys* and *Talaromyces*, respectively, using the appropriate levels of the variables. Marginal random effects (u_{jf}) only entered the linear combinations above when solving log D^* for specific fruits. The computation of z values was performed in two stages: first, by solving the linear combinations $(\beta_1 + \beta_2 Preserv_p + v_{jf})$ and $(\gamma_1 + \gamma_{2t}Type_t)$ for *Neosartorya*, $(\beta_1 + \beta_{3t}Type_t)$ and $(\gamma_1 + \gamma_{2t}Type_t)$ for *Byssochlamys* and $(\beta_1 + \beta_2 Preserv_p)$ for *Talaromyces*; and second, by performing Monte Carlo simulations to propagate the uncertainty of these linear combinations (represented by their mean and standard errors as parameters of a normal distribution) onto their reciprocal functions (Eqs. (2), (3), (4)), producing thereby distributions of z_T and z_{Brix} for *Neosartorya*, z_T and z_{Brix} for *Byssochlamys*, and z_T for *Talaromyces*. Estimates of z_{pH} for the three moulds and z_{Brix} for *Talaromyces* were also computed by Monte Carlo simulation, yet directly using the fitted parameters δ_1 (Eqs. (5), (6), (7)) and γ_1 (Eq. (7)). The number of iterations was set to 30,000, and the estimate values of mean, standard error and 95% CI were calculated from the simulated data. The final models were adjusted by the restricted maximum likelihood estimator using the 'nlme' package (Pinheiro et al., 2013) in the R software (version 3.6.1, R Development Core Team).

3. Results and discussion

3.1. Description of meta-analytical data sets

The distribution of the number of D values by the aforementioned study characteristics is separately presented for *Neosartorya*, *Byssochlamys* and *Talaromyces* in the Supplementary Material (Table S1). The inactivation data of *Neosartorya* spores of age between 10 and 90 days, was tested at temperatures of inactivation of 80–98 °C in 14 primary studies. The *Neosartorya* data set was comprised of 44 entries for model media, 216 entries for fruit juices, and 65 entries for concentrates. Juices and concentrates were made of apple, blueberry, cherry, cranberry, grape, grapefruit, mango, orange, papaya, peach, pear, pineapple, prune, raspberry or strawberry. In 106 entries, preservatives such as lactic acid, malic acid, citric acid, tartaric acid, and fructose/sucrose in high concentrations, were added to the model/food matrices; although for the meta-analytical model, preservative was codified as a binary variable (yes/no), as explained in Methodology. The inactivation methods used to determine D values in the *Neosartorya* data set were: CAP ($n = 21$), Erlen ($n = 45$), PTB ($n = 26$), SCW ($n = 144$), TDT ($n = 51$) and TNR ($n = 39$).

The *Byssochlamys* meta-analytical data set was extracted from 9 primary studies, which presented inactivation outcomes for *B. fulva* ($n = 57$) and *B. nivea* ($n = 28$) spores of age 28–84 days inoculated in model media ($n = 50$), juice ($n = 18$) and concentrates ($n = 17$) made of apple, grapes, pineapple and tomato, tested at constant temperatures between 77.5 and 100 °C. For the *Byssochlamys* data, the addition of preservatives could not be considered as a moderating variable in the meta-regression model since only one primary study (King et al., 1979) tested the effect of tartaric acid, producing 4 entries out of the 85 D values recovered. The inactivation methods used to quantify *Byssochlamys* thermal resistance were only CAP (n = 21), SCW ($n = 16$) and TDT ($n = 48$).

The inactivation data of *Talaromyces* spores of age between 30 and 60 days, evaluated at constant temperatures of inactivation of 80.0–92.5 °C in 6 primary studies, included 53 entries for model media, 113 entries for juice of °Brix between 12.0 and 19.5°, and 30 entries for concentrates of 25.2–33.4°Brix. Juices and concentrates were made of apple, blueberry, cherry, grapefruit, orange, pineapple, peach, pear, pineapple, raspberry or strawberry. In 90 entries, preservatives such as citric acid, lactic acid, malic acid, tartaric acid, sodium benzoate, and fructose/sucrose in high concentrations, were added to the matrices; whereas the inactivation methods were Erlen ($n = 30$), PTB ($n = 6$), SCW ($n = 111$) and TDT ($n = 49$).

Some data sparseness was identified in the variables related to the intrinsic characteristics of the medium (°Brix and pH) across the types of medium. pH of juices and concentrates were well within the acid range (between 2.4 and 4.3) while the pH of model foods presented a wider range from 3 to 7.4. In relation to °Brix, ranges within type of medium were more variable across moulds: in the *Neosartorya* data set, °Brix ranges were 0°–12.5° for model food, 9.0°–37.2° for juices and 25.2°–47.3° for concentrates; °Brix for the *Byssochlamys* data set were 0°–40.0° for model, 5.0°–54.0° for juices and 4.0°–40.0° for concentrates; and for the *Talaromyces* data set, °Brix values were in the range of 0.0°–60.0° for model foods, 12.0°–37.2° for juices and 25.2°–33.4° for concentrates. It was due to the different domains of pH and °Brix between types of medium that the meta-regression models ascertained whether specific z_{pH} and z_{Brix} could be determined for model food, juices and concentrates. In fact, nested arrangements of continuous variables of different range within categorical variables constitute a valid approach to deal with unbalanced data, and this data dependency structure can be efficiently dealt with by mixed effects modelling (Harrison et al., 2018). Interestingly, the effect of pH on log D (δ_1) was not regulated by the type of medium in any fungi; on the contrary, the effect of °Brix on log D (γ_1) turned out to be dependent on the type of medium for *Neosartorya* and *Byssochlamys*. Collinearity was not found between the continuous variables, temperature, pH, °Brix and the log-transformed age of spores. For further appraisal of parameter estimates reliability, the correlation matrices of fixed effects are given in the Supplementary Material (Tables S2, S3 and S4) for the meta-regressions of the three fungi.

3.2. Sources of variability in the behavior of fungal spore inactivation

The data set displayed great variability in the behavior of fungal spore inactivation (Fig. 1). The variations in D values are explained by differences in fungal genera, food matrices, temperature deviations, age of spores, inactivation methods and the particular physicochemical characteristics of the substrates, such as pH and °Brix (Souza et al., 2017; Tournas, 1994a, 1994b). Nevertheless, Fig. 1 evidences that the D values presented similar trends, allowing few patterns to be characterized. Data for *Neosartorya* and *Talaromyces* were more homogeneous (for 85 °C, log D ranged from approximately 0 to 2) than the data recovered for *Byssochlamys* (for 85 °C, log D ranged from −1 to 3, approximately). The segregation found for *Byssochlamys* data into two lines, is due to the presence of two different species (*B. nivea* and *B. fulva*). Ascospores from different species show a difference in their response to time/temperature combinations. In general, *B. fulva* has higher D values than *B. nivea*: whereas ascospores of *B. fulva* can survive at 85 °C for 120 min (Splittstoesser and Splittstoesser, 1977), *B. nivea* ascospores are inactivated at 80 °C for 20 min (Sant'Ana et al., 2009a, 2009b).

Although the arrangement of variables in the meta-analytical models for *Neosartorya*, *Byssochlamys* and *Talaromyces* were data-driven, the analyses of variance of the meta-regressions showed some similarities among genus in the significance of the fixed effects of the physicochemical and moderating variables on the D values (Table 1). As expected, the thermal resistance of the three genera was strongly affected by the inactivation temperature ($p < 0.0001$; F = 84.9–1101). In the case of *Neosartorya* and *Talaromyces*, the effect of temperature on D was further modulated by the use of preservatives ($p < 0.0001$; F = 23.3–34.7), and, in the case of *Byssochlamys*, by the type/consistency of medium ($p < 0.0001$; F = 33.6). For the three fungus genus, the medium pH was significant ($p = 0.006$–0.019; F = 4.74–8.04), yet to a lesser extent than the soluble solids ($p < 0.0001$; F = 24.8–71.0). The type of medium also regulated the effect of °Brix on the log D-values of *Neosartorya* ($p = 0.001$; F = 7.01) and *Byssochlamys* ($p = 0.012$; F = 3.94).

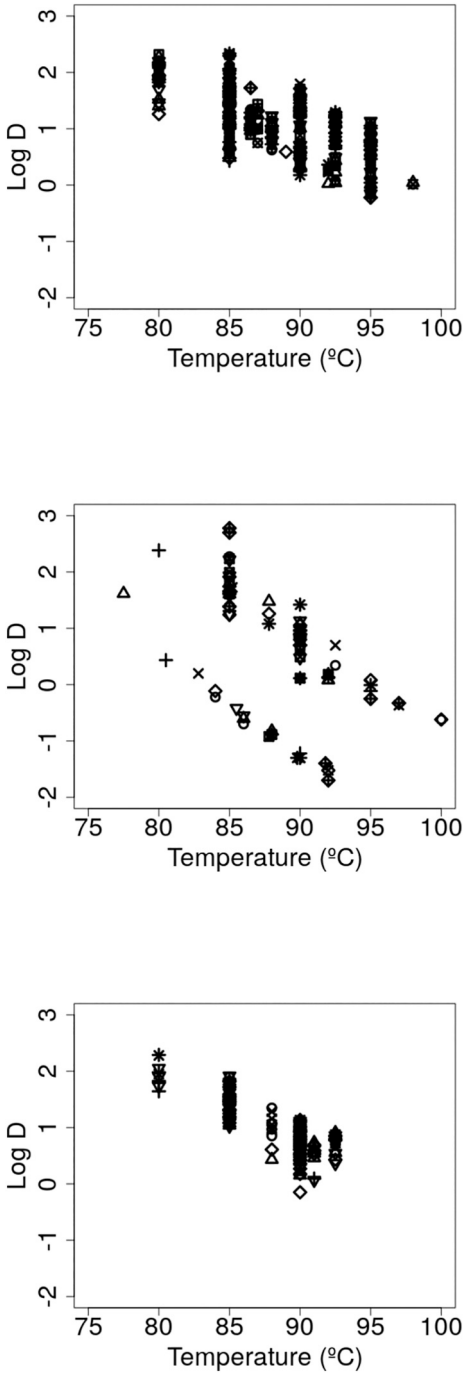

Fig. 1. Scatter plots of log D and heating temperature from the raw meta-analytical data of thermal resistance of spores of *Neosartorya* (top), *Byssochlamys* (middle) and *Talaromyces* (bottom) in liquid media. Different markers indicate different inactivation experiments.

Furthermore, the heat resistance parameter was strongly influenced ($p < 0.0001$) by the type of inactivation method employed ($F = 10.4–44.4$), while the age of the spores used in the inoculation experiments was found to regulate the *Neosartorya* D-values thereof derived ($p = 0.023$; $F = 5.23$).

In assessing the fitting quality of the meta-analytical models, it was found that the Studentized residuals fell between −3.0 and +3.0, and according to the Shapiro-Wilk test, no evidence was found to state that the residuals did not follow a normal distribution (p values from 0.34 to 0.45). Furthermore, the residuals versus the fitted values (i.e., log D) were well behaved, not revealing any singular pattern, as they were randomly spread with a coefficient of correlation below 0.1 in all cases (Fig. S1). In addition, there was good agreement between the fitted and the observed log D values for the three models with coefficients of correlation higher than 0.980 (Fig. S2).

Table 1
Analyses of variance of the fixed effects of the Bigelow-type meta-regression models predicting the logarithm of the decimal reduction time D (min) of spores of *Neosartorya*, *Byssochlamys* and *Talaromyces* in liquid media and beverages as a function of temperature, pH, °Brix and moderating variables.

Model	Fixed effect	Num/Den DF[a]	F value	Pr > F
Neosartorya	Inactivation method	5/297	2.47	0.032
	Log10 (spores age)	1/297	5.23	0.023
	Temperature	1/9	84.9	<0.0001
	Temperature * preservative	1/297	23.3	<0.0001
	°Brix	1/297	24.8	<0.0001
	°Brix*type	2/297	7.01	0.001
	pH	1/297	5.56	0.019
Byssochlamys	Species	1/64	113.6	<0.0001
	Inactivation method	2/64	44.4	<0.0001
	Type	2/64	6.11	0.004
	Temperature	1/4	1101	<0.0001
	Temperature*type	2/64	33.6	<0.0001
	°Brix*type	3/64	3.94	0.012
	pH	1/64	8.04	0.006
Talaromyces	Inactivation method	3/167	10.4	<0.0001
	Temperature	1/11	787.8	<0.0001
	Temperature*preservative	1/167	34.7	<0.0001
	°Brix	1/167	71.0	<0.0001
	pH	1/167	4.74	0.030

[a] Degrees of freedom of the numerator and denominator.

Table 2
Parameter estimates of the Bigelow-type meta-regression model predicting the logarithm of the decimal reduction time D (min) of *Neosartorya* spores in liquid media as a function of temperature, pH, °Brix and moderating variables (parameters of Eq. (5)).

Parameters	Mean	Standard error	Pr > |t|, Z	Heterogeneity
Predictors of log D*				
α_{1m} (inactivation method)				Null model
Method: CAP	0.282[a]	0.229	0.219	$\tau^2 = 0.0930$
Method: Erlenmeyer	0.076[a]	0.279	0.785	$I^2 = 64.6\%$
Method: PTB	0.309[a]	0.220	0.161	
Method: SCW	0.454[a]	0.187	0.016	With moderators
Method: TDT	0.159[a]	0.191	0.407	$\tau^2_{res} = 0.0524$
Method: TNR	1.021[b]	0.275	0.003	$R^2 = 43.6\%$
α_2 (log spores age)	0.188	0.083	0.023	
Predictors of $(1/z_T)$				
β_1 (temperature)	−0.137	0.015	<0.0001	
β_{2p} (temperature × preservative)				
Preservative: yes	0.038	0.008	<0.0001	
Preservative: no	0	–	–	
Predictors of $(1/z_{Brix})$				
γ_1 (°Brix)	0.023	0.007	0.001	
γ_{2t} (°Brix × type)				
Type: concentrate	−0.020	0.007	0.003	
Type: juice	−0.015	0.007	0.046	
Type: model	0	–	–	
Predictors of $(1/z_{pH})$				
δ_1 (pH)	0.021	0.009	0.019	
Variances				
s^2_u (log D^*_{mean})	0.0505	0.0261	0.026	
s^2_v (temperature)	0.0019	0.0010	0.031	
s^2 (residual)	0.0462	0.0037	<0.0001	

Different superscript letters in the same column indicate significant difference according to anova two way ($p < 0.05$).

3.3. Meta-regression analysis for Neosartorya spores

The main parameters which influenced the log D* predictors of *Neosartorya* spores estimated by the Bigelow-type meta-regression model were: inactivation method and spores age (Table 2). Notwithstanding, use of preservative, type of medium, temperature, °Brix, and pH were the parameters determining z values of *Neosartorya* spores (Table 2). Two-way comparisons of means at $\alpha = 0.05$ was not able to find any significant differences in log D between the other methods CAP, Erlen, PTB, SCW and TDR. Nonetheless, the TNR method tended to produce higher values of log D*.

In practice, all inactivation methods may show some limitations. Method such as Erlenmeyer and SCW may lead to under or over-estimation of D* due to the heating exchange mechanisms that occurs within the systems. When a glass flask containing an aqueous solution is heated in a bath, changes in the pressure and temperature may induce evaporation. It may increase the pressure within the system and causes the microbial inactivation in a temperature under the temperature the experiment is being tested (Pflug, 2003; Pflug et al., 2001). Moreover, the heat conduction transfer from the glass wall to the aqueous solution may bias the D value thus it cannot be neglected. Therefore, a correction of any heating or cooling lag must be considered to avoid bias in D value estimates (Davies et al., 1977; Haas et al., 1996; Pflug, 2003; Pflug et al., 2001).

In addition, the reference D* was significantly influenced by spores age ($p = 0.023$): the older the spores the greater their thermal resistance. The significant and positive temperature × preservative interaction term ($\beta_{2p} = 0.038$; $p < 0.0001$ in Table 2) indicates that adding preservatives to the liquid media changes the effect of the temperature for *Neosartorya*. Similarly, the significant °Brix × type interaction terms ($p = 0.003$ for concentrate, and $p = 0.046$ for juice) revealed that the z_{Brix} of *Neosartorya* is affected by the type or consistency of the medium. The decreasing estimate values of γ_{2t} for model ($\gamma_{21} = 0$), juice ($\gamma_{22} = -0.015$) and concentrate ($\gamma_{23} = -0.020$) suggest that the higher the consistency of the medium, the higher the z_{Brix}. However, while z_T and z_{Brix} were found to be regulated by the addition of preservatives and the type of medium, respectively, z_{pH} only depended on the medium pH ($p = 0.019$) and was not moderated by any study characteristic. Analysis of heterogeneity indicated that 64.6% of the total variability in the D estimates retrieved from the literature was due to the different primary studies. Nonetheless, the strategic incorporation of the various moderators to the basic Bigelow equation was able to explain 43.6% of the variability in log D between studies. Greater between-study variability was extracted from log D* ($s^2_u = 0.0505$) than from z_T ($s^2_v = 0.0019$; Table 2).

With the parameters of the meta-regression, the overall D* of *Neosartorya* spores was estimated at 5.345 min (95% CI: 4.102–7.084 min; Table 3). The pooled D* values of *Neosartorya* for each of the inactivation methods was solved by fixing the spores age to 30 days (median of the data). As deduced from the two-way comparisons of inactivation methods (Table 2), the TNR method tended to produce higher D* values (19.81 min; 95% CI: 6.456–60.81 min) than the SCW (5.383 min; 95% CI: 2.944–9.840 min), PTB (3.846 min; 95% CI: 1.746–8.492 min), CAP (3.622 min; 95% 1.535–8.531 min), TDT (2.729 min; 95% CI: 1.466–5.070 min) and Erlen (2.254 min; 0.767–6.607 min) methods. Solving the meta-regression for the random effects u_{jf} due to study × fruit, it was possible to estimate overall reference D* values for the different fruits as produced by any inactivation method. For instance, using TDT method, the overall reference D* values of juices were 2.223 min for apple, 2.735 min for grape, 2.786 min for papaya, 2.870 min for peach, 3.119 min for berries, 3.483 min for pineapple and 3.776 min for orange. Numerically higher pooled D* values would be obtained for the same fruits when determined by the SCW method (Table 3).

From the meta-regression parameter estimates (Table 2), the addition of any common preservative such as citric, malic, tartaric and lactic acid, sorbates and benzoates, significantly regulated the effect of temperature on log D ($p < 0.0001$ for β_{2p}), which, in turn, produced overall z_T-values for *Neosartorya* spp. of 6.944 °C (95% CI: 6.166–7.723 °C) and 8.913 °C (95% CI: 6.994–10.83 °C) for the non-addition and the addition of preservatives in the liquid media (Table 4). As mathematically inferred from the Bigelow equation, this increase in the z_T due to

Table 3
Estimates of log D* (log base 10 of D-value [min] at 90 °C, pH 3.5 and 12° Brix) of *Neosartorya* spores in juice for different combinations of the moderating variables: fruit, inactivation method and addition/no addition of preservatives.

Parameter	Mean	Standard error	95% CI
Overall mean (any method)	0.728	0.059	[0.613–0.848]
Inactivation method[b]			
CAP	0.559	0.189	[0.186–0.931]
Erlen	0.353	0.238	[−0.115–0.820]
PTB	0.585	0.175	[0.242–0.929]
SCW	0.731	0.133	[0.469–0.993]
TDT	0.436	0.133	[0.166–0.705]
TNR	1.297	0.247	[0.810–1.784]
With inactivation method SCW			
Orange juice	0.981	0.106	[0.773–1.189]
Pineapple juice	0.947	0.103	[0.744–1.150]
Berry juice[a]	0.899	0.124	[0.656–1.143]
Peach juice	0.863	0.144	[0.578–1.147]
Apple juice	0.752	0.112	[0.531–0.973]
Grape juice	0.842	0.111	[0.625–1.059]
Papaya juice	0.850	0.150	[0.555–1.146]
Buffer	0.798	0.137	[0.527–1.068]
With inactivation method TDT			
Orange juice	0.577	0.143	[0.296–0.857]
Pineapple juice	0.542	0.102	[0.341–0.742]
Berry juice[a]	0.494	0.134	[0.229–0.758]
Peach juice	0.458	0.154	[0.154–0.762]
Apple juice	0.347	0.122	[0.105–0.589]
Grape juice	0.437	0.116	[0.209–0.666]
Papaya juice	0.445	0.077	[0.294–0.596]
Buffer	0.393	0.143	[0.112–0.675]

[a] Berry encompasses strawberry, raspberry, cranberry and blueberry.
[b] Estimates solved for the median of the spores age (30 days) in all methods.

Table 4
Estimates of z-values of *Neosartorya* spores as affected by fruit, type of beverage and addition/no addition of preservatives.

z-Value		Mean	Standard error	95% CI
z_T	Preservatives			
(°C)	No addition	6.944	0.393	[6.166–7.723]
	Addition	8.913	0.969	[6.994–10.83]
	No addition of preservative			
	Orange juice	6.645	0.654	[5.350–7.940]
	Pineapple juice	6.468	0.486	[5.507–7.430]
	Berry juice[a]	6.431	0.481	[5.479–7.383]
	Peach juice	6.684	0.884	[4.393–8.436]
	Apple juice	7.321	0.698	[5.938–8.703]
	Grape juice	9.042	0.982	[7.098–10.98]
	Papaya juice	6.176	0.428	[5.328–7.025]
	Buffer	7.236	0.583	[6.082–8.390]
	Preservative added			
	Orange juice	8.425	1.198	[6.051–10.79]
	Pineapple juice	8.143	0.958	[6.245–10.04]
	Berry juice[a]	8.084	0.975	[6.153–10.01]
	Peach juice	8.489	1.575	[5.371–11.61]
	Apple juice	9.551	1.455	[6.671–12.43]
	Grape juice	12.69	2.483	[7.773–17.60]
	Papaya juice	7.692	0.869	[5.971–9.413]
	Buffer	9.395	1.285	[6.854–11.94]
$z_{°Brix}$	Type			
	Model (buffer/water)	46.82	15.20	[27.72–87.90]
	Juice	133.5	40.68	[80.92–242.1]
	Concentrate	384.9	200.8	[173.3–986.2]
z_{pH}	Overall	7.073	1.463	[5.045–10.84]

[a] Berry encompasses strawberry, raspberry, cranberry and blueberry.

the addition of a preservative translates into higher D values at temperatures higher than 90 °C (T*) and lower D values at temperatures lower than 90 °C. Global z_T-values by type of fruit were also estimated by solving the meta-regression for the random effects v_{jf} due to fruits. To mention only some fruits, overall z_T-values for juices without preservatives were 6.431 °C for berries, 7.321 °C for apple and 9.042 °C for grape, whereas, when a chemical preservative was added, overall z_T-values increased up to 8.084 °C, 9.551 °C and 12.69 °C, respectively.

The global z_{pH} of *Neosartorya* spp. was estimated at 7.073 (95% CI: 5.045–10.84), and was not found to be affected by any of the moderators or study characteristics extracted (Table 4). As explained before, this meta-analysis characterized, for the first time, the influence of °Brix on the thermal resistance of the fungus by defining a $z_{°Brix}$ parameter. The type of medium influenced the variation of this parameter. Usually, Brix values for juice ranged from 8° Brix (strawberry) to 30°Brix (mango), while for juice concentrate or puree they varied from 20 to 70°Brix (FAO, 2005). The global $z_{°Brix}$ value for model food (46.82; 95% CI: 27.72–87.90), juices (133.5; 95% CI: 80.92–242.1) and concentrates (384.9; 95% CI: 173.3–986.2). Although the uncertainty about these estimates was high, a trend could be observed that increasing soluble solids in juices causes a greater increase in the fungus' thermal resistance than increasing soluble solids in concentrates/pastes/purees, probably because the thermal resistance of spores in media of high osmotic pressure is already high (Berni et al., 2017a, 2017b).

3.4. Meta-regression analysis for Byssochlamys spores

Although the D values retrieved for *Byssochlamys* were not obtained under the various methods of inactivation, still the parameter estimates of the *Byssochlamys* meta-regression revealed that the CAP method would produce lower log D values than the TDT method, and, in turn, the latter would produce lower log D values than the SCW method. Haas et al. (1996) and Pflug (2003) argued that capillary tubes may be considered to render the closest to the ideal square-wave heating and cooling curve. On the other hand, the lower lag heating time of TDT may be associated to physiological changes in fungal ascospores, which activate the Heat Shock Proteins (HSPs) that end up increasing the D values (Wyatt et al., 2013).

On average, D* values for *B. fulva* were lower than those of *B. nivea* in 0.725 log units (α_3; $p < 0.0001$ in Table 5). Thus, a higher reference thermal resistance was estimated for *B. nivea* (overall D* = 10.33 min; 95% CI: 5.808–18.40) than for *B. fulva* (overall D* = 1.945 min; 95% CI: 1.213–3.111 min) (Table 6). It was also found that the effects of juice ($\alpha_4 = -0.204$; $p = 0.001$) and concentrate ($\alpha_4 = -0.264$; p = 0.001) on log D* were significantly lower than the effect of liquid model (water/buffer, reference set at zero in Table 5). These effects allowed the calculation of overall log D* values for liquid models, juices and concentrates, which were all significantly lower for *B. fulva* (2.382 min, 95% CI: 1.211–4.677 min; 1.297 min, 95% CI: 0.714–2.355 min; and 1.489 min, 95% CI: 0.766–2.904 min, respectively) than for *B. nivea* (12.68 min, 95% CI: 5.781–27.79 min; 6.886 min, 95% CI: 3.573–13.30 min; and 7.762 min, 3.622–17.34 min, respectively) (Table 6). Other examples of predictions that can be obtained by the meta-regression model are the overall D* values of *B. fulva* and *B. nivea* in the three types of medium, as would be measured employing the SCW and the TDT inactivation method. D* values quantified by the SCW would be in all cases higher (Table 6).

The type of medium regulated both the effect of temperature on D (β_{3t}) and the effect of °Brix on D (γ_{2t}), with a parallel trend; this is, the higher the consistency of medium, the higher the z_T and the $z_{°Brix}$, respectively (Table 7). As occurred with *Neosartorya*, the overall $z_{°Brix}$ of *Byssochlamys* numerically increased with increasing consistency of the medium ($z_{°Brix}$ = 74.38 for model, 130.7 for juices and 165.8 for concentrates; Table 7), although the variability around the $z_{°Brix}$ estimates was so high that significant differences between the types of media could not be determined. By contrast, the influence of type of medium on the

Table 5
Parameter estimates of the Bigelow-type meta-regression model predicting the logarithm of the decimal reduction time D (min) of *Byssochlamys* spores in liquid media as a function of temperature, pH, °Brix and moderating variables (parameters of Eq. (6)).

Parameters	Mean	Standard error	Pr > |t|, Z	Heterogeneity
Predictors of log D*				
α_{1m} (inactivation method)				
Method: CAP	−0.186[a]	0.375	0.622	Null model
Method: SCW	1.985[c]	0.147	<0.0001	$\tau^2 = 0.0005$
Method: TDT	1.497[b]	0.083	<0.0001	$I^2 = 0.73\%$
α_3 (species)				
Species: *B. fulva*	−0.725	0.068	<0.0001	With moderators
Species: *B. nivea*	0	–	–	$\tau^2_{res} \sim 0.0000$
α_4 (type)				$R^2 \sim 100\%$
Type: concentrate	−0.204	0.072	0.006	
Type: juice	−0.264	0.077	0.001	
Type: model	0	–	–	
Predictors of ($1/z_T$)				
β_1 (temperature)	−0.194	0.008	<0.0001	
β_{3t} (temperature × type)				
Type: concentrate	0.079	0.009	<0.0001	
Type: juice	0.043	0.012	0.001	
Type: model	0	–	–	
Predictors of ($1/z_{Brix}$)				
γ_{2t} (°Brix × type)				
Type: concentrate	0.007	0.003	0.003	
Type: juice	0.009	0.005	0.062	
Type: model	0.016	0.009	0.079	
Predictors of ($1/z_{pH}$)				
δ_1 (pH)	−0.058	0.020	0.006	
Variances				
s^2_u (log D^*_{mean})	0.0000	–	–	
s^2_v (temperature)	0.0000	–	–	
s^2 (residual)	0.0613	0.0094	<0.0001	

Different superscript letters in the same column indicate significant difference according to anova two way ($p < 0.05$).

Table 6
Estimates of log D* (log base 10 of D-value [min] at 90 °C, pH 3.5 and 12° Brix) of *Byssochlamys* spores in juice for different combinations of the moderating variables: species, inactivation method and type of liquid medium.

Parameter	Mean	Standard error	95% CI
Overall	0.652	0.109	[0.433–0.870]
Model	0.739	0.156	[0.429–1.051]
Juice	0.476	0.132	[0.211–0.739]
Concentrate	0.536	0.154	[0.228–0.844]
B. fulva overall	0.289	0.102	[0.084–0.493]
B. nivea overall	1.014	0.156	[0.764–1.265]
B. fulva in model	0.377	0.146	[0.083–0.670]
B. fulva in juice	0.113	0.129	[−0.146–0.372]
B. fulva in concentrate	0.173	0.145	[−0.116–0.463]
B. nivea in model	1.103	0.171	[0.762–1.444]
B. nivea in juice	0.838	0.143	[0.553–1.124]
B. nivea in concentrate	0.890	0.170	[0.559–1.239]
With inactivation method SCW			
B. fulva in model	1.259	0.104	[1.051–1.467]
B. fulva in juice	0.995	0.070	[0.855–1.134]
B. fulva in concentrate	1.055	0.095	[0.865–1.246]
B. nivea in model	1.985	0.147	[1.690–2.279]
B. nivea in juice	1.720	0.108	[1.503–1.938]
B. nivea in concentrate	1.780	0.143	[1.496–2.066]
With inactivation method TDT			
B. fulva in model	0.766	0.060	[0.645–0.886]
B. fulva in juice	0.568	0.107	[0.354–0.781]
B. fulva in concentrate	0.617	0.105	[0.407–0.827]
B. nivea in model	1.113	0.147	[0.819–1.406]
B. nivea in juice	0.914	0.104	[0.712–1.117]
B. nivea in concentrate	0.964	0.180	[0.605–1.322]

Table 7
Estimates of z-values of *Byssochlamys* spores as affected by type of medium.

z-Value		Mean	Standard error	95% CI
z_T (°C)	Type			
	Model (buffer/water)	5.168	0.199	[4.795–5.578]
	Juice	6.642	0.432	[5.867–7.560]
	Concentrate	8.730	0.457	[7.895–9.686]
$z_{°Brix}$	Type			
	Model (buffer/water)	74.38	46.47	[29.52–215.5]
	Juice	130.7	78.79	[52.83–368.5]
	Concentrate	165.8	85.85	[77.03–422.6]
z_{pH}	Overall	4.344	0.900	[3.199–6.732]

effect of temperature could be more precisely quantified (Table 5), which allowed a clear differentiation in pooled z_T values for model food (5.168; 95% CI: 4.795–5.578), juices (6.642 °C; 95% CI: 5.867–7.560 °C), and concentrates (8.730 °C; 95% CI: 7.895–9.686) (Table 7).

The overall z_{pH} was estimated at 4.344 (95% CI: 3.199–6.732). Unlike the meta-regression for *Neosartorya*, estimates of D* and z_T of *Byssochlamys* were not produced for the different fruits, because the variance of their random effects were not significant. Since there were only juices/concentrates of four fruits (apple, grape, pineapple and tomato) in the *Byssochlamys* data set, it is probable that the few fruits did not confer high variability to log D, or alternatively, that these substrates can be sufficiently described by pH and °Brix. With regards to the heterogeneity analysis (Table 5), the moderators introduced to the basis Bigelow equation accounted for practically all of the variability in D between studies ($R^2 \sim 100\%$), probably because the between-study variance was already low ($\tau^2 = 0.0005$; $I^2 = 0.73\%$).

3.5. Meta-regression analysis for Talaromyces spores

In relation to the inactivation methods available in the meta-analytical data for *Talaromyces* spores, on average, the log D* values determined by the SCW (D* = 5.248 min; 95% CI: 4.246–6.501 min) and Erlen methods (D* = 3.917 min; 95% CI: 3.055–5.035 min) did not differ significantly from one another; yet these methods produced higher log D* values than those of the TDT method (D* = 2.512 min; 95% CI: 1.866–3.388 min), which in turn generated higher log D* values than the PTB method (D* = 1.655 min; 95% CI: 1.132–2.415 min) (Table 9). A similar trend was also observed for *Byssochlamys*, where SCW method led to higher log D* estimates than the TDT method. Since the between-fruit × study variance of the random effects placed on log D* ($p = 0.056$ in Table 8) was very close to reach significance, the clustering variable was maintained in the meta-regression model. This allowed the estimation of fruit-specific D* values obtained under a given inactivation method. As an example, pooled D* estimates of *Talaromyces* for the different fruit juices are predicted for the SCW and the TDT inactivation methods (Table 9).

As occurred with *Neosartorya* spores, the addition of a preservative agent increases the effect of a given change in temperature on the D value of *Talaromyces* ($\beta_{2p} = 0.047$; $p < 0.0001$ in Table 8). As such, the overall z_T of *Talaromyces* in liquid media with preservatives ($z_T = 10.13$ °C; 95% CI: 8.980–11.50 °C; Table 10) was significantly higher than that of liquid media without preservatives ($z_T = 6.875$ °C; 95% CI: 6.416–7.375 °C). After verifying the significant effects of medium °Brix and pH on the D value of *Talaromyces* ($\gamma_1 = 0.011$ with $p < 0.0001$, and $\delta_1 = 0.020$ with $p = 0.030$; Table 8), the pooled $z_{°Briz}$ and z_{pH} were estimated at 86.65 (95% CI: 69.38–110.7) and at 7.566 (95% CI: 5.134–13.55), respectively (Table 10). While the fraction of the total variability in D arising from the different fruits/primary studies was low ($I^2 = 35.9\%$), the addition of moderators to the basis Bigelow equation explained 72.5% of such variability (Table 8).

Table 8
Parameter estimates of the Bigelow-type meta-regression model predicting the logarithm of the decimal reduction time D (min) of *Talaromyces* spores in liquid media as a function of temperature, pH, °Brix and moderating variables (parameters of Eq. (7)).

Parameters	Mean	Standard error	Pr > |t|, Z	Heterogeneity
Predictors of log D*				
α_{1m} (inactivation method)				
Method: Erlenmeyer	0.593[c]	0.054	<0.0001	Null model
Method: PTB	0.218[a]	0.083	0.010	$\tau^2 = 0.0255$
Method: SCW	0.720[c]	0.047	<0.0001	$I^2 = 35.9\%$
Method: TDT	0.400[b]	0.066	<0.0001	
Predictors of $(1/z_T)$				With moderators
β_1 (temperature)	−0.146	0.005	<0.0001	$\tau^2_{res} = 0.0070$
β_{2p} (temperature × preservative)				$R^2 = 72.5\%$
Preservative: yes	0.047	0.008	<0.0001	
Preservative: no	0	–	–	
Predictors of $(1/z_{Brix})$				
γ_1 (°Brix)	0.011	0.001	<0.0001	
Predictors of $(1/z_{pH})$				
δ_1 (pH)	0.020	0.009	0.030	
Variances				
s^2_u (log D^*_{mean})	0.0070	0.0044	0.056	
s^2 (residual)	0.0295	0.0031	<0.0001	

Different superscript letters in the same column indicate significant difference according to anova two way ($p < 0.05$).

4. Conclusions

This meta-analysis compiled data published from 1969 to 2017, and fitted Bigelow-based meta-regression models to synthesize the thermal destruction kinetic parameters of the three most important HRM in liquid media. The fungi studied exhibited different thermal resistance, with *B. fulva* (pooled D* = 1.95 min; 95% CI: 1.21–3.11 min) presenting lower heat resistance than *Talaromyces* spp. (pooled D* = 4.03 min; 95%

Table 9
Estimates of log D* (log base 10 of D-value [min] at 90 °C, pH 3.5 and 12° Brix) of *Talaromyces* spores in juice for different combinations of the moderating variables: fruit or model liquid and inactivation method.

Parameter	Mean	Standard error	95% CI
Overall mean (any method)	0.605	0.035	[0.535–0.676]
Inactivation method			
Erlen	0.593	0.054	[0.485–0.702]
PTB	0.219	0.083	[0.054–0.383]
SCW	0.720	0.047	[0.628–0.813]
TDT	0.400	0.066	[0.271–0.530]
With inactivation method SCW			
Apple juice	0.661	0.077	[0.510–0.813]
Blueberry juice	0.818	0.073	[0.673–0.963]
Buffer	0.629	0.053	[0.524–0.734]
Cherry juice	0.702	0.076	[0.551–0.853]
Glycose solution	0.836	0.077	[0.682–0.990]
Grapefruit juice	0.769	0.033	[0.702–0.836]
Orange juice	0.691	0.032	[0.626–0.756]
Peach juice	0.681	0.076	[0.530–0.832]
Pineapple juice	0.818	0.032	[0.752–0.883]
Raspberry juice	0.631	0.077	[0.479–0.782]
Strawberry juice	0.714	0.077	[0.562–0.865]
With inactivation method TDT			
Apple juice	0.341	0.053	[0.238–0.445]
Blueberry juice	0.498	0.080	[0.339–0.657]
Buffer	0.309	0.087	[0.138–0.479]
Cherry juice	0.382	0.088	[0.208–0.555]
Glycose solution	0.516	0.039	[0.440–0.593]
Grapefruit juice	0.449	0.080	[0.291–0.607]
Orange juice	0.371	0.080	[0.213–0.528]
Peach juice	0.361	0.088	[0.187–0.535]
Pineapple juice	0.498	0.080	[0.340–0.656]
Raspberry juice	0.311	0.088	[0.136–0.485]
Strawberry juice	0.394	0.088	[0.219–0.568]

Table 10
Estimates of z-values of *Talaromyces* spores as affected by fruit and addition/no addition of preservatives.

z-Value		Mean	Standard error	95% CI
z_T (°C)	Preservatives			
	No addition	6.875	0.245	[6.416–7.375]
	Addition	10.13	0.643	[8.980–11.50]
$z_{°Brix}$	Overall	86.65	10.50	[69.38–110.7]
z_{pH}	Overall	7.566	2.089	[5.134–13.55]

CI: 3.43–4.74 min), *Neosartorya* spp. (pooled D* = 5.35 min; 95% CI: 4.10–7.08 min), and *B. nivea* (pooled D* of 10.32 min; 95% CI: 5.81–18.4 min). Comparative analysis of the meta-regressions for the three HRM showed that the inactivation method significantly affects the measured value of D*, since methods such as the screw tubes and three-neck round tend to produce higher D* than the thermal death tubes, polyethylene bags and capillary methods. For the three HRM, the concentration of soluble solids and pH increase the heat resistance of the spores, and furthermore, the addition of food preservatives such as benzoic or sorbic acid to the menstruum significantly modified the effect of temperature on log D, as evidenced for *Neosartorya* and *Talaromyces*. The meta-analysis of *Neosartorya* and *Byssochlamys* revealed the significant impact of the consistency of the medium on $z_{°Brix}$ (p = 0.001–0.012): increasing the soluble solids in concentrates tend to cause a lower decrease in the mold's heat resistance than increasing the soluble solids in model liquid or juices. Furthermore, it appeared that *Talaromyces* (pooled z_{pH} = 7.566; 95% CI: 5.134–13.55) and *Neosartorya* (pooled z_{pH} = 7.073; 95% CI: 5.045–10.84) tend to be less resistant to a decrease in pH of the liquid medium than *Byssochlamys* (pooled z_{pH} = 4.344; 95% CI: 3.199–6.732). This meta-analysis also demonstrated that older spores exhibit greater D* (p = 0.023 from *Neosartorya* data set), and that spores have higher z_T (hence lower heat resistance) in liquid media of greater consistency ($p < 0.0001$ from the *Byssochlamys* data set).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

V.O. Alvarenga, L.P-Silva and A.S. Sant'Ana acknowledge CYTED Program (Network 115 RT0488) for the financial support. A.S. Sant'Ana acknowledges the financial support from CNPq (Grant #302763/2014-7, #305804/2017-0) and to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. U. Gonzales-Barron and V. Cadavez are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). U. Gonzales-Barron also thanks the national funding by FCT, P.I., through the institutional scientific employment programme contract her contract.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2020.108985.

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