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Development of a predictive model of the microbial inactivation of L. monocytogenes during low thermal treatment of fruit juices in combination with carvacrol as aroma compound

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ABSTRACT

Mild heat treatment of fruit juices in combination with natural aroma compounds has been reported as an alternative to conventional pasteurization to better preserve their nutritional value. However, its antimicrobial efficiency varies from one juice to another. This study aims at developing a secondary predictive model of microbial inactivation scale during such combined process. Carvacrol was used as aroma compound and acid-adapted *L. monocytogenes* as target microorganism. The inactivation kinetics of this bacteria were followed in simulated fruit juices using a Central Composite Design with pH (2-6), °Brix (0–24), temperature (55–65 °C), and carvacrol concentration (0–60 μL/L) as independent variables. Curves were fitted to the Weibull inactivation model, and data collected used to generate two predictive models of the inactivation scale parameter through multiple regression analysis following an empirical and a mechanistic (based on Gamma concept) approach. The best of the two models was then validated using real fruit (orange, pineapple, and watermelon) juices. The empirical model where only the four variables tested were considered showed a lower statistical performance compared to the mechanistic model where octanol-water partition coefficient (Ko/w) and vapour pressure (Vp) of carvacrol at the treatment temperature were integrated (R^2 0.6 and 0.9; Accuracy factor 1.5 and 1.3; Sum of Squared Error 3.6 and 1.1, respectively). No significant difference was observed between inactivation scale values obtained with real juices and the predicted values calculated using this mechanistic model. The Ko/w and Vp of the aroma compound used are key parameters that determine the efficiency of the above-described combined treatment.

1. Introduction

Mild thermal treatment of fruit juices in combination with natural aroma compounds (essential oils or their pure aroma compounds) has been reported as an alternative to conventional pasteurization. The latter is known to result in products with poor sensory and nutritional quality and requires a high energetic consumption (Espina et al., 2014; Ngang et al., 2014; Torregrosa et al., 2006). Recent studies showed that supplementation of fruit juices with a natural aroma compound is an effective way of reducing the mild thermal tolerance of acid-adapted microorganisms as well as ensuring the safety of fruit juices (5-Log pathogen reduction: FDA HACCP rule) while preserving their colour and nutritional value (Tchuenchieu et al., 2018a–c). Furthermore, essential oils or their pure aroma compounds are Generally Recognised As Safe (GRAS) and are known for their multiple other positive biological properties (Calo et al., 2015; Lucera et al., 2012). In comparison to other low thermal processes (pulsed electric fields, ultrasounds, or high-pressure technologies, etc), this combined process does not require a particular technology. However, many aspects of this new processing strategy have not yet been systematically studied, which makes it difficult to predict its antimicrobial efficiency. The study of this efficiency is generally performed through primary modelling of inactivation

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with time, and this at fixed temperature and antimicrobial concentration (Ait-Ouazzou et al., 2013; Char et al., 2009; Espina et al., 2012, 2014; Ngang et al., 2014; Sado et al., 2011; Tchuenchieu et al., 2016). Indeed, it is the Weibull inactivation model which is the most used one since it fits well to the experimental microbial inactivation data. The effect of aroma compounds on microbial thermal inactivation is therefore appreciated by comparing the inactivation curves obtained at the different tested concentrations. However, recent studies have shown that for the same treatment condition, inactivation varied from one juice to another (Ait-Ouazzou et al., 2013; Tchuenchieu et al., 2018a). Unfortunately, primary inactivation models obtained have not been exploited to produce secondary models that could help in predictions. They have time as the only independent variable, and do not take into consideration the fruit juice characteristics (like pH and °Brix) variability or the change of the physicochemical properties of the associated aroma compounds with treatment temperature. It is therefore not yet possible to predict the antimicrobial efficiency of this combined treatment of juices and thus the stability of the treated products. As an example, carvacrol which is a natural aroma compound appeared in previous studies, as deeply reducing the time required to ensure the safety of fruit juices when treated at mild temperature (Tchuenchieu et al., 2018a,c). This study aimed at developing for the first time a secondary predictive model of the Weibull scale parameter during combined treatment of fruit juices, that will integrate the possible antimicrobial mechanism of aroma compounds during the treatment. This shall help in making the prediction more applicable at an industrial level. For this purpose, *Listeria monocytogenes,* which is a ubiquitous pathogenic bacteria already isolated from fruit juices and more heat tolerant than the commonly tested non spore-forming pathogen like *E. coli* or *Salmonella* (Doyle et al., 2001; Mazzotta, 2001), was chosen as the target microorganism.

2. Material and methods

2.1. Experimental design

The effects of heating temperature, pH, soluble solids (°Brix), and carvacrol (natural, 99%, Food Grade, Sigma-Aldrich, St. Louis, USA) concentration on the inactivation kinetic of acid-adapted *Listeria monocytogenes* were determined in a simulated fruit juice medium (SFJM). A Central Composite Design (CCD) reinforced at its extremities composed of 29 combinations was used. This was to characterize and quantify the individual (linear and quadratic) and interactive effects of the 4 tested factors on the estimated scale parameter of the heat inactivation kinetic of the strain. The tested levels of pH and °Brix were chosen based on the values generally reported for fruit juices in the literature, and those of carvacrol on the results obtained from the analysis of its acceptance in these food matrices (Tchuenchieu et al., 2018a).

2.2. Simulated fruit juice medium preparation

Fruit juices are mainly made of water and carbohydrates. That's the reason why simulated fruit juice medium (SFJM) corresponding to each of the 29 combinations of the CCD experimental design was prepared as described by Gabriel (2012). Indeed, sucrose was first dissolved in distilled water at an amount corresponding to the °Brix value. The mixture was heated for total dissolution, cooled and the pH adjusted with a 1 N HCl or NaOH solution. A portable pH meter (pH Meter HI 98103, Hanna Instruments, Inc., USA) and an ATAGO refractometer (model 2313; ATAGO Co. Ltd., Tokyo, Japan) were used for that.

2.3. Strain preparation and acid-adaptation

Listeria monocytogenes 56 LY used as the target strain in this study was provided by the Food Microbiology Laboratory of the University of Bologna (Cesena, Italy). It was firstly sub-cultured thrice in Nutrient Broth (CM0001, Oxoid Ltd., Basingstoke, UK) at 37 °C for 24 h. This was followed by an acid-adaptation through a new culture in Nutrient Broth adjusted at pH 6.5 using citric acid. This specific condition was observed in a previous study to strongly enhance the mild thermal tolerance of this strain (Tchuenchieu et al., 2018c).

2.4. Inactivation kinetics

Inactivation kinetics of the obtained acid-adapted cells were followed in the 29 SFJM. The thermal treatment was conducted in a thermostatically controlled water bath as described in a previous study (Ngang et al., 2014). Globally, for each run, 100-μl amounts of *L. monocytogenes* culture were transferred into vials containing 9.9 ml of SFJM at the desired carvacrol concentration and already preheated to the programmed temperature. This ratio of inoculated volume to preheated volume has shown not to cause sensible variation of the temperature profile that can be considered as isothermal. The initial concentrations of cells and survivors were periodically evaluated during thermal treatment by the most-probable-number method. The kinetics were fitted to the Weibull equation (Eq. (1)):

$$\text{Log } S(t) = \text{Log } (Nt/N_0) = -bt^n \quad (1)$$

Where Nt/N0 is the survival ratio S(t) after the treatment time t. The parameters "b" and "n" are the inactivation scale and shape, respectively.

2.5. Theory/calculation

To characterize the individual and interactive influences of heating temperature, pH, °Brix, and carvacrol concentration on the inactivation scale parameter of cells; the results obtained were subjected to multiple regression analysis by Response Surface Methodology (RSM). The results were fitted in a general quadratic model (Eq. (2)) that measured the influences of the tested factors (X_1, X_2, X_3, and X_4) on the measured response (Y). The interactive effects ($x_1 \bullet x_2$; $x_1 \bullet x_3$; $x_2 \bullet x_3$ etc); as well as the quadratic effects $(x_1)^2$, $(x_2)^2$, $(x_3)^2$, and $(x_4)^2$ of the factors on the response were also accounted for in the equation. The C_i corresponded to regression coefficients.

$$Y = C_0 + C_1X_1^2 + C_2X_2^2 + C_3X_3^2 + C_4X_4^2 + C_5X_1 + C_6X_2 + C_7X_3 + C_8X_4 + C_9X_1X_2 + C_{10} \times 1 \times {}_3 + C_{11} \times 1 \times {}_4 + C_{12} \times 2 \times {}_3 + C_{13} \times 2 \times {}_4 + C_{14} \times 3 \times {}_4 + C_{15}X_1X_2X_3 + C_{16}X_1X_2X_4 + C_{17}X_1X_3X_4 + C_{18}X_2X_3X_4 + C_{19}X_1X_2X_3X_4 \quad (2)$$

Two approaches were used to generate this polynomial equation. In the first case, an empirical approach was used. The model was generated on a simple mathematical basis. The best model was generated without any transformation of the data before the multiple regression analysis. In the second case, the model was built including a biological hypothesis (mechanistic approach). Before the multiple regression analysis, data on carvacrol concentration were transformed in what was considered as the "Active Fraction Index" (AFI) which was calculated using Eq. (Abraham et al., 1999). This AFI takes into consideration the tested concentration of carvacrol, its octanol/water partition coefficient (Ko/w (T°c)), and its vapour pressure (Vp(T°c)) at the tested temperature.

$$AFI_{carv} = 10^{[carv] * Ko/w(T°c) * Vp(T°c)} \quad (3)$$

This formula was written based on the hypothesis that only a certain fraction of the carvacrol concentration supplemented to the medium was effectively active at the microbial level during the treatment. Sado et al. (2009) had already suggested that Ko/w and Vp have a significant effect on microbial thermal inactivation. Carvacrol has been reported to target primarily the cell membrane increasing its fluidity and permeability. The possibility of an interaction with membrane proteins and periplasmic enzymes has also been suggested (Hyldgaard et al., 2012; Nostro and Papalia, 2012). Considering Ko/w as an indicator of the lipophilic characteristic of the compound, and thus of its capacity to enter the

lipidic system of the target cell membrane or to stay in the aqueous matrix at the tested temperature; Vp as the strength with which the compound enters the cells at the tested temperature, one could therefore assume that the fraction of carvacrol which is effectively active at the cell level is dependent on these two parameters together with the supplemented concentration in the medium. By extending the Gamma concept of Zwietering et al. (1992) which states that all measurable factors influencing growth rate (μ) are independent with a multiplicative effect [μ = f (temperature) x f (A_w) x f (pH) x f (X_1) x f (X_2)x … x (X_n)], we also hypothesize an interactive effect between Ko/w, Vp and carvacrol concentration during the heat treatment. Besides, considering the possibility of a saturation point where the compound stops entering cells, this AFI, therefore, takes a logarithmic form. One could therefore write the following equation (Eq (4)) which is the equivalent of the previous one.

$$\text{Log } (AFI_{carv}) = [carv]*Ko/w(T°c)* \ Vp(T°c) \quad (4)$$

The Ko/w and Vp of carvacrol at the different tested temperatures were obtained using Goss (2005) and Antoine equation, respectively. The reference Log Ko/w at 25 °C was estimated based on works performed by Abraham et al. (1994a,b, 1999). Table 1 presents the different values obtained.

Response fit analyses, regression coefficient estimations, and model significance evaluation were conducted using Statistica.10 software of Statsoft (Dell, TX, USA). To compare the two generated models, their Accuracy factor (A_f) and Biais factor (B_f) were calculated as described by Baranyi et al. (1999).

2.6. Model validation

The validation of the better of the two generated models consisted in assessing the predictive performances of inactivation scale "b" of the target strain into real fruit juices. The inactivation kinetics at 55 and 60 °C of acid-adapted *L. monocytogenes* cells were assessed in 100%, 50%, and 25% orange, pineapple, and watermelon juices supplemented or not with carvacrol at 30 μL/L. This aimed at evaluating if the model could be valid both for 100% fruit juices and nectar fruit juices (≤50%). On the other hand, the concentration of carvacrol here was defined based on an acceptance test in fruit juices that was done in a previous study (Tchuenchieu et al., 2018 a). Once juices were produced at the laboratory, their pH and °Brix were measured before the heat treatment. The "b" values obtained in real juices were compared to the ones predicted by the model for the tested conditions.

3. Results

3.1. Inactivation parameters in the experimental conditions and modelling

Table 2 presents the b and n values obtained from Weibull inactivation kinetics modelling in the different experimental conditions. The Weibull model was adequate to fit these curves with an R^2 varying between 0.8 and 1, and very low SSE values. All kinetics showed a concave shape (n < 1). The best antimicrobial interaction between mild heat and carvacrol was observed at 62.5 °C where the highest b values were noticed.

The multiple regression analysis of these data through the empirical and the mechanistic approaches lead to equations (5) and (6), respectively, which were written based on the regression coefficients obtained (Tables 3 and 4). In both cases, all the tested factors appear to have a significant effect on the inactivation scale, this latter being individual or interactive.

$$b = 0.73–0.49*°Brix + 0.008*°Brix*T°c + 0.00006*pH*T°c*[carv] \quad (5)$$

From the empirical approach, a negative effect of °Brix was noticed. As it could be observed in Table 2, inactivation scale values tended to decrease with the rise of °Brix values, especially at 57.5 °C. This °Brix effect appeared to depend on temperature. Indeed, this effect was neither observed at 60 °C nor 62.5 °C. An interactive effect between pH, temperature, and carvacrol concentration also came out. Globally, the increase of each of these three factors led to an increase of the inactivation scale, the effect of carvacrol being more important at 62.5 °C than at 57.5 °C, and rising with the pH of the medium. Figs. 1–3 generated from the model illustrate these observations. However, the generated model with this approach helped explain only 60% (R^2 = 0.6) of the inactivation scale data obtained.

In contrast, with the mechanistic approach where carvacrol concentrations were firstly transformed in the correspondent AFI before the multiple regression analysis, a model explaining up to 90% (R^2 = 0.9) of the data was obtained. In addition to the negative °Brix effect and the interaction of this factor with temperature already observed in the empirical approach, a low quadratic effect of AFI was noticed, as well as an interaction between this latter and pH (Fig. 4). As this semi-mechanistic model was showing a higher R^2, a higher A_f, and a lower SSE, it was retained as the model to be validated.

$$b = 0.99–0.29*°Brix\text{-}1.08*10^{-21}*(IFA_{carv})^2 + 1.33*10^{-10}*pH*IFA_{carv} + 4.55*10^{-3}*°Brix*T°c \quad (6)$$

3.2. Predictive model validation

The "b" values obtained from modelling the inactivation kinetics of acid-adapted *L. monocytogenes* cells into real fruit juices (100%; 50% and 25%), as well the predicted values with the semi-mechanistic model for the pH and °Brix characteristics of the juices tested are presented in Table 5. Taking into consideration the estimated error of the "b" values with the Weibull model, we observed that there was no significant difference between the obtained and predicted b values. Besides, a similar inactivation scale was noticed in all the three tested dilutions of each juice.

As the modelling of the shape parameter "n" is not possible, the use of the mean of the values obtained with the CCD (n = 0.135) was helpful to predict the inactivation kinetics with the predicted "b" values as shown in Fig. 5 as an example.

4. Discussion

The physicochemical characteristics of fruit juices are known to affect microbial thermal inactivation during heat treatment. As observed in this study, a negative effect was noticed on the inactivation scale. This may be the result of the use of saccharose to adjust the °Brix value of the synthetic media. This sugar has been reported to reduce microbial thermal inactivation through a decrease in water activity (Coroller et al., 2001; Syamaladevi et al., 2016; Török and Reichart, 1983). The positive interaction with temperature tends to confirm this hypothesis. Indeed, the rise of temperature leads to an increase of water activity, and thus an easier dissociation of this disaccharide in an acidic medium. No effect of °Brix was observed at the higher tested temperatures. pH also appeared to play a key role in the inactivation through the positive interaction noticed with carvacrol. Many research works had already shown that the antimicrobial activity of a compound is dependent on the pH of the

Table 1
Ko/w and Vp of carvacrol at the tested temperatures.

Temperature (°C)	Ko/w	Vp (mm Hg)
55	3.529	0.050
57.5	3.527	0.053
60	3.524	0.056
62.5	3.521	0.059
65	3.519	0.062

Table 2
« b » and « n » values obtained from the inactivation kinetics of acid-adapted *L. monocytogenes* cells in the different tested conditions.

Run	pH	°Brix	Temperature (°C)	Carvacrol (μL/L)	b			n			Model	
					Value	SE	p	Value	SE	p	SSE	R^2
1	4.5	12	55	0	0.5	0.1	0	0.2	0	0	0	0.9
2	4.5	12	55	30	0.8	0.1	0	0.2	0	0	0.3	0.9
3	3.5	6	57.5	15	0.7	0.1	0	0.1	0	0	0.1	1
4	5.5	6	57.5	15	0.8	0.2	0	0.1	0.1	0.3	0.2	0.8
5	3.5	18	57.5	15	0.3	0	0	0.3	0	0	0	1.0
6	5.5	18	57.5	15	0.4	0.1	0	0.3	0.1	0	0.1	0.9
7	3.5	6	57.5	45	1.1	0.1	0	0.1	0	0	0.3	0.9
8	5.5	6	57.5	45	1.5	0.1	0	0.1	0	0	0	1
9	3.5	18	57.5	45	0.8	0.1	0	0.3	0	0	0.2	1
10	5.5	18	57.5	45	1.0	0.2	0	0.3	0.1	0	0.5	0.9
11	4.5	12	60	0	0.5	0.1	0	0.2	0.1	0	0.1	0.9
12	4.5	12	60	30	0.8	0.1	0	0.1	0.1	0.2	0.2	0.8
13	4.5	12	60	30	0.7	0.1	0	0.1	0.1	0.1	0.1	0.9
14	4.5	12	60	30	0.7	0.1	0	0.1	0.1	0.2	0.2	0.8
15	4.5	12	60	60	0.8	0.1	0	0.0	0	0.6	0.1	0.9
16	2.5	12	60	30	0.8	0.1		0.2	0.1	0	0.2	0.9
17	6.5	12	60	30	0.9	0	0	0.1	0	0	0	1
18	4.5	0	60	30	0.8	0.1	0	0.1	0	0.2	0.1	0.9
19	4.5	24	60	30	0.8	0.1	0	0.2	0.1	0	0.1	0.9
20	3.5	6	62.5	15	1.3	0.2	0	0.1	0.1	0.1	0.5	0.8
21	5.5	6	62.5	15	1	0.1	0	0.1	0	0	0.1	0.9
22	3.5	18	62.5	15	1.4	0	0	0.1	0	0	0	1
23	5.5	18	62.5	15	1.0	0.1	0	0.2	0	0	0.1	1
24	3.5	6	62.5	45	2.2	0.1	0	0.1	0	0	0.1	1
25	5.5	6	62.5	45	2.3	0.2	0	0.1	0	0	0.4	1
26	3.5	18	62.5	45	1.8	0.1	0	0.1	0	0	0.1	1
27	5.5	18	62.5	45	2.4	0.2	0	0.1	0	0	0.5	1
28	4.5	12	65	0	1	0.1	0	0.1	0	0	0.1	1
29	4.5	12	65	30	1.1	0.1	0	0.1	0	0.1	0.2	0.9

*R^2: coefficient of determination; SE: Standard error; SSE: Sum of squared errors; p: level of significance (value significative at $p \leq 0.05$).

Table 3
Regression coefficients of significant factors obtained generating the *L. monocytogenes* inactivation scale model with the empirical approach.

Intersection	°Brix	°Brix*T°C	pH*T°C*[carv]	Model				
				R^2	p	SSE	A_f	B_f
0.73	−0.49	0.008	0.00006	0.6	0	3.6	1.5	1

*R^2: coefficient of determination; p: level of significance (value significative at $p \leq 0.05$); SSE: Sum of squared errors; A_f: Accuracy factor; B_f: Biais factor.

Table 4
Regression coefficients of significant factors obtained generating the *L. monocytogenes* inactivation scale model with the mechanistic approach.

Intersection	°Brix	$[carv]^2$	pH*[carv]	°Brix*T°C	Model				
					R^2	p	SSE	A_f	B_f
0.99	−0.29	$-1.08*10^{-21}$	$1.33*10^{-10}$	$4.55*10^{-3}$	0.9	0	1.1	1.3	1

*R^2: coefficient of determination; p: level of significance (value significative at $p \leq 0.05$); SSE: Sum of squared errors; A_f: Accuracy factor; B_f: Biais factor.

medium. In the case of carvacrol, Ultee et al. (1998) observed a bactericidal activity on *Bacillus cereus* which was 2 and 6 times higher at pH 5.5 and 8, respectively, compared to pH 7. Thompson (2016) reported this molecule to have a higher antifungal activity on *Aspergillus* sp. at pH 4 and 8 than at 6. As mentioned by Ultee et al. (1998), this effect cannot be linked to the dissociated form of the compound which is in minority at our tested pH values since the pKa of phenolic compounds is at 10. Even if weak, the supplementation of carvacrol in the medium had a significant positive impact on the inactivation scale of the targeted acid-adapted *L. monocytogenes* cells. This increase in the mild thermal inactivation of microorganisms in presence of carvacrol had already been observed in previous studies (Tchuenchieu et al., 2016, 2018 a,c). The development of the predictive model taking into consideration for carvacrol only its concentration (empirical approach) showed that in addition to pH, the activity of this compound is also dependent on temperature. The change of the tested concentrations into the corresponding AFI (which takes into consideration its octanol/water partition coefficient and its vapour pressure at the tested temperature) before generating the model helped to explain not only 60% of the inactivation scale data collected but up to 90%. This shows how important the octanol/water partition coefficient of a natural aroma compound and its vapour pressure at the tested temperature determine the antimicrobial efficiency of the combined process. Ngang et al. (2014) also suggested that there is an equilibrium point during heat treatment between compounds in solution and those in the headspace under the effect of the vapour pressure. High temperature may therefore lead to a partition of the compound in the headspace, while mild temperatures contribute to maintaining it inside the solution. This explains the more obvious effect

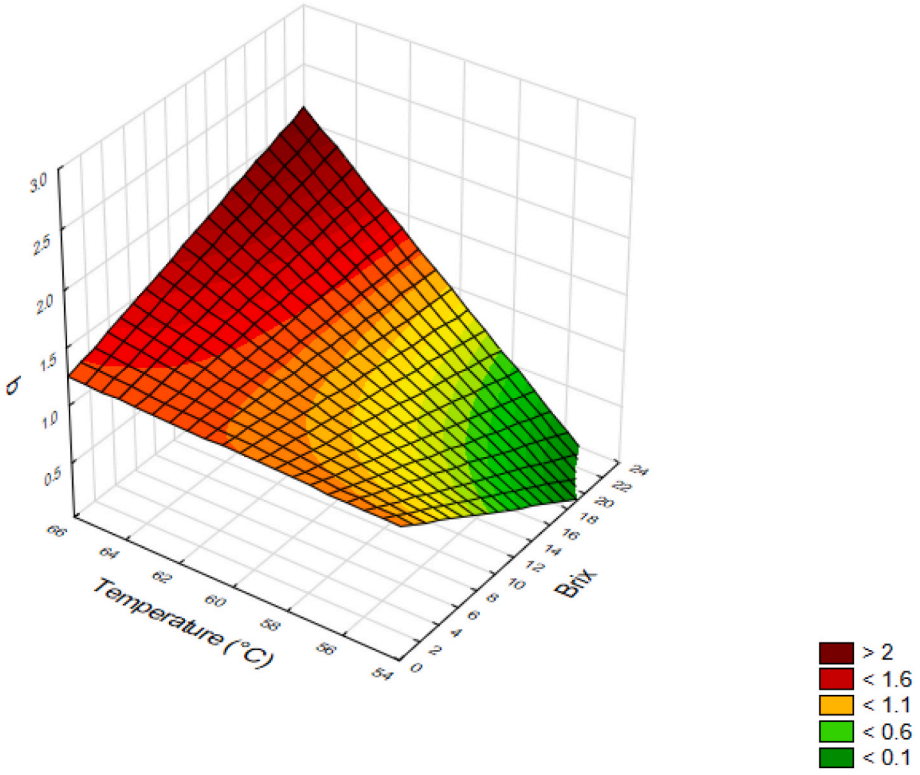

Fig. 1. Change of inactivation scale with temperature and °Brix at pH 4.5 and concentration of carvacrol of 30 μL/L.

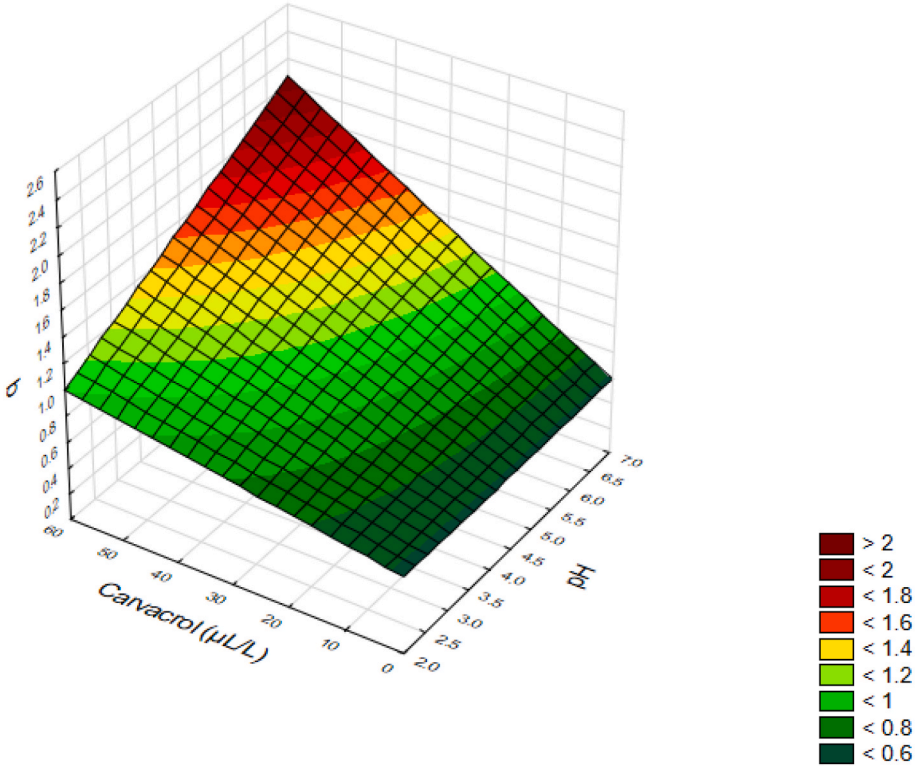

Fig. 2. Change of inactivation scale with pH and concentration of carvacrol at temperature 60 °C and °Brix 12.

of carvacrol on inactivation in conditions tested between 55 and 62.5 °C than at 65 °C. Solubility of aroma compounds in microbial cell membranes has also been reported as important for their antimicrobial activity, a solubility that is dependent on the hydrophobicity of the compound (Sado et al., 2011). Thus, in addition to its presence inside the solution, the compound should enter the cell membrane to be active, a capacity which is appreciated through its Ko/w at the tested temperature. The quadratic effect observed with AFI suggests that there is a

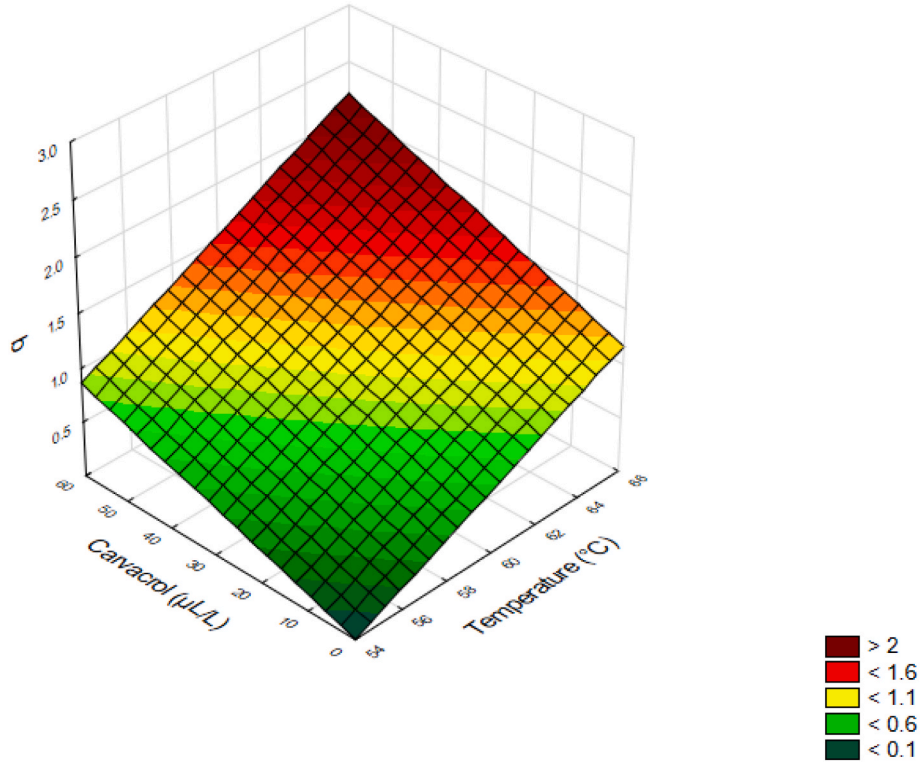

Fig. 3. Change of inactivation scale with temperature and concentration of carvacrol at pH 4.5 and °Brix of 12.

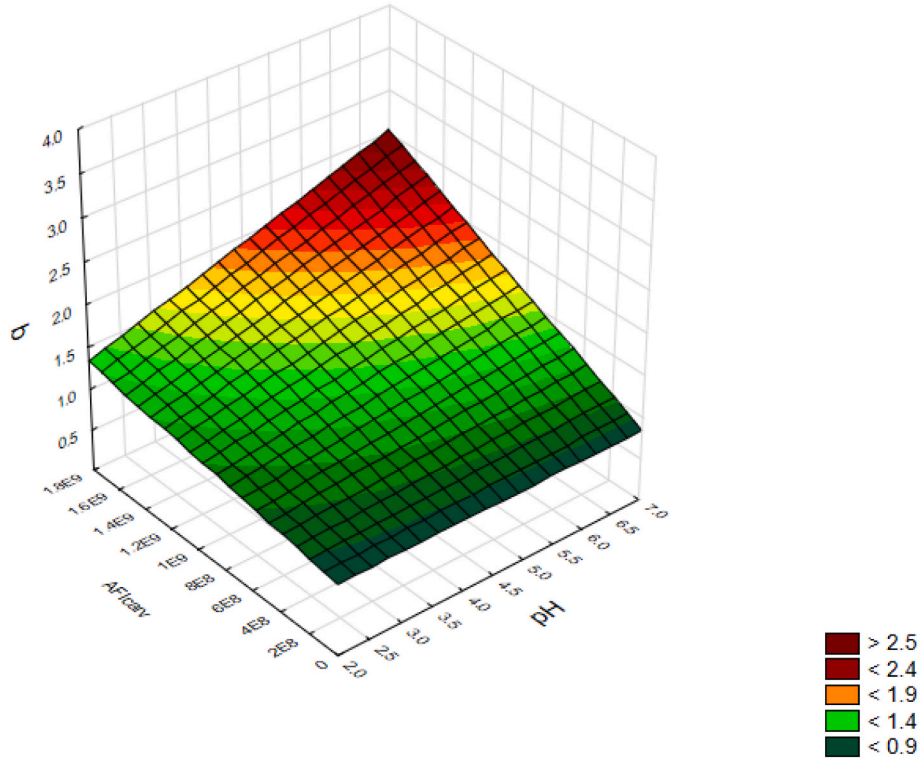

Fig. 4. Change of inactivation scale with pH and AFI_{Carv} at temperature 60 °C and °Brix of 12.

minimum concentration required inside the cell membranes to impact the inactivation scale. The other extrinsic factor which is temperature also significantly impacted the inactivation scale. This lethal effect of heat treatments on microorganisms is widely documented. They target ribosomes, proteins and enzymes, nucleic acids, and cell membranes where they increase fluidity and permeability (Smelt et al., 2002).

The non-significant difference observed between the predicted "b" values and the real values obtained in the validation phase shows that the factor taken into consideration to develop the model are important to explain the phenomenon. Besides, the similarity of the inactivation

Table 5
Comparison of the inactivation scale values of acid-adapted *L. monocytogenes* cells obtained with real fruit juices to those predicted for the different tested conditions.

Juice		pH	°Brix	Temperature (°C)	Carvacrol (μL/L)	« b » predicted	« b » obtained			Difference
							Value	Min	Max	
Pineaple	100%	4.51	13	55	0	0.54	0.69	0.59	0.80	NS
	50%	4.58	7	55	0	0.75	0.47	0.23	0.70	NS
	25%	4.64	3	55	0	0.89	0.42	0.30	0.54	NS
Orange	100%	4.71	11.8	55	0	0.58	0.68	0.27	1.09	NS
	50%	4.76	6	55	0	0.78	0.70	0.50	0.89	NS
	25%	4.81	2.5	55	0	0.90	0.74	0.39	1.10	NS
Watermelon	100%	5.81	8	55	0	0.71	0.74	0.52	0.95	NS
	50%	5.90	4	55	0	0.85	0.53	0.20	0.87	NS
	25%	5.86	1.7	55	0	0.93	0.41	0.22	0.60	NS
Pineaple	100%	4.51	13	55	30	0.54	0.70	0.47	0.92	NS
	50%	4.58	7	55	30	0.75	0.70	0.68	0.72	NS
	25%	4.64	3	55	30	0.89	0.78	0.57	1.00	NS
Orange	100%	4.71	11.8	55	30	0.58	0.58	0.39	0.77	NS
	50%	4.76	6	55	30	0.78	0.55	0.25	0.85	NS
	25%	4.81	2.5	55	30	0.90	0.78	0.64	0.93	NS
Watermelon	100%	5.81	8	55	30	0.71	0.64	0.37	0.90	NS
	50%	5.90	4	55	30	0.85	0.52	0.35	0.69	NS
	25%	5.86	1.7	55	30	0.93	0.62	0.39	0.86	NS
Pineaple	100%	4.51	13	60	0	0.83	0.68	0.43	0.92	NS
	50%	4.58	7	60	0	0.91	0.74	0.42	1.06	NS
	25%	4.64	3	60	0	0.96	0.77	0.47	1.07	NS
Orange	100%	4.71	11.8	60	0	0.85	0.61	0.50	0.73	NS
	50%	4.76	6	60	0	0.92	0.60	0.26	0.94	NS
	25%	4.81	2.5	60	0	0.96	0.61	0.48	0.75	NS
Watermelon	100%	5.81	8	60	0	0.89	0.71	0.58	0.83	NS
	50%	5.90	4	60	0	0.94	0.70	0.60	0.80	NS
	25%	5.86	1.7	60	0	0.97	0.81	0.42	1.20	NS
Pineaple	100%	4.51	13	60	30	0.83	0.60	0.44	0.75	NS
	50%	4.58	7	60	30	0.91	0.69	0.40	0.97	NS
	25%	4.64	3	60	30	0.96	0.60	0.46	0.75	NS
Orange	100%	4.71	11.8	60	30	0.85	0.55	0.45	0.66	NS
	50%	4.76	6	60	30	0.92	0.58	0.36	0.79	NS
	25%	4.81	2.5	60	30	0.96	0.70	0.35	1.06	NS
Watermelon	100%	5.81	8	60	30	0.90	0.70	0.41	1.00	NS
	50%	5.90	4	60	30	0.94	0.67	0.47	0.88	NS
	25%	5.86	1.7	60	30	0.97	0.74	0.47	1.01	NS

*NS: Not significant.

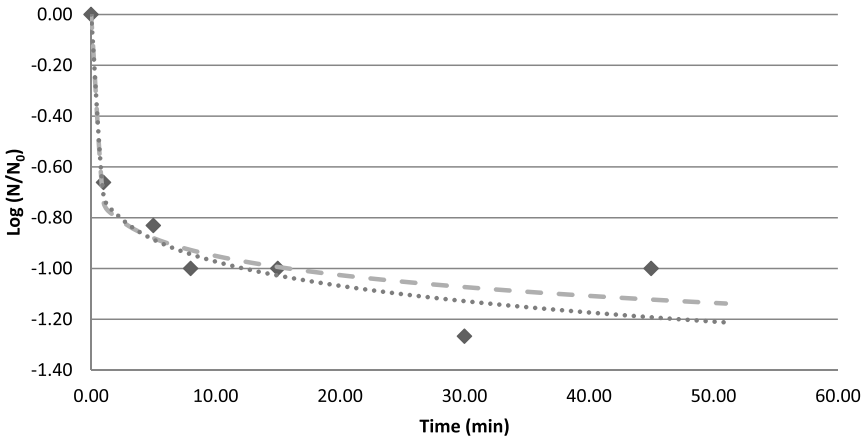

Fig. 5. Obtained inactivation kinetic (experimental data ◆ and model —) of acid-adapted *L. monocytogenes* cells in 100% watermelon juice (pH 5.81-°Brix 8) treated at 55 °C without carvacrol, and predicted kinetic (***) in this condition.

scales obtained in the different dilutions of each juice shows that acid-adapted *L. monocytogenes* cells that were targeted were no more sensitive to the organic acids contents of juices. This is certainly due to the membrane rigidity induced by the prior acid-adaptation (Álvarez-Ordóñez et al., 2008; Annous et al., 1999).

5. Conclusion

In addition to pH and °Brix of the matrix, the concentration of the natural aroma compound supplemented and its Octanol/water partition coefficient, as well as its vapour pressure characteristics at the treatment temperature, are parameters that should be considered in the development of this new low thermal treatment approach of fruit juices.

CRediT authorship contribution statement

Alex Tchuenchieu: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft. **Sylvain Sado Kamdem:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – review & editing. **Annamaria Bevivino:** Supervision, Writing – review & editing. **Francois-Xavier Etoa:** Supervision, Writing – review & editing. **Jean-Justin Essia Ngang:** Conceptualization, Supervision, Writing – review & editing.

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.

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