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Statistical modeling of the inactivation of spoilage microorganisms during ohmic heating of sour orange juice

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Abstract

To reduce food pathogens, increase shelf life of fruit juices and maintaining bioactive properties preservation technologies as ohmic heating have gained interest. This study sought to investigate the effect of four ohmic resistance variables, temperature (Temp), voltage (V), current (AMP) and electric conductivity (EC), on the population of two yeasts, an ascospore and three fermentative bacteria, by inoculation into sour orange juice. The incidence of the variables was evaluated through different regression models. The results of the simple linear regression (SLR) indicated that Temp, AMP and EC had a significant negative effect on the population of all microorganisms, while V had no effect on the population of any microorganism. The results of the stepwise linear regression (SWLR) showed that, for each microorganism, the variables Temp and AMP were considered to be significant being the only ones included in the model. Temperature had the highest negative effect on the population of each microorganism, explaining more than 87 % of the variability of the microorganism. A full quadratic multiple linear regression (FQMLR) model fitted to the dataset such that all significant variables and interactions between variables were considered. Diverse statistical analysis confirmed the goodness of the model.

Keywords

Ohmic heating, orange juice, temperature, current, FQMLR

Nomenclature

Abbreviation	Description	Units
Amp	Electric current	A
EC	Electrical conductivity	S/m
N	Population of microorganism	log CFU/mL
Temp	Temperature	ºC
V	Voltage	V

1. INTRODUCTION

Fruit juices are very popular worldwide due to their taste, content of bioactive compounds and consumers' awareness of their contribution to beneficial effects on human health (Hashemi *et* al., 2017a; Persic *et* al., 2017). However, several studies regarding food-borne illness outbreaks, reported that fruit juices can carry different food-borne pathogens and spoilage organisms (Simforian, Nonga, & Ndabikunze, 2015; Sanz-Puig *et* al., 2016; Barbosa, Mantovani, & Jain, 2017). Therefore, adequate control of pathogens is of great significance to the fruit juice industry.

Conventional processing usually involves heat treatment, which might result in the decrease of the organoleptic and nutritional quality of juices. Nowadays, there are many novel preservation methods that have emerged aiming to decrease the deleterious effects of heat on fruit juices, while still assuring microbial safety (Jiménez-Sánchez *et* al., 2017). Ohmic heating is such a novel technology that can heat food products by the passage of electric currents, as the food materials behave as an electrical resistance. Consequently, this technology can heat a material rapidly and homogeneously, without jeopardizing the quality, since foods are so subjected to heat for shorter periods, as the heat is produced internally inside the product (Knirsch *et* al., 2010). The potential uses of ohmic heating in the food industry are rather abrangent, including microbial inactivation for pasteurization, sterilization and enzymes inactivation for blanching (Knirsch *et* al., 2010; Hashemi *et* al., 2017b). For instance, Lee, Kim & Kang (2015) reported inactivation of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* by ohmic heating in orange juice and Leizerson & Shimoni (2005) showed that ultrahigh-temperature continuous ohmic heating of orange juice could considerably inactive bacteria, yeast and molds to below the detection limit.

To adequately and precisely estimate the microbial inactivation effect of a thermal treatment for processing optimization, suitable mathematical models are of interest to be used, since can estimate the parameters describing microbial inactivation and be used to predict results of microbial inactivation, using processing conditions different from those used experimentally to obtain results to use the models. The implementation of mathematical models can provide the researchers with significant information about the numerous commonly affecting mechanisms present in microbial inactivation processes such as pasteurization and sterilization (Hashemi and Roohi, 2019).

Careful analysis, using appropriated statistical procedures, is conducted by proving the adequacy of these models fitting the inactivation effect. There are several statistical methods of data analysis that might be used to examine and model the effects of one or more predictor variables $X_1, \dots, X_k$ on a quantitative response variable Y. For example, simple linear regression (SLR) is applied to examine the effect of a predictor variable X, on a quantitative response variable Y (Montgomery *et* al., 2012), while more robust methods like stepwise linear regression (SWLR) or full quadratic multiple linear regression (FQMLR) are applied for a deeper examination or modelling, of the effects on a quantitative response variable Y, that depends on not one, but several predictor variables, $X_1, \dots, X_k$ (Montgomery *et* al., 2012).

In this work SLR, SWLR, and, FQMLR were applied to examine and model the effects of ohmic heating (voltage, temperature, amperage, and electrical conductivity) on inactivation of several spoilage microorgamisms (*Leuconostoc mesenteroides* subsp. *mesenteroides*, *Lactobacillus acidophilus*, *Lactobacillus plantarum* subsp. *plantarum*, *Saccharomyces cerevisiae*, *Byssochlamys fulva* and *Zygosaccharomyces rouxii*) on sour orange juice, using as quantitative response variable the quantification of the number of survival microorganisms. The objectives of this paper were so: i) To study the for each of the above microorganisms, the effects of

92 V, Temp, AMP and EC on the population (N) inactivation; ii) To identify the factors with most
93 important effects on the population inactivation; iii) To model the population inactivation based on
94 the most important effects.

95

96 ## 2. MATERIALS AND METHODS

97 ### *2.1.Chemicals and microorganisms*

98 *Leuconostoc mesenteroides* subsp. *mesenteroides* PTCC 1591, *Lactobacillus acidophilus*
99 PTCC1643, *Lactobacillus plantarum* subsp. plantarum PTCC1745, *Saccharomyces*
100 *cerevisiae* PTCC5269, *Byssochlamys fulva* PTCC 5062, and *Zygosaccharomyces*
101 *rouxii* PTCC5206 were purchased from the Iranian Research Organization for Science and
102 Technology, Tehran, Iran. All chemicals were of analytical reagent grade and purchased from
103 Sigma (ST. Louis, MO, USA).

104

105 ### *2.2.Sample preparation*

106 Sour oranges were purchased in a local market in Shiraz city, Fars province, Iran. After
107 the selection of mature fruits, the fruits were washed and manually peeled. The juice was
108 obtained using a domestic juicer (Pars Khazar, JC-700P model, Gilan, Iran) under aseptic
109 conditions and subsequently, the sour orange juice was heat treated at 85 °C for 15 min for
110 pasteurization (no microbial cells were detected after the heat treatment). After reactivation of *L.*
111 *mesenteroides* subsp. *mesenteroides*, *L. acidophilus*, and *L. plantarum* subsp. *plantarum* in de
112 Man, Rogosa and Sharpe (MRS) broth (Oxoid, UK), 1 % (v/v) of suspended physiological saline
113 (0.9 % NaCl solution, pH 6.8) cell cultures were added separately into 100 mL of pasteurized

sour orange juice aliquots at a level of, respectively, 6.1, 6.2 and 6.2 log CFU/mL. *B. fulva* culture was cultivated with potato dextrose agar slants (Oxoid, UK) for 7 days at 25 °C to harvesting of the spores while *S. cerevisiae* was cultured with yeast extract dextrose chloramphenicol agar (Lab M, UK) at 27 °C for 48 h, being afterwards the cells separated by centrifugation (Hanil, Union 55R, South Korea) for 15 min (3500×*g*, 4 °C). Approximately, 1 mL of *B. fulva* spore's solutions was inoculated into 15 mL of juice to yield an initial spore concentration of 6.3 log CFU/mL in the juice sample and *S. cerevisiae* was inoculated at the level of 6.0 log CFU/mL. *Z. rouxii* culture was cultivated with agar medium slants containing (g/L) glucose (10), peptone (5), yeast extract (3), and malt extract (3) and the inoculated slants grown in an incubator for 48 h at 35 °C and further inoculated into the juice at the level of 6.4 log CFU/mL. After each treatment, the samples were taken for microbial enumeration. There were three replicates per treatment, and the experiment was conducted three times.

2.3.Ohmic heating

Ohmic heating was carried in a 1000 mL laboratory capacity scale reactor at three voltages (100, 150 and 200 V), in a teflon chamber of cylindrical shape (7 cm internal diameter and 25 cm length), with two titanium electrodes, Figure 1 shows a schematic representation of the used system. The completely automated system with controlled temperature (21 - 86 ºC), current (0 - 16 A), voltage (0 - 300 V) and electrical conductivity (0 - 0.054 S/m) allowed recording data during heating.

For all samples, 500 mL of sour orange juice was poured into the chamber and experiments were performed for 120 s (26 - 86 °C) (Figure 1). After thermal treatment, the

136 chamber was quickly cooled in ice/water bath and temperature dropped immediately (each
137 treatment was applied in triplicate).

138

139 ***2.4.Enumeration of microorganisms***

140 1 mL of sour orange juice was serially diluted in 0.1 % peptone water and 0.1 mL of
141 appropriate diluents was spread plated onto each corresponding medium. MRS agar was used for
142 enumeration of *L. mesenteroides* subsp. *mesenteroides*, *L. acidophilus* and *L. plantarum* subsp.
143 *plantarum.* After incubation at 37 °C for 48 h, colonies were counted. The cell counts of *B.*
144 *fulva* and *S. cerevisiae* in the juice samples were carried out onto potato dextrose agar by spread
145 plating and incubation of the plates at 27 °C for 5 to 7 days. *Z. rouxii* was counted by spread
146 plating to supplemented (100 mg/L chloramphenicol and 10 % w/v NaCl) potato dextrose agar
147 after a 4 days incubation period at 30 °C.

148

149 ***2.5.Data Analysis***

150 The data gathered from the experiments were analyzed using SPSS version 24, Minitab
151 version 18. A set of simple linear regressions (SLR) was applied, to determine the importance of
152 each factor (voltage, temperature, amperage, and electrical conductivity) on the surviving
153 population (N) of each microorganism, followed by a stepwise linear regression (SWLR), to
154 identify the factors with higher effect on N. Finally, a set of full quadratic multiple linear
155 regressions (FQMLR) were used to model the effects of the different factors on N. The results

were analyzed with a Student's *t*-test model and at 95% confidence interval, responses were considered as significant.

2.6.Simple Linear Regression

The general equation of SLR is presented by:

$$Y = \beta_0 + \beta_1 X + \varepsilon, \quad \text{Equation (1)}$$

where X is the predictor and β_0 and β_1 are model parameters (coefficients) and ε is the random component of the model that follows an independent normal distribution.

The estimated equation of SLR model is:

$$\hat{Y} = b_0 + b_1 X, \quad \text{Equation (2)}$$

where, b_0 and b_1 are estimations of model parameters, and $\hat{Y}$ is the predicted value of Y.

2.7.Stepwise Linear Regression

In SWLR, the effective parameters are included step by step and the non-effective parameters are excluded. With this stepwise procedure, the final model has a lower number of parameters and the accuracy is improved. The general equation of SWLR is:

$$Y = \beta_0 + \beta_i X_i + \cdots + \beta_j X_j + \varepsilon, \quad \text{Equation (3)}$$

Where $X_i, \ldots, X_j$ are the predictors and $\beta_0, \ldots, \beta_j$ are model parameters.

The estimated equation of SLR model is:

$$\hat{Y} = b_0 + b_i X_i + \cdots + b_j X_j, \quad \text{Equation (4)}$$

where b_0, …, b_j are estimations of model parameters, and $\hat{Y}$ is the predicted value of Y.

2.8.Full Quadratic Multiple Linear Regression

The general equation of FQMLR is:

$$Y = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k + \beta_{1,1} X_1^2 + \cdots + \beta_{k,k} X_k^2 + \beta_{1,2} X_1 X_2 + \cdots + \beta_{k-1,k} X_{k-1} X_k + \varepsilon, \quad \text{Equation (5)}$$

Where $X_1, X_1^2, \ldots X_k, X_k^2$ are the predictors and $\beta_0, \beta_1, \ldots, \beta_k, \beta_{1,1}, \ldots, \beta_{k,k}, \beta_{1,2}, \ldots, \beta_{k-1,k}$ are model parameters (coefficients).

The estimated equation of FQMLR model is:

$$\hat{Y} = b_0 + b_1 X_1 + \cdots + b_k X_k + b_{1,1} X_1^2 + \cdots + b_{k,k} X_k^2 + b_{1,2} X_1 X_2 + \cdots + b_{k-1,k} X_{k-1} X_k, \quad \text{Equation (6)}$$

where, $b_0, b_1, \ldots, b_k, b_{1,1}, \ldots, b_{k,k}, b_{1,2}, \ldots, b_{k-1,k}$ are estimations of model parameters, and $\hat{Y}$ is the predicted value of Y.

2.9.Backward Full Quadratic Multiple Linear Regression (BFQMLR)

As mentioned in the above section, the FQMLR model includes effects of linear $(X_i, \ldots, X_k)$, quadratic $(X_1^2, \ldots, X_k^2)$ and interactional $(X_1, X_2, \ldots, X_{k-1} X_k)$ nature.

193 Step by step the non-significant parameters ($p > 0.05$) are discarded when the backward
194 method (BFQMLR) is applied, the final model will so have a lower number of parameters and
195 improved accuracy. It is noteworthy to highlight that: i) in FQMLR, when a square effect is
196 significant for one variable, a linear effect in the model (significant or not significant) must be
197 assumed for that variable; ii) in FQMLR, when an interaction effect is significant for a set of
198 variables, a linear effect in the model (significant or not significant) must be assumed for the
199 variables of the set under question; iii) in FQMLR, in the case of a categorical predictor, the
200 importance of this variable must be evaluated and if the effect is significant, FQMLR must be
201 applied separately for each category or otherwise the categorical variable will be removed from
202 FQMLR (Montgomery et al., 2012).

203

204

205 ## 3. RESULTS AND DISCUSSION

206 ### *3.1.Simple Linear Regression*

207 Considering N (the population of surviving microorganisms) as the response variable and
208 the other variables voltage (V), temperature (Temp), current (AMP), and electric conductivity
209 (EC) as continuous predictors, Tables 1- 4 summarize the results of the SLR models obtained for
210 the variables, respectively. As can be seen in Table 1, the voltage (V) had no significant effect on
211 N in any of the tested microorganisms ($p > 0.05$).

212 On the contrary, Table 2 shows the significant ($p < 0.05$) negative effect that the
213 temperature has on N of all microorganisms (all negative coefficients), with highest incidence on
214 *Z. rouxii* (highest absolute value of standardized coefficients, β = -0.963). The values of non-
215 standardized coefficients (B ± SD) show that by increasing temperature in 1 unit, N of *L.*
216 *mesenteroides* subsp. *mesenteroides, L. acidophilus, L. plantarum* subsp. *plantarum, S.*
217 *cerevisiae, B. fulva,* and *Z. rouxii* decrease 0.093, 0.097, 0.096, 0.096, 0.094 and 0.099 CFU/mL,
218 respectively.

219 Tables 3 and 4 indicate that the variables AMP and EC also had a negative effect on N of
220 all microorganisms, particularly on *Z. rouxii* (β = -0.902 and -0.771, respectively). The results of
221 non-standardized coefficients (B ± SD) of the regression model reported in Table 3 show that, by
222 increasing the electric current (Amp) in 1 unit, N of *L. mesenteroides* subsp. *mesenteroides, L.*
223 *acidophilus, L. plantarum* subsp. *plantarum, S. cerevisiae, B. fulva,* and *Z.rouxii* decrease 2.23,
224 2.34, 2.35, 2.33, 2.32 and 2.41 CFU/mL, respectively. The increase of electrical conductivity
225 (EC) in one unit causes a decrease of 118, 121, 122, 122, 120 and 132 CFU/mL in the population

of *L. mesenteroides* subsp. *mesenteroides, L. acidophilus, L. plantarum* subsp. *plantarum, S. cerevisiae, B. fulva,* and *Z. rouxii*, respectively (Table 4).

Baysal & İçier (2010) reported a significant effect ($p < 0.05$) of the heating time (0, 10, 15, 20, and 30 min) and temperature (70, 80, and 90 °C) on the inactivation of *Alicyclobacillus acidoterrestris* spores in orange juice by ohmic heating. These authors also found that the voltage gradient caused an additional inactivation effect, significant at 70 °C ($p > 0.05$). Park & Kang (2013) reported inactivation of *L. monocytogenes, E. coli* O157:H7 and *S.* Typhimurium in apple juice of 0.70, 2.42 and 3.21 decimal reductions and 3.40, 3.59 and 3.48 decimal reductions, when treated, respectively, with ohmic heating at 58 °C for 30 s and 60 °C for 30 s. Reduction of these pathogens at 58 °C and 60°C in combination with the electric treatment, were 2- to 3-fold higher than the reduction resulting from conventional heating only (Park and Kang, 2013). On the contrary, Palaniappan *et* al., (1992) evaluated the effect on the population of *E. coli* and *Zygosaccharomyces bailii* treated with conventional and ohmic heating. The authors found that the reduction of the population was achieved at the same level regardless of the technology used; implying that the electrical current has an insignificant effect compared with the effect of heat (Palaniappan, Sastry, and Richter 1991). Lee *et* al. (2013) studied the inactivation of *Escherichia coli* O157:H7 and *Salmonella enteric serovar* Typhimurium in salsa during ohmic heating and observed that the application of a frequency above 1 kHz, led to an efficient inactivation of these two food-borne pathogens, with a dependence not only on frequency, but also on conductivity and time. Increase of electric field strength (25 - 40 V cm^{-1}) or treatment time resulted in a greater reduction of *L. monocytogenes, S.* Typhimurium, and *E. coli* O157:H7 in tomato and orange juice during ohmic heating (Lee et al., 2012).

*3.2.**Stepwise Linear Regression***

Table 5 summarizes the results of Stepwise Linear Regression (SWLR) model on N of the different microorganisms studied. For each microorganism, the variables Temp and AMP were considered to be significant ($p < 0.05$) and were so included in the SWLR model, while the other variables (V and EC) were excluded from the regression ($p > 0.05$).

It is important to outline, that temperature (Temp) was the factor with the highest negative effect (highest negative coefficient) on the population of each microbe (Table 5). This factor explained 87.4 %, 88.9 %, 91.3 %, 90.2 %, 91.0 % and 92.7 % of the variability of N for *L. mesenteroides* subsp. *mesenteroides, L. acidophilus, L. plantarum* subsp. *plantarum, S. cerevisiae, B. fulva,* and *Z. rouxii*, respectively. On the other hand, the electrical current (Amp) factor had a positive effect on the population of all microbes (positive coefficient). Both Temp and AMP variables explained 94.5 %, 93.0 %, 93.7 %, 94.9 %, 93.4 % and 95.8 %, of the variability of N for, respectively, *L. mesenteroides* subsp. *mesenteroides, L. acidophilus, L. plantarum* subsp. *plantarum, S. cerevisiae, B. fulva,* and *Z. rouxii*. Some results found in literature indicate that regardless of the thermal treatment and other possible variable factors, inactivation of microorganisms is achieved mainly by the thermal effect itself. Hashemi and Roohi (2019) applied numerical modeling for inactivation of pathogenic bacteria during ohmic heating. They found the consistently distributed vortices in ohmic heating lead to a more consistent and quick temperature rise in temperature from 26 to 99.4 °C compared to the conventional method. Based on the evaluation of temperature rise, the inactivation time was reduced about 20–30% for the investigated pathogens. Sant'Ana *et* al. (2012) modeled the growth parameters (growth rate, μ and lag time, λ) and their changes as a function of temperature, of three different strains of *Salmonella enterica* and *Listeria monocytogenes* in minimally processed lettuce. The average growth curves of the three strains of these pathogens, presented higher R^2

values (>0.93) than those found in the separated curves for each strain (0.83 and 0.90, respectively). Alber *et* al. (1992) compared two mathematical models, the square root and Schoolfield models, for the prediction of growth rate of *Yersinia enterocolitica* as a function of temperature. These authors found that the correction of the heterogeneity of variance was more efficient by using a natural logarithm than by the use of the square root transformation on the growth rate. The square root model was found to be more precise than the Schoolfield model, when the natural logarithm transformation was used in both models.

3.3.Full Quadratic Multiple Linear Regression

At first, all variables and interaction between variables were considered in the FQMLR model and Table 6 presents the results of the significance obtained. In the second step, the most not significant variable ($p >> 0.05$) of the second run (V* type of microbe) was removed, and the operation was run again. The process was continued step by step, until only significant variables remained in the model.

Table 7 presents the summary of the excluded variables in the FQMLR method in each step and Table 8 presents the results of the final run (showing the variables that showed a significant effect ($p < 0.05$) on N). Even though the-*p* values were higher than 0.05 for Temp and type of microbe, they were considered into the model due to the significance of the interaction with other variables and the quadratic term (see i and ii) in Materials and Methods.

The listed variables (EC, V, and Temp) and their square effects (EC*EC), (V*V) and (Temp*Temp) were the ones to be included in the final model of the FQMLR. The resulting equations for each type of microorganism are presented below:

L. mesenteroides subsp. *mesenteroides:*

N = -7.81 + 0.3044 Temp + 0.1019 V - 6.13 AMP + 439.1 EC + 0.006287 Temp*Temp - 0.000091 V*V + 2071 EC*EC - 0.002960 Temp*V - 0.1378 Temp*AMP - 17.66 Temp*EC + 0.05106 V*AMP + 266.9 AMP*EC

L. acidophilus:

N = -8.15 + 0.3274 Temp + 0.1019 V - 6.84 AMP + 439.1 EC + 0.006287 Temp*Temp - 0.000091 V*V + 2071 EC*EC - 0.002960 Temp*V - 0.1378 Temp*AMP - 17.66 Temp*EC + 0.05106 V*AMP + 266.9 AMP*EC

L. plantarum subsp. *plantarum:*

N = -8.77 + 0.3512 Temp + 0.1019 V - 7.45 AMP + 439.1 EC + 0.006287 Temp*Temp - 0.000091 V*V + 2071 EC*EC - 0.002960 Temp*V - 0.1378 Temp*AMP - 17.66 Temp*EC + 0.05106 v*AMP + 266.9 AMP*EC

S. cerevisiae:

N = -7.92 + 0.3220 Temp + 0.1019 V - 6.68 AMP + 439.1 EC + 0.006287 Temp*Temp - 0.000091 V*V + 2071 EC*EC - 0.002960 Temp*V - 0.1378 Temp*AMP - 17.66 Temp*EC + 0.05106 V*AMP + 266.9 AMP*EC

B. fulva:

N = -8.96 + 0.3549 Temp + 0.1019 V - 7.51 AMP + 439.1 EC + 0.006287 Temp*Temp - 0.000091 V*V + 2071 EC*EC - 0.002960 Temp*V - 0.1378 Temp*AMP - 17.66 Temp*EC + 0.05106 V*AMP + 266.9 AMP*EC

Z. rouxii:

N = -8.22 + 0.3385 Temp + 0.1019 V - 7.19 AMP + 439.1 EC + 0.006287 Temp*Temp - 0.000091 V*V + 2071 EC*EC - 0.002960 Temp*V - 0.1378 Temp*AMP - 17.66 Temp*EC + 0.05106 V*AMP + 266.9 AMP*EC

As the type of microbe showed to be not significant in the FQMLR model ($p > 0.05$), this variable was not evaluated separately (see iii in Material and Methods), which results in a single model for all microorganisms. Three parameters of the model (constant [b_0], temperature [Temp] and electric current [Amp]) had only a slight difference when applying the model to each microorganism specifically, which may suggest that the decrease in the population depends mostly on the conditions chosen for temperature (Temp) and electric current (Amp) during ohmic heating.

3.4.Goodness of the fitted model

A lower root mean square error (RMSE) and higher coefficient of determination (R^2) values, and independent normal residuals with stable variance, were the parameters chosen to evaluate the goodness of the fitted predictive models. The values of *RMSE* and R^2 are presented in Table 9. Since BFQMLR model has the maximum R^2 and the minimum *RMSE,* it is considered as the best model to model and predicts the effect of ohmic heating on the population (N) of the studied microorganisms.

To investigate the normality of residuals, a probability plot and different statistical tests (Anderson-Darling, Kolmogorov-Smirnov, Shapiro-Wilk) were used. As can be seen in Figure 3, the normal probability plot satisfied the normality of residuals, as the points were close to the

line. The normality was also verified with the different statistical tests ($p > 0.05$). As presented in Figure 4, the independence was satisfied, since randomization of the residuals around zero was verified.

Figure 5 shows the plot of residuals versus fitted values. As it can also be seen, the points are distributed randomly around the horizontal axis and the stability of the variance is satisfied. Hence, it can be concluded that the FQMLR is an accurate model to model and predict the effect of ohmic heating on N of the studied microorganisms (N).

4. CONCLUSIONS

The FQMLR model appeared to have a good fitness on modeling the response of the survival population (N) of several microorganisms, after ohmic heating was applied on sour orange juice between the tested ranges: Temp (21 – 86 ºC), Amp (0 - 16 A) and V (0 - 300 V). The temperature and electric current were the variables with higher effect on reduction of population and more important these parameters showed different effect levels, whether the microorganism is a spore, a yeast or a bacterium. A series of single and step-wise lineal regressions also provided information regarding the effect of each variable on the variability and reduction of the population. For instance, the temperature showed a variability of more than 90 % and an increase in 1 % will result on a reduction of more than 9 % of the original population. Meanwhile, the voltage did not have a significant ($p > 0.05$) effect on any of the populations. The electric current also showed a variability above 90 % and an increase in 1 % will result in a reduction of more than 20 % of the original population. The population of *Z. rouxii* was the one most affected by all variables, which may indicate that ohmic heating has a greater incidence on yeasts.

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Conflict of interest statement

None of the authors has a known conflict of interest to declare

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Table 1. Results of the influence of voltage (V and standardized coefficient, β), on the population (N) on each of the microorganisms studied using a simple linear regression (SLR) model. Values calculated from the non-standardized coefficients (B) of the predictor variable (V) and the constant (b_0) (mean ± SD, n=3). Statistical analysis performed with Student's *t*-test considering no significant differences for $p > 0.05$.

Microorganism	Model	Non-standardized coefficient	Standardized coefficient	t	*p*-value
		B ± SD	β		
L. mesenteroides subsp. *mesenteroides*	b_0	4.864 ± 1.612		3.017	0.005
	V	-0.007 ± .010	-0.116	-0.712	0.481
L. acidophilus	b_0	5.306 ± 1.642		3.231	0.003
	V	-0.011 ± .011	-0.169	-1.041	0.305
L. plantarum subsp. *plantarum*	b_0	5.008 ± 1.607		3.116	0.004
	V	-0.10 ± .010	-0.159	-0.982	0.332
S. cerevisiae	b_0	5.040 ± 1.635		3.083	0.004
	V	-0.008 ± .011	-0.128	-0.783	0.439
B. fulva	b_0	4.973 ± 1.587		3.133	0.003
	V	-0.010 ± 0.010	-0.165	-1.017	0.316
Z. rouxii	b_0	4.551 ± 1.659		2.743	0.009
	V	-0.006 ± 0.011	-0.090	-0.548	0.587

Table 2. Results of the influence of temperature (Temp and standardized coefficient, β), on the population (N) on each of the microorganisms studied using a simple linear regression (SLR) model. Values calculated from the non-standardized coefficients (B) of the predictor variable (Temp) and the constant (b_0) (mean ± SD, n=3). Statistical analysis performed with Student's *t*-test (*p*-values for all data were below 0.001).

Microorganism	Model	Non-standardized coefficients	Standardized coefficients	t	*p*-value
		B ± SD	β		
L. mesenteroides subsp. *mesenteroides*	b_0	9.658 ± 0.399		24.227	< 0.001
	Temp	-0.093 ± 0.006	-0.935	-16.004	< 0.001
L. acidophilus	b_0	9.766 ± 0.384		25.435	<0.001
	Temp	-0.097 ± 0.006	-0.943	-17.202	<0.001
L. plantarum subsp. *plantarum*	b_0	9.535 ± 0.332		28.691	<0.001
	Temp	-0.096 ± 0.005	-0.955	-19.681	<0.001
S. cerevisiae	b_0	9.893 ± 0.357		27.720	<0.001
	Temp	-0.096 ± 0.005	-0.950	-18.440	<0.001
B. fulva	b_0	9.387 ± 0.333		28.159	<0.011
	Temp	-0.094 ± 0.005	-0.954	-19.365	<0.011
Z. rouxii	b_0	9.914 ± 0.310		31.956	<0.011
	Temp	-0.099 ± 0.005	-0.963	-21.743	<0.011

Table 3. Results of the influence of electric current (Amp, standardized coefficient, β), on the population (N) on each of the microorganisms studied, using a simple linear regression (SLR) model. Values calculated from the non-standardized coefficients (B) of the predictor variable (Amp) and the constant (b_0) (mean ± SD, n=3). Statistical analysis performed with Student's t-test (*p*-values for all data were below 0.001).

Microorganism	Model	Non-standardized coefficients	Standardized coefficients	t	*p*-value
		B ± SD	β		
L. mesenteroides subsp. *mesenteroides*	b_0	7.624 ± 0.445		17.125	< 0.001
	Amp	-2.225 ± 0.222	-0.855	-10.012	< 0.001
L. acidophilus	b_0	7.727 ± 0.424		18.222	<0.001
	Amp	-2.342 ± 0.212	-0.876	-11.064	<0.001
L. plantarum subsp. *plantarum*	b_0	7.563 ± 0.378		20.032	<0.001
	Amp	-2.346 ± 0.188	-0.898	-12.450	<0.001
S. cerevisiae	b_0	7.847 ± 0.414		18.958	<0.001
	Amp	-2.325 ± 0.207	-0.880	-11.255	<0.001
B. fulva	b_0	7.444 ± 0.374		19.901	<0.011
	Amp	-2.318 ± 0.187	-0.898	-12.413	<0.011
Z. rouxii	b_0	7.865 ± 0.380		20.715	<0.011
	Amp	-2.410 ± 0.189	-0.902	-12.720	<0.011

Table 4. Results of the influence of electric conductivity (EC, standardized coefficient, β), on the population (N) on each of the microorganisms studied, obtained with a simple linear regression (SLR) model. Values calculated from the non-standardized coefficients (B) of the predictor variable (EC) and the constant (b_0) (mean ± SD, n=3). Statistical analysis performed with Student's t-test (*p*-values for all data were below 0.001).

Microorganism	Model	Non-standardized coefficients	Standardized coefficients	t	*p*-value
		B ± SD	β		
L. mesenteroides subsp. *mesenteroides*	b_0	6.679 ± 0.561		11.909	< 0.001
	EC	-118.285 ± 19.193	-0.712	-6.163	< 0.001
L. acidophilus	b_0	6.643 ± 0.578		11.484	<0.001
	EC	-120.863 ± 19.794	-0.708	-6.106	<0.001
L. plantarum subsp. *plantarum*	b_0	6.496 ± 0.546		11.891	<0.001
	EC	-121.886 ± 18.696	-0.731	-6.519	<0.001
S. cerevisiae	b_0	6.823 ± 0.559		12.198	<0.001
	EC	-122.132 ± 19.141	-0.724	-6.381	<0.001
B. fulva	b_0	6.387 ± 0.541		11.801	<0.011
	EC	-120.244 ± 18.519	-0.730	-6.493	<0.011
Z. rouxii	b_0	6.924 ± 0.522		13.270	<0.011
	EC	-131.519 ± 17.855	-0.771	-7.366	<0.011

Table 5. Results of the effect of the temperature (Temp) and the electric current (Amp) on the population (N) of each of the microorganisms studied using stepwise linear regression (SWLR). The standardized coefficient (β) represents the influence on N, calculated from the non-standardized coefficient (B) of the predator variables and the constant (β_0) (mean ± SD, n = 3). Statistical analysis performed with Student's t-test (*p*-values for all data were below 0.001). The coefficient of determination (R^2) indicates the fitness of the regression).

Microorganism	Model		Non-standardized coefficients	Standardized coefficients	t	*p*-value	R^2
			B ± SD	β			
L. mesenteroides subsp. *mesenteroides*	1	b_0	9.658 ± 0.399		24.227	< 0.001	0.874
		Temp	-.093 ± 0.006	-0.935	-16.004	< 0.001	
	2	b_0	11.664 ± 0.399		29.242	< 0.001	0.945
		Temp	-0.213 ± 0.018	-2.131	-11.798	< 0.001	
		Amp	3.190 ± 0.470	1.225	6.785	< 0.001	
L. acidophilus	1	b_0	9.766 ± 0.384		25.435	< 0.001	0.889
		Temp	-0.097 ± 0.006	-0.943	-17.202	<0.001	
	2	b_0	11.334 ± 0.461		24.606	<0.001	0.930
		Temp	-0.190 ± 0.021	-1.853	-9.121	<0.001	
		Amp	2.492 ± 0.543	0.933	4.590	<0.001	
L. plantarum subsp. *plantarum*	1	b_0	9.535 ± 0.332		28.691	<0.001	0.913
		Temp	-0.096 ± 0.005	-0.955	-19.681	<0.001	
	2	b_0	10.722 ± 0.425		25.224	< 0.001	0.937
		Temp	-0.166 ± 0.019	-1.661	-8.657	< 0.001	
		Amp	1.888 ± 0.501	0.723	3.768	< 0.001	
S. cerevisiae	1	b_0	9.893 ± 0.357		27.720	< 0.001	0.902
		Temp	-0.096 ± 0.005	-0.950	-18.440	< 0.001	
	2	b_0	11.558 ± 0.388		29.810	< 0.001	0.949
		Temp	-0.195 ± 0.018	-1.928	-11.148	< 0.001	
		Amp	2.648 ± 0.457	1.002	5.795	< 0.001	
B. fulva	1	b_0	9.387 ± 0.333		28.159	< 0.001	0.910
		Temp	-0.094 ± 0.005	-0.954	-19.365	< 0.001	
	2	b_0	10.532 ± 0.433		24.344	< 0.001	0.934

		Temp	-0.163 ± 0.020	-1.643	-8.314	< 0.001	
		Amp	1.821 ± 0.510	0.706	3.572	< 0.001	
Z. rouxii	1	b_0	9.914 ± 0.310		31.956	< 0.001	0.927
		Temp	-0.099 ± 0.005	-0.963	-21.743	< 0.001	
	2	b_0	11.264 ± 0.357		31.537	< 0.001	0.958
		Temp	-0.179 ± 0.016	-1.747	-11.086	< 0.001	
		Amp	2.146 ± 0.421	0.803	5.096	< 0.001	

Table 6. Results for the initial step in the full quadratic multiple linear regression (FQMLR) model. The significance ($p < 0.05$) of the variables temperature (Temp), current (Amp), electric conductivity (EC), type of microorganism and interaction between variables on the population (N) was analyzed with a Student's t-test and the obtained *p*-value shown in the table.

Variable	*p*-value
Temp	0.079
V	<0.001
AMP	0.007
EC	<0.001
type of microorganism	0.563
Temp* Temp	<0.001
V*V	0.103
AMP*AMP	0.315
EC*EC	0.006
Temp*V	<0.001
Temp*AMP	0.005
Temp*EC	<0.001
Temp* type of microorganism	0.014
V*AMP	0.001
V*EC	0.169
V* type of microorganism	0.652
AMP*EC	0.007
AMP* type of microorganism	0.111

Table 7. Summary of the results obtained during the steps performed to the full quadratic multiple linear regression (FQMLR) method and the excluded variables at each step ($p > 0.05$), analyzed with Student's *t*-test (in the first step all variables were considered, and the results are displayed in Table 6).

Step	Excluded Variable	*p*-value
2	V* type of microorganism	0.652
3	AMP*AMP	0.313
4	V*EC	0.319
5	EC* type of microorganism	0.196

Table 8. Final step of the full quadratic multiple linear regression (FQMLR) model with all variables showing a significant ($p < 0.05$) effect on N, using the Student's *t*-test.

Variables	*p*-value
Temp	0.089
V	<0.001
AMP	0.001
EC	<0.001
type of microorganism	0.480
Temp* Temp	<0.001
V*V	0.003
EC*EC	<0.001
Temp*V	<0.001
Temp*AMP	<0.001
Temp*EC	<0.001
Temp* type of microorganism	0.021
V*AMP	<0.001
AMP*EC	<0.001
AMP* type of microorganism	<0.012

Table 9. Parameters that predict the goodness of fitted the FQMLR model by backward full quadratic multiple linear regression (BFQMLR) after exclusion of the variable V* type of microorganism, AMP*AMP, V*EC, and EC* type of microorganism.

Microorganism	Model											
	SLR (V)		SLR (Temp)		SLR (AMP)		SLR (EC)		SWLR		BFQMLR	
	R^2	*RMSE*	R^2	*RMSE*	R^2	*RMSE*	R^2	*RMSE*	R^2	*RMSE*	R^2	*RMSE*
L. mesenteroides subsp. *mesenteroides*	0.014	2.644	0.874	0.946	0.730	1.382	0.507	1.870	0.945	0.635	0.979	0.404
L. acidophilus	0.028	2.693	0.889	0.911	0.768	1.317	0.502	1.929	0.930	0.734		
L. plantarum subsp. *plantarum*	0.025	2.636	0.913	0.788	0.807	1.172	0.535	1.822	0.937	0.677		
S. cerevisiae	0.016	2.681	0.902	0.847	0.774	1.285	0.524	1.865	0.949	0.617		
B. fulva	0.027	2.603	0.910	0.791	0.806	1.161	0.533	1.804	0.934	0.689		
Z. rouxii	0.008	2.721	0.927	0.736	0.814	1.179	0.595	1.740	0.958	0.569		

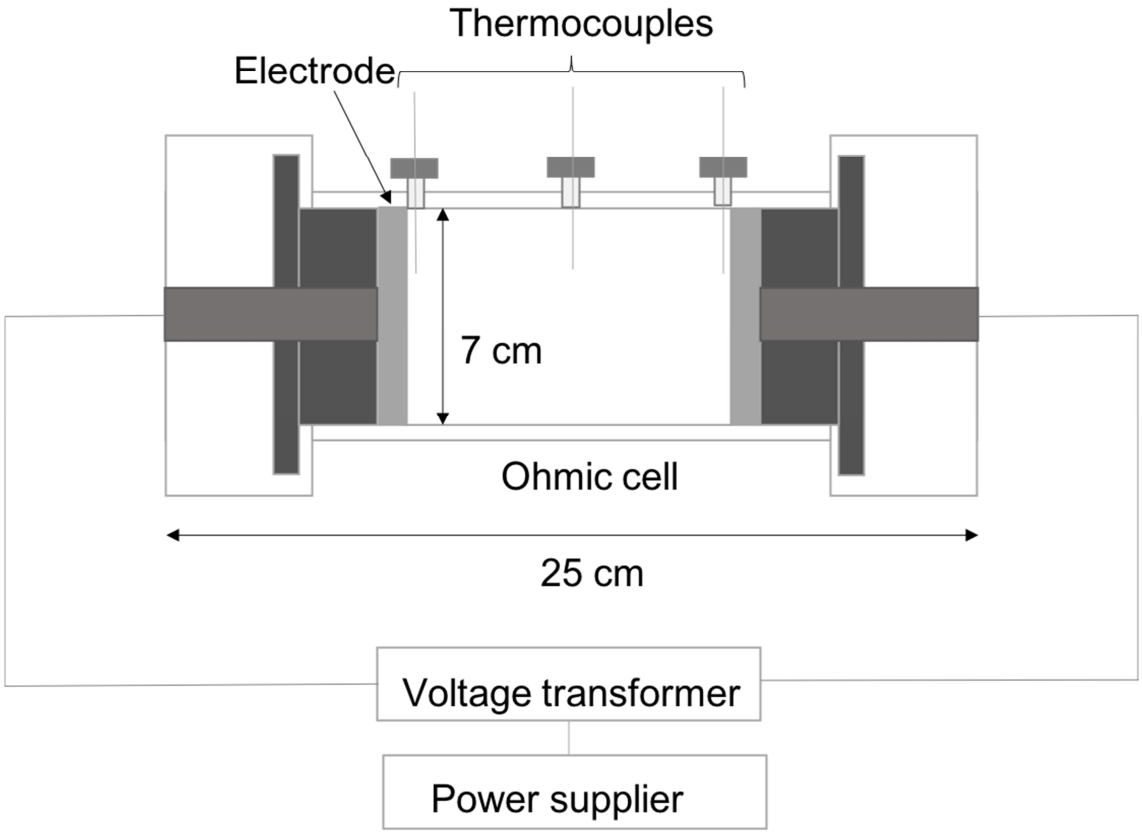

Figure 1. Schematic representation of the ohmic heating set-up used for the treatment of sour orange juice. The three main studied variables temperature (21 - 86 ºC), current (0 - 16 A) and voltage (0 - 300 V) were controlled through an automated system.

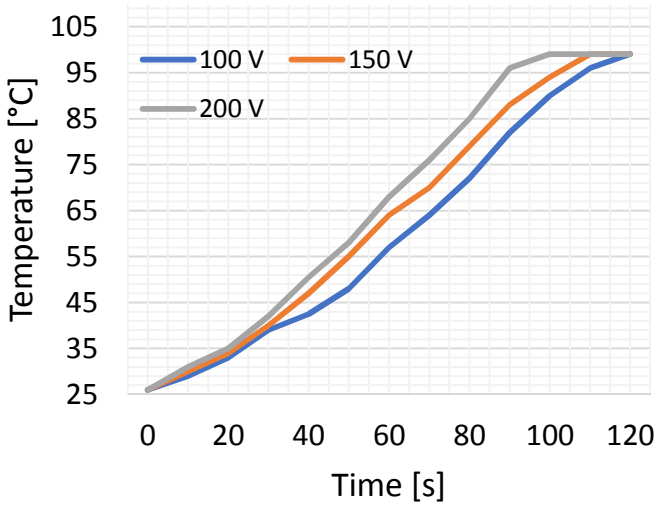

Figure 2. Temperature profile of sour orange juice during ohmic heating for 120 s and three different voltages (100, 150 and 200 V).

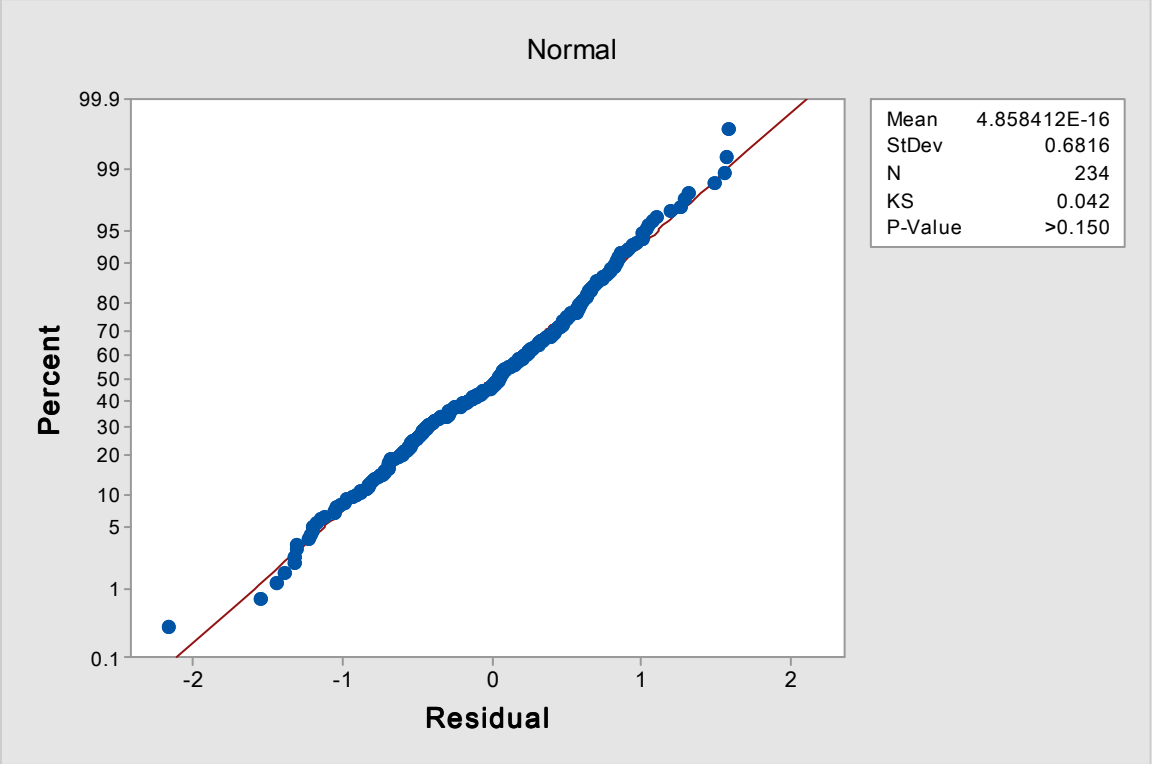

Figure 3. Normal probability plot of residuals for the full quadratic multiple linear regression (FQMLR) model.

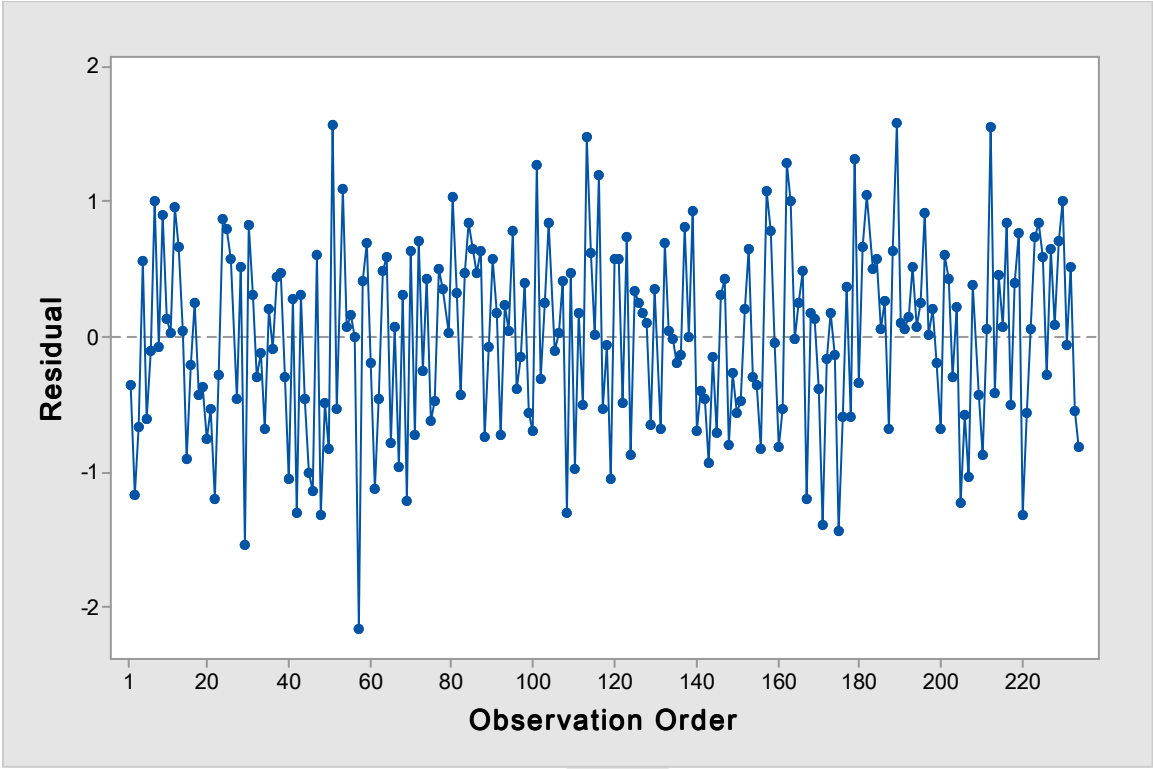

Figure 4. Plot of residuals versus the order observation for the full quadratic multiple linear regression (FQMLR) model.

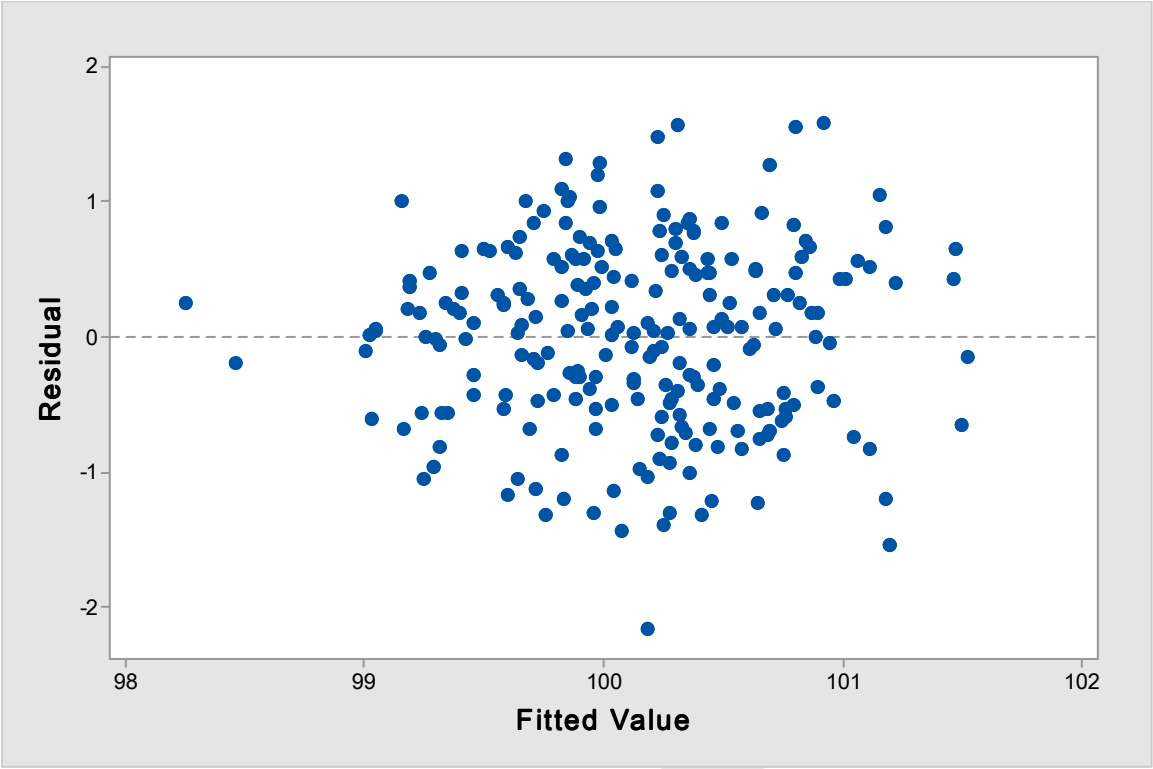

Figure 5. Plot of residuals versus fitted values for the full quadratic multiple linear regression (FQMLR) model.

Highlights

- Ohmic heating inactivated several spoilage microorganisms in sour orange juice
- Temperature and electric current were the parameters that most influenced the microorganisms inactivation
- Voltage had no effect in the population's reduction after ohmic heating
- Full Quadratic Multiple Linear Regression (FQMLR) modelled microorganisms inactivation
- *Z. rouxii* was the microorganism most affected by ohmic heating