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Quantitative assessment of the shelf-life of ozonated apple juice

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Abstract

Sterile apple juice inoculated with *S. cerevisiae* ATCC 9763 (10^3 CFU/mL) was processed in a bubble column with gaseous ozone of flow rate of 0.12 L/min and concentration of 33-40 µg/mL for 8 min. The growth kinetics of *S. cerevisiae* as an indicator of juice spoilage was monitored at 4, 8, 12 and 16 ºC for up to 30 days. The kinetics were quantitatively described by the primary model of Baranyi and Robert's and the maximum specific growth rate was further modeled as a function of temperature by the Ratkowsky type model. The developed model was successfully validated for the microbial growth of control and ozonated samples during dynamic storage temperature of periodic changes from 4 to 16 °C. Two more characteristic parameters were also evaluated, the time of spoilage of the product under static temperature conditions and the temperature quotient, Q_{10}. At lower static storage temperature (4 ºC) no spoilage occurred either for unprocessed or ozone processed apple juice. In the case of ozone processed apple juice, the shelf life was increased when compared with the controls and the Q_{10} was found to be 7.17, which appear much higher than that of the controls, indicating the effectiveness of ozonation for the extension of shelf-life of apple juice.

Keywords: yeast, ozone, apple juice, shelf-life, dynamic modeling

1. Introduction

Acidic products such as fruit juices contain substantial amounts of fermentable sugars. Spoilage of fruit and vegetable juices is primarily due to the proliferation of its natural acid tolerant and osmophilic micro flora [1]. Yeasts, lactic acid bacteria and moulds may account for the fermented taste, production of the carbon dioxide and the buttermilk off-flavour production, as well as the spoilage of juices [2]. Yeasts predominate in spoilage of acid food products as they have the ability to grow at low pH, high sugar concentration and low water activity conditions and resist inactivation by heat processing which enables them to survive or grow in fruit or fruit products [3, 4]. Fruit juices are generally rich in simple carbohydrates and complex nitrogen sources, and hence are ideal substrates for yeasts. More than 110 species of yeasts have been listed as associated with food and food products, of which large proportions occur on fruits, and more than 40 are associated with soft drinks [5]. The contamination of fruit juices with yeasts is normally indicative of highly contaminated raw materials, failure in fruit juice pasteurization, in sanitation practices or the presence of preservative resistant yeasts [6].

Saccharomyces cerevisiae is one of the most important yeasts causing spoilage of fruit juices and soft drinks [5, 7-9] and can be considered as shelf-life indicator [10, 11]. Several authors reported that fruit juice concentrates, fruit pulps, packaged fruit juices and soft drinks are particularly prone to fermentative spoilage with *S. cerevisiae, S. bayanus* and to a lesser extent *S. pastoranious* [4, 12-18]. Therefore, numerous heat inactivation studies have been conducted with *S. cerevisiae* because of its significance in the spoilage of heat pasteurized fruit juices and carbonated beverages [8, 17, 19]. Fermentation of sugars such as glucose, fructose, and sucrose is the principal spoilage

reaction of *Saccharomyces* species. Growth of yeasts is usually accompanied by formation of carbon dioxide and alcohol. Carbon dioxide gives the product a gassy, frothy appearance and causes a packaged product to swell and explode. In addition, the products develop a distinctive alcoholic, fermentative smell and taste [20]. Spoilage of fruit juice makes it unacceptable for human consumption.

Heat treatment is the most widely used method for preservation of fruit and vegetable juices due to its effectiveness in microbial inactivation [21] although it has certain disadvantages for nutritional and organoleptic values [22, 23]. There is consumer demand for a wider range of less heavily processed foods of improved quality with longer shelf-life and negligible changes in the organoleptic and nutritional values. This has enhanced interest in non-thermal technologies which could be effective on the inactivation of the undesired microorganisms [24].

Alternatives to thermal pasteurization such as ozone treatment are under investigation for potential application in fruit juice preservation. Apple juice (or apple cider in North America) is one of these products which is consumed by people of all ages for its sensory and nutritional qualities. The FDA's approval of ozone as a direct additive to food in 2001 triggered interest in ozone applications development, and industry guidelines for apple juice and cider were published by the USFDA in 2004, which also highlighted gaps in the scientific knowledge [25].

Ozone is a powerful antimicrobial agent due to its potential oxidizing capacity and it appears to be active against bacteria, fungi, viruses, protozoa, as well as bacterial and fungal spores [26, 27]. Ozone destroys microorganisms by progressive oxidation of vital cellular components. Oxidation reactions are caused by either dissolved molecular ozone

or free radical species formed during auto-decomposition of ozone [28]. Activated oxygen species resulting from ozone decomposition include singlet oxygen, hydroxyl radical, superoxide anion (perhydroxyl radical at low pH) and hydrogen peroxide which elicit potent cidal activity against a broad-spectrum of microorganisms [29].

The objective of this study was to investigate the effect of ozone as a non-thermal treatment to extend the shelf life of an apple juice system. Modeling approaches that describe the growth dynamics of *S. cerevisiae* in previously inoculated ozone processed apple juice under static (isothermal) and dynamic storage temperature conditions are also developed in order to quantitatively assess the effect of ozonation on the shelf life of the product.

2 Materials and Methods

2.1 Yeast strain and growth conditions

S. cerevisiae ATCC 9763 was obtained from microbiology stock culture of the School of Food Science and Environmental Health of the Dublin Institute of Technology, Dublin, Ireland. This strain was maintained as frozen stock at -70 ºC in the form of protective beads, which were plated onto potato dextrose agar (PDA, Scharlau Chemie) and incubated at 30 ºC for 48 h to obtain single colonies before storage at 4 ºC. Working cultures were prepared by inoculating a single colony into malt extract broth (MEB, Scharlau Chemie) and incubating at 30 ºC for 24 h.

2.2 Apple juice inoculation

S. cerevisiae cells grown for 24 h were harvested by centrifugation (SIGMA 2K15, Bench Top Refrigerated Ultracentrifuge, AGB scientific LTD) at 10,000 rpm for 10min at 4 ºC. The cell pellet was suspended in sterile phosphate buffered saline (PBS, Oxoid

LTD, UK) re-centrifuged twice as described above. Finally, after two washes with PBS, the cell pellet was re-suspended in PBS and the yeast density was determined by measuring absorbance at 550 nm using McFarland standard (BioMérieux, Marcy - l'Etoile, France).

Sterile, commercially prepared apple juice was obtained from a local retailer. This juice was chosen as a food system that could serve for performing controlled microbial experiments (e.g., [10, 30, 31]). The inoculum was then diluted in the juice to obtain approximately 10^6 CFU/mL. For each investigation, the cell concentration was further diluted in apple juice to yield a final working concentration of 10^3 CFU/mL. The inoculated apple juice with *S. cerevisiae* sample was then processed with ozone.

Soluble solids content of untreated apple juice was measured using a hand held refractometer (Bellingham and Stanley Ltd., UK). One drop of the juice was placed on the refractometer glass prism and soluble solid content was obtained as Brix. The measured °Brix was 11 ± 0.001. The pH of untreated product was measured using a pH meter with a glass electrode (Orion Model, England) and was 3.23 ± 0.015. Titratable acidity was determined by titrating 20 mL of the untreated apple juice sample diluted in 80 mL distilled water with 0.1N NaOH using phenolphthalein as an indicator. The volume of NaOH was converted to g malic acid per 100 mL of juice. The measured titratable acidity was 0.45 ± 0.009.

2.3 Ozone treatment

Ozone gas was generated using an ozone generator (Model OL80, Ozone services, Canada, Fig. 1). Ozone was produced by a corona discharge generator. Pure oxygen was supplied via an oxygen cylinder (Air Products Ltd., Dublin, Ireland) and the flow rate

was controlled using an oxygen flow regulator. Apple juice samples (90 mL) inoculated with *S. cerevisiae* (10^3 CFU/mL) were processed in a 100 mL ozone bubble column with a diameter of approximately 3.7 cm and height of around 21.7 cm. A previously determined optimum flow rate of 0.12 L/min [32] with an ozone concentration of 33-40 µg/mL was applied for each treatment for 8 min at ambient temperature (15-18ºC) [33]. In that study quality (color, phenolic content) and microbial parameters (*E. coli* strains ATCC 25922 and NCTC 12900) during ozone processing were assessed [33]. The ozone concentration was recorded using an ozone analyzer. Excess ozone was destroyed by an ozone destroyer unit. It should be mentioned that the apple juice contains large amount of organic matter which does not permit measurement of dissolved ozone in the liquid phase but also there was not any residual ozone effects as all ozone not targeting on the microbial cells is consumed by the organic matter. All experiments were carried out in duplicate.

2.4 Storage study

Storage studies were performed for the following three types of samples. Apple juice inoculated with 10^3 CFU/mL served as an unprocessed control 1. The second sample was the ozonated apple juice. Subsequently an unprocessed control 2 was prepared by inoculating *S. cerevisiae* cells with an inoculum level of 10^1 CFU/mL in order to start with a similar inoculum level that was attained after 8 min of ozone treatment.

2.4.1 Static storage temperature study (SST)

Unprocessed control samples of apple juice and ozone processed apple juice samples (45 mL each) were stored at constant temperatures of 4, 8, 12, and 16 °C respectively in incubators (LMS cooled incubators, Lennox Laboratory Supplies, Dublin, Ireland) for a

period up to 30 days. Aliquots of unprocessed and processed samples were taken daily for analysis.

2.4.2 Dynamic storage temperature study (DST)

For the DST study, unprocessed and processed apple juice samples were stored in an incubator where the lowest and the highest temperatures were set to 4 and 16 °C. The temperature was programmed to fluctuate according to a profile consisting of 4 °C for 12 h, followed by an increase of temperature from 4 to 16 °C and maintained at 16 °C for a further 12 h. The actual temperature profiles were recorded every 10 min using a temperature sensor connected to a data logger (Grant 1000 series Squirrel meter/data logger, UK). This specific profile was chosen in order to create a scenario of temperature abuse enhancing the microbial growth on which the developed modeling approaches could be validated.

2.5 Microbiological analysis

Yeast populations were determined by plating onto PDA. Aliquots (1mL) were withdrawn every day from ozone processed and unprocessed juice stored at each different temperature, serially diluted in MRD and 0.1mL of appropriate dilutions were surface plated on PDA in duplicate. Plates were incubated at 30 ºC for 48 h and colony forming units were counted. Results were reported as Log_{10}CFU/mL.

2.6 Microbial modeling

2.6.1 Parameter identification under static conditions

S. cerevisiae growth data in ozone processed apple juice stored under SST conditions were fitted to the explicit version of the Baranyi, and Roberts [34] model (Eq.1-3).

188 Regression was performed by using the DMFit Excel add-in software, version
189 2.1(www.ifr.ac.uk/safety/DMFit). The model reads as follows
190
191 $$N(t) = N(0) + \mu_{\max} A(t) - \ln\left(1 + \frac{e^{\mu_{\max} A(t)} - 1}{e^{(N_{\max} - N(0))}}\right) \quad (1)$$
192 with $$A(t) = t + \frac{1}{\mu_{\max}} \ln\left(\frac{e^{(-\mu_{\max} t)} + q(0)}{1 + q(0)}\right) \quad (2)$$
193
194 and $$\lambda = \ln\left(\frac{1 + \frac{1}{q(0)}}{\mu_{\max}}\right) \quad (3)$$
195 The kinetic parameters of maximum specific growth rate (μ_{max}) (1/days), lag phase (λ)
196 (days), initial microbial population ($N(0)$) (Log_{10}CFU/mL) and maximum population
197 density (N_{max}) (Log_{10}CFU/ml) have then been estimated. $q(0)$ (-) denotes the
198 concentration of substance critical to the microbial growth and is related to the
199 physiological state of the cells.
200 The maximum specific growth rates estimated under SST conditions were further
201 modeled as a function of storage temperature by using the Square root model [35, 36]:
202
203 $$\mu_{\max} = b\left(T - T_{\min}\right)^2 \quad (4)$$
204 where b is a constant, T is the storage temperature (ºC), T_{min} is the theoretical minimum
205 temperature for the growth of the organism. Eq. (4) has been used without the commonly
206 applied square root transformation of the μ_{max} value. This required the performance of a
207 non-linear regression which is available from the DMFit software. A (geometric) mean

208 value for $h_0 = \lambda * \mu_{\max}$ for each of the experimental set-ups (Control 1, Control 2,
209 Ozonated) was estimated from the individual growth curves, considering that the
210 parameter is constant, independent of the storage temperature [34, 37, 38] and the fact
211 that the resulting h_o was derived from the 3 levels of temperatures (refer to results). $q(0)$
212 is related to the parameter h_0 by the following equation [34]:

213

214 $$q(0) = \frac{1 - e^{-h_0}}{e^{-h_0}} \quad (5)$$

215 *2.6.2 Model validation under dynamic storage temperature* (DST) *conditions*

216 The validation of the yeast growth model was performed under DST conditions based on
217 the time temperature profile of apple juice samples during storage (control and ozone
218 processed), in conjunction with the square root model Eq. (4). The predictions were
219 performed with the differential equation of Baranyi and Roberts model (Eq. (6), (7)) in
220 which the Runge-Kutta method (ode23s, Matlab, The Mathworks) was applied for the
221 approximation of solutions of these ordinary differential equations:

222 $$\frac{dN(t)}{dt} = \left(b\left(T(t) - T_{\min}\right)^2\right)\left(\frac{q(t)}{q(t)+1}\right)\left(1 - \frac{N(t)}{N_{\max}}\right)N(t) \quad (6)$$

223 $$\frac{dq(t)}{dt} = \left(b\left(T(t) - T_{\min}\right)^2\right)q(t) \quad (7)$$

224 The root mean squared error (Eq. 8) [39] was used for evaluating the model fitting while
225 the accuracy and the bias factors presented by Baranyi et al. [40](Eq. 9, 10) were
226 considered in order to assess the prediction capability of the developed model.

227 $$\text{RMSE} = \sqrt{\sum_{i=1}^{n_t} \frac{\left(y_{\exp i} - y_{pre}\right)^2}{n_t - n_p}} \quad (8)$$

228 Where y_{expi} are experimental observations, y_{pre} are model predictions, n_t are number of
229 data points and n_p are number of estimated model parameters.

230 $$A_f = 10^{\sqrt{\frac{\sum_{i=1}^{n}\left(\log_{10}\hat{N}_i - \log N_i\right)^2}{n}}} \quad (9)$$

231 $$B_f = 10^{\frac{\sum_{i=1}^{n}\left(\log_{10}\hat{N}_i - \log_{10} N_i\right)}{n}} \quad (10)$$

232 Where $\log_{10}\hat{N}_i$ is the predicted microbial load and n is the number of the experimental
233 measurements.

234 *2.6.3 Calculation of the Q_{10} value*

235 The temperature quotient (Q_{10}) was also calculated from the information obtained in
236 Section 2.6.1 (parameter identification under static conditions). Q_{10} shows the effect of
237 temperature on the shelf-life and it is given as follows [41, 42]:

238

239 $$Q_{10} = \frac{\text{shelf life at } T^0\text{C}}{\text{shelf life at } (T + 10^0\text{C})} \quad (11)$$

240

241 Observe that this parameter was developed for a zero order reaction when the influence
242 of temperature on the reaction rate is described by using the Arrhenius relationship [43].
243 Nevertheless this approach is proposed and applied for the current microbial kinetic study
244 as an alternative method to assess the efficacy of the ozonated juice.
245 This Q_{10} value can be easily calculated by performing a regression between the ln shelf
246 life (days) versus the temperature which yields a straight line. Consequently, Q_{10}=exp
247 ($10 \cdot k$) with k the slope of the regression line. The estimation of the time of the shelf-life
248 (t_s) was calculated considering that a microbial level > 10^6 CFU/mL resulted in a failure

(spoilage) of the product (see for similar examples in other products: Al-Kadamany, et al. [44]). The shelf-life time, t_s, was obtained by solving Eq. (1-3) (*solve* command in Matlab, The Mathworks) for the estimated parameters of the two controls and the ozonated growth kinetics when log $N(t_s)$ = 6 log(CFU/mL).

3 Results

The growth of *S. cerevisiae* in unprocessed and ozone processed apple juice was assessed at SST conditions from 4 °C to 16 °C. Representative growth curves of the yeast population are shown in Fig. 2. The initial inoculum of control 1 was similar to previously reported levels of 10^3 CFU/mL [30, 45], while this level has also been reported in sound apples [46]. Finally, Kisko et al. [47] recorded *ca.* 10^3 CFU/mL level of *S. cerevisiae* in unprocessed apple juice. In the case of the unprocessed control samples 1 and 2 (i.e., initial inoculum level of 3.0 and 1.30 log CFU/mL, respectively) the lag phase was not obvious when the juice was stored under high SST (12 °C and 16 °C) (Fig. 2a and 2b). However, a typical growth pattern of *S. cerevisiae* was observed in the ozone processed apple juice stored under SST of 12 °C and 16 °C, consisting of an initial lag phase, an exponential growth phase followed by a stationary phase (Fig.2c).

The estimated kinetic parameters and statistical indices resulting from the regression of the microbial data by the Baranyi and Roberts model are shown in Table 1. The values of μ_{max} and λ varied according to the storage temperature. The μ_{max} of the unprocessed control samples increased from 0. 35 log CFU /day to 1.23 log CFU /day and for ozone processed apple juice increased from 0.275 log CFU /day to 1.270 log CFU /day with increase of the temperature from 8 to 16 °C. However, the lag phase for ozone processed apple juice was decreased from 15.07 days at 8 °C to 2.84 days at 16 °C. For both

unprocessed and ozone processed apple juice, the maximum population density (N_{max}) was found to be unaffected when stored under high SST (12 °C and 16 °C). The effect of storage temperature on μ_{max} was further modeled as a function of temperature by using the secondary square root model. The estimated parameters of the model are shown in Table 2. The model described satisfactorily the effect of temperature on the growth of *S. cerevisiae*. The calculated value for the theoretical minimum temperature of growth in ozone processed apple juice was 0.28 °C. The h_0 values obtained for the static environments studied were 0.336, 0.671 and 3.417 for unprocessed control 1, unprocessed control 2 and ozone processed apple juice samples, respectively.

The model developed under SST conditions was validated under DST conditions by using a periodically changing temperature profile and performing predictions with Eq. (6) and (7). As the maximum population density was independent of the applied storage temperature it was fixed at 7.5 logs CFU/mL (average of N_{max} estimated during isothermal conditions for which microbial stationary phase was reached). For the initial concentration *N(0)*, a nominal value was taken from the measured plate count result, i.e., 3.02 (for control 1), 1.32 (for control 2), 1.24 (for ozonated) log (CFU/mL). Finally, the nominal values for *q(0)* were 2.49, 1.05 and 0.03 for control 1, control 2 and ozonated apple juice respectively, calculated using Eq.(5) and after estimation of the h_o from the parameters derived under static environmental conditions. The comparison between the predicted and observed growth of *S. cerevisiae* in unprocessed apple juice and ozone processed apple juice samples are shown in Fig. 3. The performance of the model was evaluated statistically by the calculation of the bias (B_f) and accuracy (A_f) factors.

294 Two more characteristic parameters were evaluated, the Q_{10} and the time of spoilage of
295 the product under SST conditions (Fig. 4). At the lowest SST (4 ºC) no spoilage occurred
296 either for unprocessed or ozone processed apple juice. However, with the higher SST's
297 used product spoilage was observed in 9.45, 3.78, and 2.35 days for unprocessed control
298 1 at 8, 12 and 16 ºC, respectively. For unprocessed control 2, the spoilage occured after
299 15.08, 6.30 and 4.29 days at 8, 12 and 16 ºC respectively. In the case of ozone processed
300 apple juice, the shelf life was increased when compared with both type of controls and
301 resulted in 34.26, 10.34 and 7.08 days at 8, 12 and 16 ºC, respectively. Finally the Q_{10}
302 was found to be 7.17 in the case of ozonated juice. This was much higher than that
303 obtained for the controls, i.e., 5.68, 4.81, indicating the effectiveness of ozonation for
304 extension of the shelf-life of apple juice.

305 **4. Discussion**

306 The results of the present study showed that *S. cerevisiae* ATCC 9763 is able to grow in
307 apple juice stored within a temperature range of 8 to 16 ºC. The Baranyi, and Roberts
308 model as well as the square root model described the growth of yeast populations in
309 unprocessed and ozone processed apple juice. Based on the static data, a new model was
310 developed that described the growth of *S. cerevisiae* population well in unprocessed and
311 ozone processed apple juice under dynamic conditions that simulated a storage
312 temperature abuse. At the lower SST's (4 and 8 ºC), the longer lag phase indicates that
313 the yeast population needed longer time to adapt to the environment. However, at higher
314 storage temperatures this effect was not evident, indicating the ability of yeasts to grow at
315 these temperatures with a reduced or seemingly absent lag time. By comparison, in the
316 case of ozone processed apple juice stored at 8, 12 or 16 ºC, the lag phase (λ) was

317 increased, indicating the effect of temperature and applied ozone stress on growth of *S.*
318 *cerevisiae* populations. Panagou et al. [10] reported a very short lag phase in different
319 pasteurized fruit juices even at the lowest storage temperatures, suggesting that
320 inoculated yeasts' adaptation time was unaffected by these temperatures (4, 8, 12 and16
321 ºC) . However, in this study a lag phase was observed for all ozone processed samples.
322 This could be due to the oxidizing action of the applied ozone treatment, which may exert
323 additional stress prior to allowing growth. Ozone has been reported to inactivate cytosolic
324 enzymes, with the most drastic inactivation for glyceraldehyde 3 phosphate
325 dehydrogenase and to lesser extent to other cytosolic enzymes. It also affects the quantity
326 of ATP and other nucleoside triphosphates, reducing to about 50% of its initial level [48].
327 The performance of the developed model was validated under dynamic conditions. Ross
328 et al. [49] reported that predictive models should ideally have an A_f and B_f = 1.00,
329 indicating a perfect model fit where the predicted and actual response values are equal
330 and satisfactory. B_f limits are more difficult to define because limits of acceptability are
331 related to the specific application of the model. Ranges of 0.6-3.99 have been reported for
332 the growth pathogen and spoilage microorganisms when compared with independent
333 published data [49]. The values of B_f and A_f indicated good agreement between observed
334 data and predicted data points. Nevertheless, in the case of Control 2 some discrepancy
335 was evident (Table 3). This could be attributed to the effect of the inoculum size on the
336 microbial adaptation phenomena. This observation may require further evaluation of the
337 inoculum size effects which could elucidate if different values of h_0 should be considered
338 for each of the performed microbial predictions.

Different technologies have been applied for inhibiting the growth of spoilage microorganism in fruit juices. Patrignani et al. [30] evaluated the potential of high pressure homogenization (HPH) for inactivation of *S. cerevisiae* 635 inoculated in apricot and carrot juice and its shelf life extension. Four or more repeated passes at 100 MPa of HPH to the apricot juice samples inoculated at a level of 3 $\log_{10}$ CFU/mL showed that *S. cerevisiae* population remained under the detection limit at least up to 144 h at 25 ºC. For carrot juice samples subjected to five or more repeated HPH passes, the *S. cerevisiae* cell load was lower than 5 $\log_{10}$ CFU/mL after 144 h at 25 ºC. However, refrigerated storage (4 ºC) indicated satisfactory extension of shelf life of HPH processed juices. Qin et al. [50] reported over 3 weeks extension of standard shelf life of pulsed electric field (PEF) processed apple juice when stored at 4 ºC and 25 ºC. Ferrentino et al. [51] concluded that high pressure carbon dioxide (HPCD) treatment proved to be a promising alternative technique yielding juices with fresh-like characteristics and extension of shelf life with safety. Suarez-Jacobo et al. [52] reported the efficacy of ultra high pressure homogenization to develop fresh apple juice with an equivalent shelf life to pasteurized apple juice with respect to the microbiological characteristics. Valdramidis et al. [53] observed that no spoilage of apple juice was evident at storage temperatures of 4, 8 and 12 ºC for 36 days after treatment with high hydrostatic pressure at 500 MPa and 550 MPa. From the present work it is evident that ozone is another non thermal technology which can be employed for extending the shelf life of apple juice. The present results proved an increase of the shelf-life of the ozonated product that varied between 2.79 to 24.81 days depending on the storage temperatures when compared with the control samples.

Validation of the developed modeling approaches will be expanded based on the suggestions by Pin et al. [54]. More specifically, kinetic data that come from competition of inoculated *S. cerevisiae,* pathogenic microorganism with a naturally occurring microflora of fresh apple juice will be incorporated in future model developments while comparative studies between ozonated and other treated technologies will be applied. This will permit the application of this model to apple juice products with different properties. Further studies will focus on defining the failure (spoilage) of processed apple juice based on the effect of ozone on additional to previously reported quality parameters (e.g., color, phenolic content) [33] including volatiles responsible for flavor, odour and sensory evaluation. Effect of the different inoculums levels on the microbial adaptation phenomena will also be assessed to interpret possible modeling discrepancies.

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