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INFLUENCE OF RESPIRATION ON PREDICTIVE MICROBIAL GROWTH OF *AEROBIC MESOPHILIC BACTERIA* AND *ENTEROBACTERIACEAE* IN FRESH-CUT APPLES PACKAGED UNDER MODIFIED ATMOSPHERE

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ABSTRACT

Objectives in this study were to model/predict simultaneous influences of apple O_2-consumption/CO_2-production rates (R_{O_2/CO_2}) and modified atmosphere packaging (MAP) (from volumetric concentrations of O_2/CO_2(y_{O_2/CO_2})on microbial growth (*Aerobic mesophilic bacteria, AMB; Enterobacteriaceae, EBac)* in 2 fresh-cut apple cultivars treated with five anti-browning treatments and stored in refrigerator. Growth of both microorganisms was best predicted by length of shelf-life, R_{O_2/CO_2}, and y_{O_2/CO_2}.With *AMB/EBac* growth, cultivar's R_{O_2} decreased together with y_{O_2} in packaging. Similarly, with microbial growth R_{CO_2} dropped, while simultaneously y_{CO_2} increase was observed in package. Regression coefficients ratios for *AMB*-respiration models were similar to ratios for stoichiometric coefficients for respiration equation with malic acid as main substrate. Hence, presented models likely captured natural relations between predictors, and gave good estimate of *AMB* growth and its association with respiration in MAP. Apple browning had weak or no association with bacterial growth. Obtained models may be utilized to optimize industrial parameters with least *AMB*/*EBac* growth, hence facilitate extension of fresh-cut apple shelf-life.

PRACTICAL APPLICATIONS

Relationships between fresh-cut apples O2-consumption/CO2-production rates, volumetric concentrations of O2/CO2 in modified atmosphere packaging (MAP) and microbial growth (Aerobic mesophilic bacteria, *AMB*; Enterobacteriaceae EBac) were established and yielded mathematical models which may be utilized to optimize industrial parameters with least *AMB/EBac* growth, hence facilitate extension of fresh-cut apple shelf-life. All equations and models are publicly accessible at apple.pbf.hr or 31.147.204.87, and can be used to calculate almost every parameter relevant for apple respiration and apple browning (only for permeable systems). Additionally, users can choose their own production settings (size of package, initial volumetric concentration of gases, apple mass), and calculate optimal values (with least browning) for their fresh-cut processes (taking into account their own packaging film and others). Therefore, results presented in this paper will likely be useful to the fresh-cut industry.

INTRODUCTION

Fresh-cut industry extended their market share in produce business, as increased number of consumers became aware of nutritional principles that promote increased consumption of fresh fruits and vegetables (USDA 2013). Fresh-cut apples are very popular produce, convenient for

consumption and commonly found on the market (Lata *et al.* 2009), and their production is expected to increase in the future (Wang *et al.* 2007). Industrial processing of fresh produce induce plant tissue injury that stimulates various degenerative changes (e.g., browning, softening, sensorial changes), promotes respiration, enzymatic and microbial activity, and other negative influences on the length of the shelf-life (SL) (Mahajan *et al.* 2008; Calu *et al.* 2009; Brody *et al.* 2011; Caleb *et al.* 2012).

MAP is one of the most popular solutions to extend the short SL of fresh-cut apples (Torrieri *et al.* 2009), as it employs the concept of selective barrier (packaging film with different permeances for gases) that has role to prevent anaerobic respiration by modifying atmosphere (MA) within the package (by selective transmission of O_2 inside and CO_2 outside of the package). Difference in fluxes of MA gases through the film plus apple respiration will produce atmosphere richer on CO_2 and poorer on O_2 within package, and consequentially that will impede apple respiration (Mahajan *et al.* 2007).

Microbial contamination of food is one of the primary concerns and major limiting factor in fresh-cut apple storage, and it was found that altering concentration of MA gases can lead to inhibition of bacterial growth (Caleb *et al.* 2013). However, the role of each gas (e.g., CO_2) in respiration process is not yet clarified (Caleb *et al.* 2013) as respiration process is simultaneously affected by numerous factors (e.g., time, volumetric concentration of gases, size of package, mass of produce etc.), and their interaction with MA (Fonseca *et al.* 2002). Complexity multiplies when one wants to observe influences of apple respiration on other intricate processes such as bacterial growth.

In food engineering, mathematical modeling is good solution to lower experimental costs and construct mathematical equations that can be used to describe (or be good estimate of) naturally occurring processes from large number of parameters (Torrieri *et al.* 2009; Croarkin 2012). Currently, semifundamental (Michaelis–Menten enzyme kinetics) and fundamental approaches are employed for such purpose (Torrieri *et al.* 2009). Semifundamental modeling often assumes that some variables are constants or their influence are insignificant, hence offer simplification of naturally occurring process (Caleb *et al.* 2013). On the other hand, fundamental models are complex to build (difficult to statistically assess relations among large number of predictor variables), but offer more flexibility and preciseness while they are tailored for each particular set of circumstances (e.g., MAP modeling for each particular fresh produce).

Enterobacteriaceae (*EBac*) and *AMB* are microorganisms commonly present in industrial environments. With certain exceptions, they possess no threats to human health unless they reach particular concentration in foods. Legal requirements vary with particular country, while in Croatia fresh-cut apples with *EBac* is considered unsafe for consumption if it exceeds upper microbial contamination limit (M) above or equal to 10^3 CFU/g (Ministry of Agriculture, 2007; Ministry of Agriculture, 2008; Ministry of Agriculture, 2011). Microbial spoilage in food safety is defined as objectionable number of microorganisms that cause off-flavors in foods (Benner 2014). Level of acceptance for such spoilage varies with cultural and legal settings and presence of *AMB* is regularly used as indicator for non-hazardous produce spoilage (Benner 2014). Croatian non-legal limit that defines fresh-cut fruits as spoiled is set to $M(AMB)>10^5$CFU/g (Benussi *et al.* 2011).

Currently there is need for development of mathematical models that will describe association between microbial growth due to change in MA gases in fresh-cut packaging (Caleb *et al.* 2013). Such models can help produce industry with defining HACCP, therefore providing consumers with safer and better foods while decreasing financial losses associated with produce spoilage and expenses associated with self-monitoring.

Therefore the objectives of this study were to mathematically model/predict influences of apple's O_2-consumption/CO_2-production rates and volumetric concentration of gases in MAP on microbiological growth for: (i) *AMB*, and (ii) *Enterobacteriaceae*.

MATERIALS AND METHODS

Raw Material

From our laboratory experiments we identified the most suitable apple cultivars (*Malus domestica* Borkh) for industrial experiments (data not shown). Selected apple cultivars were determined as highly favorable for consumption, had stable physical and chemical characteristics (soluble solids content and pH) in laboratory fresh-cut experiments, together with good synergy with the best selected anti-browning treatment (ABT) (e.g., had least amount of browning with the best ABT). Hence, we selected Cripps Pink (CP) and Golden Delicious (GD) cultivars. Apple fruits ($m = 20$ kg/apple cultivar) had to be of similar size without blemishes, and uniform maturity. Apples were hand washed with water in industrial facility, peeled, cut, and halved in pieces by clean stainless-steel knifes. Slices were 1 cm thick and removed from the area between apple core and apple surface.

Production of the Packaged Fresh-Cut Apples in MA

Exposure to each ABT was $t = 3$ min with following treatments for both apple cultivar: (i) no treatment; (ii) ascorbic (10 g/dm^3) and citric acid (2 g/dm^3); (iii) ultrasound

(USND) (3 min) ascorbic (10 g/dm^3) and citric acid 2 g/dm^3); (iv) Ca-ascorbate (10 g/dm^3), and (v) USND (3 min) and Ca-ascorbate (10 g/dm^3) (Pizzocaro *et al.* 1993; Aguayo *et al.* 2010; Jang and Moon 2011). Apple pieces were dipped in 30 dm^3/per anti-browning solution ($T = 4$–7°C) in plastic basins. Ultrasonic bath with frequency of 40 kHz (Bandelin Sonorex, Germany) provided USND treatment ($t = 3$ min). After the treatments, pieces were drained and immediately packaged in 20 × 20 cm bags with the MA. The MAP was done by weighing ($m = 200 \pm 10$ g) and packaging with industrial equipment for fruit and vegetables (AV 65/CX GNA, Sorma Group S&B Verpackungsmaschinen GmbH, Germany). Bags were made of semipermeable, heat-sealable polypropylene film with 35 μm thickness with following specifications: O_2 and CO_2 transmission rates were 633 ± 11 cm^3/m^2⋆d⋆atm and 331 ± 11 cm^3/m^2⋆d⋆atm, respectively, at $T = 23$°C and RH = 0%; packaging area $A = 0.04$ m^2; outside volumetric concentration $O_2 y_{O_2}^{out} = 20.95\%$; outside volumetric concentration $CO_2 y_{CO_2}^{out} = 0.03\%$; apple density $M_\rho = 464$ kg/m^3 (1cp of apple without skin = 236.59 cm^3 = 110 g) (USDA 2014); total volume of package was $V_{\mathrm{TOTAL}} = 854.7$ cm^3. The MA gases were generated by quaternary gas mixer, and bags were sealed by compensated vacuum packing welding machine. The MA gas composition settings imputed in command console of industrial packaging machinery was $N_2 = 90.5\%$, $CO_2 = 2.5\%$ and $O_2 = 7\%$. The bags were made in six replicates (all combinations of selected apple cultivar and ABT), totaling to 60 packages that were stored at $T_{\mathrm{SL}} = 4 \pm 2$°C. Samples were randomly exempt from 4 bags and used for analyses provided. Remaining two bags represented periodic backup in case something went unplanned, and were not used for any analysis. Total experimental time was 14 days with data collection points at 1, 7, and 14 days. Data collection included: colorimetry, gas measurements, SSC [Bx°] and pH.

Colorimetric Evaluation

Superficial browning (change of color) in apples was evaluated by Konica Minolta colorimeter (Model CM 3500d, designed for use in food industry) at CIE Standard Illuminant D65 by using 8 mm thick plate. Colorimetric variables $L^\star$, $a^\star$, $b^\star$ were obtained, and ΔE_b^*, was calculated from:

$$\Delta E_{ab}^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

where all $\Delta L^\star 2$, $\Delta a^\star 2$ and $\Delta b^\star 2$ were calculated in reference to the SL = 1 d.

Gas Composition Inside Package

Changes in gas composition in package free volume were monitored using a hand held gas analyzer for MAP, OXYBABY® O_2/CO_2 (Oxybaby, HTK, Hamburg, Germany) at predefined time intervals. Headspace gas concentration was determined by inserting a needle of gas meter into the package through a septum. One package was used for a single measurement. Respiration rates for the permeable system were calculated from Eqs. (2)–(5) for unsteady-state material balance in MAP obtained from literature (Torrieri *et al.* 2009; Caleb *et al.* 2013):

$$R_{O_2} = \frac{\frac{kP_{O_2}}{x} \times A \times \frac{\left(y_{O_2}^{out} - y_{O_2}^{in}\right)}{100} - \frac{V_f}{100} \times \left(\frac{dy_{O_2}}{dt}\right)}{m} \quad (2)$$

$$R_{CO_2} = \frac{\frac{V_f}{100} \times \left(\frac{dy_{CO_2}}{dt}\right) - \frac{kP_{CO_2}}{x} \times A \times \frac{\left(y_{CO_2}^{in} - y_{CO_2}^{out}\right)}{100}}{m} \quad (3)$$

$$V_f = V_{\mathrm{TOTAL}} - \frac{m}{m_\rho} \quad (4)$$

$$A = \text{length} \times \text{width} \quad (5)$$

where R = respiration (consumption/production) rate for permeable system [cm^3/kg⋆day⋆atm]; STP = standard temperature and pressure; $kP/x = P$ = permeance (at STP cm^{3}m^{-2}day^{-1}atm^{-1}); A = package surface area [m^2]; m = apple cultivar packaged mass [kg]; x = film thickness [cm]; y = volumetric concentration [% v/v O_2 and CO_2]; M_ρ = fresh-cut apple density; V_{TOTAL} = total volume inside the package [cm^3]; and V_f = free volume inside of the package.

Determination of Soluble Solids Content and pH

The soluble solid contents (°Brix) and pH values were determined for each apple cultivar. Prior to analysis, samples were homogenized with blender (Mixy, Zepter International, Switzerland), then used for determination. °Brix in sample was measured at 22 ± 0.5°C using a digital hand-held refractometer (ATAGO, PAL-3, Japan). All measurements were performed in triplicate and the prism surface was washed with distilled water after each analysis. The pH was determined using a digital pH meter (S20 SevenEasy, Mettler Toledo Instruments (Shanghai) Co., Ltd., China). Ten grams of sample were placed in a beaker and stirred continuously with magnetic stirrer (at $T = 22 \pm 0.5$°C), and the pH meter was calibrated with commercial buffer solutions of pH 7.0 and 4.0.

Microbial Analyses

Microbiological assessment was similar to common EU/Croatian food business operator requirements for fresh-cut production. The sampling covered nine types of food safety/spoilage microorganisms (*Salmonella spp., Staphylococcus aureus, Sulfite-Reducing Clostridium, EBac*, *Escherichia coli*, *AMB*, yeast, mold, *Listeria monocytogenes*) for

each apple variety (2), for each ABT (5), and for each data collection point (3), for a total of 270 samples. The results were shown using CFU/g. Apples were tested at Food Control Center at Faculty of Food Technology and Biotechnology, University of Zagreb, that is accredited laboratory for official controls, approved for testing food and feed by Croatian Ministry of Agriculture (compliant with HRN ISO/IEC 17025).

Statistical Analysis

Experiments were designed as full factorial randomized experimental design (Croarkin 2012). Descriptive statistics was used to assess the basic information about the experimental dataset (e.g., to obtain sample basic metrics, check for normality of distribution, transform the variables if necessary). Discrete variables were analyzed using χ^2 tests and continuous variables using independent t-tests, analysis of variance (ANOVA), and multivariate analysis of variance (MANOVA). Pearson's linear correlation test was used to assess the relation between the pairs of continuous variables. Marginal means were compared with Tukey's multiple comparison tests. Analyses were performed with IBM SPSS Statistics (v.20).

RESULTS AND DISCUSSION

Of all nine tested microorganisms, only *EBac* (HRN EN ISO 178 21528-2:2008) and *AMB* were detected (HRN EN ISO 4833:2008) during experiments. Therefore they were the target for modeling their growth in fresh-cut apples. Linear regression was employed to build and compare mathematical models. The significance levels for all tests were $\alpha \leq 0.05$, while all variance inflation factors were ≤ 5. Lack of fit tests and Durbin-Watson statistic used to test goodness-of-fit were insignificant. Only statistically significant predictors ($\alpha \leq 0.05$) were retained in the models.

Predicting *AMB* Growth in Fresh-Cut Apples Treated With Anti-Browning Treatments and Packaged in MAP From Their Respiration Rates and Volumetric Concentrations of Modified Atmosphere Gases

To predict *AMB* growth (dependent variable) as a function of respiration for various combinations of two apple cultivar and five ABT, the linear regression model was created, with statistically significant predictors listed in Table 1. Predictors were L^*, a^*, b^*, ΔE_b^* SSC, pH, R_{O_2}, $y_{O_2}^{in}$, R_{CO_2}, and $y_{CO_2}^{in}$. Similar statistical approach was previously published by our research group for various purposes (Bursać Kovačević *et al.* 2015a,b; Obranović *et al.*, 2015; Putnik *et al.*, 2016b; Putnik *et al.*, 2015). From Table 2 it can be seen that number of *AMB* growth increased with time in the package, while concentration of O_2 and respiration rate decreased. On the other hand, concentrations of the CO_2 and corresponding respiration rates decreased. Similar relation for the gases were found from previous research (Chiabrando and Giacalone 2013). Respiration rates for the permeable system were calculated for unsteady-state material balance in MAP from equations obtained from literature (Torrieri *et al.* 2009; Caleb *et al.* 2013). Conclusion to separate influence of gasses into two models was derived from calculating variance inflation factors for models that had both O_2 and CO_2 and other statistical tests. General linear regression models were:

$$\begin{gathered}\textbf{O}_2\textbf{influence}:\\ \log(AMB\ [\mathrm{CFU/g}]) = \beta_0 + \beta_1 \times L^* + \beta_2 \times a^* + \beta_3 \times\ b^* \\ + \beta_4 \times \Delta E_{ab}^* + \beta_5 \times \mathrm{SL\ [days]} \\ + \beta_6 \times R_{O_2} + \beta_7 \times y_{O_2}^{in} + \beta_8 \times (\mathrm{GD}) + \beta_9 \times (\mathrm{CP}) + \\ \beta_{10} \times (\text{no treatment}) \\ + \beta_{11} \times (\text{asorbic + citric acid}) + \\ \beta_{12} \times (\text{Ca-ascorbate}) \\ + \beta_{13} \times (\text{USND + Ca-ascorbate}) + \\ \beta_{14} \times (\text{USND + asorbic + citric acid}) \\ + \beta_{15} \times \mathrm{pH} + \beta_{16} \times \mathrm{SSC} + \mathrm{error}\end{gathered} \tag{6}$$

$$\begin{gathered}\textbf{CO}_2\textbf{influence}:\\ \log(AMB\ [\mathrm{CFU/g}]) = \beta_0 + \beta_1 \times L^* + \beta_2 \times a^* + \beta_3 \times\ b^* \\ + \beta_4 \times \Delta E_{ab}^* + \beta_5 \times \mathrm{SL\ [days]} \\ + \beta_6 \times R_{CO_2} + \beta_7 \times y_{CO_2}^{in} + \beta_8 \times (\mathrm{GD}) + \beta_9 \times (\mathrm{CP}) + \\ \beta_{10} \times (\text{no treatment}) \\ + \beta_{11} \times (\text{asorbic + citric acid}) + \\ \beta_{12} \times (\text{Ca-ascorbate}) \\ + \beta_{13} \times (\text{USND + Ca-ascorbate}) + \\ \beta_{14} \times (\text{USND + asorbic + citric acid}) \\ + \beta_{15} \times \mathrm{pH} + \beta_{16} \times \mathrm{SSC} + \mathrm{error}\end{gathered} \tag{7}$$

All continuous variables in analysis were log-transformed in order to improve model fitness. Variables apple cultivar, ABT, L^*, b^*, ΔE_b^*, pH, and interaction of ABT x apple cultivar were excluded from the models due to insignificance (Table 2, Fig. 1). All models are available online (Pizent *et al.* 2014). Calculated models were:

$$\begin{gathered}\log(AMB\ [\mathrm{CFU/g}]) = 36.99 + 1.27 \times \log\ a^* \\ + 5.65 \times \log(\mathrm{SL[days]}) - 5.16 \times \log\ (y_{O_2}^{in}[\%]) \\ - 11.56 \times \log\ (R_{O_2}) - 14.03 \times \log(\mathrm{SSC})\end{gathered} \tag{8}$$

TABLE 1. STATISTICAL PARAMETERS FOR MATHEMATICAL EQUATIONS

Equation No.	n (exp. measurements)	Predicting dependent variable	Predictors	*P* (model)	Model R^2_{noad}	Model R^2_{adj}	Influence of predictors' number on explained variance (R^2_{noad} vs. R^2_{adj})	Explanation of variance by single predictor (%)	Lack of fit (pure error/*P*)
(8)	30	Bacterial growth *AMB* [logCFU/g]	**a***; $y^{in}_{O_2}$, **R(O$_2$)**; **3 SL** (1, 7 and 14 days); **SSC**	$P \leq 0.05$	0.93	0.92	0.01	**a***=1%; **SL**=78%; $y^{in}_{O_2}$=21%; **R(O$_2$)**=13%; **SSC**=8%	0.00/+∞
(9)	30	Bacterial growth *AMB* [logCFU/g]	**a***; $y^{in}_{CO_2}$, **R(CO$_2$)**; **3 SL** (1, 7 and 14 days); **SSC**	$P \leq 0.05$	0.92	0.91	0.01	**a***=1%; **SL**=78%; $y^{in}_{CO_2}$=76%; **R(CO$_2$)**=28%; **SSC**=8%	0.00/+∞
(12)	30	Bacterial growth *EBac* [logCFU/g]	$y^{in}_{O_2}$=21%; **R(O$_2$)**=8%	$P \leq 0.05$	0.90	0.89	0.01	**SL**=65%; $y^{in}_{O_2}$, **R(O$_2$)**; **3 SL** (1, 7 and 14 days)	0.00/+∞
(13)	30	Bacterial growth *EBac* [logCFU/g]	$y^{in}_{CO_2}$, **R(CO$_2$)**; **3 SL** (1, 7 and 14 days)	$P \leq 0.05$	0.89	0.85	0.04	**SL**=65%; $y^{in}_{CO_2}$=71%; **R(CO$_2$)**=19%	0.00/+∞

$$\log(AMB\ [\mathrm{CFU/g}]) = 13.49 + 0.81\times\log\ a^{*} + 4.62\times\log(\mathrm{SL[days]}) + 5.84\times\log\ (y^{in}_{CO_2}[\%]) - 2.57\times\log\ (R_{CO_2}) - 13.49\times\log(\mathrm{SSC}) \quad (9)$$

Currently no models were found that associate respiration and any microbial growth in fresh-cut apples. However, models that only predict respiration are available (Torrieri *et al.* 2009). Most of them are exponential or rely on Michaelis–Menten and Arrhenius equations (Torrieri *et al.* 2009; Tornuk *et al.* 2014). Judging by the difference in adjusted vs. no-adjusted R^2, number of predictors played negligible role in explanation of the variance for both models (Table 1). As observed from statistical parameters in Table 1 and data from Fig. 2 models fitted well with experimental data. Figure 2 showed that modeled log CFU/g values for the *AMB* that were calculated by the mathematical models (8) and (9) matched to those that were observed by the experimental assessment.

Mathematically, from model 8 follows that if *AMB* growth expressed as log [CFU/g] would increase for one unit in 5.65 log days, log ($a^{\star}$) would rise for 1.27 units while logarithmic values for $y^{in}_{O_2}$[%], R_{O_2}, and log (SSC) would decrease for 5.16, 11.56, and 14.03 units respectively. In model 9, log ($a^{\star}$) and $y^{in}_{CO_2}$[%] will rise for 0.81 and 5.84 log units in 4.62 log days, while log of R_{CO_2} will drop for 2.57 units. Three strongest predictors for growth of *AMB* in both models were length of SL, R_{O_2/CO_2}, and $y^{in}_{O_2/CO_2}$ that is consistent with their importance in aerobic respiration (Torrieri *et al.* 2009). As expected, O_2 volumetric concentration and consumption rate (defined as use of mL of O_2 per kg of apple cultivar at 1 atm) were both negatively correlated with growth of *AMB*, since with their increased growth there is less available oxygen within the package for apple segments, hence O_2 consumption decreases as well as available O_2 within the package. With bacterial growth SSC [Bx°] drops indicating that *AMB* consume available SSC for their physiological processes.

This relation is consistent with relations from the model 9 where CO_2 volumetric concentration is positively correlated with growth of *AMB* while CO_2 production rate (defined as production of mL of CO_2 per kg of apple cultivar at 1 atm) drops with bacterial growth. As with *AMB* growth and impeded O_2 consumption rate, CO_2 production rate drops, while at the same time *AMB* growth increases volumetric concentration of CO_2 in the package and decreases available SSC. In other words, *AMB* and packaged apple cultivar "compete" for the free oxygen in the package, likely until *AMB* increases in numbers to the extent that oxygen becomes in short supply and respiration is shifted towards the anaerobic conditions.

By observing both predictive models it can be indirectly inferred that oxygen has higher impact on the growth of *AMB* in comparison to the carbon-dioxide (as slope is much higher in case of O_2 than CO_2), while ratio of change in volumetric

TABLE 2. AVERAGE VALUES FOR MICROBIAL GROWTH AND RESPIRATION PARAMETERS

Independent variables	n	Log CFU/g *AMB*	Log CFU/g *EBac*	y_{O_2} %	R_{O_2}	y_{CO_2} %	R_{CO_2}
Apple Cultivar		$P = 0.81$‡	$P = 0.19$‡	$P = 0.09$‡	$P = 0.86$‡	$P = 0.06$‡	$P = 0.96$‡
Cripps Pink	15	5.4 ± 0.1^{a}	2.3 ± 0.1^{a}	5.8 ± 0.4^{a}	23.0 ± 1.3^{a}	7.3 ± 0.5^{a}	1.2 ± 0.4^{a}
Golden Delicious	15	5.4 ± 0.1^{a}	2.2 ± 0.1^{a}	6.8 ± 0.4^{a}	22.7 ± 1.3^{a}	6.3 ± 0.5^{a}	1.2 ± 0.4^{a}
Treatment		$P = 0.32$‡	$P = 0.85$‡	$P = 0.14$‡	$P = 0.71$‡	$P = 0.06$‡	$P = 0.32$‡
No treatment	6	5.3 ± 0.2^{a}	2.3 ± 0.1^{a}	5.4 ± 0.6^{a}	24.3 ± 2.1^{a}	6.9 ± 0.5^{a}	0.7 ± 0.6^{a}
Ascorbic a. + Citric a.	6	5.4 ± 0.2	2.3 ± 0.1^{a}	5.9 ± 0.6^{a}	23.3 ± 2.1^{a}	8.1 ± 0.5^{a}	1.7 ± 0.6^{a}
Ca-ascorbate	6	5.6 ± 0.2^{a}	2.2 ± 0.1^{a}	5.8 ± 0.6^{a}	23.9 ± 2.1^{a}	6.3 ± 0.5^{a}	0.7 ± 0.6^{a}
USND + Ca-ascorbate	6	5.8 ± 0.2^{a}	2.3 ± 0.1^{a}	7.6 ± 0.6^{a}	20.7 ± 2.1^{a}	6.0 ± 0.5^{a}	2.0 ± 0.6^{a}
USND + Ascorbic a. + Citric a.	6	5.3 ± 0.2^{a}	2.2 ± 0.1^{a}	6.8 ± 0.6^{a}	21.9 ± 2.1^{a}	6.6 ± 0.5^{a}	0.8 ± 0.6^{a}
Shelf-life in days		$P \leq 0.01$†	$P \leq 0.01$†	$P \leq 0.01$†	$P \leq 0.01$†	$P \leq 0.01$†	$P \leq 0.01$†
1	10	2.8 ± 0.1^{a}	1.0 ± 0.1^{a}	8.2 ± 0.5^{a}	16.2 ± 1.6^{a}	4.0 ± 0.4^{a}	2.6 ± 0.4^{a}
7	10	4.7 ± 0.1^{b}	1.6 ± 0.1^{b}	5.3 ± 0.5^{b}	28.3 ± 1.6^{b}	6.7 ± 0.4^{b}	3.9 ± 0.4^{b}
14	10	8.8 ± 0.1^{c}	4.2 ± 0.1^{c}	5.4 ± 0.5^{b}	23.9 ± 1.6^{c}	9.7 ± 0.4^{c}	2.3 ± 0.4^{c}
Apple Cultivar by Treatment		$P = 0.99$‡	$P = 0.95$‡	$P = 0.26$‡	$P = 0.21$‡	$P = 0.45$‡	$P = 0.75$‡
Cripps Pink, Ca-ascorbate	3	5.6 ± 0.2^{a}	2.2 ± 0.2^{a}	5.1 ± 0.9^{a}	20.7 ± 2.9^{a}	6.9 ± 0.7^{a}	0.2 ± 0.8^{a}
Cripps Pink, No treatment	3	5.3 ± 0.2^{a}	2.4 ± 0.2^{a}	4.5 ± 0.9^{a}	27.4 ± 2.9^{a}	7.4 ± 0.7^{a}	1.1 ± 0.8^{a}
Cripps Pink, USND + Ca-ascorbate	3	5.6 ± 0.2^{a}	2.4 ± 0.2^{a}	8.4 ± 0.9^{a}	18.8 ± 2.9^{a}	5.9 ± 0.7^{a}	2.3 ± 0.8^{a}
Cripps Pink, USND + Ascorbic a. + Citric a.	3	5.3 ± 0.2^{a}	2.3 ± 0.2^{a}	5.9 ± 0.9^{a}	23.9 ± 2.9^{a}	7.1 ± 0.7^{a}	0.9 ± 0.8^{a}
Cripps Pink, Ascorbic a. + Citric a.	3	5.3 ± 0.2^{a}	2.5 ± 0.2^{a}	5.2 ± 0.9^{a}	24.0 ± 2.9^{a}	9.2 ± 0.7^{a}	1.3 ± 0.8^{a}
Golden Delicious, Ca-ascorbate	3	5.5 ± 0.2^{a}	2.2 ± 0.2^{a}	6.6 ± 0.9^{a}	27.1 ± 2.9^{a}	5.7 ± 0.7^{a}	1.2 ± 0.8^{a}
Golden Delicious, No treatment	3	5.3 ± 0.2^{a}	2.3 ± 0.2^{a}	6.4 ± 0.9^{a}	21.1 ± 2.9^{a}	6.4 ± 0.7^{a}	0.2 ± 0.8^{a}
Golden Delicious, USND + Ca-ascorbate	3	5.7 ± 0.2^{a}	2.2 ± 0.2^{a}	6.7 ± 0.9^{a}	22.5 ± 2.9^{a}	6.2 ± 0.7^{a}	1.8 ± 0.8^{a}
Golden Delicious, USND + ascorbic a. + citric a.	3	5.3 ± 0.2^{a}	2.1 ± 0.2^{a}	7.6 ± 0.9^{a}	19.9 ± 2.9^{a}	6.2 ± 0.7^{a}	0.7 ± 0.8^{a}
Golden Delicious, ascorbic a. + citric a.	3	5.3 ± 0.2^{a}	2.2 ± 0.2^{a}	6.6 ± 0.9^{a}	22.6 ± 2.9^{a}	6.9 ± 0.7^{a}	2.0 ± 0.8^{a}
MEAN	30	5.4	2.3	6.3	22.8	6.8	1.2

* Results are expressed as mean ± standard error. Values represented with different letters are statistically different marginal means at $P \leq 0.05$.

† Statistically significant variable at $P \leq 0.05$.

‡ Statistically insignificant variable at $P \leq 0.05$.

concentration [%] for both gases is close 1:1.13. In other words, if O_2 decreased in package for 1 unit, CO_2 would increase for 1.13 units, and this takes into account cumulative respiration for apple, *AMB* and exchange of gases between package interior and atmosphere. Simultaneously, consumption of the nutrients (contained in SSC) will decrease with similar rate regardless of the influence of gasses. Ratio of total drop in O_2 vs. drop in SSC was 1:2.77 and ratio of total rise in CO_2 vs. drop in SSC 1:2.3 that is reasonably close to the equation for respiration with malic acid as main substrate (Torrieri *et al.* 2009):

$$C_4H_6O_5 + 3O_2 \rightarrow 4CO_2 + 3H_2O$$

Hence, giving additional argument that presented mathematical equations captured true relations between predictors, and that they give a good estimate of *AMB* growth and its association with the respiration.

The CIELab variable a^* explained small amount of variance, implying weak but significant positive association between *AMB* growth. Interestingly, *AMB* growth was not predicted by L^*, b^*, and ΔE_b^*, meaning that growth of this microorganisms does not affects browning of apples. However, weak association between microbial growth and colorimetric variable a^* suggests that *AMB* may alter environmental conditions during storage that favor apple browning. This was indicative by the regression coefficient and was more expressed with O_2 then with the CO_2. For instance *AMB* can consume phenolic compounds as sources of energy (Müller *et al.* 1998) and metabolites from such biochemical processes (brown polymers) can be associated with enzymatic or non-enzymatic apple browning (Corzo-Martinez *et al.* 2012). If this hypothesis is true this result might be good start for the future research. Also industry might be provided with good alternative for expensive monitoring of microbial growth for particular microorganism in fresh-cut apple production. For instance, food business operators would need to measure color and be able to immediately calculate degree of microbial contamination with target microorganism.

Similar to our previous findings (data not shown), apple cultivar and ABT showed no significant influence on growth of *AMB*, likely due to genetic similarity among studied apple cultivars (Velasco *et al.* 2010), and absence of antimicrobial influence of ABT on this group of microorganisms. Periodic change of pH with bacterial growth was not detected in this set of data.

This model was created to describe respiratory waning and waxing of oxygen and carbon-dioxide for fresh-cut apple with MAP, and its relation to the growth of *AMB*. Further, it can be useful for optimization of production parameters that will

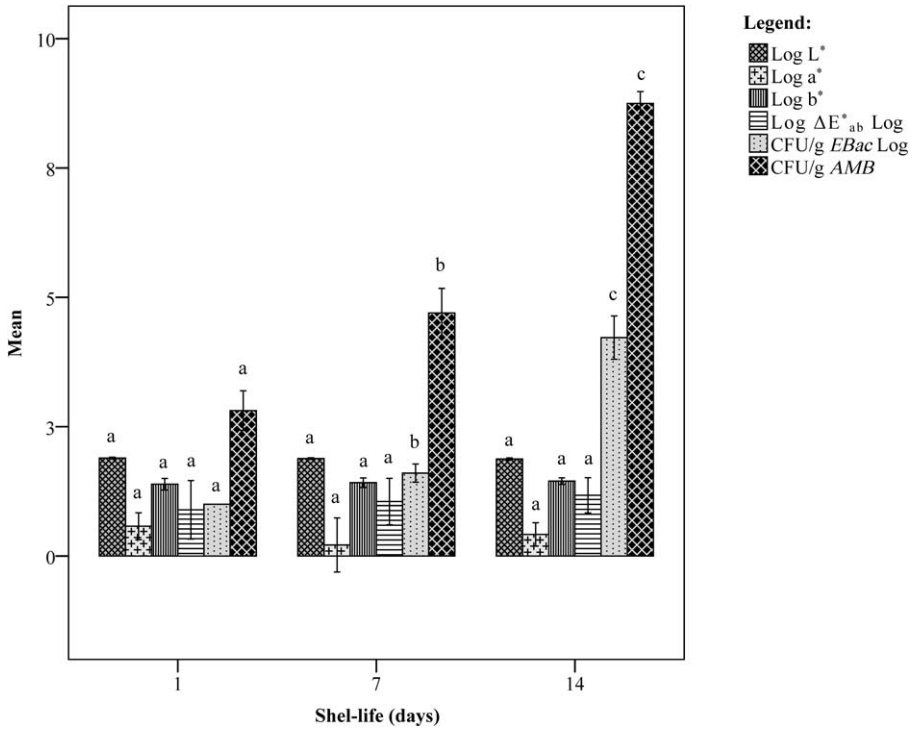

FIG. 1. AVERAGES FOR COLORIMETRY, AND MICROBIAL GROWTH

yield least growth of *AMB* and will facilitate extension of fresh-cut apples SL stored at refrigerator temperature (4°C). For instance, it was suggested by the literature that models similar to Eqs. (8) and (9) can be helpful in selecting optimal parameters for polymer engineering and converting technology to manufacture packaging film that will optimally impede bacterial growth (Caleb *et al.* 2013). Another approach suggested from above equations would be to maximally eliminate *AMB* from MAP by applying some form of sterile packaging conditions as studied microbes significantly interfere with apple respiration. Indeed, that will most certainly give more control over respiratory conditions and likely extend SL for fresh-cut apple in MAP. Due to absence of difference in bacterial growth among our apple cultivars it is likely that above mentioned models can be used for various fresh-cut apples. Although this should be tested in practice, but relying on apple physiology derived from genetics, it is reasonable to expect small variation in respiration among various apple cultivars.

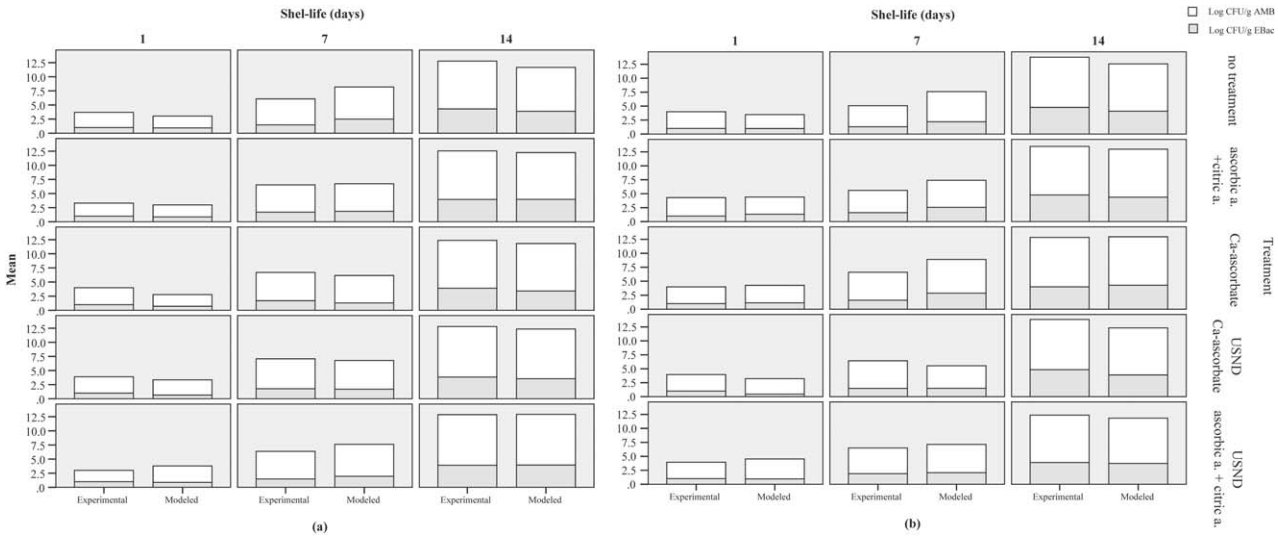

FIG. 2. GOODNESS-OF-FIT EXPERIMENTAL VS. MODELED DATA FOR (A) GOLDEN DELICIOUS AND (B) CRIPPS PINK

Predicting *EBac* Growth in Fresh-Cut Apples Treated With Anti-Browning Treatments and Packaged in MAP From Their Respiration Rates and Volumetric Concentrations of Modified Atmosphere Gases

Growth of *Enterobacteriaceae* (dependent variable) was measured as a function of respiration for two apple cultivars, treated with five ABT, with regards to $L^{\star}$, $a^{\star}$, $b^{\star}$, ΔE_b^*, SSC, pH,R_{O_2}, $y_{O_2}^{in}$, R_{CO_2}, and $y_{CO_2}^{in}$. The regression model was created, with predictors showed in Table 1. Construction of the models was consistent with the approaches previously reported in literature (Bursać Kovačević *et al.* 2015a,b; Obranović *et al.* 2015; Putnik *et al.* 2016b; Putnik *et al.* 2015). Similar as for the *AMB*, with *EBac* growth over the SL concentration of the oxygen decreased, apple O_2 expenditure and production of CO_2 increased (respiration rates) together with CO_2 concentrations within the package (Table 1). Similar as before influences of gasses was separated into two models to avoid negative influence of multicollinearity. General linear regression models were:

$$\begin{aligned}&\textbf{O}_2\textbf{influence}:\\ &\log(EBac\ [\mathrm{CFU/g}])=\beta_0+\beta_1\times L^*+\beta_2\times a^*+\beta_3\times\ b^*\\ &+\beta_4\times\Delta E^*_{ab}+\beta_5\times\mathrm{SL\ [days]}\\ &+\beta_6\times R_{O_2}+\beta_7\times y^{in}_{O_2}+\beta_8\times(\mathrm{GD})+\beta_9\times(\mathrm{CP})+\\ &\beta_{10}\times(\mathrm{no\ treatment})\\ &+\beta_{11}\times(\mathrm{asorbic\ +\ citric\ acid})+\\ &\beta_{12}\times(\mathrm{Ca\text{-}ascorbate})\\ &+\beta_{13}\times(\mathrm{USND\ +Ca\text{-}ascorbate})+\\ &\beta_{14}\times(\mathrm{USND\ +asorbic\ +\ citric\ acid})\\ &+\beta_{15}\times\mathrm{pH}+\beta_{16}\times\mathrm{SSC}+\mathrm{error}\end{aligned}\tag{10}$$

$$\begin{aligned}&\textbf{CO}_2\textbf{influence}:\\ &\log(EBac\ [\mathrm{CFU/g}])=\beta_0+\beta_1\times L^*+\beta_2\times a^*\\ &+\beta_3\times\ b^*+\beta_4\times\Delta E^*_{ab}+\beta_5\times\mathrm{SL\ [days]}\\ &+\beta_6\times R_{CO_2}+\beta_7\times y^{in}_{CO_2}+\beta_8\times(\mathrm{GD})+\\ &\beta_9\times(\mathrm{CP})+\beta_{10}\times(\mathrm{no\ treatment})\\ &+\beta_{11}\times(\mathrm{asorbic\ +\ citric\ acid})+\\ &\beta_{12}\times(\mathrm{Ca\text{-}ascorbate})\\ &+\beta_{13}\times(\mathrm{USND\ +Ca\text{-}ascorbate})+\\ &\beta_{14}\times(\mathrm{USND\ +asorbic\ +\ citric\ acid})\\ &+\beta_{15}\times\mathrm{pH}+\beta_{16}\times\mathrm{SSC}+\mathrm{error}\end{aligned}\tag{11}$$

Literature reports with predicting microbial growth based on respiration rates were not detected in current literature (Torrieri *et al.* 2009). Variables apple cultivar, ABT, pH, SSC and interaction of ABT × apple cultivar were excluded from the models due to insignificance (Table 1, Fig. 2). Additionally, change of color (measured by the $L^{\star}$, $a^{\star}$, $b^{\star}$, ΔE_b^*) was not significant (Figure 1). All continuous variables were log-transformed to improve model fitness. Figure 2 showed virtually no difference between modeled log [CFU/g] values for the *EBac* calculated by the mathematical models (10) and (11) and those that were measured experimentally.

$$\begin{aligned}\log\ (EBac\,[\mathrm{CFU/g}])=18.13+2.94\times\log\ (\mathrm{SL[days]})\\ -5.69\times\log\ (y^{in}_{O_2}[\%])-10.02\times\log\ (R_{O_2})\end{aligned}\tag{12}$$

$$\begin{aligned}\log\ (EBac\,[\mathrm{CFU/g}])=-3.18+2.09\times\log\ (\mathrm{SL[days]})\\ +5.48\times\log\ (y^{in}_{CO_2}[\%])-2.04\times\log\ (R_{CO_2})\end{aligned}\tag{13}$$

From model 12 follows that if log [CFU/g] of *EBac* increased for 1 unit in 2.94 units of log days, volumetric concentration and consumption rate of O_2 will drop for 5.69 and 10.02 units respectively. Similarly as with modeling *AMB*, model 13 proposed that growth of *EBac* may deplete available O_2 in a package, and that will impede consumption of O_2. Regression coefficients in model 13 suggested that if log [CFU/g] of *EBac* increased for one unit in 2.09 log days, volumetric concentration CO_2 will rise for 5.48 log [%] units, whereas production of CO_2 will drop for 2.04 log units. Roughly, ratio of volumetric concentrations CO_2 vs. O_2 is 1:1, meaning how much O_2 is decreased in the package, almost same amount of CO_2 increased. Interestingly, *EBac* growth showed no association with the SSC. This might be explained with difference in growth rate *AMB* vs. *EBac* in our samples. We previously estimated that $\mu_{max}(EBac)=0.25\pm0.02$ $\mathrm{logCFUg^{-1}day^{-1}}$ and $\mu_{max}(AMB)=0.46\pm0.02$ $\mathrm{logCFUg^{-1}day^{-1}}$ (Putnik *et al.* 2016a), Meaning that *EBac* grows almost as twice slower than *AMB* (and presumably with proportional slower nutrient consumption rate) and it is possible that periodic change in SSC was too small to be captured by our modeling. Judging by the size of the slope, *EBac* showed higher average growth in the presence of oxygen vs. carbon-dioxide. It is important to note that these explanations for mathematical relation among regression coefficients for the *EBac* should be taken with care. The *EBac* are facultative anaerobic microbes, and as such they have tendency to "switch" between anaerobic and aerobic respiration with regards to the presence of the oxygen (Hogg 2013). For our samples this mathematical relation (Eq. (13)) showed statistical significance, but interfering any concrete causality is rather difficult due to changing complexity of biological interaction between microbial and apple respiration.

Since growth of *EBac* did not alter color of apple cultivar segments and microbiological analysis revealed only presence of *EBac* and *AMB* within the package, it is likely that responsibility for color change during storage time is sole

responsibility of intrinsic enzymatic activity of particular apple cultivar (such as polyphenol oxidase) or it can have non-enzymatic origins (as Malliard reactions). Browning of apples may be slightly associated with the growth and respiration of *AMB* and their consequential change of volumetric concentrations of MA gases.

Consistent with previous results, apple cultivars and ABT showed no significant influence on growth of *EBac*. That is likely due to genetic relatedness among apple cultivars and corresponding similarity in nutrient content that is available for *EBac* growth in studied apples. Additionally, ABT did not facilitate or impede growth of these microorganisms. Similarly as before, periodic change of pH with *EBac* growth was not observed (Putnik *et al.* 2016a).

Models 8 and 9 can be used to calculate growth of *EBac* with regards to content of atmosphere (volumetric concentrations of O_2/CO_2) for two apple cultivars treated with 5 ABT and to set atmospheric conditions that are not suited for bacterial growth. Alternatively, fresh-cut apples may be packaged under some form of sterile conditions to eliminate *EBac* from packaging, as they are additional significant factor in MAP that is difficult to control.

CONCLUSIONS

Results of this study yielded 4 predictive microbiological models that described changes in microbial growth of *AMB* and *Enterobacteriaceae (EBac)* with volumetric concentrations of $O_2/CO_2(y_{O_2/CO_2})$ and O_2-consumption/CO_2-production rates(R_{O_2/CO_2}) for fresh-cut apples packaged in MAP stored in refrigerator at 4C.

Apple cultivars and anti-browning treatments (ABT) showed no significant influence on any microbial growth likely due to genetic similarity among studied apples and absence of antimicrobial influence of ABT on any microorganisms in our study.

All models fitted well with experimental data. Three strongest predictors for microbial growth (for *AMB/EBac*) were length of SL, R_{O_2/CO_2}, and y_{O_2/CO_2} that is consistent with their importance in aerobic respiration.

Models suggest that *AMB* and packaged apple cultivars compete for the free oxygen in the package, while apple browning may be slightly modified by *AMB* growth and their consequential influence on change of volumetric concentrations of MA gases.

Oxygen had higher impact on growth of *AMB* in comparison to the carbon-dioxide while relations among regression coefficients from *AMB*-respiration models bear close resemblance to relation for stoichiometric coefficient observed from equation for respiration with malic acid as the main substrate. Indicating that associations among predictors from our models likely encompass biological relations of complex naturally occurring respiratory processes in fresh-cut apples.

Obtained models may be utilized to optimize production parameters that will yield least *AMB*/*EBac* growth and will facilitate extension of fresh-cut apple SL while providing safer and quality foods. All created mathematical models were incorporated to on-line application ("Anti-browning Apple Calculator – C.A.P.P.A.B.L.E.©") that will serve to the food industry professionals and scientific community (apple.pbf.hr or 31.147.204.87).

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REFERENCES

AGUAYO, E., REQUEJO-JACKMAN, C., STANLEY, R. and WOOLF, A. 2010. Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices. Postharvest Biol Technol. *57*, 52–60.

BENNER, R.A. 2014. Organisms of concern but not foodborne or confirmed foodborne: Spoilage Microorganisms. In: *Encyclopedia of Food Safety,* Vol. 1, (Y. Motarjem, G. Moy, Todd, E.C., eds.), Academic Press, San Diego.

BENUSSI, A., BOROŠ, K., BRLEK, D., GRIZELJ, N., HEGEDUŠIĆ, P., HENGL, B., HUMSKI, A., KARAČIĆ, T., KOVAČEK, I., MAJIĆ, K., PALČIĆ-JAKOPOVIĆ, K., PUTNIK, P. and VAZDAR, R. 2011. Guide for microbiological criteria for food. (C.M.o. Agriculture, ed.) pp. 58, Ministry of Agriculture, Zagreb.

BRODY, A., ZHUANG, H. and HAN, J.H. 2011. Modified Atmosphere Packaging for Fresh-Cut Fruits and Vegetables, Blackwell Publishing Ltd., West Sussex.

BURSAĆ KOVAČEVIĆ, D., PUTNIK, P., DRAGOVIĆ-UZELAC, V., PEDISIĆ, S., REŽEK JAMBRAK, A. and HERCEG, Z. 2015a. Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. Food Chem *190*, 317–323.

BURSAĆ KOVAČEVIĆ, D., PUTNIK, P., DRAGOVIĆ-UZELAC, V., VAHČIĆ, N., SKENDEROVIĆ BABOJELIĆ, M. and LEVAJ, B. 2015b. Influences of organically and conventionally grown strawberry cultivars on anthocyanins content and color in purees and low-sugar jams. Food Chem. *181*, 94–100.

CALEB, O., MAHAJAN, P., AL-SAID, F.-J. and OPARA, U. 2013. Modified atmosphere packaging technology of fresh and fresh-cut produce and the microbial consequences—A review. Food Bioprocess Tech. *6*, 303–329.

CALEB, O.J., OPARA, U.L. and WITTHUHN, C.R. 2012. Modified atmosphere packaging of pomegranate fruit and arils: A review. Food Bioprocess Tech. *5*, 15–30.

CALU, M., BONCIU, C., IORDACHESCU, G. and TOFAN, I. 2009. The effect of controlled atmosphere storage conditions on some physico-chemical and sensorial characteristics of apples. J. Process Energ. Agric. *13*, 232–234.

CHIABRANDO, V. and GIACALONE, G. 2013. Maintaining Quality of Fresh-Cut Apple Slices Using Calcium Ascorbate and Stored under Modified Atmosphere. Acta Aliment Hung. *42*, 245–255.

CORZO-MARTINEZ, M., CORZO, N., VILLAMIEL, M. and DEL CASTILLO, M.D. 2012. *Food Biochemistry and Food Processing,* Blackwell Publishing, Oxford, UK.

CROARKIN, C. 2012. NIST SEMATECH e-Handbook of Statistical Methods: http://www.itl.nist.gov/div898/handbook/ (accessed: January 19, 2014), NIST/SEMATECH.

FONSECA, S.C., OLIVEIRA, F.A.R. and BRECHT, J.K. 2002. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: A review. J Food Eng. *52*, 99–119.

HOGG, S. 2013. Microbial metabolism. In: *Essential Microbiology,* pp. 115–169, Wiley-Blackwell, West Sussex, UK.

JANG, J.H. and MOON, K.D. 2011. Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. Food Chem. *124*, 444–449.

LATA, B., TRAMPCZYNSKA, A. and PACZESNA, J. 2009. Cultivar variation in apple peel and whole fruit phenolic composition. Sci Hortic-Amsterdam. *121*, 176–181.

MAHAJAN, P.V., OLIVEIRA, F.A.R., MONTANEZ, J.C. and FRIAS, J. 2007. Development of user friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce. Innov. Food Sci. Emerg. *8*, 84–92.

MAHAJAN, P.V., RODRIGUES, F.A.S. and LEFLAIVE, E. 2008. Analysis of water vapour transmission rate of perforation-mediated modified atmosphere packaging (PM-MAP). Biosyst Eng. *100*, 555–561.

MINISTRY OF AGRICULTURE. 2007. Zakon o hrani. In: *Narodne novine,* Vol. 46/07, Zagreb, Croatia.

MINISTRY OF AGRICULTURE. 2008. Uredba o izmjeni Zakona o hrani. In: *Narodne novine,* Vol. 155/08, Zagreb, Croatia.

MINISTRY OF AGRICULTURE. 2011. Zakon o izmjeni Zakona o hrani. In: *Narodne novine*, Vol. 55/11, Zagreb, Croatia.

MÜLLER, R., ANTRANIKIAN, G., MALONEY, S. and SHARP, S. 1998. Thermophilic degradation of environmental pollutants. In: *Biotechnology of Extremophiles: 61 (Advances in Biochemical Engineering/Biotechnology),* Vol. *61*, (G. Antranikian, ed.) pp. 156–167, Springer Berlin Heidelberg, Verlag Berlin.

OBRANOVIĆ, O., ŠKEVIN, D., KRALJIĆ, K., POSPIŠIL, M., NEĐERAL, N., BLEKIĆ, M. and PUTNIK, P. 2015b. Influence of Climate, Varieties and Production Process on Tocopherols, Plastochromanol-8 and Pigments in Flaxseed Oil. Food Technol Biotech. *54*, 496–504.

PIZENT, G., PUTNIK, P., BURSAĆ KOVAČEVIĆ, D. and HERCEG, K. 2014. Anti-browning Apple Calculator—CAPPABLE: http://apple.pbf.hr (IP: 31.147.204.87) (accessed August 25, 2015), Faculty of Food Technology and Biotechnology, University in Zagreb.

PIZZOCARO, F., TORREGGIANI, D. and GILARDI, G. 1993. Inhibition of apple polyphenoloxidase (Ppo) by ascorbic-acid, citric-acid and sodium-chloride. J. Food Process. Preserv. *17*, 21–30.

PUTNIK, P., BURSAC KOVACEVC, D., HERCEG, K. and LEVAJ, B. 2016a. Optimizing length of shelf-life for fresh-cut apples packaged in modified atmosphere from food quality. J Food Safety Manuscript submitted for publication.

PUTNIK, P., BURSAC KOVACEVC, D., PENIĆ, M., FEGEŠ, M. and DRAGOVIC-UZELAC, V. 2016b. Microwave-assisted extraction (MAE) of Dalmatian sage leafs for the optimal yield of polyphenols: HPLC-DAD identification and quantification. Food Anal Method. In press., 10.1007/s12161-12016-10428-12163.

PUTNIK, P., BURSAĆ KOVAČEVIĆ, D., and DRAGOVIĆ-UZELAC, V. 2015. Optimizing acidity and extraction time for polyphenolic recovery and antioxidant capacity in grape pomace skin extracts with response surface methodology approach. J Food Process Pres. In Press, 10.1111/jfpp.12710.

TORNUK, F., OZTURK, I., SAGDIC, O., YILMAZ, A. and ERKMEN, O. 2014. Application of predictive inactivation models to evaluate survival of *Staphylococcus Aureus* in fresh-cut apples treated with different plant hydrosols. Int. J. Food Prop. *17*, 587–598.

TORRIERI, E., MAHAJAN, P.V., CAVELLA, S., GALLAGHER, M.D.S., OLIVEIRA, F.A.R. and MASI, P. 2009. Mathematical modelling of modified atmosphere package: An engineering approach to design packaging systems for fresh-cut produce. In: *Advances in Modeling Agricultural Systems, Vol.* 25*, (P.J. Papajorgji and P.M. Pardalos, eds.), Springer Science+Business Media, New York.*

USDA. 2013. Dietary Guidelines for Americans 2013. Vol. 2014, U.S. Department of Health and Human Services.

USDA. 2014. National Nutrient Database for Standard Reference, entry ID:09004. United States Department of Agriculture.

VELASCO, R., ZHARKIKH, A., AFFOURTIT, J., DHINGRA, A., CESTARO, A., KALYANARAMAN, A., FONTANA, P., BHATNAGAR, S.K., TROGGIO, M., PRUSS, D., *ET AL.* 2010. The genome of the domesticated apple (Malus x domestica Borkh.). Nat. Genet. *42*, 833–839.

WANG, H., FENG, H. and LUO, Y.G. 2007. Control of browning and microbial growth on fresh-cut apples by sequential treatment of sanitizers and calcium ascorbate. J Food Sci. *72*, M1–M7.