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Can metabolites at harvest be used as physiological markers for modelling the softening behaviour of Chilean "Hass" avocados destined to local and distant markets?

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Abstract

The aim of this study was to model Chilean "Hass" avocado softening behaviour, destined to local and distant markets, taking into account the biological variation given by growing location and harvest stages. A total of 24 batches were obtained during the season 2018-2019 from different agro-climatic zones (coast, intermediate and interior) and two harvest stages (based on dry matter content). Fruit softening during either regular air (RA) or controlled atmosphere (CA) storage at 5 °C followed by shelf-life at 20 °C was modelled using a simplified mechanistic model. Most of the model parameters were treated as being generic for all fruit except for two fruit specific parameters, F_0 (firmness at harvest) and E_0 (amount of enzyme complex at harvest) that characterized the fruit at harvest and thus postharvest ripening behaviour. The model was able to describe 87.6 % of the observed variation of all 24 fruit batches studied from different agro-climatic zones at the batch averaged level, but 93.5 % of the observed variation at the fruit individual level. Since measured at harvest when most fruit are highly firm, initial fruit firmness by itself was not able to discriminate among the various batches as they all showed similar normal distributions among the different agro-climatic zones, in addition, the estimated E_0 values for each individual fruit were correlated to key metabolites to identify potential metabolite biomarkers discriminating among the different regions and batches. The developed model can be utilized to predict the batch specific ripening behaviour of "Hass" avocado under different postharvest logistic chains given the distribution of E_0 is known.

Keywords: *Persea americana*, heterogeneity, firmness, modelling, ripening, metabolites

1. Introduction

Chile is currently the fifth world exporter of “Hass” avocado. Strong competition for export volumes between neighbouring markets may result in a price reduction due to high simultaneous market volumes (Muñoz, 2018). In addition, the central part of Chile, being the main area of “Hass” avocado production, has experienced the consequences of climate change events (e.g., drought, elevated temperatures, etc.) impacting on the sector (Garreaud et al., 2017). Therefore, it is necessary that the Chilean “Hass” avocado market can provide high- quality differentiated product, even after several days of shipping and storage considering its distant markets (e.g., 30 d to Europe and up to 55 d to Asia). In addition, their main challenge is to provide homogeneous product in terms of quality and ripening attributes given the high variability encountered in fruit coming from the same agro-climatic zone, or even within a single orchard. This situation is critical as it complicates the prediction of postharvest fruit behaviour (Rivera et al., 2017; Pedreschi et al., 2019).

Fruit firmness and skin colour are considered the main quality attributes characterising avocado ripening during postharvest cold storage largely determining the acceptability of the batch at distribution centres or retailer stores (Magwaza and Tesfay, 2015).

Loss of firmness results from the activity of enzymes involved in cell wall remodelling (Bower, 1988; Defilippi et al., 2018). At harvest, the firmness of the mesocarp determined as a non-destructive compression force is generally in the range of 80-100 N and decreases at a moderate speed during cold storage. The softening rate increases during the shelf life period resulting in firmness levels lower than 5 N (Ochoa-Ascencio et al., 2009; Uarrota et al., 2019). The possibility to characterize the softening process of “Hass” avocado would allow the development of an objective criterion at harvest to segregate fast ripening from slow

ripening batches to contribute to adequate logistics and marketing strategies (Ochoa-Ascencio et al., 2009; Pedreschi et al., 2014).

Most attempts to model the loss of firmness of some fruit have been based on purely empirical models. Although these models are capable of describing biological systems, their parameters have no logical biological significance, therefore, their generic and predictive value is generally limited. Recent studies have used mechanistic or kinetic-based models to describe the loss of firmness in avocado (Hertog et al., 2003; Ochoa-Ascencio et al., 2009), apples (Gwanpua et al., 2013) and kiwifruit (Hertog et al., 2016). Hertog et al. (2003) developed a mathematical model to describe the impact of the atmospheric gas composition on the rate of quality change of "Hass" avocado, assuming that the rate of change of firmness and colour were proportional to the metabolic respiration rate. Ochoa-Ascencio et al. (2009) assumed a simple logistic model based on an autocatalytic enzymatic system catalysing postharvest softening of "Hass" avocado. Recently, Gwanpua et.al. (2018) developed a mathematical model that, together with avocado softening, describes the change in skin colour linked to the autocatalytic production of ethylene and its dependence on temperature and exogenous ethylene but ignoring the high biological variability already reported in avocado. Hertog et al. (2016) incorporated biological variation during the softening of kiwifruit by linking parameters measured at harvest to the modelled enzyme complex catalysing softening of kiwifruit.

The present study aims to model the loss of firmness of "Hass" avocado batches from different agro-climatic areas of Chile and harvest stages covering a wide range of biological variation through a mechanistic model based on simplified physiological concepts. The biological age of each fruit reflected by the E_0 fruit specific parameter provided by the model is correlated to key metabolites to predict ripening heterogeneity and behaviour. The ultimate

aim is to develop an approach that allows the industry to predict the ripening behaviour of each batch of avocado fruit given the distribution of its biological age and at harvest measured firmness. In this approach, information regarding the physiological status of the batch is obtained through correlations with key metabolites.

2. Material and methods

2.1. Fruit sampling and storage conditions

Two hundred avocado fruit cv. "Hass" of export quality from 4 orchards for each agro-climatic zone (coast, intermediate and interior) resulting in a total of 12 orchards were sampled. The orchards selected by agro-climatic area were defined based on the following criteria: (i) interior zone: distance from the orchard to the sea ≥ 45 km and at 300–900 meters above sea level (m.a.s.l); (ii) intermediate zone: distance from the orchard to the sea between 20 and 45 km and between 300 - 400 m.a.s.l and (iii) coastal zone: distance from the orchard to the sea ≤ 10 km and between 100 - 250 m.a.s.l. Sampling considered two harvests: early harvest (>23 – 26 % dry matter content) and middle harvest (> 26 - < 30 % dry matter content) resulting in a total of 24 different batches. One hundred fruit from each batch were stored under controlled atmosphere conditions (CA) of 4 kPa O_2 and 6 kPa CO_2 at 5 °C for 30 d. The other 100 remaining fruit from each orchard were stored under regular air (RA) at 5 °C for 30 d. After RA or CA storage, the fruit were brought to shelf life at 20 °C until each fruit reached the ready to eat stage (RTE) (4-8 N). Edible ripeness or RTE was recorded for each of the 4200 fruit. For early harvest, only 6 out of the 12 orchards (4 coastal zone and 2 interior zone) were sampled for CA but all for RA. For middle season, fruit from all 12 orchards were stored at both storage conditions (CA and RA). In total 4200 fruit were used for the

experiments. Climatic information and other relevant information about the fruit used for the experiments is displayed in Table 1.

2.2.At harvest biopsy sampling and fruit firmness measurements

A mesocarp biopsy (5 mm diameter) was taken from each fruit at harvest, then sealed with petroleum jelly and wax as previously reported by Pedreschi et al. (2014). Each biopsy was snap frozen in liquid nitrogen and stored at -80 °C for further metabolomics analysis. Firmness of each fruit was measured non-destructively at harvest, during CA and RA storage and during the shelf life storage period. A fruit texture analyser (TAXT Plus, Stable Micro Systems, UK) was used. A cylindrical probe was used with a convex tip (10 mm diameter), a trigger force of 0.50 N and a travel speed of 10 mms^{-1}. The compression force was recorded in Newtons (N) at a deformation of 2 mm and determined at two equidistant points in the equatorial region of each fruit.

2.3.Metabolite analysis at harvest

The extraction and derivatization of polar metabolites was performed according to Hatoum et al. (2014) with modifications as detailed in Uarrota et al. (2019). Focus was placed on main primary metabolites (sugars, amino acids and organic acids) and the relative amounts of these metabolites were used to correlate them with its corresponding E_0 of the model. A total of 10 biopsies from the E_0 distribution of each batch were analysed resulting in a total of 420 individual samples submitted to polar metabolite analysis.

2.4.Data analysis

As indicated above, the experimental design was the same for all orchards of both harvests, with the exception of early harvest fruit that only included 6 orchards for storage in CA. However, the data of all the batches were analysed taking into account differences between batches of each harvest stage, in terms of dry matter content (as indicated in Table 1) and in storage (30 d) plus shelf-life days (37 and 50 d for early harvest fruit and 37 and 52 d for middle harvest fruit). The model proposed in this research was implemented and model parameters were estimated using OptiPa, an interface created for the development of ordinary differential equations (ODE) based models (Hertog et al., 2007). In order to generate the stochastic model output, Monte Carlo simulations were performed based on the parameter distributions obtained for E_0 and F_0, estimated from the experimental data (Hertog et al., 2009). Metabolites were correlated to E_0 values using partial least squares regression analysis. The analysis was performed in the statistical computing program R (version 3.6.3) (R Core Team, 2020).

2.5. Model development

2.5.1. Model formulation

Loss of firmness in avocado cv. "Hass" has been modelled in previous studies through a simple logistic model (Ochoa-Ascencio et al. 2009). This model is based on a simplified representation of an autocatalytic process composed of two sub-processes acting in parallel: (i) an exponential increase in enzymatic activity during the ripening process and (ii) the action of this enzymatic complex catalysing firmness breakdown of the fruit.

It is known that this softening process occurs due to the complex interaction of several enzymes. In this model these various enzymes are lumped into a single enzyme complex (E in arbitrary units) responsible for the breakdown of firmness (F in N):

159

$$F + E \xrightarrow{k_f} E \quad (1)$$

160

161 With rate constant k_f (d^{-1})

162 To mimic the ethylene driven autocatalytic climacteric process, driving fruit softening, the
163 model adopts the simplified approach of an enzyme complex that induces its own activation
164 from a limited inactive precursor resource (E_{pre}):

165 $$E_{pre} + E \xrightarrow{k_e} 2 \cdot E \quad (2)$$

166 with rate constant k_e (in d^{-1})

167 From equations (1) and (2) three ordinary differential equations (ODE) were derived that
168 describe the changes of F and E over time (with t in d) through the following coupled system
169 of ODEs:

170 $$\frac{d}{dt}F(t) = -k_f\, E(t)\, (F(t) - F_{fix})$$

171 $$\frac{d}{dt}E(t) = k_e\, E(t)\, E_{pre}(t) \quad (3)$$

172 $$\frac{d}{dt}E_{pre}(t) = -k_e\, E(t)\, E_{pre}(t)$$

173 where $t \in (0, t_f]$, $t_f > 0$ is the final time (to be determined on each experiment) and then,
174 we finally define an initial value problem from the setting of the following initial conditions
175 (harvest values) at $t = 0\ (d)$:

176 $$E(0) = E_0$$

177 $$F(0) = F_0 \quad (4)$$

178 $$E_{pre}(0) = E_{tot} - E_0\ ,$$

where E_0, F_0, E_{tot} are real numbers. In these equations it is considered that avocados do not soften until 0 N, but present a firmness value at edible ripeness around 4 - 8 N (F_{fix} in N). E_{tot} is in arbitrary units.

Rate constants k_f and k_e are temperature dependent following Arrhenius's law,

$$k = k_{ref} \cdot e^{\frac{R}{Ea}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} . \quad (5)$$

with k_{ref} the reference rate value, valid at T_{ref}, a reference temperature of 288.15 K, Ea (J mol^{-1}) being the activation energy and R the universal gas constant (8.314 $Jmol^{-1} K^{-1}$).

In addition, it is assumed that the second differential equation is affected by atmospheric conditions, therefore, depending on the level of O_2 and CO_2 (CA or AIR) $k_{e,ref}$ takes a different value (k_{eAIR} or k_{eCA}).

2.5.2. Model assumptions

Throughout the analysis, several a priori assumptions were made in relation to some of the model parameters to avoid over parameterization of the model. As already indicated, the temperature reference (T_{ref}) was selected at 288.15 K in the middle of the experimental temperature range applied during storage and shelf life. Both rate constants (k_e and k_f) were assumed to obey Arrhenius' law with their own activation energies (Ea_{kf} and Ea_{ke}). The rate constant governing the enzyme turnover was assumed to be affected by atmospheric conditions which were implemented by introducing two parameter values, k_{eAIR} and k_{eCA}, one for each O_2 condition (CA or RA).

The total size of the available enzyme pool (E_{tot}) was set at an arbitrary value of 100 % with the initial amount of active enzyme system present at harvest (E_0) being a measure of fruit maturity. Assuming the kinetic parameters are fixed properties of the enzymatic systems

involved, these parameters were kept in common for all batches. F_0 and E_0 were estimated for each of the individual 4200 fruit.

2.5.3. Model calibration

Based on preliminary analysis of the data and previous work of Ochoa-Ascencio et al. (2009) but with some modifications based on our own generated data, it was decided for each of the model parameters if they would be treated as generic (T_{ref}, E_{akf}, $E_{ake,}$ k_{fref}, k_{eCAref}, $k_{eAIRref}$, E_{tot}) or fruit dependent (E_0 and F_0), some of them will be set at a priori values as already indicated (T_{ref}, E_{tot}) and others will be estimated from experimental data (E_{ake}, E_{akf}, k_{fref}, k_{eCAref}, $k_{eAIRref}$, F_{fix}, F_0 and E_0). These parameters were estimated in a two-step approach as described below. In a first analysis, data was categorized based on their origin and harvest stage resulting in 24 batches with the individual fruit data treated as ordinary replicate measurements. Based on these data, the generic parameters of the model E_{ake}, E_{akf}, k_{fref}, k_{eCAref}, $k_{eAIRref}$, F_{fix} were estimated in common for all 24 batches while batch specific values were estimated for E_0 and F_0.

In a second execution, the data of the individual fruit were analysed one by one keeping the generic model parameters from the first analysis at their appropriate estimated values, this time only estimating the fruit specific parameters E_0 and F_0 for the 4200 fruit.

To facilitate estimating valid values for E_0 within the range of 0-100 a $\tan^{-1}$ transformation was used. So, starting from an unrestricted positive parameter value $E_{0,tan}$ equation 6 was applied to turn this value into the restricted model input value for $E_0 \in (0,100)$ as defined as follows:

$$E_0 = 100 \cdot \tan^{-1}\left(E_{0,tan}\right) . \qquad (6)$$

225 *2.5.4. Monte Carlo simulations*

226 Starting from the 4200 estimated value pairs for E_0 and F_0, three subsets of parameter value
227 combinations were defined according the geographical origin of the fruit (coast, intermediate
228 and interior). Applying the algorithm developed by Hertog et al. (2009) implemented in
229 OptiPa, new random parameter sets were generated with the same distribution and correlation
230 properties as the ones obtained from the individual fruit analyses. In total three sets of 1000
231 parameter value combinations were generated, one per region. These random sets were used
232 to simulate six different logistic chains that were defined based on practical considerations
233 and current industry practices (Table 3). To mimic fruit sorting at harvest, data from the
234 Monte Carlo simulations for the coastal agro-climatic zone was separated in two sub-batches
235 one with $E_0<5$ and one with $E_0\geq5$. In this way the potential of sorting fruit based on
236 physiological age (represented by E_0) and its impact on ripening performance under different
237 chain conditions was evaluated in more detail.

238

239 **3. Results and discussion**

240 *3.1. General avocado softening behaviour*

241 Softening rate was highly affected by the storage type (RA vs CA) as expected (Figure 1).
242 Fruit exposed to regular air at 5 °C for 30 d displayed a greater ripening synchronization
243 during the shelf life period than fruit exposed to controlled atmosphere (4 kPa O_2 and 6 kPa
244 CO_2) at 5 °C for 30 d (Figure 1). No big differences in softening patterns were observed for
245 batches from the 3 climatic evaluated zones and harvest stages but storage type. CA storage
246 for 30 d favoured firmness retention as compared to RA storage for 30 d (Figure 1), in
247 addition to controlling the external physiological disorder known as "black spot"

248 (Supplementary Table 1). Internal disorders such as flesh browning seemed to be orchard
249 dependent. Middle harvest fruit in general presented lower incidence of internal disorders
250 after CA than RA storage.

251 Our results, revealed almost zero incidence of rots (body and stem end rots) either after 30 d
252 storage in RA or CA conditions (Supplementary Table 1), so no association between rot
253 incidence and softening rate can be established. The incidence of internal physiological
254 problems (internal flesh browning) and external browning (black spot) was lower or totally
255 controlled under CA storage, even though it is clear that these disorders are orchard
256 dependent (Uarrota et al., 2020).

257 *Model results*

258 Experimental data were analysed as described above using the ODE-based model developed
259 that describes the softening of cv. Hass avocado from different agro-climatic zones and
260 harvest stages under the model assumptions indicated in Section 2.5.2.
261 The approach differed from the one used by Ochoa-Ascencio et al. (2009), in that they only
262 collected experimental data after storage, while in the current investigation, firmness was
263 measured non-destructively on all 4200 fruits from harvest onward. Based on our
264 experimental data and previous work (Pedreschi et al., 2014 and Uarrota et al., 2019)
265 indicating that the measured firmness and dry matter at harvest of individual fruit do not
266 correlate well with postharvest ripening behaviour, we now assume that E_0 is the main at
267 harvest factor correlated with the maturity of the fruit at harvest (physiological age), and
268 being the main determinant of the postharvest ripening behaviour.
269

3.1.1. Batch based analysis

By interpreting the data at the batch level treating the individual fruit data as ordinary replicate measurements the generic kinetic parameters (k_{fref}, E_{akf}, E_{ake}, k_{eCAref}, $k_{eAIRref}$ and F_{fix}) were determined in common for all batches while batch specific average values were estimated for E_0 and F_0 (Table 2).

All of the generic model parameters were accurately estimated with acceptable standard deviations. Only k_{eCAref} was estimated extremely small and not to be significantly different from zero. With other words, during CA storage the enzyme conversion was not noticeable present, only during air storage (and shelf life) there was a noticeable autocatalytic increase in the active enzyme system. The energy of activation of the softening step (Ea_{kf}) was about two times the energy of activation of the enzyme activation step (Ea_{kf}) indicating that softening itself was the most temperature sensitive. The averaged final firmness level fruit softened to (F_{fix}) was estimated at about 12 N. In practice, individual fruit might soften to even lower values with the lowest registered value in the current experimental data being 3.9 N. Per batch a value was estimated for both E_0 and F_0. To prevent estimation problems related to a possible over-parameterisation of the model, the $E_{0,tan}$ value for the first batch was fixed at an arbitrarily value of 10 (so E_0=6.35 %; see Table 2). For the other batches $E_{0,tan}$ was estimated to be in the range of 5.4 to 31.2 (so E_0 ranging from 3.4 % to 19.3 %; see Table 2). The estimated batch averaged initial firmness values ranged from 86 N to 118 N (Table 2).

Overall, the batch based model was able to explain 87.6 % of the experimental data with the lack of fit mainly due to the large biological variation within each batch which was not yet accounted for. Looking at the observed versus predicted values for the batch averaged data

(Figure 2) no systematic deviations were observed from the line X=Y indicating a proper fit of the generic model. A detailed model fit for three selected batches of "Hass" avocado from different agro-climatic zones is displayed in Figure 3. All curves show an initial slow softening during the cold storage phase (5 °C) followed by accelerated softening during shelf life at 20 °C. Typically, the softening rate during CA is slightly slower than during air storage. Also the acceleration during shelf life after CA goes less fast as compared to shelf life after air storage. This can be explained by the fact that during cold air storage the enzyme system continues to be activated resulting in more active enzyme at the start of shelf life. At this point in time, when temperature is raised, this higher level of active enzyme will trigger fruit softening to a larger extend as compared to CA stored fruit that did not accumulate any active enzyme during the cold storage phase.

3.1.2. Individual fruit based analysis

Using the generic model parameters from Table 2, the data were re-analysed but this time fruit by fruit, to estimate the fruit specific E_0 and F_0 parameter values to fully account for the biological variation. Afterwards, the estimates were grouped per climate zone and harvest stage to look at their distributions (see Figure 4 for E_0 distributions). The fruit individual F_0 values ranged from 55 N to 155 N while the E_0 values ranged from 0 % to 100 %. A clear difference in the average of E_0 from both harvests (early and middle) can be observed in Figure 4. Average values of E_0 were higher for middle harvest fruit compared to early harvest fruit independent of the agro-climatic zone. This might explain the differences in the average time to reach the edible ripeness between early and middle harvest fruit. This behaviour supports previous reports that state that the physiological development of early harvest fruit might not be complete (Uarrota et al., 2019). From Figure 4, it can be observed that fruit from

315 Bartolillo interior orchard (batch number 2 for early and 14 for middle harvests) presented
316 an opposite behaviour of E_0 (higher for early than middle harvest fruit), but contrary to the
317 other orchards, the average dry matter content was higher for early harvest fruit than middle
318 harvest fruit (26.5 % and 25.8 % respectively). Despite the fact that middle harvest fruit
319 remained at least two more months on the tree and exposed to higher temperatures compared
320 to the early harvest, no increase of dry matter content for Bartolillo was observed as
321 previously indicated. Differences in E_0 between harvests translate into different states of
322 functioning of the biochemical machinery of the fruit to trigger ripening. A study carried out
323 by Uarrota et al. (2019) reported that early and middle harvest "Hass" avocado subjected to
324 heat treatment behaved differently at the proteomic and metabolomic level which could be
325 linked to the physiological status of the batch. Results related to the agro-climatic zone,
326 showed similar distributions of E_0 for the orchards except for Bartolillo (interior zone) as
327 already explained. Among agro-climatic zones, the coastal zone presented in average lower
328 E_0 values being evident for the early harvest being slightly at a more immature fruit stage.
329 Differences within and among agro-climatic zones will affect the physiological state at
330 harvest through its E_0 values impacting on the subsequent postharvest ripening behavior of
331 the fruit.

332 The developed model was able to explain 93.5 % of the biological fruit-to-fruit variation
333 during postharvest softening (Supplementary Figure 1). Main systematic deviation of the
334 model from the measured data is related to the final firmness F_{fix} which was estimated at an
335 in common value of about 12 N while the individual fruit showed a range of divergent values.
336 In other words, not every fruit softened to the same final firmness value.

337 Unlike the study carried out by Ochoa-Ascencio et al. (2009), the current study included
338 measurements of at harvest firmness on each individual fruit. However, the measured at
339 harvest firmness is not a good indicator of postharvest softening rate and ripening
340 heterogeneity as previously reported (Pedreschi et al., 2014; Uarrota et al., 2019). Some
341 batches presented higher values of firmness at harvest but ripened faster than batches
342 presenting lower values of firmness at harvest (Figure 3). Recently, Shezi et al. (2020) have
343 reported that "Hass" avocado fruit sampled from outside the canopy presented higher dry
344 matter and oil content and lower amounts of C_7 sugars than fruit sampled from inside the
345 canopy. The same authors claimed that these differences in biochemical constituents between
346 outside and inside sampled fruit are responsible of the differences in observed ripening
347 behaviour. However, conclusions were drawn on batch behaviour and not on individual fruit
348 behaviour. Commercially, fruit harvest is based on measurement of dry matter content on
349 few samples (10 to 20 fruit) representative of an orchard sector. Other previous studies based
350 on fruit semi-destructive evaluations (mesocarp biopsies) at harvest have shown differences
351 in metabolites and enzymes at harvest between "Hass" avocado fruit displaying different
352 ripening heterogeneity and behaviour (Pedreschi et al., 2014; Fuentealba et al., 2017; Uarrota
353 et al., 2019).

354 *3.3. Monte Carlo simulations*

355 The original objective of the model is to predict the softening behaviour of different batches
356 of cv. "Hass" avocado from different agro-climatic zones. To predict the behaviour of a
357 representative batch of fruit, Monte Carlo simulations were applied. To this end random
358 correlated sets of the fruit specific parameters E_0 and F_0 were generated with the same
359 statistical properties as the ones determined from the experimental data. For each agro-

climatic zone, 1000 combinations were drawn and used to simulate batch behaviour for different postharvest logistic chains based on ideal and real storage and transport conditions for the national and international markets (Table 3). Based on the simulations, confidence intervals and mean values are presented in Figure 5. Due to the lack of difference among zones in terms of F_0 distribution and the limited difference with regard to E_0 (see Figure 4) the simulated differences among the agro-climactic zones was rather limited (Supplementary Figure 2). Main differences were observed due to storage type (RA and CA) and storage temperature (5 and 7 °C) and the combination of both. Condition 1 represents extreme storage conditions for the national market (up to 30 d at 5 °C in regular air). It was observed that fruit stored in this condition synchronized ripening during the shelf-life period as compared to the other five conditions in which during the shelf-life period a higher ripening heterogeneity was observed. Incidence of internal and external physiological disorders were more pronounced in RA storage specially for middle harvest fruit (Supplementary Table 1). However, to reach international markets with excellent "Hass" avocado quality (without internal and external disorders) (Uarrota et al., 2020; Fuentealba et al., 2017), the use of regular air storage at low temperature is not an option for Chilean exporters, as their markets are distant (up to 30 and 55 d to Europe and Asia, respectively). Therefore, "Hass" avocado fruit is typically transported under controlled atmosphere conditions of 4 kPa O_2 and 6 kPa CO_2 at 5 °C and/or other suitable combinations. An increase of transport temperature from 5 °C to 7 °C (condition 3 vs 6) under CA conditions resulted in much lower firmness values at destination (Figure 5). This temperature increase resulted in lower firmness values at destination for all simulated conditions. Even when comparing condition 6 to condition 4, that includes ten more days to reach the Asian market, a lower firmness at destination was predicted for the shorter but warmer chain. Instead, if conditions of controlled atmosphere

transport at the same temperature (5 °C, 4 kPa O_2 and 6 kPa CO_2) but with different travel times are compared, firmness at distant markets are not largely affected (conditions 2 and 5). Similar results are obtained for comparison of chain conditions that mix atmospheric conditions but with the same temperature (conditions 3 and 4). Thus, to maintain and extend firmness retention during transport to distant markets besides controlled atmosphere application, the minimum transport temperature allowed by the fruit is critical. This minimum critical temperature is dependent on the growing conditions that allow fruit to develop adaptations mechanisms to withstand low temperatures, besides harvest maturity. Clear differences already in the fatty acid profiles of Peruvian and Chilean "Hass" avocados have been reported and attributed to the climatic conditions (differences in growth temperatures) (Campos et al., 2020; Pedreschi et al., 2016) with also implications in the storage temperature tolerated by the fruit from different origin. Thus, our simulations of the various chain configurations, have practical relevance regarding firmness retention of Chilean fruit under different export scenarios.

One of the aims of this research is to facilitate early segregation of fruit at harvest based on E_0. Thus, the results from the Monte Carlo simulations for coastal fruit was sorted *in silico* with the simulated unsorted 1000 fruit, segregated based on $E_0<5$ (observed for 267 fruit) and $E_0>5$ (observed for 733 fruit). For these sub-batches, 95 % confidence intervals for conditions 1 and 2 (30 d storage in RA or CA storage followed by shelf-life at 20 °C) are displayed in Figure 6. Based on this sorting at harvest, fruit can be classified as either slow ripening (low E_0 – "premium fruit") or fast ripening fruit (high E_0 - "mainstream fruit"). From Figure 6, it can be observed that after 30 d CA (chain 2) or RA (chain 1) storage, all fruit considered to be "premium" displayed firmness values within the 125-70 N and 110-70 N

ranges, respectively; while “mainstream fruit” displayed firmness values within the ranges of 110-50 N and 90-45 N for CA and RA storage. In addition, mainstream fruit reached its bottom firmness up to 5 days faster than premium fruit, this last category displaying longer storage capability. By performing this sorting at harvest, besides segregating for storage capabilities, ripening heterogeneity during shelf life conditions of the two sorted batches has been reduced as compared to the original unsorted batch. For the fast ripening mainstream fruit any fruit-to-fruit variation by the end of storage is more rapidly removed during the shelf life period while for the slow ripening premium fruit this variation tends to remain for longer. On one hand one could argue that this remaining ripening heterogeneity during shelf life is a negative trait while at the same time it allows for marketing the individual fruit over a longer timespan without the whole batch going off at once like with the batch of mainstream fruit.

3.4. At harvest measured metabolites and its correlation with E_0

One of the objectives of this research is to correlate the key metabolites at harvest with the estimated parameter (E_0) provided by the model for each individual fruit. A partial least squares analysis was performed to find correlations between key metabolites and E_0 as described in the materials and methods. A total of 50 metabolites were used to perform the partial least squares regression (PLS-R) and this model was able to explain with the first two latent variables 8.81 % and 12.82 % of X variance and 17.35 % and 16.85 % of Y variance. The VIP analysis revealed 17 important metabolites, of which 7 showed a negative correlation with the parameter E_0, being palmitic acid, nonanoic acid, quinic acid, threonine, galactitol, myoinositol and α-linolenic acid. Therefore, the remaining 10 metabolites (oxalic acid, dodecane, 4 aminobutanoic acid, lauric acid, stearic acid, perseitol, linoleic acid, 4-

429 methyl-2-oxovaleric acid, 6 hexadecenoic acid and oleic acid) showed a positive correlation
430 (Supplementary Figure 3).

431 Recent studies have reported for "Hass" avocado, malic acid as the predominant organic acid
432 at harvest and during postharvest followed by quinic and succinic acid in lower amounts
433 (Campos et al., 2020; Yahia. 2012). However, in this study the content of malic acid at
434 harvest was not found to be significantly correlated to E_0. One of the significant organic acids
435 was quinic acid, showing a negative correlation with the E_0 value. Few recent works have
436 reported on organic acids in avocado, Defilippi et al. (2015) determined the profile of organic
437 acids in Chilean "Hass" avocado and reported a decrease in the total amount of tartaric, malic,
438 ascorbic, citric and succinic acids as ripening progressed at 20 ° C, with a drastic decrease in
439 the malic acid content. In the particular case of quinic acid, it has been reported that it did
440 not present a significant variation during ripening (Campos et al., 2020). But previous studies
441 (Hurtado-Fernández et al., 2013) reported that quinic acid along with other metabolites
442 decreased during the ripening process. This decrease may be due to the fact that some organic
443 acids can act as substrates in the Krebs cycle. Therefore, the significant negative correlation
444 of quinic acid with the value of E_0, suggest it as a potential contributor of the physiological
445 state of the fruit at harvest (E_0). Another organic acid that was significant was oxalic acid
446 that presented a positive correlation with E_0. Oxalic acid has not been widely reported in
447 "Hass" avocado due to its low contribution to the organic acid profile. However, Yahia and
448 Woolf (2011) reported that oxalic acid represents 0.03 % of the organic acids in "Hass"
449 avocados ready to eat.

450 The second group of metabolites that was classified among the most relevant in terms of
451 correlations with E_0 corresponded to polyols (galactitol and myo-inositol). These metabolites

452 have not been reported to show much relevance in avocado development and ripening,
453 however, several studies have shown that the accumulation of different polyols, such as
454 galactitol and myo-inositol, increases in response to stress (Abebe et al., 2003; Macaluso et
455 al., 2007) and are capable of protecting various proteins from denaturation and deactivation
456 processes by high temperatures (Jaindl and Popp, 2006). In a study using thermal treatment
457 of "Hass" avocados to synchronize ripening, Uarrota et al. (2019) reported one day after
458 harvest galactinol content increases in middle harvest avocado fruit that ripened in a more
459 homogenous form.

460 The third group showing significant correlations to E_0 corresponded to fatty acids. Some of
461 the fatty acids that presented significance have already been reported for their abundance in
462 "Hass" avocado, such as palmitic, α-linolenic, linoleic and oleic. But other fatty acids of low
463 importance for "Hass" avocado such as stearic acid, lauric acid, nonanoic acid and 6-
464 hexadecenoic acid were also found significant. The fatty acid content of avocado increases
465 as the harvest seasons elapse and it has been observed that the profile of these fatty acids
466 changes according to the geographical location and climatic conditions in which the orchards
467 develop (Mpai et al. 2020). Other studies have reported that fruit from the same batch of
468 "Hass" avocado displayed no differences in the profile and content of fatty acids during
469 postharvest (Blakey al., 2012; Hernández et al., 2017). Although there are studies that have
470 revealed some relationships between fatty acids and ripening behaviour, such as the study of
471 Pedreschi et al. (2014) who reported a relationship between the linoleic acid content and fast
472 ripening avocados, the literature is not clear about the content of fatty acids at harvest and
473 participation in the ripening behaviour of the fruit.

The fourth group of metabolites displaying significant correlations with E_0 corresponded to amino acids, with threonine only presenting a negative correlation as revealed by PLS-R, whereas 4 aminobutanoic acid (GABA) presented a positive correlation in both analyses performed. Amino acids and their derivatives are known to be closely related to the quality of a fruit, but to date there is no study that describes the behaviour and content of amino acids in "Hass" avocado during its development and ripening (Pedreschi et al., 2019). But previous works have reported high content of different amino acids at harvest as important metabolites potentially related to the physiological age of the fruit (Pedreschi et al., 2014; Uarrota et al., 2019). Although previous studies have shown that amino acids are related to the ripening behaviour of "Hass" avocado batches, the amino acids GABA and threonine have not been specifically reported. But in other climacteric fruit such as tomato and papaya, they were reported as amino acids closely related to the ripening process (Pal et al., 2019; Der Agopian et al., 2020). This could explain the behaviour of these amino acids obtained in this study with respect to the physiological age of the fruit.

Another metabolite corresponding to a sugar alcohol that was chosen as important in the partial least squares regression (PLS-R) analysis was perseitol. Perseitol is an important sugar alcohol that together with mannoheptulose represent the highest proportion of sugars in avocado, in addition to playing a fundamental role during storage and ripening due to its functions as an energy source and antioxidant (Campos et al. 2020). Landahl et al. (2009) reported even differences in the spatial distribution of these C_7 sugars in avocado, being perseitol present in higher amounts in the middle part of the fruit and mannoheptulose presented high heterogeneity in concentration towards the stem end and base of the fruit with the largest concentration in the apical region. In our study, as described in materials and

497 methods, the biopsy was taken from the equatorial part of the fruit where industry and
498 consumers account for firmness in avocados. Recently, Shezi et al. (2020) indicated that the
499 content of perseitol was responsible for the avocado fruit taking longer to ripen. Other studies
500 have indicated that there is no (strong) correlation between perseitol and the time it takes an
501 individual fruit and/or batch to reach edible ripeness (Pedreschi et al., 2014; Meyer et al.,
502 2017). Other metabolites (amino acids and fatty acids) and proteins have been correlated
503 instead (Fuentealba et al., 2017; Uarrota et al., 2019) to differences in ripening speed and
504 heterogeneity.

505 To conclude this section, 4-methyl-2-oxovaleric acid or α-ketoisocaproic acid showed a
506 positive correlation with E_0. This metabolite is an important intermediary in the metabolism
507 of leucine and in tomato its concentration is related to the physiological stage of this fruit
508 (Yu and Spencer, 1969). A study by our group reported a greater increase in norleucine
509 (leucine isomer) as storage time (controlled atmosphere) was increased in batches of middle
510 harvest fruit "Hass" avocado subjected to a heat treatment which was an efficient treatment
511 to synchronizing ripening (Uarrota et al., 2019). In general, ripening being a high energy
512 demanding process involves active participation of different metabolic pathways. The
513 synthesis of cell wall modifying enzymes involved in softening requires energy and carbon
514 sources and the interplay of primary metabolites (e.g., sugars, organic acids, amino acids,
515 sugar alcohols) and related enzymes might determine the speed of the ripening process. For
516 instance, amino acids act as substrates in primary metabolism pathways (e.g., respiration,
517 protein synthesis), can enter directly to the tricarboxylic acid cycle and its higher content at
518 harvest might explain the faster ripening (Pedreschi et al., 2014). Although, the pool of
519 metabolites at harvest are indicative of the biochemical machinery of the fruit to trigger the

different pathways associated to the ripening process, from our results and based on previous works that involved mainly amino acids and a whole set of proteins as indicators of potential biological age of the fruit (Fuentealba et al., 2017; Uarrota et al., 2019), it may be that deeper levels of cellular control such as the expression of transcripts at harvest can provide greater information and better correlations with the biological age of the fruit (E_0). Future works of our group will focus on correlations of E_0 with gene expression levels at harvest. Future studies, could incorporate in the model the application of either external ethylene and its effect on hastening the average softening of the batch and heterogeneity. Even though, previous studies have reported ethylene external application to have an effect on hastening the average softening time of the batch, results are contradictory regarding its effect on reducing the spread of ripening or heterogeneity (Hernández et al., 2016). Avocado ripening heterogeneity has been extensively reported be related to the physiological age of the fruit (Blakey et al., 2009; Pedreschi et al., 2014; Uarrota et al., 2019).

4. Conclusions

The model built in this research, based on the work of Ochoa-Ascensio et al. (2009) presents several improvements in applying the approach to the Chilean "Hass" avocado situation. These improvements involved the incorporation of real measured from at harvest onwards, and the correlation of the parameter (E_0) to key metabolites of interest. Monte Carlo simulations of relevant scenario's revealed storage temperature to be critical in the maintenance of firmness retention, having more effect than extension of storage time under controlled atmosphere conditions. Segregation of fruit based on physiological age

(represented by E_0) as evaluated using Monte Carlo simulations would allow to separately market "premium" (slow ripening fruit, higher firmness retention) and "mainstream" fruit (fast ripening fruit, lower firmness retention) either transported in RA or CA conditions with positive impact on storage capabilities (longer storage time) and on reduction of ripening heterogeneity during shelf-life conditions as compared to the unsorted fruit.

PLS-R VIP analysis revealed a total of 17 key metabolites correlated to E_0. Organic acids (oxalic acid, quinic acid and phosphoric acid), polyols (galactitol and myo-inositol), fatty acids (palmitic, α linolenic, oleic, linoleic), amino acids (4 aminobutanoic acid and threonine), and perseitol displayed correlations at harvest with the E_0 parameter. Thus, GC-MS metabolic profiling has potential as biochemical phenotyping technique to early assess the physiological age of avocado fruit.

Further studies will focus on finding stronger correlations between the E_0 parameter of the model with the level of gene expression in fruit at harvest. We believe, transcriptomics data will provide a better overview of the physiological status of the fruit at harvest not as clearly reflected at the polar metabolite level.

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Table 1: Overview of all 42 experiments including in total 24 batches of fruit from different origin, dry matter content, mean growing temperature, the duration of the storage conditions preceding the shelf-life period at 20°C.

ID	Orchard	Zone	Dry matter content (%)	Mean T_{growth} (°C)	Storage cond.	Storage time (d)	Shelf-life* (d)	ID	Orchard	Zone	Dry matter content (%)	Mean T_{growth} (°C)	Storage cond.	Storage time (d)	Shelf life* (d)
1	4 Palmas	intermediate	24.1	12.9	RA	30	16	13	4 Palmas	intermediate	29.2	13.5	RA	30	16
2	Bartolillo	interior	26.5	14.2	RA	30	8	14	Bartolillo	interior	25.8	15.1	RA	30	13
3	Ensenada	interior	22.3	13.8	RA	30	11	15	Ensenada	interior	28.9	15.4	RA	30	13
4	Inversiones	coast	25.3	12.2	RA	30	7	16	Inversiones	coast	29.1	12.6	RA	30	8
5	Los Angeles	intermediate	23.4	13.8	RA	30	20	17	Los Lilenes	coast	27.7	13.1	RA	30	22
6	Los Lilenes	coast	23.5	12.3	RA	30	13	18	Los Ángeles	intermediate	29.4	14.9	RA	30	10
7	El Peumo	coast	23.6	12.9	RA	30	10	19	El Peumo	coast	32.8	13.2	RA	30	14
8	Pililén	interior	22.3	13.1	RA	30	20	20	Pililén	interior	26.8	14.3	RA	30	7
9	Quilhuica	intermediate	25.1	13.4	RA	30	12	21	Quilhuica	intermediate	25.6	14.5	RA	30	11
10	Quillay	interior	23.0	14.0	RA	30	15	22	Quillay	interior	26.4	15.0	RA	30	11
11	El Rancho	coast	26.9	13.3	RA	30	8	23	El Rancho	coast	32.5	13.6	RA	30	14
12	Resguardo	intermediate	26.8	12.4	RA	30	13	24	Resguardo	intermediate	28.9	12.4	RA	30	11
102	Bartolillo	interior	26.5	14.2	CA	30	10	113	4 Palmas	intermediate	29.2	13.5	CA	30	11
104	Inversiones	coast	25.3	12.2	CA	30	17	114	Bartolillo	interior	25.8	15.1	CA	30	13
106	Los Lilenes	coast	23.5	12.3	CA	30	13	115	Ensenada	interior	28.9	15.4	CA	30	13
107	El Peumo	coast	23.6	12.9	CA	30	10	116	Inversiones	coast	29.1	12.6	CA	30	8
110	Quillay	interior	23.0	14.0	CA	30	15	117	Los Angeles	intermediate	27.7	13.1	CA	30	21
111	El Rancho	coast	26.9	13.3	CA	30	19	118	Los Lilenes	coast	29.4	14.9	CA	30	10
								119	El Peumo	coast	32.8	13.2	CA	30	16
								120	Pililén	interior	26.8	14.3	CA	30	10
								121	Quilhuica	intermediate	25.6	14.5	CA	30	7
								122	Quillay	interior	26.4	15.0	CA	30	17
								123	El Rancho	coast	32.5	13.6	CA	30	16
								124	Resguardo	intermediate	28.9	12.4	CA	30	11

RA: regular air storage at 5 °C for 30 d followed by shelf-life period at 20 °C; CA: controlled atmosphere storage at 4 kPa O_2 and 6 kPa CO_2 at 5 °C for 30 d followed by shelf-life period at 20 °C. Mean T_{growth} corresponds to the average temperature from full bloom until harvest. *Shelf-life time corresponds to the days to reach edible ripeness at 20°C after RA or CA storage.

Table 2: Generic model parameters obtained from the 24 batches only ignoring fruit to fruit variation

Generic parameters[a]	Estimate (s.e)[b]		Generic statistics[c]	
kf_{ref} (d^{-1})	0.0089	(0.00069)	R^2_{adj}	87.55
Ea_{kf} (J mol^{-1})	1.63E+05	(1962.2)	n	4200
ke_{CAref} (d^{-1})	2.29E-10	(9.26E-05)		
ke_{AIRref} (d^{-1})	0.0019	(0.00014)		
Ea_{ke} (J mol^{-1})	87916	(4897.7)		
E_{0_1} (%)	6.35			
E_{0_2} (%)	14.88	(1.02)		
E_{0_3} (%)	9.38	(0.66)		
E_{0_4} (%)	8.30	(0.49)		
E_{0_5} (%)	3.41	(0.20)		
E_{0_6} (%)	13.52	(0.87)		
E_{0_7} (%)	7.64	(0.56)		
E_{0_8} (%)	4.69	(0.30)		
E_{0_9} (%)	10.25	(0.72)		
E_{0_10} (%)	5.43	(0.31)		
E_{0_11} (%)	5.51	(0.48)		
E_{0_12} (%)	10.02	(0.73)		
E_{0_13} (%)	9.92	(0.58)		
E_{0_14} (%)	11.11	(0.76)		
E_{0_15} (%)	9.13	(0.57)		
E_{0_16} (%)	12.10	(0.75)		
E_{0_17} (%)	12.50	(0.75)		
E_{0_18} (%)	9.06	(0.54)		
E_{0_19} (%)	8.91	(0.59)		
E_{0_20} (%)	19.26	(1.38)		
E_{0_21} (%)	11.20	(0.71)		
E_{0_22} (%)	12.83	(0.81)		
E_{0_23} (%)	9.14	(0.70)		
E_{0_24} (%)	11.39	(0.70)		
F_{fix} (N)	11.77	(0.15)		
F_{0_1} (N)	118.31	(0.77)		
F_{0_2} (N)	102.85	(0.75)		
F_{0_3} (N)	111.54	(0.90)		
F_{0_4} (N)	97.15	(0.61)		
F_{0_5} (N)	103.20	(0.64)		
F_{0_6} (N)	93.29	(0.69)		
F_{0_7} (N)	100.55	(0.70)		
F_{0_8} (N)	98.12	(0.70)		
F_{0_9} (N)	103.38	(0.88)		
F_{0_10} (N)	103.53	(0.58)		
F_{0_11} (N)	86.44	(0.65)		
F_{0_12} (N)	104.24	(0.91)		
F_{0_13} (N)	108.63	(0.66)		

F_{0_14} (N)	102.36	(0.73)	
F_{0_15} (N)	97.17	(0.65)	
F_{0_16} (N)	100.09	(0.68)	
F_{0_17} (N)	113.44	(0.69)	
F_{0_18} (N)	98.38	(0.64)	
F_{0_19} (N)	102.94	(0.69)	
F_{0_20} (N)	85.59	(0.74)	
F_{0_21} (N)	91.17	(0.67)	
F_{0_22} (N)	96.56	(0.69)	
F_{0_23} (N)	88.94	(0.72)	
F_{0_24} (N)	100.27	(0.68)	

Table 3: Description condition file for Monte Carlo simulations.

Number	Conditions	Description
1	30 d regular air at 5 °C + shelf life at 20 °C	Chilean storage conditions for the national market
2	30 d controlled atmosphere (4 kPa O_2 and 6 kPa CO_2) at 5 °C + shelf-life at 20 °C	Ideal transport conditions to the European market (main market). However, in practice fruit remain several days at regular air
3	7 d regular air at 5 °C + 30 d CA at 5 °C + 7 d at regular air at 5 °C + shelf life at 20 °C	Real transport conditions to European market
4	7 d regular air at 5 °C + 40 d CA at 5 °C + shelf life at 20 °C	Real transport conditions to Asian market (growing market)
5	40 d controlled atmosphere (4 kPa O_2 and 6 kPa CO_2) at 5 °C + shelf-life at 20 °C	Ideal transport conditions to the Asian market
6	7 d regular air at 7 °C + 30 d CA at 7 °C + 7 d at regular air at 7 °C + shelf life at 20 °C	Ideal transport conditions to Europe by a strong competitor (Peru)

Figure Captions

Figure 1: Softening of avocado fruit for three batches from different agro-climatic areas and storage conditions (regular air at 5 °C for 30 d + shelf life at 20 °C and controlled atmosphere at 4 kPa O_2 and 6 kPa CO_2 for 30 d + shelf life period at 20 °C). a) RA and b) CA for early harvest fruit, c) RA and d) CA for middle harvest fruit. No data was available for CA conditions of early harvest fruit for the intermediate orchards.

Figure 2: Experimentally observed firmness values as compared to the predicted model firmness values coming from the batch analyses. Each point represents a single sampling point with the experimental data averaged out over the replicate fruit measurements.

Figure 3: Model fit of the firmness model for three batches of Hass avocado from different agro-climatic zones (Bartolillo, Quilhuica and Inversiones) during regular air or controlled atmosphere storage. The dots represent the measured averaged firmness while the lines represent the fitted model outcome for each of the batches describing the averaged batch behaviour. Generic parameters used are given in Table 2 including the batch specific model parameters E_0 and F_0. Group (a) correspond to early harvest and group (b) to middle harvest. No data was available for CA conditions of early harvest fruit for the intermediate orchards.

Figure 4: Calculated values for active enzyme level (E_0) at harvest using the fruit specific parameters. The average calculated values are displayed per batch and per agro-climatic zone. Figure (a) corresponds to early harvest data and (b) for middle harvest data.

Figure 5: Results of the Monte Carlo simulation of six artificial fruit chain conditions for the coast agro-climatic zone. Condition 1: storage in regular air at 5 ° C for 30 d plus a shelf life at 20 °C. Condition 2: storage in a controlled atmosphere at 4 kPa O_2 and 6 kPa CO_2 at 5 °C for 30 d followed by shelf life period at 20 °C. Condition 3: 7 d storage in normal air at 5 °C

followed by storage in controlled atmosphere at 4 kPa O_2 and 6 kPa CO_2 at 5 °C for 30 d plus 7 d in normal air at 5 °C followed by a shelf life period at 20 °C. Condition 4: 7 d storage in normal air at 5 °C followed by storage in controlled atmosphere at 4 kPa O_2 and 6 kPa CO_2 at 5 °C for 40 d followed of a shelf life period at 20 °C. Condition 5: storage in a controlled atmosphere at 4 kPa O_2 and 6 kPa CO_2 at 5 °C for 40 d followed by a shelf life period at 20 °C. Condition 6: storage of 7 d in normal air at 7 °C followed by storage in a controlled atmosphere at 4 kPa O_2 and 6 kPa CO_2 at 7 °C for 30 d plus 7 d in normal air at 7 °C followed by an shelf life period at 20 °C. The 4x1000 Monte Carlo simulations are summarized by the 95 % confidence interval and its mean.

Figure 6: Monte Carlo simulation of batch sub-sets using data of coastal agro-climatic zone and chain conditions 1 and 2 of Table 3. In this analyses, the 1000 fruit were segregated at harvest into slow ripening fruit (low $E_0 < 5$ – "premium fruit") and fast ripening fruit (high $E_0 \geq 5$ - "mainstream fruit") and their ripening behavior was simulated.

Figure 1

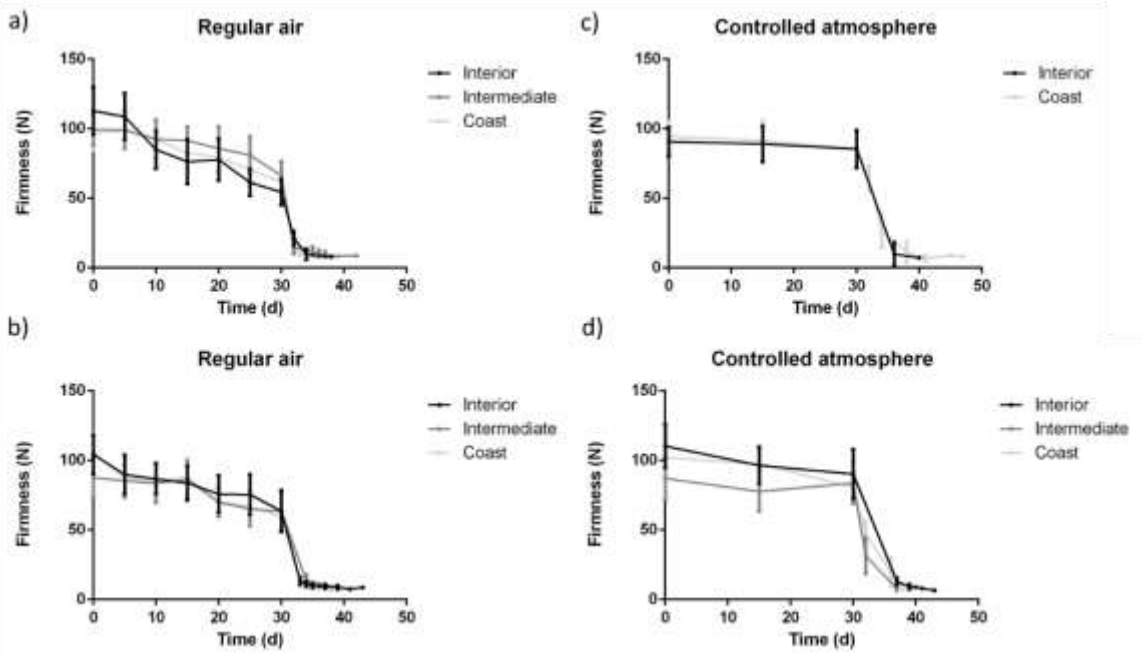

a)
Regular air
Firmness (N)
Time (d)
Interior
Intermediate
Coast
b)
Regular air
Firmness (N)
Time (d)
Interior
Intermediate
Coast
c)
Controlled atmosphere
Firmness (N)
Time (d)
Interior
Coast
d)
Controlled atmosphere
Firmness (N)
Time (d)
Interior
Intermediate
Coast

Figure 2

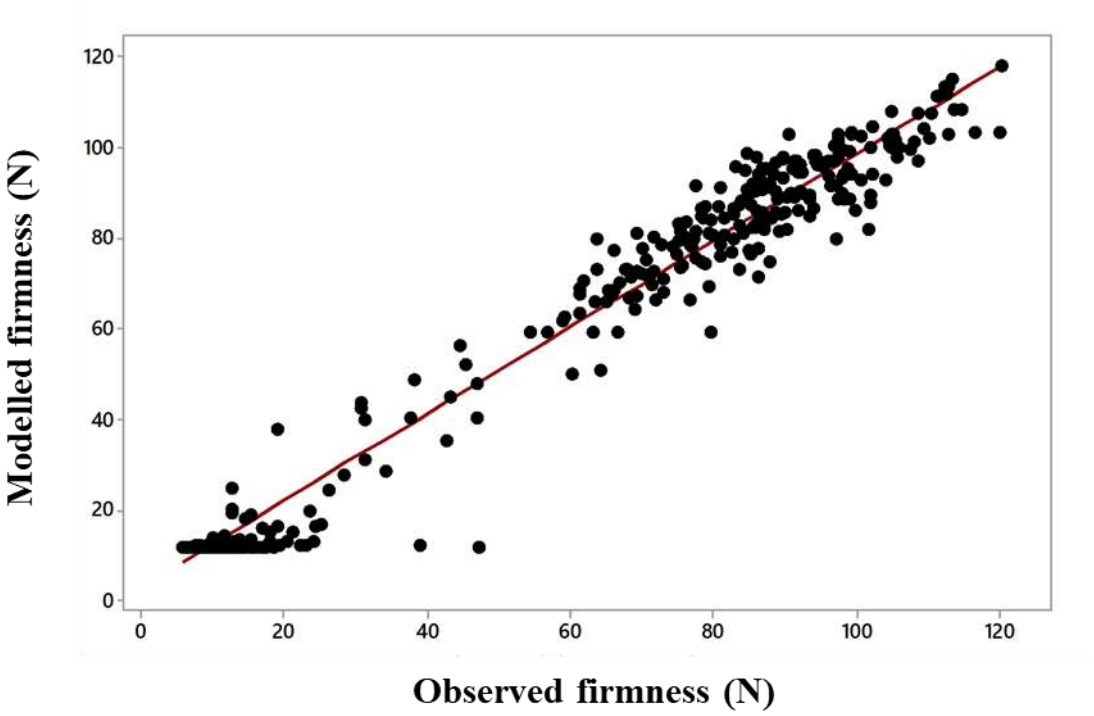


Figure 3

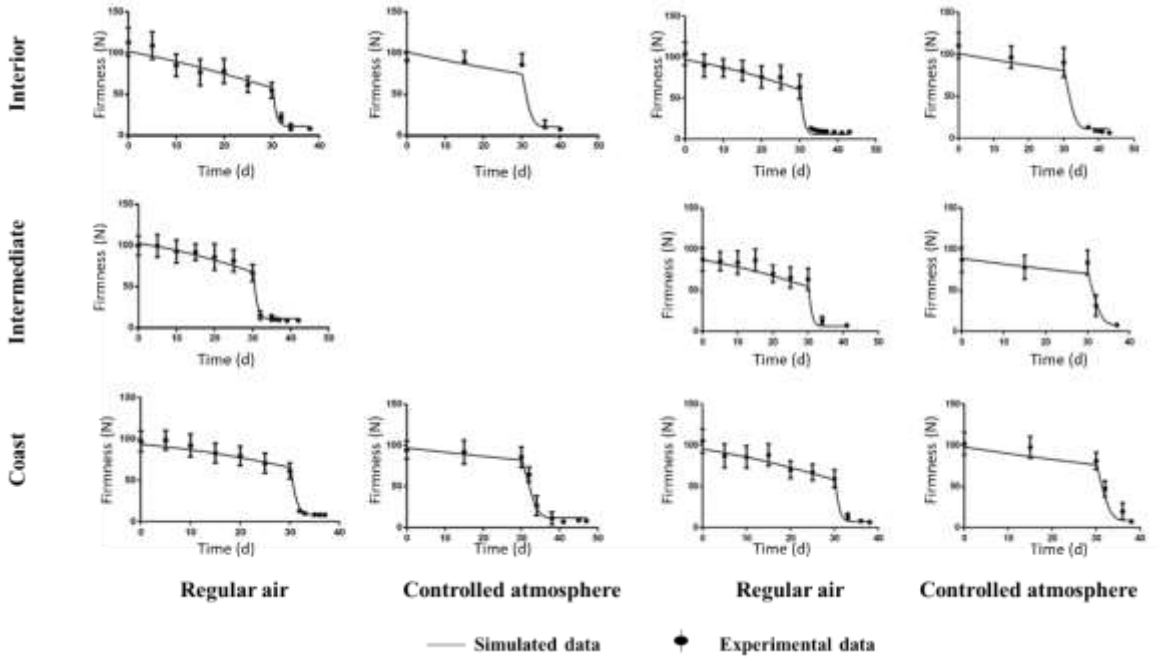

Interior
Intermediate
Coast
Firmness (N)
Time (d)
Regular air
Controlled atmosphere
Regular air
Controlled atmosphere
Simulated data
Experimental data

Figure 4

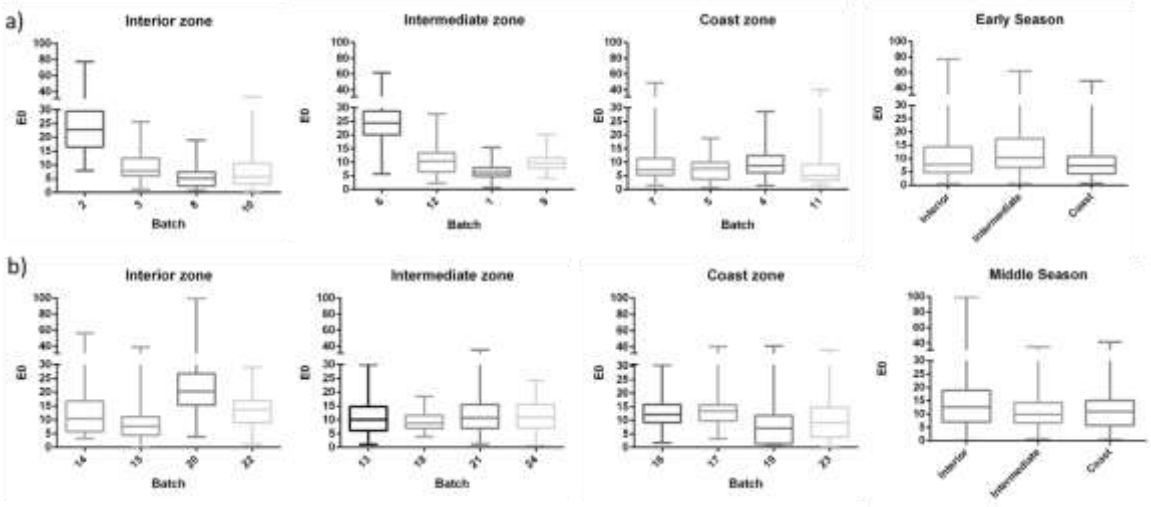

a)
Interior zone
Intermediate zone
Coast zone
Early Season
b)
Interior zone
Intermediate zone
Coast zone
Middle Season
E0
Batch

Figure 5

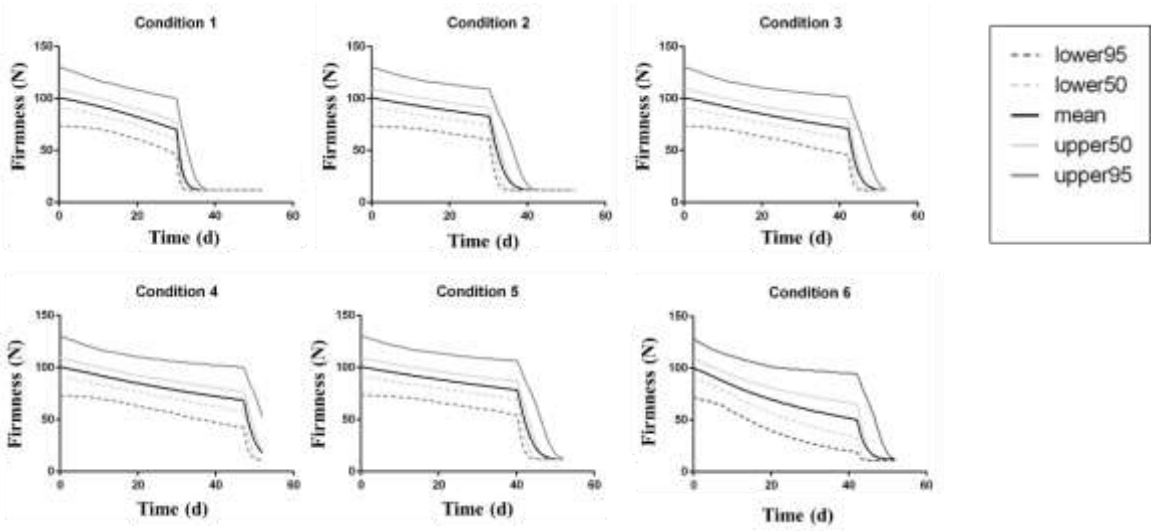
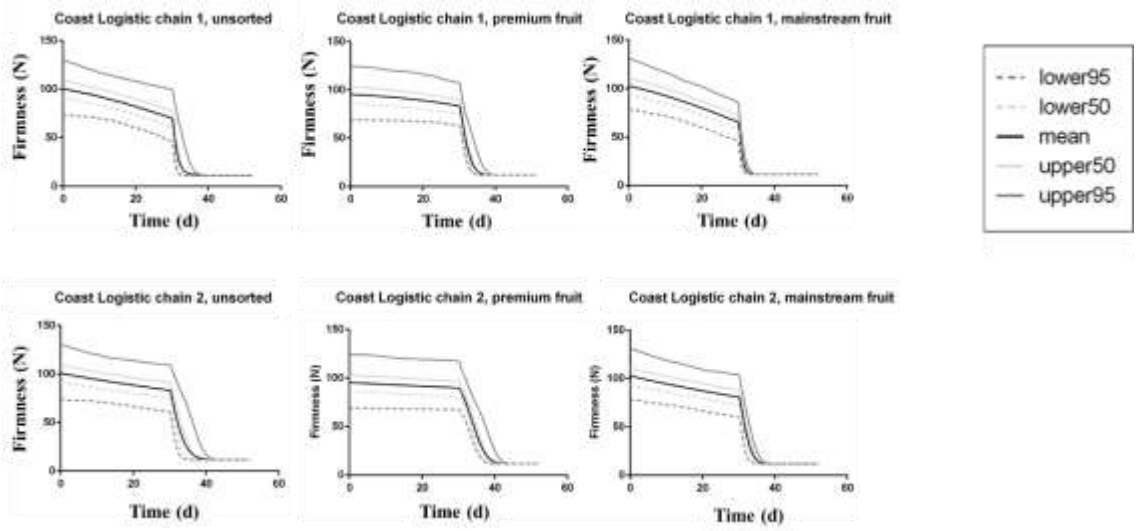

Condition 1
Condition 2
Condition 3
Condition 4
Condition 5
Condition 6
Firmness (N)
Time (d)
lower95
lower50
mean
upper50
upper95

Figure 6


Coast Logistic chain 1, unsorted
Coast Logistic chain 1, premium fruit
Coast Logistic chain 1, mainstream fruit
Coast Logistic chain 2, unsorted
Coast Logistic chain 2, premium fruit
Coast Logistic chain 2, mainstream fruit
Firmness (N)
Time (d)
lower95
lower50
mean
upper50
upper95

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