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Modelling the biochemical and sensory changes of strawberries during storage under diverse relative humidity conditions

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Abstract

Many studies stress the importance of keeping strawberries at high relative humidity conditions during postharvest storage. However, the effect of deviations occurring across the supply chain on the appearance, acceptability and biochemical properties of strawberries has not been adequately explored or quantified to date using kinetic modelling applications. This study investigated the effect of relative humidity (RH) on degradation kinetics of quality and biochemical properties of 'Strawberry Festival', during 7 days of storage at 2 °C, using zero, first-order and Weibull models. The strawberries were stored at 40, 60, 70, 80 or 90 % RH and were evaluated using subjective quality evaluation, weight loss monitoring and biochemical analysis. The shelf life was established based on current industry practices using subjective quality evaluation, namely shrivelling and colour scores. The Weibull model was found to better fit the experimental chemical analysis data compared to zero and first order kinetics models. The analysis of the rate constants quantified the significant effect of RH conditions on the weight loss and degradation rate of chemical components. Storage at low RH conditions accelerated the loss of ascorbic acid, and anthocyanins and negatively affect the in vitro antioxidant activity. The overall appearance of strawberries was modelled with zero-order kinetic model and the results revealed that lower RH conditions can limit the remaining shelf life of fresh strawberries by increasing the rate of appearance deterioration. Using RH and time as predictors in a logistic regression model, the waste occurring due to unacceptable strawberry quality, was predicted; highlighting the importance of using RH in predictive modelling when designing supply chains with the view to minimise losses.

1. Introduction

Fresh fruits and vegetables are among the most perishable food products. Due to their high perishability, they are also amongst the most frequently wasted foods (Kelly et al., 2018). Food waste has a devastating impact on food security affecting all people regardless of geography or social groupings. With a predicted increase of 1.7 billion in world population between now and 2050, mankind is placing more and more pressure on the shrinking finite resources used to produce our food (McCarthy et al., 2018). Yet it is estimated that globally 30–50% of all food produced never reaches its intended destination, i.e. the consumer. Under-addressed supply chain issues are a key cause of commercial food waste (White, 2007). Emphasis has been placed over the years on temperature management that often deviates from optimum during storage, handling and distribution, as one of the major causes of fresh fruit and vegetable overall quality deterioration (van Boekel, 2008, Taoukis et al., 1997; Nunes et al., 2003; Lai et al., 2011; .

Strawberry is a popular fruit around the world due its physical characteristics and its sweet flavour; but is also considered an important source of bioactive compounds such as vitamin C and diverse phenolic compounds such as phenolic acids, flavonoids and tannins (Cayo et al., 2016; Kelly et al., 2016b, Flores-Felix et al., 2018) for human nutrition. At the same time, strawberries are one of the fruits most often discarded throughout the supply chain, due to their short shelf life and high perishability and due to poor management during distribution (Kelly et al., 2018; Nunes et al., 2009, Pan et al., 2014). As the strawberry fruit is metabolically active after harvest, the rate at which its quality will deteriorate depends on both genetic factors and on pre- and post-harvest conditions (Castro et al., 2002). The recommended storage conditions for strawberries are 0 °C and 90-95 % Relative Humidity (RH). However, Nunes et al. (2009) found that during retail storage strawberries are kept at temperatures ranging from 5.8 °C to 7.1 °C and RH conditions ranging from 56 % to 93 %. This range does not consider the common practice of storing and displaying strawberries outside refrigerated compartments or displays at open markets where temperatures and RH conditions can deviate at a larger scale from the optimum conditions. Furthermore, Lai et al. (2011) studied real strawberry supply chain conditions and their effect on quality and found that RH varies from 33.8 % to 87.2 % along the different supply chain stages (harvest to retail). Many studies stress the importance of keeping strawberries at 90-95 % RH but the effect of the deviations occurring during the supply chain on the appearance, acceptability and nutritional value of strawberries has not been adequately explored to date. Shin et al. (2007) studied the effect of three RH conditions (75 %, 85 %, and 95 %) and the effect of temperature (0.5 °C, 10 °C, and 20 °C) on the quality and nutritional properties of strawberries and found no effect of RH

on these properties. In a later study, Shin et al. (2008) looked at a lower RH condition (65 %) and found that weight loss increased, whereas total flavonoids and total phenols decreased in fruit stored at 65 % compared to samples stored at 95 % RH. Both studies evaluated their nutritional analysis results in fresh weight basis, which could fail to consider the concentration effect due to water loss and hence obscure the effect of RH on these properties.

To better define the effect of storage conditions on deterioration of quality, the kinetics of sensory and chemical properties should be examined. Traditionally, degradation of nutrients and quality characteristics are modelled by 0, 1st or 2nd order kinetic models (Amodio et al., 2013; Oms-Oliu et al., 2009). Also studies have emerged that used the Weibull model, the cumulative form of the Weibull distribution function, to study several degradation reactions (Ong et al., 2011; Odriozola-Serrano et al., 2009; Oms-Oliu et al., 2009; Amodio et al., 2013). The Weibull model is flexible due to the inclusion of a shape constant in addition to the rate constant (Oms-Oliu et al., 2009). Odriozola-Serrano et al. (2009) studied the degradation kinetics anthocyanins, total phenols and ascorbic acid in fresh cut strawberries using the Weibull model and used it to explain the changes occurring in anthocyanins and antioxidant capacity of the fruits under storage.

The present study focuses on a broad range of RH conditions (40 % - 90 %) that can be encountered along the supply chain of strawberries. The main objective is to examine the effect of the different RH conditions during storage on the shelf life, sensory and nutritional quality of strawberries; emphasising on properly describing the changes occurring in both physical and chemical properties of the fruit. This paper aims: i) to examine the effect of RH conditions on the shelf life of strawberries including both the sensory and nutritional quality, ii) to study the kinetics of sensory and chemical changes occurring in strawberries during storage by comparing three kinetic models; and iii) to examine and predict through modelling the waste that would occur depending on the storage conditions.

2. Materials & Methods

2.1. Plant material and experimental setup

‘Strawberry Festival’ strawberries were harvested twice during 2012 and 2013 (March 2012 and February 2013) from a commercial field in Florida, USA. The samples were brought to the USF-Food Quality Laboratory in Tampa, Florida, USA, with minimal delay after harvest (Max delay of 30 minutes). The fruit were then sorted by colour, size, and absence of defects and packed into clamshells (capacity $\approx$ 0.453 kg). In total, 210 clamshells (containing 10 fruit each) were prepared

and distributed equally to five RH-controlled chambers (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) at 2 °C and 40, 60, 70, 80, and 90 % RH. Three clamshells were removed daily from each chamber and used for sensory quality evaluation and weigh loss monitoring. Three additional clamshells were used for chemical analysis. Temperature and RH were monitored throughout storage using battery-powered data loggers (Hobo® U10 Temp/RH data logger, Onset Computer Corporation, Pocasset, MA, USA).

One the day of harvest (day 0) three replicates of 10 fruit each, were used for sensory quality evaluation (30 fruits in total) and weight loss (3 clamshells of 10 fruits each). Three replicates of 10 fruit each were immediately frozen to preserve their biochemical properties and used later for chemical analysis.

2.2. Sensory quality evaluation

Sensory quality evaluation was performed every day during a 7-day storage period, always by the same trained personnel. Colour, shrivelling, decay severity, firmness, taste, and aroma were determined subjectively using 1–5 rating scales (where 1 = very poor, 3 = acceptable and 5 = excellent) (Nunes, 2015; Nunes and Delgado, 2014). A score of 3 was considered as the lower limit of acceptability for sale.

2.3. Weight loss

Weight loss was calculated from the initial weight of each of the three clamshells containing 15 fruits each, and every day during a seven-day storage period. The following formula was used for water loss corrections: $[\text{chemical components (fresh weight)} \times 100 \text{ g}/(\text{dry weight}) + \text{weight loss during storage (g)}]$. The dry weight was determined by drying three weighed aliquots of homogenized fruit tissue at 80 °C, until weight stabilized. All chemical components were expressed on a dry weight basis to compensate for water loss during storage.

2.4. Biochemical analysis

Three replicated samples of fifteen individual fruit per treatment were homogenized in a laboratory blender at high speed for 120 s and the resulting puree immediately frozen and kept at -30 °C until used. The samples were evaluated on days 0, 2, 3, 4, 5, 6, and 7. The following analyses were performed:

2.4.1. Total ascorbic acid

Total ascorbic acid (AA) analysis was conducted using a Hitachi LaChromUltra UHPLC system with a diode array detector and a LaChromUltra C18 2 μ m column (2 $\times$ 50 mm) (Hitachi, Ltd., Tokyo, Japan), as described in Kelly et al. (2016b). The retention time of AA peak was 0.35 min. After comparison of retention time with the AA standard, the peak was identified. The amount of total AA in strawberry was quantified using calibration curves obtained from different concentrations of AA standards. The total ascorbic acid content was expressed in g kg⁻¹ on a dry weight basis.

2.4.2. Total anthocyanin content

Total anthocyanins (ANC) were extracted in 0.5% (v/v) HCl methanol and measured using the procedure described by Nunes et al. (2005). Total anthocyanin content (ANC) is expressed as g kg⁻¹ pelargonidin 3-glucoside equivalents on dry weight basis.

2.4.3. Total phenolic content

In vitro antioxidant activity of the samples was determined using the total phenols assay by Folin–Ciocalteu reagent as described by Nunes et al. (2005). The total phenolic content (TPC) of the samples was measured and expressed as g kg⁻¹ gallic acid equivalents (GAE) on dry weight basis.

2.4.4. Sugar analysis

Quantification of sucrose, fructose and glucose was conducted using a Hitachi HPLC with a refractive index detector and a 300 mm $\times$ 8 mm Shodex SP0810 column (Shodex, Colorado Springs, CO) with an SP-G guard column (2 mm $\times$ 4 mm) as described by Kelly et al. (2016b). Total sugars were calculated as the sum of the individual sugars (sucrose and glucose) values and expressed as g kg⁻¹ on a dry weight basis.

2.4.5. Titratable acidity and soluble solids content

Titrateable acidity (TA) and soluble solids content (SSC) were determined according to (Nunes et al., 2006a) and expressed in g kg⁻¹ on a dry weight basis.

2.5. Mathematical Modelling

2.5.1. Kinetics modelling

Three kinetic models were evaluated to relate and explain the changes in appearance (colour and shrivelling) and the changes in chemical properties that occurred to the strawberries during storage under different RH. Zero (eq.1) and first order (eq.2) kinetics models have been applied to describe degradation reactions in foods and the Weibull model (eq.3) has been shown to fit the degradation of chemical compounds in strawberries (Odriozola-Serrano et al., 2009), sensory and ascorbic acid changes in melon (Amodio et al., 2013), and also colour changes in dried salak fruit (Ong et al., 2011).

$$Q(t) = Q_0 - kt \quad \text{eq.1}$$

$$Q(t) = Q_0 e^{-kt} \quad \text{eq.2}$$

$Q(t)$ is the value/concentration of the quality index at time t , Q_0 is the intercept of the curve, and k is the rate constant.

The cumulative distribution of the Weibull distribution function may be re-written as follows (Amodio et al., 2013):

$$Q(t) = Q_0 \left[1 - \exp\left(-\left(\frac{t}{a}\right)^\beta\right)\right] \quad \text{eq.3}$$

Where $Q(t)$ is the value/concentration of the quality index at time t , Q_0 is value/concentration of the quality index at initial time, and a and β are the scale factor and the shape parameter, respectively. The inverse of a ($1/a$) may be considered as the kinetic constant of the process (Corradini and Peleg, 2004). The β parameter indicates concavity or convexity of the curve when it takes values below and above 1 respectively (Odriozola-Serrano et al., 2009). The constants a and β were estimated and the initial value of the samples was used for Q_0 .

Statistical parameters such as root mean square error (RMSE), Akaike information criterion (AIC), and the regression coefficient (R^2) were the criteria for goodness of fit of the models. The models with the highest R^2 and lowest RMSE and AIC were chosen as the best fit for the data.

2.5.2 Classification models

The average of the shriveling and the colour scores, was used to evaluate the acceptability of the strawberries. A score of 3 was the lower limit of acceptability for sale. Samples were divided in two categories (Pass/Fail) depending on that score ($\text{score} > 3 = \text{Pass}$, $\text{score} \leq 3 = \text{Fail}$). Data from both harvests were merged and the following classification models were fitted to the experimental data: binary logistic regression model, decision tree, support vector machine, and k-nearest neighbours. The independent variables were Time (days) and RH (%). The models were evaluated

using the k-fold cross validation method. The model with the highest average accuracy and the lowest standard deviation of the accuracies between the folds was the binary logistic regression model. Therefore, it was considered the best fit for our data (see Results and Discussion section). The general formula for the binary logistic regression model can be written as in eq.4:

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 * RH + \beta_2 * Time \quad \text{eq.4}$$

Where p is the probability that the sample is a “Pass”, 1-p is the probability that a sample is a “Fail”, logit(p) is the odds ratio of the two probabilities, β are logistic coefficients, RH is the relative humidity, and Time is the days after harvest.

2.6. Statistical and Data Analysis

One-way ANOVA analysis was carried out using Minitab (Minitab version 16, Minitab Inc., USA). Where ANOVA indicated significant differences were present, the Tukey pairwise comparison of the means (and 95% confidence intervals) was used to identify where the sample differences occurred. Significant differences were identified between the two harvests and the results were treated separately. The kinetic models were fitted to the data using non-linear regression procedures in R version 3.4.4 (R Core Team, 2017). The following packages were used to perform the data analysis in R: Caret (Kuhn, 2018), e1071 (Meyer et. al, 2017), caTools (Tuszynski, 2014), ElemStatLearn (Halvorsen, 2015), ROCR (Sing et al., 2005), Metrics (Hammer & Frasco, 2017), writexl (Ooms, 2018), class (Venables, 2002), and rpart (Therneau & Atkinson, 2018).

3. Results and Discussion

3.1. Effect of RH and time on weight loss

Weight loss is an important parameter in determining the shelf life of fresh fruits and vegetables, because it is responsible not only for visual appearance deterioration (discolouration, shrivelling etc) but also for objectionable changes in texture, flavour and nutritional value (Nunes and Emond, 2007, Nunes et al., 1998). Robinson et al. (1975) reported previously that the maximum acceptable weight loss in strawberries is 6 %, before they become unacceptable for sale. A more recent study (Nunes and Emond, 2007) found that a loss of 2.5 %-3 % in weight of strawberries was the maximum acceptable loss when kept at 20 °C and occurred within 2-3 days. In our study, weight

loss increased with storage time and it was dependant on the RH conditions during storage. The total weight loss from harvest to day 7 of storage, varied from 7.2 % (90 % RH) to 19 % (40 % RH) in 2012; and from 5.7 % (90 % RH) to 15.5 % (40 % RH) in 2013 (Figure 1).

Both the zero-order model and the Weibull model fit the data. The Weibull model was chosen to interpret the changes in weight loss because of the highest coefficient of determination (R^2) and lower RMSE and AIC in both harvests. Table 1 shows the rate constants and the goodness of fit of the Weibull model for the weight loss data. Strawberries kept at lower RH conditions, were found to have higher rate constants; proving that weight loss occurred faster when fruit was kept at 40, 60 and 70 % RH compared to 80 and 90 %. Water vapor pressure deficit (VPD) will increase at any given temperature as RH of air decreases (Hosahalli, 2015). Exposure of fruits and vegetables to conditions that lead to an increase in the VPD, will increase the transpiration rate and can result in increased water loss (Laurin et al., 2005). In 2012, higher rates of water loss were observed than in 2013 and hence the total weight loss in 2012 was overall higher in all storage RH. The shape factor γ is less than 1 in all cases indicating that water loss is more intense during the first days of storage.

If we adopt the more conservative approach of 6 % being the maximum acceptable weight loss, it can be observed that shelf life of strawberries harvested in 2012 would be limited to 1.5, 2, 3, 4, and 5.5 days for fruit kept at 40, 60, 70, 80 and 90 % respectively (Figure 1). Keeping strawberries nearer optimum RH conditions (i.e., 80-90 %) can extend the shelf life by 1- 4 days compared to fruit kept to sub-optimum RH conditions (40-60 %).

3.2. Effect of RH and time on sensory quality

Colour became less acceptable (darker and less vibrant) with time and significant differences were observed between day 0 and day 7 at all RHs (results not shown). However, colour was not considered a limiting factor of strawberry shelf life (i.e., rating score was always higher than 3).

Shrivelling increased with time, and it was more intense for strawberries kept at 40 % and 60 % RH (Figure 2). In 2012, shrivelling was more pronounced on the day of harvest (average score of 4.5) than in 2013 (average score of 5). Shrivelling increased more rapidly in strawberries stored at 40 % RH for both harvests, limiting the shelf life to approximately 2.5 days in 2012 and to 4 days in 2013. Strawberries kept at 60, 70 and 80 % RH had a shelf life of approximately 5.5 days in 2012 based on shrivelling scores; whereas for fruit kept at 90 % RH, shrivelling was not a limiting shelf life factor. In 2013, shrivelling was not a limiting shelf life factor for strawberries

kept at 70, 80 and 90 % RH; whereas fruit kept at 60 % RH received a score below 3 just after day 6. The differences between harvests are due to the initial significant differences in shrivelling scores (2012 samples received a lower score on the day of harvest) and to the higher rate of weight loss observed during storage at all RHs in 2012 (Table 1). Nunes (2015) studied the correlation between subjective measurements (such as sensory quality evaluation) and physicochemical attributes, and found strong correlation between shrivelling and weight loss ($R^2=0.92$) for strawberries when kept at different RH regimes.

The average score between colour and shrivelling was used as the overall appearance score. To better understand the changes in the overall appearance, the three kinetic models were fitted to the data. All models fitted the data well (Table 2). In most cases the zero-order model had the highest coefficient of determination and the smallest AIC and it was chosen as the best fit in both harvest years. The rate constant shows that the rate of appearance degradation increased as the storage RH decreased. For example, in 2013, the rate of appearance score decrease, for samples kept at 40 % RH, is 0.44 per day, double the rate for samples kept at 90 % RH (0.22 /day). This can be linked to the higher rate of weight loss at lower RHs, which affects the discolouration and drying rates and hence the shrivelling and colour scores. One-way ANOVA analysis showed that the score of strawberries kept at 90 % RH was significantly higher on most days (and on day 7) for both harvests, than the scores of fruit kept at the lower RHs (40-80%). As a result, based on the acceptability scores, shelf life as being evaluated to date in the industry based on visual appearance will be significantly shorter when samples are kept at RH conditions lower than 90 %.

3.3. Biochemical Analysis

Significant differences were observed on the initial values of all the chemical compounds studied between the two harvests. Most of the differences can be attributed to the difference in temperature at the time of harvest observed in 2012 and 2013 (Table 3). To better understand the effect of RH during storage on chemical properties of strawberries, the three kinetic models mentioned earlier were applied. The Weibull model was the most suitable for fitting the changes in the chemical properties described below. The goodness of fit of the model was judged based on the coefficient of determination, RMSE and AIC. Table 4 shows the results of the goodness of fit for all the models applied to the sugar retention data. The Weibull model had the highest coefficient of determination, lowest RMSE and lowest AIC. The same was observed for all chemical properties

and hence the results for the AA, ANC, TPC, SSC and TA only show the goodness of fit parameters for the Weibull model.

3.3.1. Total Ascorbic Acid

Significant differences were observed between the initial concentrations of AA for the two harvests. The concentration of AA varied from 8.25 g kg⁻¹ in 2012 to 10.92 g kg⁻¹ in 2013. Concentration of AA is influenced by environmental factors such as temperature, light intensity, and maturity at harvest (Cayo et al., 2016). Wang and Camp (2000) found that strawberry cultivars grown at cooler temperatures had higher concentration of AA possibly due to decreased metabolism. The higher temperatures observed in 2012 (Table 3) could explain the lower content of ascorbic acid that year. In general, AA decreased with storage time for all RH conditions. Strawberries kept at higher RHs showed better retention of AA throughout storage. For example, the concentration of AA on day 7 for strawberries kept at 40 % RH was 1.68 g kg⁻¹ compared to 3.55 g kg⁻¹ for samples kept at 90 % RH in 2012.

The Weibull model was the most suitable model to describe the changes in AA retention during storage (Table 5). The degradation constant decreased as the RH increased, indicating the significant effect RH has on degradation kinetics of AA. In 2012, the degradation rate varied between 0.15 (80 % RH) to 0.66 (40 % RH) per day. These results show that the retention rate decreased 4 times faster for strawberries kept at 40 % RH compared to samples kept at 80 % RH. The γ parameter was below 1 indicating that AA degraded during the first days of storage at all RH conditions. Similar results were observed for strawberries harvested in 2013. Loss of AA in fresh fruit and vegetables during storage has been attributed to various causes, such as tissue degradation as the product becomes overripe, cell wall damage and enzymatic oxidation caused by bruising, but also due to water loss which can intensify AA oxidation (Nunes et al., 1998; Klein, 1987). Many other studies have also reported the loss of AA during storage of strawberries (Kelly et al., 2018; Cayo et al., 2016; Kelly et al., 2016a; Kelly et al., 2016b; Odrizola-Serrano et al., 2009). Our results show that the rate of AA degradation is linked to water loss and highlight the fast deterioration than can occur when fruit are stored in suboptimal RH conditions.

3.3.2. Total Anthocyanin Content

Strawberries harvested in 2012 had significantly higher ANC (4.04 g kg⁻¹) compared to strawberries harvested in 2013 (1.69 g kg⁻¹), possibly due to the higher temperatures in 2012 on

the week before harvest. Differences in ANC between years were reported by Cayo et al. (2016), also observing that higher temperatures around time of harvest led to higher accumulation of anthocyanins. Total anthocyanins content decreased with time with significant differences among the different RH conditions. At the end of storage (day 7), strawberries stored at 40 % RH had the lowest ANC both in 2012 (0.72 g kg^{-1}) and 2013 (0.48 g kg^{-1}). On the contrary, strawberries stored at 90 % RH had nearly double the concentration of ANC on day 7.

To better demonstrate the effect of RH on the retention of anthocyanins, the kinetics models were used on the retention data. The degradation rate constants obtained from the Weibull model, for the different RH conditions for both harvests are shown in Table 5. Consistent with the results of AA, the rate of ANC degradation was lower for samples kept at higher RH. In 2013, the rate of decrease was lower than in 2012 for most RHs, leading to the higher retentions observed. In 2012, the retention of ANC varied from 18.3 % (at 40 % RH) to 46.4 % (at 80 % RH), compared to 28.6 % (at 40 % RH) to 64.6 % (at 80 % RH), in 2013. The differences in the rate constants among the RH conditions and between the harvests can be explained by the highest rate of weight loss observed under the same conditions. Nunes et al. (2005) have reported a decrease in ANC in strawberries kept uncovered at 1°C and 90-95 % RH over storage. The authors explained how water loss in combination with oxidative mechanisms can contribute to degradation and changes in the anthocyanin pigments. Water loss is not the only reason for ANC degradation during storage (other factors such as ascorbic acid oxidation, pH changes, changes in sugar content and presence of other phenolics also contribute to loss of ANC (Kader et al., 1999; Holcroft and Kader, 1999); however as Nunes et al. (2005) mentions water loss can make the degradation more prominent. Despite the higher degradation rate observed in 2012 the concentration of ANC was still higher at the end of storage than the content measured in 2013, due the higher ANC in strawberries on the day of harvest.

3.3.4. Total phenol content

Significant differences were observed in TPC between the two harvests. The total phenolic content varied between 26.92 g kg^{-1} GAE, in 2012 to 23.72 g kg^{-1} GAE in 2013. The higher temperatures observed in 2012, could have assisted in the accumulation of compounds with antioxidant activity (i.e., anthocyanins) and hence contribute to the higher antioxidant activity of the samples as measured with the TPC assay. Also the strawberry plants from the two harvests were most likely exposed to different levels of stress, leading to different levels of bioactive compounds (i.e.,

polyphenols) to be present as a response to that stress (Kelly et al., 2016b). Total phenols content decreased with time of storage. Strawberries stored at 90 % RH had the highest TPC on all days of storage in 2013 whereas in 2012, strawberries stored at 80 % and 90%, both had the highest content. An increase in TPC was observed on days 2 and 3 in samples harvested in 2013 and stored at 90 % RH. According to Van de Velde et al. (2017), an increase in TPC at specific periods during storage can attributed to de novo synthesis as a result to stress. This has also been reported before (Oms-Oliu et al., 2009). However, such an increase in TPC on those days was not consistently observed in all strawberry samples.

The Weibull model was the best fit to describe the retention of total phenols data (Table 5). The degradation constants varied from 0.09 (in 2012) and 0.1 (in 2013) per day for samples stored at 90 % RH, to 0.30 (in 2012) and 0.26 (in 2013) per day for samples stored at 40 % RH (Table 5). The constants increased as the RH decreased, indicating that the degradation of bioactive compounds is faster at lower RH conditions, indicating a possible link to the faster rate of weight loss. Shin et al. (2008) found a decrease in TPC in strawberries after 12 days of storage at 10 °C, and observed a simultaneously increase in water loss and decrease in anthocyanins content. The loss of water leads to membrane collapse which allows for enzymes like PPO to come in contact with polyphenols and hence a reduction in TPC is observed (Nunes et al., 2005). This in combination with the degradation of anthocyanins will lead to the decrease in antioxidant activity measured with the TPC assay.

3.3.5. Total sugar content

Total sugar content was significantly higher in strawberries harvested in 2013 (1068 g kg⁻¹) than in 2012 (865 g kg⁻¹), most likely due to the lower temperatures at the time of harvest in 2013. Lower temperatures result in lower metabolic rate and higher accumulation of soluble carbohydrates and starch (Wang and Camp, 2000). Loss of sugars in strawberries postharvest has been reported elsewhere (Kelly et al., 2016b; Kelly et al., 2018) and the degradation occurs regardless of harvest or genotype effects (Cayo et al., 2016). Caleb et al. (2016) also observed a depletion of sucrose and glucose in strawberries during storage; highlighting that the role of sucrose, postharvest, is to sustain the energy demand during respiration and support fruit maintenance and metabolic processes. In our study, total sugar content decreased with storage time for all RH conditions, with significant differences in sugar content on day 7 among strawberries kept at 80 and 90 % RH and the lower RH conditions (Figure 4).

The Weibull model was the best fit for the retention of total sugars data, considering the high coefficient of determination (R^2), low RMSE and low AIC (Table 4). Based on the rate constants shown in Table 4, sugar content reduced faster for strawberries kept at 40 and 60 % RH in both harvests. Kader and Salveit (2003) stated that water stress caused by low storage RH conditions can stimulate the rate of respiration. The higher rate of weight loss experienced in these RH conditions, attributed to water loss, could potentially have increased the rate of respiration, consequently increasing the rate of simple sugars depletion. In both years the parameter γ was below 1 indicating that degradation takes place the first days of storage. Sweetness intensity has been linked to consumers' acceptance of strawberries (Schwieterman et al., 2014), hence a higher rate of decrease in sugar content during storage under low RH conditions, will become a limiting shelf life factor in strawberries at consumer level.

3.3.6. Titratable Acidity

Titrateable acidity (TA) was significantly higher in fruit harvested in 2012 (22.7 %) than in 2013 (16.9 %). These results cannot be attributed to the weather conditions during harvests, as it has been shown that higher temperatures during harvest (as observed in 2012) will lead to lower TA values (Wang and Camp, 2000). These differences could be due to possible differences in maturity at harvest or due to differences in the growing conditions between the two harvests. A reduction in TA during storage was observed for all RH conditions in both years. Conflicting results have been reported in the literature regarding TA during storage. A reduction in TA with time was observed by Kelly et al. (2016b) and Cayo et al. (2016), whereas an increase in TA was reported during storage by (Nunes et al. (2006a) and Caleb et al. (2016). Shin et al. (2007) found no effect of RH in TA of strawberries over a storage period of 4 days. The RH conditions in their study varied from 75 % to 95 %. Using the Weibull model to fit our data, it was found that higher rates of TA degradation were observed when strawberries were kept at lower RHs and varied between 0.08 to 0.23 per day (Table 6). The reduction in TA observed in our study is possibly due to the use of organic acids as substrates in respiration (Wang and Camp, 2000); and as mentioned earlier a relationship exists between the respiration rate and the weight loss rate.

3.3.6. Soluble Solids Content

Soluble solids content (SSC) is considered a good indicator of sweetness (Jouquand et al., 2008). The SSC in strawberries harvested in 2013 was significantly higher than that of strawberries

harvested in 2012 (1370 g kg^{-1} and 1300 g kg^{-1} respectively), possibly due to the variation in temperature at the time of harvest observed between the two years. A reduction in SSC during storage was observed for all RH conditions and the rate of reduction was modelled using the Weibull model. The rate of decrease in SSC increased as the storage RH decreased (Table 6). The same was observed for both harvests and varied between 0.12 and 0.51 per day depending on the harvest and the RH conditions. Higher rates of decrease were observed in 2012 for samples stored in 40 % and 60 % RH due to the higher rate of weight loss also taking place.

3.4. Classification model

The logistic regression model was the best fit to the experimental data when compared to other classification models as mentioned earlier. The results of the K-fold validation showed that the logistic regression had the highest mean accuracy when compared to other classification models (0.93 ± 0.02). Data from both harvests were merged and the dataset was split to a training set and a test set and these were used to train and validate the model respectively. The results of the logistic regression on the test set showed that both RH and time and their interaction had a significant effect on the score (Pass/Fail) of the strawberry samples. The confusion matrix produced by the model is shown in Table 7.

Table 7 shows that the model predicted 488 samples (out of 600) in the test set would pass and 112 samples would fail based on the acceptability criterion. The actual number of samples that passed or failed was 508 and 92 respectively., resulting in a 96.6 % accuracy in prediction. The accuracy calculated from the confusion matrix tables can sometimes be misleading due to what is known as the accuracy paradox (a model with higher accuracy has less predictive power than a model with lower accuracy). To better estimate the accuracy of the model the Receiving Operating Characteristic (ROC) curve was constructed (Figure 5) and the area under the curve was calculated. A ROC curve is commonly plotted to illustrate the diagnostic ability of a binary classifier. It shows the sensitivity (the proportion of correctly classified positive observations) and specificity (the proportion of correctly classified negative observations) as the output threshold is moved over the range of all possible values (Robin et al., 2011). A popular way of summarizing the discrimination ability of a model is to report the area under the ROC curve (AUC) (Prasad, 2016). The AUC value for the model was 0.97, showing that the logistic regression using the RH and time after harvest as predictors, has a strong discriminating power between strawberry samples that would score above or below the acceptability threshold.

4. Conclusions

Strawberries are metabolically active during postharvest storage, and their quality and shelf life is influenced among other things by the storage conditions they experience. Temperature and RH are environmental factors with major impact on strawberries postharvest quality. However, the RH effect on strawberries sensory and nutritional properties has not been explored sufficiently. This study emphasised on studying the kinetics of the physicochemical changes occurring during storage under RH conditions experienced by strawberries under simulated commercial situations. The results provided a comprehensive description and explanation of these changes, information which is useful when designing supply chain aiming at minimising food losses. One of the most significant impacts of RH on strawberry quality during storage, was on weight loss. Weight loss significantly increased when the storage RH decreased. In turn, the weight loss was correlated with the changes that occurred in visual appearance and chemical properties. Commercially, strawberry quality and shelf life are determined by visual inspection. Overall appearance (i.e., average score of colour and shrivelling), was modelled with a zero-order kinetics model for the various RH conditions. It was found that the lower RH increased the rate of appearance deterioration and hence limited the remaining shelf life of strawberries. The Weibull model was the best fit for the data related to the chemical properties and it was found to be an important tool in explaining the changes occurring and the effect of the various RH conditions during storage. Our results showed that improper RH storage conditions have a significant impact on the rate of degradation of chemical components and affects negatively nutritional and biochemical properties such as retention of AA, TPC, and ANC. In addition, sugar content, a major quality attribute in strawberries that determines acceptability by the consumer, was negatively affected by lower RH storage conditions. Overall, the Weibull model showed the rate of deterioration was the highest when strawberries were kept at 40 or 60 % RH.

Furthermore, significant differences in quality were observed between harvests. Higher contents of ANC and TPC were found in strawberries from the 2012 harvest possibly due to the higher temperatures occurring around the time of harvest. On the contrary, the higher temperatures in 2012, may have affected negatively the sugar content and the ascorbic acid contents. Arising from the Weibull model the degradation rate of many chemical properties was different between the two harvests, following the same pattern as the rate of weight loss for these harvests. Pre-harvest conditions can affect postharvest quality and shelf life. This study showed the significant interaction between pre-harvest and postharvest conditions on the overall concentration of

nutritional properties over storage. Higher initial concentrations due to favourable conditions, resulted in higher concentrations after 7 days of storage even when the degradation rate was higher compared to a harvest with lower initial concentrations. Similarly, the study showed that higher RH storage ($RH \geq 80\%$) can extend the shelf life and aid in the retention of overall quality even in strawberries with lower initial levels of AA, TPC, ANC and sugars due to pre-harvest conditions.

Finally, using the logistic regression classification method, our results showed that RH and time after harvest have a strong predictive power in forecasting the number of samples that would be unacceptable based on the visual appearance. This highlighted the importance of RH in determining the shelf life of fresh strawberries and therefore it be considered when predictive modelling is used for designing the supply chain with the view to minimising unnecessary losses.

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Figure Captions

654 Figure 1 Effect of storage under various RH conditions on the weight loss (%) of fresh strawberries
655 harvested (a) in 2012 and (b) in 2013. Error bars represent the standard deviation of the mean, plotted
656 lines correspond to the values estimated by the Weibull model

657 Figure 2 Changes observed in shrivelling of fresh strawberries during storage under various RH
658 conditions (a-2012 harvest, b-2013 harvest). Error bars represent the standard deviation of the mean
659 score. Dashed straight line represents the minimum acceptable quality before strawberries become
660 unmarketable (rating 3).

661 Figure 3 Effect of storage under various RH conditions on the AA retention (%) of fresh strawberries
662 harvested in 2012. Error bars represent the standard deviation of the mean, plotted lines correspond to
663 the values estimated by the Weibull model.

664 Figure 4 Effect of storage under various RH conditions on the total sugar retention (%) of fresh
665 strawberries harvested (a) in 2012 and (b) in 2013. Error bars represent the standard deviation of the
666 mean, plotted lines correspond to the values estimated by the Weibull model

667 Figure 5 Receiver operating characteristic (ROC) curve of logistic regression model predicting “ Pass-Fail”
668 score of fresh strawberries kept under various RH conditions. The area under the ROC curve, is 0.97
669 (p=0).

670

Table 1 Kinetic constants and goodness of fit parameters of zero-order, 1st-order and Weibull models of weight loss (%) in fresh strawberries kept at various RH conditions

Harvest	Model	RH	Rate constants (per day)	R ²	RMSE	AIC
2012	M0	40	-2.66	0.98	0.83	65
	M0	60	-2.54	0.99	0.59	49
	M0	70	-1.83	0.99	0.46	37
	M0	80	-1.40	0.97	0.58	48
	M0	90	-1.03	0.99	0.19	-4
	M1	40	-0.23	0.90	1.99	107
	M1	60	-0.24	0.91	1.84	103
	M1	70	-0.25	0.91	1.28	86
	M1	80	-0.24	0.88	1.14	80
	M1	90	-0.26	0.93	0.67	55
	MW	40	0.03, $\gamma=-0.37$	0.99	0.74	60
	MW	60	0.03, $\gamma=-0.38$	0.99	0.49	40
	MW	70	0.02, $\gamma=-0.35$	0.99	0.47	38
	MW	80	0.01, $\gamma=-0.32$	0.97	0.55	45
	MW	90	0.01, $\gamma=-0.30$	0.99	0.22	2
2013	M0	40	-2.15	0.99	0.5	41
	M0	60	-1.80	0.99	0.4	35
	M0	70	-1.34	0.99	0.3	17
	M0	80	-1.29	0.99	0.2	4
	M0	90	-0.87	0.94	0.5	43
	M1	40	-0.24	0.91	1.5	93
	M1	60	-0.24	0.91	1.3	85
	M1	70	-0.25	0.92	0.9	68
	M1	80	-0.25	0.92	0.9	67
	M1	90	-0.25	0.85	0.8	64
	MW	40	0.022, $\gamma=-0.35$	0.99	0.5	41
	MW	60	0.015, $\gamma=-0.33$	0.99	0.4	34
	MW	70	0.009, $\gamma=-0.31$	0.99	0.4	26
	MW	80	0.008, $\gamma=-0.31$	0.99	0.2	6
	MW	90	0.004, $\gamma=-0.28$	0.94	0.5	41

M0 = Zero-order (eq. 1), M1 = 1st-order (eq. 2), MW = Weibull model (eq. 3)

Table 2 Kinetic constants and goodness of fit parameters of zero-order, 1st-order and Weibull models of the overall appearance of fresh strawberries kept at various RH conditions

Harvest	Model	RH	Rate constants (per day)	R ²	RMSE	AIC
2012	M0	40	0.39	0.94	0.23	-17
	M0	60	0.24	0.89	0.19	-109
	M0	70	0.23	0.80	0.27	52
	M0	80	0.26	0.78	0.31	129
	M0	90	0.21	0.88	0.18	-137
	M1	40	0.14	0.94	0.22	-30
	M1	60	0.07	0.91	0.17	-168
	M1	70	0.06	0.80	0.27	52
	M1	80	0.07	0.77	0.32	133
	M1	90	0.05	0.88	0.18	-145
	MW	40	0.16, $\gamma=0.82$	0.92	0.34	162
	MW	60	0.06, $\gamma=0.58$	0.90	0.23	-15
	MW	70	0.06, $\gamma=0.67$	0.76	0.34	168
	MW	80	0.07, $\gamma=0.80$	0.75	0.35	184
	MW	90	0.05, $\gamma=0.78$	0.86	0.21	-65
	M0	40	0.44	0.93	0.27	58
	M0	60	0.31	0.85	0.30	114
	M0	70	0.26	0.83	0.28	70
	M0	80	0.23	0.83	0.24	-3
	M0	90	0.22	0.78	0.26	44
2013	M1	40	0.13	0.91	0.31	130
	M1	60	0.08	0.86	0.30	105
	M1	70	0.07	0.81	0.29	89
	M1	80	0.06	0.83	0.24	3
	M1	90	0.05	0.78	0.26	49
	MW	40	0.14, $\gamma=1.26$	0.93	0.28	82
	MW	60	0.09, $\gamma=0.97$	0.85	0.30	107
	MW	70	0.07, $\gamma=0.99$	0.81	0.30	102
	MW	80	0.05, $\gamma=0.77$	0.81	0.27	56
	MW	90	0.05, $\gamma=0.91$	0.77	0.28	72

M0 = Zero-order (eq. 1), M1 = 1st-order (eq. 2), MW = Weibull model (eq. 3)

Table 3 Daily mean temperatures for the week of harvest during 2012 and 2013 periods in Floral City, Florida (data from weather underground <https://www.wunderground.com>)

Harvest	Temperature °C		
	Mean	Max	Min
March 2012	20.5	28.8	13.8
February 2013	12.1	21.6	6.8

Table 4 Kinetic constants and goodness of fit parameters of zero-order, 1st-order and Weibull models of sugar retention in fresh strawberries kept at various RH conditions

Harvest	Model	RH	Rate constants (per day)	R ₂	RMSE	AIC
2012	M0	40	9.70	0.69	14.55	178
	M0	60	9.99	0.72	13.90	176
	M0	70	10.75	0.86	9.58	160
	M0	80	8.14	0.81	8.71	156
	M0	90	8.62	0.76	10.83	166
	M1	40	0.31	0.92	7.89	152
	M1	60	0.32	0.94	6.88	147
	M1	70	0.26	0.96	4.93	133
	M1	80	0.16	0.91	6.20	142
	M1	90	0.19	0.88	7.73	151
	MW	40	0.50, $\gamma=0.37$	1.00	1.04	67
	MW	60	0.44, $\gamma=0.45$	1.00	0.97	64
	MW	70	0.28, $\gamma=0.72$	0.98	3.59	119
	MW	80	0.14, $\gamma=0.51$	0.98	3.04	112
	MW	90	0.20, $\gamma=0.41$	0.98	3.15	114
2013	M0	40	9.94	0.84	9.69	161
	M0	60	8.77	0.62	15.48	181
	M0	70	8.79	0.72	12.15	171
	M0	80	8.29	0.84	8.1	154
	M0	90	7.97	0.88	6.59	145
	M1	40	0.23	0.96	5.10	134
	M1	60	0.27	0.84	10.38	164
	M1	70	0.22	0.88	8.08	153
	M1	80	0.16	0.92	5.8	140
	M1	90	0.14	0.94	4.52	129
	MW	40	0.25, $\gamma=0.6$	0.99	2.38	102
	MW	60	0.45, $\gamma=0.3$	0.97	4.57	129
	MW	70	0.24, $\gamma=0.4$	0.98	3.21	115
	MW	80	0.14, $\gamma=0.5$	0.97	3.2	114
	MW	90	0.12, $\gamma=0.7$	0.97	3.3	115

M0 = Zero-order (eq. 1), M1 = 1st-order (eq. 2), MW = Weibull model (eq. 3)

Table 5 Kinetic constants and goodness of fit parameters of the Weibull model of ascorbic acid, anthocyanins, and total phenol retention in fresh strawberries kept at various RH conditions

Retention %	RH	2012				2013			
		Rate constants (per day)	R ²	RMSE	AIC	Rate constants (per day)	R ²	RMSE	AIC
Ascorbic acid	40	0.66, $\gamma=0.31$	0.98	3.48	172.92	0.66, $\gamma=0.31$	0.92	7.93	173
	60	0.34, $\gamma=0.42$	0.98	3.69	193.08	0.57, $\gamma=0.20$	0.82	11.53	168
	70	0.17, $\gamma=0.48$	0.95	5.95	177.06	0.17, $\gamma=0.64$	0.78	11.26	167
	80	0.15, $\gamma=0.61$	0.96	3.88	122.56	0.24, $\gamma=0.47$	0.77	12.76	173
	90	0.13, $\gamma=1.02$	0.42	17.95	112.23	0.14, $\gamma=0.66$	0.55	17.91	187
Anthocyanins	40	0.73, $\gamma=0.37$	0.99	3.27	115.35	0.17, $\gamma=0.95$	0.96	4.22	126
	60	0.80, $\gamma=0.34$	0.99	3.14	113.63	0.10, $\gamma=0.81$	0.93	4.70	131
	70	0.14, $\gamma=0.48$	0.81	9.57	160.48	0.09, $\gamma=0.85$	0.84	6.74	146
	80	0.12, $\gamma=0.91$	0.85	7.62	150.90	0.02, $\gamma=0.33$	0.83	5.67	138
	90	0.003, $\gamma=0.12$	0.67	10.94	166.06	0.10, $\gamma=1.66$	0.72	8.48	155
Total Phenols	40	0.30, $\gamma=0.56$	0.97	4.34	127.29	0.26, $\gamma=0.52$	0.99	1.79	90
	60	0.33, $\gamma=0.40$	0.99	2.84	109.44	0.20, $\gamma=0.56$	0.99	1.64	86
	70	0.26, $\gamma=0.51$	1.00	1.61	85.69	0.10, $\gamma=1.31$	0.84	6.71	146
	80	0.12, $\gamma=0.75$	0.91	5.94	140.40	0.07, $\gamma=1.42$	0.71	6.81	146
	90	0.09, $\gamma=0.76$	0.89	5.58	137.83	0.11, $\gamma=4.45$	0.60	12.52	172

Table 6 Kinetic constants and goodness of fit parameters of the Weibull model of SSC and TA contents in fresh strawberries kept at various RH conditions

Composition	RH	2012				2013			
		Rate constants (per day)	R^2	RMSE	AIC	Rate constants (per day)	R^2	RMSE	AIC
SSC	40	0.51, $\gamma=0.39$	1.00	3.01	111.92	0.21, $\gamma=0.42$	0.98	3.97	124
	60	0.46, $\gamma=0.45$	1.00	2.89	110.23	0.18, $\gamma=0.64$	0.99	3.37	117
	70	0.26, $\gamma=0.54$	0.99	3.50	118.18	0.15, $\gamma=0.64$	0.98	4.24	126
	80	0.18, $\gamma=0.47$	0.96	6.11	141.61	0.13, $\gamma=0.63$	0.98	4.03	124
	90	0.14, $\gamma=0.54$	0.99	3.89	122.60	0.12, $\gamma=0.95$	0.93	6.73	146
TA (%)	40	0.32, $\gamma=0.44$	0.99	2.20	98.73	0.23, $\gamma=0.44$	0.99	0.35	21
	60	0.25, $\gamma=0.58$	0.99	2.20	98.64	0.17, $\gamma=0.55$	0.99	0.36	23
	70	0.19, $\gamma=0.80$	0.97	2.21	98.88	0.12, $\gamma=0.58$	0.98	0.45	32
	80	0.15, $\gamma=0.81$	0.95	2.28	100.19	0.11, $\gamma=0.56$	0.93	0.87	60
	90	0.11, $\gamma=1.05$	0.92	2.20	98.68	0.08, $\gamma=0.76$	0.93	0.71	51

Table 7 Confusion matrix of the logistic regression model

Harvests 2012 & 2013				
Actual	Predicted			Total
		Fail	Pass	
—	Fail	80	12	92
	Pass	32	476	508
Total		112	488	600