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Research Paper

Optimisation of O₂ and CO₂ concentrations to retain quality and prolong shelf life of 'shelly' mango fruit using a simplex lattice mixture design



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Keywords: Controlled atmosphere fruit quality special cubic model pigments The experimental design and gas mixture selection is of great importance in the definition of optimal gas concentrations for use in storage of fresh produce. The aim of this study was to optimise O_2 and CO_2 concentrations under controlled atmosphere conditions to understand the effect on quality and shelf life of 'Shelly' mango fruit stored at 13 $^\circ$ C for 28 d. This was achieved by designing three experimental points (gas compositions $= O_2$, CO_2 and N_2 using simplex lattice mixture design to (i) determine single and interaction effects of gas compositions on selected quality parameters and (ii) determine the optimal gas combination in order to maintain quality and prolonging shelf life of 'Shelly' mango fruit. The estimated model parameters coefficients successfully categorised the single and interaction effects of O2, CO2 and N2 gas compositions. The selected quality attributes experimental data was fitted well using the canonical Scheffe type special cubic model, resulting in coefficient of Determination, $R^2 = 0.70$ to 0.97. The low O₂ and high CO₂ in CA-2 managed to retard ripening and mass loss, and reduce fruit softening and chlorophyll degradation. Positive relationship was observed for linear effect in all quality attributes, while binary and ternary interaction effects varied across all the treatments. The optimal gas compositions for storage of 'Shelly' mango fruit in terms of selected quality attributes ranged between 5 and 8% O_2 + 5–9% CO_2 + 86–91% N_2 . The results highlight the potential use of simplex lattice mixture design to optimise CA storage conditions.

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commercialisation and distribution of mango fruit in non-

producing countries is limited due to the perishable nature of the fruit, compromising quality and flavour. This occurs

mainly because mango fruit is produced in tropical and sub-

tropical regions, mostly in the developing parts of the world,

with poor and limited postharvest infrastructures (Yahia &

1. Introduction

Mango fruit is increasingly becoming important in both producing and non-producing countries (Lalel, Singh, & Tan, 2005), due to its nutritional properties, excellent flavour and <u>colour (Sivakumar, Jiang, & Yahia, 2011)</u>. However,

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Singh, 2009). In the past decades, extensive research has been carried out to optimise storage conditions and the outcome has varied significantly with cultivars and maturity stages (Ntsoane, Luca, Zude-Sasse, Sivakumar, & Mahajan, 2019; Ntsoane, Zude-Sasse, Mahajan & Sivakumar, 2019). Commercially, modified atmosphere (MA) and controlled atmosphere (CA) are used during sea shipping of mango fruit from countries such as Australia and Mexico (Yahia & Singh, 2009). In previous studies, CA storage has shown the potential to reduce respiration and ripening rates, to inhibit ethylene production and to maintain the nutritional and sensory quality of tropical fruits (Gonzalez-Aguilar, Villa-Rodriguez, Ayala-Zavala, & Yahia, 2010). However, there is a lack of information available on potential impact of CA/MA on 'Shelly' mango produced in South Africa and its commercial use; meanwhile it is becoming popular for export. Atmospheres with low O₂ and/or high CO₂ levels in CA storage have been evaluated, and it is clear that 'Tommy Atkins' mangoes can tolerate extreme atmospheres (\leq 2% O₂ and/or \geq 25% CO₂) for up to 3 weeks at 12 °C (Bender, Brecht, Baldwin, & Malundo, 2000). However, slight variation in gas composition from optimal conditions such as extremely low O2 or high CO2 result in a shift from aerobic to anaerobic respiration, which contributes to reducing shelf-life, increasing susceptibility to decay, producing off-flavours, and leading to physiological disorders (Yahia & Singh, 2009). In order to overcome this, it is important to select optimal gas mixtures based on the effect of various gases on quality attribute responses.

The storage atmospheres of $3-5\% O_2 + 5-10\% CO_2$ are recommended to maximise shelf life of stored mango fruit by delaying ripening (Brecht & Yahia, 2009). Reduction of O₂ and increase in CO₂ is reported to significantly increase polyphenolics and antioxidant capacity (Kim, Brecht, & Talcott, 2007), maintaining titratable acidity and preserving fruit firmness. Storage conditions of 2% O_2 + 3–9% CO₂ have managed to enhance the nutritional quality of 'Kensington Pride' mango after 35 days storage by increasing β -carotene, and this was attributed to its de novo synthesis that occurs under elevated CO₂ storage conditions (Lalel, Singh, & Tan, 2002). In a recent study, storage of 'Shelly' mango fruit in low oxygen storage conditions of 5-10% delayed fruit ripening process and reduced development of O2 restricted volatile compounds (Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019). However, the impact of CO₂ on quality attributes in 'Shelly' mango fruit was not considered in this study. Simplex lattice mixture design is one of the most suitable experimental designs, considered to minimise the costs of determining and analysing optimal storage conditions (Belay, Caleb, Mahajan, Fröhling and Opara, 2019a; Belay, Caleb, Mahajan, Fröhling and Opara, 2019b; Belay, Caleb, Mahajan, & Opara, 2017; Mahajan, Luca, & Edelenbos, 2014). However, no study has been conducted using the same design to establish the potential effect of CA storage conditions (O2, CO2 and N2) for mango fruits and there is little information on the CO2 effect on 'Shelly' mango fruit quality and shelf life. This approach could enable better understanding of individual gas components and/or their interaction effect on selected quality attributes by using simple interactive model coefficients and ternary graphs.

2. Materials and methods

2.1. Plant material and handling

Freshly harvested 'Shelly' mango fruit (Mangifera indica) were imported from Westfalia Marketing (Pty) Ltd, Hoedspruit, Limpopo, South Africa at commercial maturity stage. This is a new cultivar introduced to South Africa over the years from Israel and it was considered for the study because is gaining popularity in the European market. The fruits were produced at Bavaria Fruit Estate, Hoedspruit, Limpopo Province, South Africa (latitude 24°22'45.5"S, longitude 30°52'56.1"E). Immediately after harvest, the fruits were washed and treated with hot water (50 °C) for a period 2 min (as standard treatment for postharvest disease control). Thereafter, fruits were cooled and waxed with Endura (commercial treatment), (Endura-fresh®, John Bean Technologies, Foodtech, Brackenfell, South Africa). The fruits were air freighted to Berlin, Germany in well-ventilated corrugated cardboard cartons $(25 \times 40 \times 10 \text{ cm})$, each containing 7–8 fruits. The fruits were freshly delivered 5 d after harvest to the Leibniz institute for Agricultural Engineering and Bioeconomy (ATB), Department of Horticultural Engineering in Potsdam, Germany. The fruits were then transferred to a walk-in cold room and precooled to 13 °C for 3 h to reach the optimal study temperature.

2.2. Storage conditions and experimental design

Immediately after precooling, the fruits were transferred to seven metal chambers (190 L) connected to a controlled gas system and maintained at 13 °C (Frigotechnik, Germany). The metal chambers were flushed with different gas mixtures of O2, CO2 and N2. A controlled storage system was used to create and maintain the modified gas mixtures in each metal chamber. The total concentration was kept and maintained at 100% by flushing each metal chamber with a humidified gas mixture until equilibrium was reached, according to Table 1. The automatic and computerised system was used to monitor different gas mixtures of O2, CO2 and N2, temperature and relative humidity. Additionally, to monitor the gas compositions in storage chambers daily, gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark) was used. On each sampling day (0, 7, 14, 21 and 28), the chambers were opened and 6 samples from each treatment were taken out for visual quality evaluation, physiochemical and biochemical quality

Table 1 – Layout of the simplex mixture lattice experimental design for different gas components.						
Gas mixtures	Uncoded variables (%)	Pseudocomponents				

				-			
	O ₂	CO ₂	N ₂	O ₂	CO_2	N ₂	
CA-1	18	2	80	1	0	0	
CA-2	2	18	80	0	1	0	
CA-3	2	2	96	0	0	1	
CA-4	4.67	12.67	82.67	0.167	0.667	0.167	
CA-5	4.67	4.67	90.67	0.167	0.167	0.667	
CA-6	12.67	4.67	82.67	0.667	0.667	0.167	
CA-7	7.33	7.33	85.33	0.333	0.333	0.333	

analyses. The simplex lattice mixture design (SLMD) was selected to evaluate the effect of different gas mixtures, thus: oxygen (X_1) , carbon dioxide (X_2) and nitrogen (X_3) with the aim to optimise controlled atmosphere storage conditions based on selected quality attributes (mass loss, firmness, ascorbic acid, soluble solids content (SSC), titratable acidity (TA), Chroma colour value, pigment content (normalised differential vegetation index [NDVI] and normalised anthocyanin index [NAI], visual colour, texture and decay). The number of experimental runs and the proportions of the three factors (gas compositions), {3, 3} was determined using SLMD described in terms of coded (pseudo components) and uncoded variables (Table 1). The SLMD resulted in seven gas mixtures as independent components in the mixture design in the range of O₂ (2-18%), CO₂ (2-18%) and N₂ (80-96%) as previously reported by Belay et al., (2017, 2019a, 2019b).

2.3. Physiochemical properties of the fruit

The mass loss of the fruit was recorded on every sampling day and the difference between initial and final mass was calculated to define mass loss percentage (%). This was achieved by using an electronic balance CPA10035 (Sartorius, Göttingen, Germany). Fruit colour changes were measured nondestructively at two points along the equatorial region of the fruit. Fruit colour was measured and evaluated on the basis of the CIE colour system L*, a*, b*, Chroma (C) and hue (h°) angle using a digital chromameter (CM-2600d, Konica Minolta Sensing Inc., Tokyo, Japan). Calibration of the equipment was done using white and black tile, prior to taking measurements (Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019). Pigment content was evaluated using pigment indices such as normalised differential vegetation index (NDVI) and normalised anthocyanin index (NAI). The changes were measured using non-destructive hand-held photodiode array spectrophotometer device (Pigment Analyser 1101, CP, Germany) (Zude, 2003; Zude, Birlouez-Aragon, Paschold, & Rutledge, 2007; Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019). The fruit firmness was measured from a peeled flesh at the same points used for colour measurements using a texture analyser (TA-XT Plus, Stable Micro Systems, Surrey, UK) with an 8 mm diameter flathead Magness-Taylor cylindrical probe (puncture test). The results were expressed as the maximum force (N) from the mean of two measurements per fruit. To measure ethylene production rate, individual fruits were weighed and transferred to 1 L metal jars and stored for 1 h. The headspace gas was withdrawn from the metal jars with 5 ml syringe to measure ethylene production rate using ETD-300 over a period of 6 h at 2 h intervals. Ethylene production rate was calculated as μ L kg⁻¹ h⁻¹ according to Pathak et al., (2017).

2.4. Biochemical analyses

The fresh mango juice was used to determine ascorbic acid content according to the method described by Hassenberg, Geyer, Ammon, & Herppich, 2011; Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019 using a Reflectoquant Ascorbic Acid Test Strips at a range of 25–450 mg (Merck, Darmstadt, Germany). Briefly, quick test strips were dipped in the fruit juice and then inserted into the measuring device and the value expressed as mg L⁻¹. The soluble solids content (SSC) of mango fruit was measured using a hand refractometer (DR301-95, Krüss Optronic, Hamburg, Germany) and expressed as % Brix (Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019). The TA of mango fruit was measured by titrating with 0.1 mol L⁻¹ NaOH to an end-point of pH 8.2, using an automated T50 M Titrator with a Rondo 20 sample changer (Mettler Toledo, Switzerland). The TA value was expressed as g L⁻¹ of citric acid on the basis of fresh mass. The relation of SSC toTA of mango fruit was calculated and expressed as SSC/TA ratio (Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019).

2.5. Evaluation of visual quality attributes

On each of the sampling days prior to further analysis, the following visual quality attributes were evaluated: visual colour, texture and decay. This was done by six panellists who are regular consumers and familiar with mango fruit quality attributes. The initial quality of the mangoes was taken into consideration when evaluating six fruits from each storage conditions, on each sampling day. Briefly, the quality attributes were scored on the scale of 1–5 according to Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019. The score of 1 corresponds to poor acceptable quality and (<10%) yellow surface colour, score of 3 refers to moderately acceptable quality and (50–60%) yellow surface colour, and 5 was highly acceptable quality and complete (100%) yellow surface colour. The average score of less than 3 was considered as an indicator for unacceptable quality and marketability rejection.

2.6. Mathematical modelling

The results obtained from the measured quality parameters were fitted to a linear model (Eq. (1)), to investigate the potential effect of single gas components (O_2 , CO_2 and N_2) (Mahajan et al., 2014; Belay et al., 2017, 2019a, 2019b). Furthermore, the canonical Scheffe type special cubical model with linear, binary and ternary terms illustrated in Eq. (2) was used as a function of different gas components to fit measured selected quality attributes (variable response):

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$
 (1)

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3$$
(2)

where Y represents the measured variables response, $\beta_{1,2,3}$ represent the response at pure mixture compositions (O₂, CO₂, N₂ respectively) with β_{12} , β_{13} , β_{23} and β_{123} being the blending components, X variables represent: X₁ = O₂, X₂ = CO₂ and X₃ = N₂ as pseudocomponents (Belay, et al., 2019a, 2019b). The constants of both models were estimated by fitting Eq. (1) or (2) to the experimental data by nonlinear regression. The pseudocomponent variables in Table 1 were used for model parameter fitting and for ternary contour plots. The effect of CA storage on quality attributes was characterised by either positive or negative effect defined by coefficients of the model parameter estimates. Furthermore, the optimal gas composition was determined using the values obtained from the polynomial equation over the simplex region.

2.7. Statistical analysis

The statistical analysis included the design of 7 experimental runs from a simplex lattice mixture design (Mahajan et al., 2014). The statistical package design expert version STATISTICA (version 10, DOE StatSoft Inc. Tulsa, USA) was used to analyse the variance, fit the model parameters and to perform ternary contour plots. To optimise ideal gas composition for retaining quality attributes and maximising shelf life, beta values, set constraints and solver in Excel (Microsoft office, Version: 2010) was used to reduce or increase the evaluated quality attributes of fruits from each treatment. Statistical analyses were performed using the freely available chemometric statistical software package 'R and RStudio' version 1.1.442. The data was analysed by means of one way analysis of variance (ANOVA) with a 95% confidence interval to evaluate the effect of gas compositions on the quality attributes of mango fruit. The significance among the mean treatment values were determined by the least significant difference (LSD) and Tukey post hoc tests at P < 0.05 level. Six replicates were used for each treatment.

3. Results and discussion

3.1. Ethylene production

The ethylene production in this study ranged between 0.04 and $1.06 \ \mu L \ kg^{-1} \ h^{-1}$ at day 0 and 28, respectively as shown in Fig. 1. The ethylene production rate increased with storage period in all the storage conditions and the increase is due to the autocatalytic nature of ethylene during ripening (Zerbini et al., 2015). Differences in ethylene production were also observed among storage conditions. The low O₂ and high CO₂ concentrations in CA-2 (2% O₂ + 18% CO₂ + 80% N₂) was responsible for delaying ethylene biosynthesis by slowing down the autocatalytic response triggered by ethylene produced by the fruit itself and this affected the chlorophyll



Fig. 1 – Ethylene production rate of 'Shelly' mango fruit stored at 13 °C for 28 d. Error bars indicates the standard deviation from mean values (n = 3). The horizontal dashed line indicates the initial ethylene production rate.

breakdown and biosynthesis of carotenoids (de Almeida Teixeira., 2018; Zerbini et al., 2015).

3.2. Mass loss and firmness

Mango fruit are subject to an increase in substrate loss as a result of high respiration rate when stored under high O2 storage conditions and this can lead to significant mass loss (Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019). The results showed that mass loss of mango fruit from initial fruit mass of 615.93 ± 27.98 g was influenced by storage conditions (O₂ and CO₂) and varied significantly among different storage conditions. Increased O2 storage concentrations exhibited the highest mass loss, CA-1 (4.46%) followed by CA-6 (3.66%), CA-7 (3.11%) and CA-5 (3.02%) respectively and decreased towards the highest CO₂ atmospheres, CA-2 (2.35%) as shown in Table 2 and ternary contour plots (Fig. 2a). As a result, CO₂ had a significant effect on retaining mass loss of 'Shelly' mango fruit. Linear (β 1, β 2 and β 3) coefficient of the model parameter estimates resulted with positive and significant (p < 0.05) effects on mass loss in the following order (O2, N2, CO2), binary and ternary interaction effects were insignificant. Similarly, previous studies have reported positive effect of high CO2 atmospheric storage conditions (3% $O_2 + 5\% CO_2$) in restricting mass loss of 'Nam Dok Mai' mango fruit to 3%, possibly due to the reduced transpiration and respiration rates when stored for a period of 28 d at 13 °C (Kramchote, Jirapong, & Wongs-Aree, 2008). The model parameter estimates of mass loss resulted in a good coefficient of determination of 0.97 (Table 4).

The most important quality attribute to consider for optimising fruit storage conditions is fruit firmness, also known as textural softening, which is associated with fresh produce shelf life and consumer acceptability (Tharanathan, Yashoda, & Prabha, 2006). It is clear from our results that the initial fruit firmness of 139.33 \pm 38.32 N significantly and drastically decreased during 28 d of CA storage at 13 °C and varied from 20.09 \pm 5.60 to 61.17 \pm 18.33 N across the storage conditions (Table 2). The decrease in fruit firmness could be attributed to cell wall degradation, action of enzymes promoting softness such as polygalacturonase, galactosidases, pectin methylesterase and β-1,4-glucanases (Ramayya, Niranjan, & Duncan, 2012). Decreasing firmness in (CA-1) is attributed to high respiration rate in fruits stored under high O₂ concentration, which is linked to hastening of senescence. Meanwhile, high CO₂ concentration (CA-2) had an impact in delaying fruit softening (Fig. 2b). According to these results, high CO2 and low O₂ had a positive effect on retaining fruit firmness, while O₂ hardly had any impact on fruit firmness among the gas components. These findings fall in line with those reported by Ullah, Ahmad & Thompson (2010). 'Palmer' and 'Shelly' mango fruit stored in storage conditions containing < O₂ remained firmer, resulting in less starch utilisation, which is linked to soluble solids content (SSC), and reduced pectin solubilisation contributing to cell wall integrity (Henrique, Eixeira, & Urigan, 2011; Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019). The single component coefficient values were significantly equal to measured quality attributes data in comparison to interaction effects coefficient values which were either underestimated or overestimated in

Table 2 $-$ Effect of modified atmosphere storage on quality attributes of Shelly mango stored for 28 d at 13 $^\circ$ C.								
	Mass loss (%)	Firmness (N)	Ascorbic acid (mg/L)	SSC (%)	TA (g/L)	SSC/TA ratio (%)		
CA-1	4.46 ± 0.33^{a}	20.09 ± 5.60 ^c	208.33 ± 29.69^{a}	18.13 ± 0.57^{a}	1.21 ± 0.33^{a}	15.88 ± 4.40^{a}		
CA-2	2.35 ± 0.13^{e}	61.17 ± 18.33 ^a	216.67 ± 59.94^{a}	14.33 ± 0.87^{d}	1.64 ± 0.22^{a}	8.86 ± 1.33^{b}		
CA-3	2.58 ± 0.33^{de}	45.52 ± 16.11^{abc}	174.33 ± 33.00^{a}	16.55 ± 1.15^{b}	$1.58 \pm 0.37^{\rm a}$	10.99 ± 2.70^{ab}		
CA-4	2.79 ± 0.13^{cd}	49.83 ± 13.37^{ab}	207.33 ± 62.66^{a}	16.80 ± 0.45^{ab}	1.52 ± 0.52^{a}	12.35 ± 4.71^{ab}		
CA-5	$3.02 \pm 0.28^{\circ}$	44.87 ± 9.65^{abc}	157.83 ± 48.42^{a}	14.88 ± 0.39^{cd}	1.59 ± 0.32^{a}	9.72 ± 2.21^{ab}		
CA-6	3.66 ± 0.20^{b}	40.10 ± 27.29^{abc}	236.50 ± 62.89^{a}	$16.58 \pm 0.78^{\rm b}$	1.43 ± 0.32^{a}	11.98 ± 2.22^{ab}		
CA-7	3.11 ± 0.12^{c}	31.15 ± 15.37 ^{bc}	192.83 ± 62.53^{a}	16.17 ± 1.26^{bc}	1.25 ± 0.44^{a}	14.75 ± 6.53^{ab}		

Mean value \pm standard deviations in the same columns with different superscript lower case letters are significantly different (p < 0.05).



Fig. 2 – Ternary contour plots for the effect of gas components on mass loss (a), firmness (b) and ascorbic acid (c) of 'Shelly' mango fruit stored for 28 d at 13 °C.

comparison to the measured average data (Table 4). However, the coefficient of determination for firmness as good, $R^2 = 0.87$. The level of CO_2 was the most significant factor resulting in reduced mass loss and maximum firmness, and therefore CA-2 (2% $O_2 + 18\% CO_2 + 80\% N_2$) is suggested as the recommended optimal gas composition for maximising shelf life of 'Shelly' mango fruit with regards to fruit firmness.

3.3. Soluble solids content and titratable acidity

The soluble solids content (SSC) ranged from 14.33 \pm 0.87 to 18.13 ± 0.57 (Table 2) at the end of postharvest storage among storage conditions compared to initial values of 10.69 ± 1.90 . It was slightly higher towards the highest O₂ (18%) atmospheres (CA-1), while it was the least (14.33 ± 0.87) in CA-2 (Fig. 3a). The delay in SSC accumulation could be attributed to low respiratory activity due to reduced O2 and elevated CO2 in CA-2 (2% $O_2 + 18\% CO_2 + 80\% N_2$), this is because of delayed breakdown of several organic substances such as starch into glucose. The delay could be attributed to reduction in respiration rate that could have happened under reduced O2 and elevated CO2 storage conditions. Similar results have been reported for mango fruits stored under low O2 concentrations (Henrique et al., 2011; Ntsoane, Luca, et al., 2019; Ntsoane, Zude-Sasse, et al., 2019). The single components significantly (p < 0.05) influenced SSC in the following order (O₂, N₂, CO₂), as observed in values of model parameter estimates shown in Table 4. These results show that optimisation of a single gas component is vital to maintain the SSC of mango fruit, which is one of the primary taste parameters for some markets and for consumers indicating ripeness of the fruit (Sivakumar et al., 2011).

The additional indicator of fruit ripening is titratable acidity (TA) concentration, which ranged from 1.21 \pm 0.33 to 1.64 ± 0.22 (Table 2) compared to initial values of 3.42 ± 0.10 , and was reduced under high O_2 (18%) atmosphere (CA-1). In mango fruit, acidity decreases with ripening and organic acids such as citric and malic substrates are used up during storage as a result of respiration (Kim et al., 2007). This is considered to have led to an overall decline in titratable acidity across all the storage conditions with no significant difference. However, TA concentration was slightly higher under storage conditions where CO₂ was high as shown in Fig. 3b. The linear and binary interaction has shown positive effects on the coefficients of TA concentration. Meanwhile, an inverse relationship was observed for ternary (β_{123}) interaction resulting with negative effect between the concentration of gases and TA concentration. Similarly, SSC/TA ratio was significantly (p < 0.05) higher in increased O₂ and reduced CO₂ storage conditions (CA-1). This is due to high accumulation of SSC and reduction in titratable acidity which indicates a sweet taste, which is a desirable retail quality parameter perceived by consumers. Based on retained SSC and reduced titratable acidity, CA-1 (18% $O_2 + 2\% CO_2 + 80\% N_2$) is suggested as the optimal gas composition for 'Shelly' mango fruit.

3.4. Ascorbic acid

The ascorbic acid concentration ranged from 157.83 ± 48.42 to $236.50 \pm 62.89 \text{ mg L}^{-1}$ from initial values of 175.67 ± 52.31 . The ascorbic acid content was high in fruits stored under CA-6 (13% O₂ + 5% CO₂ + 82% N₂) and lowest in CA-2 (5% O₂ + 5%



Fig. 3 — Ternary contour plots for the effect of gas components on soluble sugars (a), titratable acidity (b) and chroma (c) of 'Shelly' mango fruit stored for 28 d at 13 ℃.

 CO_2 + 90% N₂). Although no significant difference was observed, the retention of ascorbic acid could be linked to stability of TA in 'Shelly' mango fruit as no significant difference was observed in TA concentration either (Sivakumar, Van Deventer, Terry, Polenta, & Korsten, 2012). According to the ternary contour plot (Fig. 2c), the ascorbic acid content was retained by reduction in gas compositions (O₂ and CO₂) concentrations. Degradation of ascorbic acid in fruits is greatly dependent on gas concentration. The reduction of O₂ storage to concentrations lower than 4% is reported to reduce ascorbic acid content as a result of avoiding oxidation. However, controversial results have been reported regarding the effect of CO₂ concentration as this varies according to commodity, CO₂ concentration, storage duration and storage temperature (Kader, 2009). Linear (β 1, β 2 and β 3) coefficient of the model parameter estimates resulted in significant effects on ascorbic acid content.

3.5. Colour and pigments

Colour is regarded as the most important indicator of fruit flavour, edibility, shelf life and nutritional value, since it is related to the physiochemical and sensory attributes of fruits and vegetables. Colour influences consumer perception more than any other quality attribute (Nagle et al., 2016). In this study, the initial surface colour parameters such as L (41.20 ± 2.75) , a^{*} (15.84 ± 4.41) , b^{*} (15.00 ± 4.83) , C (22.52 ± 3.13) and h° (43.09 \pm 11.57) were noted and slight changes were observed during CA storage. At the end of the postharvest storage period (28 d), surface colour parameters (L, a*, b* and h°) showed no significant differences. The chroma value (C) was somewhat affected by gas compositions during storage, ranging from 35.63 to 45.33. This indicated the ripening stages of the mangoes and CA-2 ($2\% O_2 + 18\% CO_2 + 80\% N_2$) resulted in the lowest chroma value despite the hue angle having the same values as for the other storage conditions. This suggests that the increased CO_2 concentration in CA-2 (2% O_2 + 18% $CO_2 + 80\% N_2$) negatively influenced accumulation of carotenoid content in the fruit (Fig. 3c). Similar to these results, Kim et al., (2007) observed that a high CO2 concentration (3% $O_2 + 10\% CO_2 + 87\% N_2$) led to reduced chroma values of ripe 'Tommy Atkins' mango. The model parameter values showed a significant effect of linear (β_1 , β_2 and β_3) and binary (β_{12} and

 β_{23}) on chroma. The negative effect was observed in binary (β_{13}) and ternary (β_{123}) interaction of the gases, indicating a negative relationship between the concentration of CA gases and chroma colour value.

The non-destructive indices such as NDVI and NAI have potential to provide adequate information in terms of fruit pigment content (chlorophylls, carotenoids and anthocyanins). The NDVI was significantly (p < 0.05) higher in CA-2 (2% O_2 + 18% CO_2 + 80% N_2) from initial values of (0.98 ± 0.01) due to a high CO₂ concentration and it decreased during storage across all storage conditions, especially with low CO₂ (Table 3). The CO₂ concentration had an influence in delaying ripening of the mangoes stored at 13 °C for 28 d (Fig. 4a). Kader (2009) reported an influence of high CO₂ concentration on delaying ripening, which resulted in a delay in biosynthesis of carotenoids, particularly β -carotene in mangoes. Furthermore, the ripening process was retarded due to delayed oxidative metabolism, which is influenced by reduced O₂ and elevated CO₂. According to these results, NDVI is linked with the chlorophyll content of the fruits (Pathak et al., 2017). Significant effects of individual gases on NDVI, and inverse relationships for binary and ternary interaction were observed with model parameter estimates shown in Table 4. Meanwhile, the NAI index ranged from 0.91 to 0.96 across all CA treatments compared to initial values of (0.97 \pm 0.00) and was well maintained at the highest O₂ concentrations in CA-1 (18% O_2 + 2% CO_2 + 80% N_2). This could be due to the colour change of the mango skin and edible flesh during fruit ripening which is mostly attributed to accumulation of carotenoid content. Additionally, anthocyanin synthesis is also reported to decrease with decrease in O2 and increase in CO2 concentration in storage of fruits (Brandenburg & Zagory, 2009). In previous studies of 'Golden Delicious' apples, NAI has shown great potential to determine the ripening stage, based on ethylene evolution. Being nondestructive, NAI index could be a good tool to be adopted in the fruit industry storage technologies such as regular, CA, and ULO conditions and 1-MCP treatment to classify fruits (Rutkowski, Michalczuk, & Konopacki, 2008). Based on the ternary contour plot (Fig. 4b), high O₂ concentration managed to retain NAI index and the coefficient of determination was $R^2 = 0.69$. Linear (β_1 , β_2 and β_3) and ternary (β_{123}) interactions were positive and, on the contrary, negative effect was

conditions for 28 d at 13 °C.								
		Р	Pigments	Pigments indexes				
	L	a*	b*	Chroma	Hue angle	NDVI	NAI	
CA-1	51.27 ± 4.20^{a}	24.01 ± 7.24^{a}	32.22 ± 6.15^{a}	41.68 ± 4.32^{abc}	52.66 ± 11.35 ^a	$0.60 \pm 0.17^{\rm b}$	0.96 ± 0.01^{a}	
CA-2	45.04 ± 4.28^{a}	23.34 ± 6.15^{a}	23.49 ± 6.51^{a}	35.63 ± 3.71 ^c	44.88 ± 14.38^{a}	0.86 ± 0.11^{a}	0.93 ± 0.2^{ab}	
CA-3	51.52 ± 6.92^{a}	23.76 ± 6.71^{a}	32.01 ± 7.41^{a}	43.14 ± 2.31^{ab}	52.87 ± 14.02^{a}	0.53 ± 0.09^{b}	0.93 ± 0.02^{ab}	
CA-4	43.93 ± 3.06^{a}	28.41 ± 3.63^{a}	22.18 ± 3.45^{a}	37.82 ± 2.20^{bc}	37.66 ± 6.73^{a}	0.70 ± 0.12^{ab}	0.92 ± 0.23^{ab}	
CA-5	52.21 ± 4.57^{a}	26.64 ± 7.70^{a}	32.42 ± 5.34^{a}	43.91 ± 3.00^{ab}	50.58 ± 12.18^{a}	0.61 ± 0.15^{b}	$0.91\pm0.04^{\rm b}$	
CA-6	51.69 ± 8.62^{a}	27.70 ± 3.53^{a}	31.67 ± 10.06^{a}	43.74 ± 6.39 ^{ab}	46.91 ± 12.86^{a}	0.51 ± 0.11^{b}	0.93 ± 0.04^{ab}	
CA-7	51.53 ± 4.61^{a}	28.77 ± 5.83^{a}	32.46 ± 6.94^{a}	45.33 ± 2.04^{a}	47.48 ± 9.68^{a}	0.55 ± 0.15^{b}	$0.92\pm0.03^{\rm ab}$	
Mean value + standard deviation in the same columns with different superscript lower case letters are significantly different ($p < 0.05$).								

Table 3 – Fruit surface colour parameters (L*, a*, b*, C*, h°) and pigments of 'Shelly' stored in different controlled storage conditions for 28 d at 13 $^{\circ}$ C.

observed for binary interaction. The ternary interaction effects were positive but the model underestimated the NAI, resulting in lower values (0.58) than the measured average data.

3.6. Visual quality analysis

Visual quality attributes such as appearance (colour), texture and decay are often used to evaluate the quality of fruits and vegetables. The CA storage conditions managed to maintain these quality attributes with an average of >3 for visual colour, >3 for texture and >4 for decay. A special cubic model fitted visual colour, texture, decay and overall acceptance experimental data well with coefficients of determination $R^2 = 0.90$, 0.81, 0.96 and 0.76, respectively. Linear (β_1 , β_2 and β_3) and binary (β_{12} , β_{23} and β_{13}) coefficient of the model parameter estimates indicated significant positive effects on quality attributes. However, binary (β_{23}) interaction of decay and overall acceptance resulted in negative effects. Meanwhile, the ternary interaction effects for all the quality attributes were negative, except for visual appearance (colour). This showed that the effect of linear (β_1 , β_2 and β_3), binary (β_{13} and β_{23}) and ternary (β_{123}) interaction on visual colour of mango fruit was positively predicted with coefficients of determination $R^2 = 0.90$, using a special cubic model in this study (Table 4).

Acceptable visual colour development was observed in high O_2 and low CO_2 storage conditions, as shown in Fig. 4c. This implies that high CO_2 in CA-2 (2% O_2 + 18% CO_2 + 80% N_2) had an impact on delaying chlorophyll degradation and yellow colour development. A similar trend has been reported for NDVI in this study (Fig. 4a). The ternary contour plot (Fig. 4e) has shown that fruits stored in CA-1 (18% O_2 + 2% CO_2 + 80% N_2) were more susceptible to decay and this resulted in a reduction in the texture due to softness of the fruit, attributed to spoilage caused by microorganisms that mainly occur



Fig. 4 – Ternary contour plots for the effect of gas components on NDVI (a), NAI (b), visual colour (c), texture (d) and decay (e) of 'Shelly' mango fruit stored for 28 d at 13 °C.

Table 4 – Estimated model parameter coefficients with standard error obtained in the linear (β_1 , β_2 and $\overline{\beta_3}$), binary and ternary effects (β_{12} , β_{13} , β_{23} and β_{123}) describing the effect of gas combinations on selected quality attributes of 'Shelly' mango fruit stored for 28 d at 13 °C.

Quality	Model parameter coefficients								
parameters	β1	β2	β_3	β ₁₂	β ₁₃	β ₂₃	β ₁₂₃		
Mass loss	4.46 ± 0.12	2.35 ± 0.12	2.58 ± 0.12	-1.19 ± 1.79	0.20 ± 1.79	2.37 ± 1.79	-4.88 ± 12.62	0.97	
Firmness	38.76 ± 9.36	39.50 ± 9.36	45.52 ± 9.36	-8.87 ± 137.60	95.38 ± 137.60	107.71 ± 137.60	-855.66 ± 986.09	0.87	
Ascorbic acid	208.33 ± 20.35	216.67 ± 20.35	174.33 ± 20.35	411.00 ± 299.03	71.00 ± 299.03	-329.00 ± 299.03	-646.50 ± 2103.83	0.59	
SSC	16.80 ± 0.42	16.17 ± 0.42	16.55 ± 0.42	9.23 ± 6.13	-8.47 ± 6.13	-2.07 ± 6.13	-5.25 ± 43.12	0.51	
ТА	1.21 ± 0.17	1.64 ± 0.17	1.58 ± 0.17	1.48 ± 2.53	2.75 ± 2.53	1.19 ± 2.53	-22.40 ± 17.82	0.46	
NDVI	0.60 ± 0.07	0.86 ± 0.07	0.53 ± 0.07	-0.81 ± 0.99	0.07 ± 0.99	0.71 ± 0.99	-2.99 ± 6.96	0.73	
NAI	0.96 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	-0.13 ± 0.16	-0.18 ± 0.16	-0.10 ± 0.16	0.58 ± 1.09	0.69	
Chroma	62.37 ± 2.55	59.84 ± 2.55	57.26 ± 2.55	32.21 ± 37.45	-46.16 ± 37.45	39.21 ± 37.45	-102.93 ± 263.47	0.51	
Visual colour	4.08 ± 0.25	2.00 ± 0.25	3.92 ± 0.25	2.83 ± 3.67	1.33 ± 3.67	0.83 ± 3.67	3.00 ± 25.85	0.90	
Texture	3.00 ± 0.24	4.50 ± 0.24	3.67 ± 0.24	3.00 ± 3.49	4.00 ± 3.49	2.00 ± 3.49	-15.00 ± 24.59	0.81	
Decay	4.17 ± 0.06	5.00 ± 0.06	5.00 ± 0.06	3.33 ± 0.93	3.33 ± 0.93	-1.67 ± 0.93	-7.50 ± 6.51	0.96	
Overall	3.33 ± 0.18	3.50 ± 0.18	3.91 ± 0.18	5.83 ± 2.70	5.33 ± 2.70	-4.67 ± 2.70	-8.25 ± 18.99	0.76	
acceptance									
Subscript 1, 2 and 3 represents oxygen, carbon dioxide and nitrogen respectively. + Standard error, SSC - Soluble solids contents, TA - Titratable									

acidity, NDVI - Normalized difference vegetation index, NAI - Normalized anthocyanin index.

under high O₂ storage. The effects of gas modification were observed for decay and resulted in good fit ($R^2 = 0.96$) between the simplex lattice experimental data and the predicted values obtained from special cubic model (Table 4). Carbon dioxide (CO₂) concentrations of more than 10% have shown the potential to suppress decay development (Brandenburg & Zagory, 2009). However, as much as the CO_2 has the ability to supress product spoilage and maintain fruit firmness, extreme CO₂ concentration in storage could result in detrimental damage such as uneven ripening, skin discolouration or greyish flesh colouration or fruit softening and off-flavour development, due to the formation of acetaldehyde and ethanol (Sivakumar et al., 2011). According to the ternary contour plot (Fig. 4d), CO₂ had a greater impact on retaining texture of the fruit. Based on the parameter estimates of the gas modification effects, the simplex lattice experimental data and the predicted values obtained from special cubic model resulted in good fit ($R^2 = 0.81$) for fruit texture. Linear and binary effects of fruit texture were positive (Table 4). The overall acceptance of the fruit depends greatly on the maximum overall score of the visual quality attributes and in this study was obtained at storage conditions containing gas compositions of 5–8% O_2 + 5–10% CO_2 + 84–87% N_2 .

4. Optimal O₂ and CO₂ concentrations based on quality attributes

Optimisation of postharvest storage conditions, particularly O_2 and CO_2 in CA storage, still remains one of the major challenges. The potential benefits of CA conditions vary among commodity, cultivar and maturity. Optimisation of O_2 and CO_2 concentrations to increase shelf life of 'shelly' mango fruit based on selected quality attributes resulted in three main optimal regions for controlled atmosphere, as shown in Fig. 5. The maximum storage life of 'Shelly' mango fruit was achieved at gas compositions of: $5-8\% O_2 + 5-9\% CO_2 + 86-91\%$ N2 according to various quality attributes. These storage conditions retained quality of 'Shelly' mango with respect to titratable acidity (1.21 g L^{-1}), ascorbic acid content (236.50 mg L^{-1}) and visual appearance (4.63). Furthermore, an increase in CO_2 concentration and reduction in O_2 concentration resulted in minimum mass loss (2.35%) and fruit texture (4.45). Meanwhile, SSC was retained in fruits stored under increased O2 concentration (18%) and reduced CO2 concentration (2%). According to the results, optimal gas composition that was ideal for retaining quality attributes of 'Shelly' mango varied with different storage conditions. The identified optimal gas compositions are closely in line with previously recommended gas mixtures of 5-10% CO₂ and 3-5% O₂ gas compositions for mango fruit (Kader, 1994). Meanwhile, the optimal storage conditions for 'Kensington' mangoes stored at 13 °C for 33 d was reported to be 4% CO₂ + 2–4% O₂ (McLauchlan & Barker, 1994). Optimal storage



Fig. 5 – Estimated optimum gas mixture for selected individual quality parameters of 'Shelly' mango fruit stored for 28 d at 13 $^{\circ}$ C.

conditions of 6% $CO_2 + 3\% O_2$ prolonged shelf-life of 'Delta R2E2' mangoes based on normal fruit ripening, good taste, high SSC and SSC/TA ratio for a period of 38 d at 13 °C (Lalel et al., 2005).

5. Conclusion

Simplex lattice mixture design was very useful to optimise the CA storage conditions for 'Shelly' mango fruit. Optimisation of CA storage based on selected quality attributes has the potential to minimise fruit ripening, fruit softening, maintaining quality and prolonging shelf life. The relationships between quality attributes and gas compositions (individual and interaction gas effects) were positively or negatively defined by special cubic model parameter estimates. The optimal gas mixtures identified in this study were: $5-8\% O_2 + 5-9\% CO_2 + 86-91\% N_2$, which maintained the quality attributes of 'Shelly' mango. Simplex lattice mixture design showed that gas concentrations (O2, CO2 and N₂) in controlled atmosphere storage are important to optimise based on the selected quality attributes for commercial storage of various mango cultivars. This new information will benefit the mango fruit industry since 'Shelly' mango fruit is gaining popularity in the international market. More research work should focus on validating the optimal gas mixtures in realistic storage conditions with extensive sensory evaluation and volatile profile measurement, since fruits stored in CA are mainly rejected due to poor aroma.

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