Improved storage and eat-ripe quality of avocados using a plant protein-based coating formulation

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Abstract
Coating comprising the plant protein, kafirin, propylene glycol (PG) and glucono-delta-lactone (GDL) have been shown to extend the quality of 'Packham's Triumph' pears. In this study, both of these coating treatments considerably extended the shelf-life and the time that the eat-ripe quality of 'Hass' avocados was maintained when compared to uncoated fruit. To determine the roles of the additives, PG and GDL in the functionality of kafirin edible coatings, different kafirin coatings were applied to 'Hass' avocados prior to storage under ripening conditions of 18ºC for up to 21 days. All the kafirin coatings were effective at extending and maintaining the shelf-life quality of avocados compared to uncoated fruit due to reduced respiration rate, 102 ml CO_2/kg/h for kafirin-PG+ GDL compared to 115 ml CO_2/kg/h uncoated avocados on day 14 and consequently reduced ethylene production of the coated fruit. Kafirin-PG+GDL coated avocados lost less weight and remained firmer than uncoated fruit. Descriptive sensory analysis showed kafirin-PG+GDL coatings were most effective, extending the quality of the avocados 7 days beyond the untreated control due to improved kafirin solvation facilitated by the GDL. Thus kafirin- PG+GDL coatings are an effective method of increasing shelf-life and extending the eat-ripe quality of climacteric fruit.

Introduction

Avocado is a sub-tropical, climacteric fruit that is exported in large quantities from sub-tropical countries such as Mexico and South Africa (Food and Agriculture Organisation, , 2014). However, the fruit has a very fast ripening rate and suffers flesh browning and softening (Seymour *et al.*, 1993), all of which reduce their quality and marketability (Wills *et al.,* 1998). Cold storage delays ripening and prolongs storage life (Zauberman *et al.*,1977), but avocados are easily prone to chilling injury (Yahia and Gonzalez-Aguilar, 1998), resulting in vascular browning and mesocarp discolouration (Scott and Chaplin, 1978). Generally the quality of any fresh fruit deteriorates when it comes out of the producers storage facilities and is transferred to the wholesalers storage (Omar and MatJafri, 2013). Also, interruptions can occur in the cold chain occur during transit and have a detrimental effect on fruit quality (Dodd *et al.,* 2007). Other techniques have been used instead of, and in addition to cold storage to extend avocado shelf –life (Wills *et al.,* 1998). These include fruit storage in a modified atmosphere or the use of modified atmosphere packaging (MAP) (Maftoonazad and Ramaswamy, 2005). When the gas composition in the storage environment is changed, either by lowering the oxygen concentration or increasing the carbon dioxide concentration, the synthesis of ethylene by the fruit is reduced (Kader, 1986). This reduction in ethylene production decreases metabolic activity and thus extends the shelf-life of the fruit.

Edible coatings are materials which are applied in thin layers to food surfaces by wrapping, immersing, brushing or spraying, with the objective of improving product quality and extending shelf-life (Dononhowe and Fennema, 1994). The materials may be consumed with the food product or may be removed with the skin or peal of the product, as is the case with avocados. Coatings are applied directly to the product surface where it is formed (Krochta, 2002). The coating acts as a barrier to prevent mechanical damage to the food product, whilst preventing moisture, oxygen, aroma and/or oil from migrating to and from the food. Thus edible coatings can be considered as a method of creating a modified atmosphere around a fruit (Maftoonazad and Ramaswamy, 2005). It has been found that various different types of edible coatings extend avocado quality and shelf-life (Aguillar-Méndez *et al.,* 2008; Jeong *et al.,* 2003; Johnston and Banks, 1998; Maftoonazad and Ramaswamy, 2008; Saucedo-Pompa *et al.,* 2009). The edible coating functions as a gas barrier, modifying the internal atmosphere of the fruit by increasing carbon dioxide and decreasing oxygen concentrations, with the consequence of retarding ripening (Park, 1999).

The effectiveness of the coating is dependent on the coating permeability and the fruit respiration (Maftoonazad and Ramaswamy, 2005). If the respiratory gas exchange through the skin is reduced excessively by a coating then off odours and off flavours may develop due to fermentation (Hagenmaier and Shaw, 1992; Banks *et al.*, 1993). A hydrophobic polyethylene-based wax coating at a concentration of 11% in aqueous solution, was found to be the best coating in terms of reducing mass loss without affecting the internal atmosphere of the fruit, when six different types of edible coating were applied to ‘Hass’ avocados (Johnston and Banks, 1998). Other hydrophobic coatings may also be effective at reducing mass loss and extending the shelf life of avocados. We have shown that a coating of the hydrophobic protein, kafirin containing propylene glycol (PG) and glucono-delta lactone (GDL) can extend the shelf-life of ‘Packham’s Triumph’ pears when stored at 20ºC (Buchner *et al.*, 2011). However, it is not known whether this coating would be effective when used on avocados, which are noted as one of the most rapidly ripening fruits (Seymour *et al.*, 1993).

Hence the objective of this study is to determine the effect of a kafirin based coating containing different additives (the plasticizer PG alone and PG in combination with the acidulant, GDL) on the respiration rate, shelf-life and sensory qualities of 'Hass' avocados.

Materials and methods

Materials

Physiologically mature, unripe 'Hass' variety avocados (*Persea americana*), weight range 125-135 g, were obtained from Westfalia Fruit Products (Politsi Tzaneen area, Limpopo, South Africa) and stored at 5.5^{o}C prior to fruit coating and analysis. Fruit maturity was confirmed on the basis of dry matter content (mean 35.3%, n=6), which is within the range (27.7-36.8%) for unripe but physiologically mature 'Hass' avocados (Clark *et al.,* 2003). Before coating, fruits were sorted and those perceived to be slightly riper in terms of colour and softness were separated and used to prepare "perfectly eat-ripe" samples for descriptive sensory evaluation.

Kafirin, protein content 83.6% (dry basis, N x 6.25) was extracted from milled, decorticated, non-tannin, red sorghum using a solvent extraction described by Emmambux and Taylor (2003) using modifications described in Buchner *et al.,* (2011).

Fruit coating

Coating solutions were prepared by dissolving 2% (w/w) kafirin in 70% (v/v) aqueous ethanol at 70 °C with vigorous stirring. Ethanol (98%, v/v) was added to replace the solvent lost during evaporation and the coating solution was then cooled to approximately 25°C. Two coating formulations were used: Kafirin-PG, kafirin coating solution with propylene glycol (1,2-propanediol) (Sigma Aldrich, Johannesburg, South Africa) 7.2 g/l, and kafirin-PG+GDL coating solution with propylene glycol and glucono-delta-lactone (3.6 g/l) (CC Immelman, Southdale Johannesburg, South Africa).

Prior to coating, avocados were equilibrated to 18°C. Avocados were dipped in the coating solution for five seconds, and left to dry at room temperature (22ºC) for 20-30 minutes. Avocados dipped in aqueous ethanol were included as uncoated control. After treatment, coated and uncoated avocados were then placed on trays (lined with tissue paper) in monolayer and stored at ripening temperature (18ºC) for up to 21 days.

Experimental design

Ethylene production, respiration rate, weight loss and texture determinations were determined on the same five groups of 6 avocados per treatment, on day 1, 2, 3, 6, 8, 10 and 14. Further texture measurements were also made on days 16 and 21 and weight loss on day 21. Additional coated and uncoated avocados were used for descriptive sensory evaluation.

Ethylene production and respiration rate

Ethylene production was measured by gas chromatography headspace analysis using a Varian 3700 GC (Palo Alto, CA) fitted with a 250 µm (internal diameter), 30 m stainless-steel column packed with fused silica (Supelco SPB-1, Sigma Aldrich, Johannesburg, South Africa) and a flame ionisation (FID) detector. The injection, column and detector temperatures were 275°C; 100°C; and 290°C, respectively. The carrier gas was H_2; flow rate ±10ml/min. A standard of 1.96% ethylene in H_2 was used for calibration. Each fruit was placed in a 1 l glass jar connected to an air flow meter (flow rate: 11.0 m/s) After 30 min. a 1 ml gas sample was taken and injected directly into the GC. The glass jar containing the fruit was then connected to a gas analyser (Varian Model 3300, Palo Alto, CA) equipped with an infrared detector (sensitivity range 0-2000 ppm) and allowed to further equilibrate before the CO_2 concentration (ppm) was measured and converted to respiration rate (ml CO_2/kg/h), taking into account the fruit mass and the ambient CO_2 concentration.

Weight loss

Moisture loss was indicated by weight loss, calculated by mass difference with respect to fruit mass on day 0 and expressed as percentage moisture loss (fresh weight basis).

Texture determination

Fruit firmness was determined using a HPR II Fff densitometer (Bareiss, Germany) calibrated in SHORE units, at two equidistant points on the equatorial region of each whole fruit. Four fruit from each treatment at each time interval were used.

Descriptive sensory evaluation

Eight sensory descriptors, as defined in Table 1S, were developed and used by a trained descriptive sensory evaluation panel (n = 7, 1 male, 6 female) to determine the sensory quality of uncoated and kafirin-coated 'Hass' avocados stored for 17 days at 18ºC. The minimum qualification for panel selection was based on the ability to recognise the basic tastes (sweet, sour, bitter, salty). Panellists who were bitter blind were eliminated from the panel. Panellists were further selected based their ability to distinguish between small flavour differences between avocados at different stages of ripeness (days 0, 3, 7, 10, 14). During a number of orientation sessions (2 h each, over a period of 3 weeks) panellists developed descriptive terms and evaluation scales. They were then assessed to determine the reproducibility and consistency of their ratings before being used to evaluate samples. Sensory appearance attributes of uncoated and coated fruit were assessed on day 0, 3, 6, 10, 13, 15 and 17. Sensory flavour and texture attributes were only analysed up until day 10 for uncoated and day 13 for kafirin-PG coated avocados. Beyond these time periods avocados were considered unfit for consumption. A perfectly ripe avocado sample, ripened for 8 days at 18°C, was evaluated at each time interval for reference A quarter of an avocado of each treatment was presented to the panellists. The panellists were trained to evaluate the fruit in a standardised manner, evaluating the same sized fruit piece, from the same part of the fruit, with the same number of chews before swallowing. The panellist tasted samples (n=4) in duplicate with a 15 min break after four samples. A nine-point intensity scale was used for evaluation, where 1 was the minimum score and 9 the maximum score. As an example, for the descriptor 'soft', a score of 1=not at all soft and 9=extremely soft. An indication of how the scores were used for green (unripe) avocados and for ripe avocados is given in supporting information Table 1S. The four samples tasted were: Uncoated, Kafirin- PG, Kafirin-PG + GDL and an eat-ripe sample. The following sensory descriptors were evaluated (Table 1S): presence of dark flesh blemishes, visual extent of ripeness, green and avocado-like flavour (flavour intensity), bitter aftertaste, creaminess, softness and oiliness (fatty).

Free-standing film formation

To determine the effect of the addition of PG and PG + GDL on the solvation of kafirin in the coating solution, free-standing films were made. Coating solutions (2% kafirin) were prepared as described above in 'Methods, Fruit coating' and PG (7.2 g/l) was added. GDL

(3.5 g/l) was added to a portion of the coating solution, resulting in two treatments, kafirin-PG and kafirin-PG+GDL. This was repeated twice. Aliquots of coating solution (4 g) were weighed into plastic petri dishes (9 cm diameter) in duplicate (a total of 4 films per treatment) and dried overnight at 50°C in an oven without a forced draft. Films were photographed using a flatbed scanner and assessed as to whether these films were whole, with no defects or fragmented.

Statistical analyses

Two-way analysis of variance (2 Way-ANOVA) including interactions, using Tukeys (HSD) test was used to analyse data with XLSTAT Pro software (Addinsoft SARL, Paris , France),where $p< 0.05$ for ethylene production, respiration rate, flesh firmnessand weight loss.

Descriptive sensory ratings were captured using Compusense® *five* (Compusense® *five*, release 4.8, Compusense Inc., Guelph, ON, Canada). Parametric testing methods were used for the sensory data. According to Næs *et al.* (2010), although sensory data is never completely normal in distribution, it can be considered at least a reasonable approximation and good enough to give useful results. This is as long as p-values are not considered as exact but as approximations of the level of significance. Thus data was analysed using two-way analysis of variance (2 Way-ANOVA) including interactions, using Tukeys (HSD) as described above. p values for each descriptor are given in Table 2S. Principal component analysis (PCA) classification of the sensory descriptors for appearance, flavour and texture and physiochemical characteristics (texture) was conducted using a correlation matrix with treatments in rows and characteristics in columns. PCA is a statistical method used to transform a large number of correlated variables into a smaller number of uncorrelated variables, called principal components. However, analysis of sensory data often includes averaged data on a small number of treatments examined on a number of sensory variables (Bower 2009), as was the case in this study. The transformation is defined such that the first principal component accounts for as much variability in the data as possible and each subsequent component in turn then accounts for as much of the remaining variability as possible, under the constraint that it is uncorrelated with the preceding components (Wold *et al.*, 1987). The authors accept the limitation of the analysis using a small number of treatments and variables and thus confine their interpretation to the first 2 dimensions of the PCA analysis, the first of which accounts for more than 90% of the variability of the data.

Results and discussion

Two-way ANOVA showed the presence of interactions between treatment and storage time ($p<0.0001$) for both respiration rate and ethylene production. The respiration rate of the kafirin coated avocados was generally lower than that of uncoated fruit (Figure 1A). However, there was no clear statistical difference between the kafirin-PG and kafirin-PG+ GDL coating at any time interval.. Respiration rate in avocados is high, with a range of CO_2 production rate of 40-150 ml/kg/h, usually reaching the climacteric peak after six days of storage at 20^{o}C (Pérez *et al.*, 2004). The respiration rate of all avocados in this study fell within this range. Maximum values were at the start of the storage period, 166 ml CO_2/kg/h - uncoated, 117 ml CO_2/kg/h - kafirin-PG, and 112 ml CO_2/kg/h - kafirin-PG+GDL. Respiration rates then decreased for all treatments before rising again on day 6 for the uncoated and kafirin-PG+GDL coated fruit and day 8 for the kafirin-PG coated fruit. At the end of the period (day 14), respiration rates were 115 ml CO_2 ml/kg/h for the uncoated, 102 ml CO_2 /kg/h kafirin-PG+GDL and kafirin-PG 75 ml CO_2/kg/h . There was only a small but not significant, respiration peak, on day 6 for the uncoated and kafirin-PG+GDL coated fruit and day 10 for kafirin-PG.. This lack of a clear maximum CO_2 production for 'Hass' avocados was also described by Hershkovitz *et al.* (2005) for control fruit and those treated with 1-Methylcyclopene and stored at 20^oC. Using hydrophilic coatings, methyl cellulose (Maftoonazad and Ramaswamy, 2005), pectin (Maftoonazad and Ramaswamy, 2008), and gelatin/starch (Aguillar *et al.*, 2008), lower respiration rates were also recorded in coated 'Hass' avocados when compared with controls.When comparing the effect of different coatings on the respiration rate of 'Hass' avocados, hydrophobic coatings such as avocado wax, have been found to be more effective at lowering the rate of respiration than other coatings (Johnston and Banks, 1998). Differences in the respiration behavior of coated avocados can be attributed to the different gas exchange and water barrier characteristics of the coatings used (Johnston and Banks, 1998). The mechanism of gas exchange for avocados is primarily through the pores and to a lesser extent through the cuticle (Ben-Yehoshua and Cameron, 1989) According to Johnston and Banks (1998) avocado wax coatings reduced cuticular water permeability but did not affect gas diffusion through the pores, whereas films made from kafirin have good gas barrier properties, but have poor water barrier properties (Emmambux *et al.*, 2004).

Overall, ethylene production of kafirin coated avocados was much lower than uncoated fruit (Figure 1B). Maximum ethylene production was measured at 6 days for uncoated avocados and at 3 days for kafirin-PG coated fruit and day 8the kafirin-PG+GDL treatment. The lower ethylene production of the coated avocados was probably due to the coatings acting as gas barriers, resulting from their somewhat reduced respiration rate (Figure 1A). As stated, kafirin films are good gas barriers (Emmambux *et al.*, 2004) so it is likely that the kafirin coatings limited oxygen availability to the fruit.

Figure 1: Effect of different kafirin based coatings on the respiration rate and ethylene production of avocados stored at 18°C for 14 days
A: Respiration Rate
B: Ethylene production
Black-No coating
Grey-Kafirin PG coating
White-Kafirin-PG+GDL coating
Mean values with different lower case letters differ significantly from each other (Treatment*Day $p < 0.0001$)

It has been observed that when avocados were subjected to a reduced oxygen atmosphere, ethylene production was reduced (Meir *et al.*, 1997). The acidification of the coating solution with GDL, probably enabled better kafirin solvation, in a similar way to acetic and lactic acids, which have been found to be good kafirin solvents (Taylor *et al.*, 2005). GDL is a food compatible, slow release acidulant widely used in the food industry (Igoe, 2011) . It slowly hydrolyses to gluconic acid in aqueous solution and has a neutral to mild acid taste (Lindsy, 1996). Whilst good solvents for kafirin, lactic acid and acetic acid (Taylor *et al.* 2005) are not suitable in this type of coating application. Lactic acid is not volatile and so does not dry on the surface of the fruit, remaining sticky. Acetic acid is volatile and coatings can be formed using acetic acid as a solvent but on drying the acid burns the surface of the fruit. In addition, both lactic acid and acetic acid have strong flavours and odours which would not be accepted sensorially (Őlmez and Kretzschmar, 2009). Free-standing films made from kafirin-PG+GDL were whole, with no defects whereas films made from kafirin-PG were fragmented and contained large holes. The holes in the fragmented films represented approximately 24% of the area of the film. This suggests the addition of GDL did enable better kafirin solvation in the aqueous ethanol coating solution. Better kafirin solvation would result in a more complete coating on the avocado surface, resulting in a coating with better gas barrier properties, reducing ethylene production which would consequently be expected to delay the ripening process.

Over the period of 21 days, there was no significant difference in avocado weight loss of any of the treatments. Avocados coated with kafirin-PG+GDL lost 8.94% of their weight over a period of 21 days, whereas kafirin-PG coated fruit lost 9.89% and the uncoated fruit 9.81%. As mentioned, although kafirin films have good gas barrier properties, they have poor water barrier properties (Emmambux *et al.,* 2004). Thus, the more complete coating of the kafirin-PG+GDL coated avocados probably accounted for their lower weight loss compared with the kafirin-PG coated and uncoated fruit. Other workers have similarly recorded reduction in weight loss during storage of 'Hass' avocadoes due to the application of edible coatings (Aguillar-Méndez *et al.,* 2008, Johnston and Banks, 1998; Maftoonazad and Ramaswamy, 2008).

Figure 2: Effect of different kafirin based coatings on flesh texture (as determined by densitometer) of avocados stored at 18°C for 21 days
-No coating
Grey-Kafirin PG coating
White-Kafirin-PG+GDL coating
Mean values with different lower case letters differ significantly from each other (Treatment*Day p 0.002)

With regard to fruit quality and possible microbial spoilage, our related work has shown that no bacteria or fungi are associated with other fruits coated with kafirin films over a storage period of 24 days (Buchner, 2008). It should be noted that in this present work all the avocado fruit were dipped in 70% ethanol. Other workers have reported that similar kafirin films exhibit antimicrobial activity (Giteru *et al.*, 2015).

Fruit quality, in terms of flesh firmness, as measured by densitometer, decreased over the storage period for all the treatments (Figure 2). Two-way ANOVA showed the presence of interactions between treatment and storage time (p 0.002). Avocado fruit softens rapidly and is completed after 7 days of storage at 20^{o}C (Jeong *et al.*, 2003). In this study, the kafirin-based coatings slowed down the rate of fruit softening. The kafirin-PG+GDL coated fruit generally remained firmer than the kafirin-PG coated avocados throughout the study. The kafirin-PG+GDL coated avocados were statistically firmer than the uncoated fruit from day 6 until day 10, whereas the kafirin-PG coated fruit were not significantly firmer than the uncoated fruit at any time interval. . This level of firmness retention shown by the kafirin-PG+GDL coating was similar to the 10 days reported for methyl cellulose based coatings when 'Hass' avocados were stored at 20°C (Maftoonazad and Ramaswamy, 2008). It has already been demonstrated that kafirin coatings are effective at delaying the softening of other climacteric fruit using 'Packham's Triumph' pears stored at 20^{o}C (Buchner *et al.,* 2011).

Of the two kafirin coatings, the kafirin-PG+GDL coating was more effective in delaying fruit softening, with little softening up to day 10. Avocado softening during ripening is attributed to degradation of cell wall components due to the activity of the enzymes, polygalacturonase, cellulase and pectin methylesterase (Joeng and Huber, 2004). It has been found that gelatine-starch based edible coatings lowered O_2 permeability and increased CO_2 retention and subsequently reduced the enzymic activity responsible for fruit softening (Aguillar-Méndez *et al.*, 2008). It is probable that the kafirin coatings behaved in a similar way and that the somewhat lower respiration rate resulting from kafirin-PG+GDL was responsible for better retention of flesh firmness.

The better gas barrier of the kafirin-PG+ GDL film also resulted in the beneficial effects of kafirin coatings on the sensory attributes of coated avocadoes (Table 2S, Figure 3B). The most important sensory quality parameter of avocado fruit is the appearance, in particular the flesh colour (Golukeu and Ozdemir, 2010). As would be expected, the presence of dark flesh blemishes increased with storage time in all treatments (Table 2S). The rate blemishing was reduced but not statistically significantly by the kafirin-PG+GDL coatings up to day 15 in comparison with the uncoated and kafirin-PG coated fruit The kafirin-PG+GDL coating also delayed the time to visual eat-ripeness to 10 days compared to 6 days for the uncoated and kafirin-PG coated avocados. In addition, kafirin-PG-GDL coated avocados maintained visual eat-ripeness until 17 days.

Figure 3: Principal component analysis (PCA) loadings for (A) sensory attributes and flesh firmness measured by densitometer during ripening and (B) all coated and uncoated avocados analysed on day 0, 3, 6, 10, 13, 15, and day 17 of the shelf-life study. NC-No coating, KP-Kafirin-PG, KG- Kafirin-PG+ GDL
R-Visual extent of ripeness
S-Softness
C-Creaminess
AF-Avocado flavour (flavour intensity)
BL-Presence of dark flesh blemishes
T-Flesh firmness
O-Oiliness (fatty)
B-Bitter aftertaste
GF-Green flavour

The panellist's perception of the flavour attributes, green flavour, bitter aftertaste and avocado-like flavour (flavour intensity) and the textural attributes, creaminess and oiliness (fatty), all followed the same pattern as that of the fruit softness for all the treatments (Figure 3B, Table 2S). The sensory textural attributes followed a similar trend to those measured by the densitometer (Figure 2, Table 2S). Sensory perception of fruit softness increased with storage time (Figure 3B, Table 2S). The uncoated and the kafirin-PG coated avocados were scored the same softness as the eat-ripe sample on day 6. The uncoated avocados remained eat ripe soft until day 10. The kafirin-PG coated avocados remained eat ripe soft until day 13, an extension of eat ripe quality by 3 days. Kafirin-PG+GDL coated avocados reached the same softness as the eat-ripe sample only on day 10 (4 days longer than the control) and stayed eat ripe soft for longer, a further 7 days until the end of the trial. Up until day 17, kafirin-PG+ GDL coated fruit did not differ significantly in softness from the eat-ripe sample. This indicated that avocados coated with kafirin-PG+GDL-based coatings both extended the ripening time and delayed senescence. After day 10, the uncoated fruit, and after day 13, the kafirin-PG coated fruit, were no longer evaluated for any of the sensory flavour or textural attributes as the avocados were considered to be unfit for consumption (Table 2S).

Considering the principal component analysis of the descriptive sensory evaluation data, component 1 (Figure 3A) described 91% of the variance in avocado sensory attributes and the measured texture during ripening. The texture in terms of measured firmness and the sensory attributes, bitterness, oiliness (fatty) and green flavour were negatively correlated to the sensory attributes softness, extent of ripeness and blemishes (Figure 3A). These parameters divided the avocados into three distinct groups (Figure 3B): those where the avocados exhibited unripe characteristics, (NC days 0-3, KP days 0-3, KG days 0-6), those consisted of eat ripe fruit (NC days 6-10, KP days 6-13, KG days 10-17) and the third group, overripe or senescent fruit (NC days 13-17, KP days 15-17). These groupings showed clearly that the kafirin coated avocados retained quality substantially longer, than uncoated avocados in respect of the important sensory attributes (Figure 3B, Tables 2S). This maintenance of sensory quality compared with uncoated fruit was for a further 3 days in the case of the kafirin-PG coating and 7 days for the kafirin -PG+GDL coating. These results emphasise the superiority of the kafirin-PG + GDL coating over the kafirin-PG coating in terms of delaying avocado ripening and the onset of senescence. The delay in the onset of senescence increased the length of time the avocados were eat-ripe. As stated previously this was probably due to the superior gas barrier properties of the kafirin-PG + GDL coating. Other workers have shown that reducing oxygen availability to ‘Hass’ avocados, using modified atmosphere conditions has resulted in significant differences in sensory characteristics (taste, flavour, texture, flesh colour and overall acceptability) when compared to control avocados when an untrained consumer panel was used (Sellamuthu *et al.*, 2013).

Conclusions

Kafirin-based coatings applied to ‘Hass’ export grade avocados were effective at extending the shelf life and sensory quality for several days at 18^{o}C compared to uncoated fruit. The kafirin-PG+GDL coating was more effective than the kafirin-PG coating. This was probably due to the GDL enabling better solvation of the kafirin during coating formulation, resulting in improved gas barrier properties. This in turn reduced ethylene production and consequently delayed fruit ripening. As these findings are similar to those obtained with ‘Packham’s Triumph’ pears, kafirin-based coatings containing PG and GDL clearly have wider potential to extend the shelf-life and maintain the eat ripe quality of other climacteric fruits.

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Supporting information

Table 1S: Sensory descriptors, scales and definitions used to describe the sensory attributes of the avocados

Table 2S: Effect of kafirin-based edible coatings on significant sensory attributes of avocados stored at 18°C

Table 1S: Sensory descriptors, scales and definitions used to describe the sensory attributes of the avocados

Descriptor	Definition	Score for Green avocados	Score for Eat-ripe avocados
Appearance			
Presence of dark flesh blemishes	Extent of black or brown blemishes on the flesh of the avocado	1	2-3
Visual extent of ripeness	Extent of overall ripeness of avocados	1	4-6
Flavour			
Green Flavour	Similar flavor to unripe avocado	3-6	1-2
Avocado-like flavour (flavour intensity)	Avocado-like flavor intensity associated with fresh ripe avocados	2-3	5-6
Bitter aftertaste	A primary taste characterised by a solution of caffeine	2-4	1-2
Texture			
Creaminess	Extent of the buttery/creamy feeling of ripe avocados when chewing	1	5-6
Softness	Extent of softness of avocado while chewing	1	5-6
Oiliness (fatty)	Oily lining in the mouth when chewing	5-6	1-2

Table 2S: Effect of kafirin-based edible coatings on significant sensory attributes of avocados stored at 18°C

Characteristic	Storage Time (days)	Uncoated	Kafirin + PG Coated	Kafirin + PG + GDL Coated	Eat-ripe control[4]	Effect of Storage Timef
Appearance						
Presence of dark flesh blemishes (Treat*Day p 0.0317)	0	1.1b[1] (0.3)	1.2b (0.4)	1.2b (0.4)	1.9ab (0.8)	1.4C[2]
	3	1.1b (0.3)	1.3ab (0.5)	1.2b (0.4)	3.7ab (1.0)	1.8C
	6	1.2b(0.6)	1.1b (0.4)	1.0b (0)	2.8ab (1.2)	1.5C
	10	2.2ab (0.8)	3.8ab (3.1)	1.8ab (0.8)	3.7ab (2.1)	2.9ABC
	13	3.6ab (2.4)	2.2ab (2.5)	1.4ab (0.5)	1.9ab (1.0)	2.3BC
	15	5.4ab (2.1)	4.3ab (2.9)	3.4ab (1.5)	2.3ab (0.6)	3.8AB
	17	6.0a (2.9)	6.0a (2.0)	3.1ab (1.5)	2.1ab (0.8)	4.3A
Effect of Treatment[3]		2.9X	2.8X	1.9X	2.6X	
Visual extent of ripeness (Treat*Day p 0.0136)	0	2.2de (1.5)	1.9e (1.1)	3.1bcde (1.5)	4.4abcde (1.3)	2.9C
	3	2.6cde (1.5)	1.8e (1.0)	2.8bcde (1.5)	6.3abcd (0.5)	3.4C
	6	5.9abcde (1.1)	4.4abcde (2.1)	1.9e (0.9)	5.2abcde (0.9)	4.4BC
	10	6.1abcd (1.0)	6.5abc (1.6)	5.1abcde (1.0)	6.1abcd (1.5)	6.0AB
	13	6.7abc (1.5)	5.9abcde (1.5)	4.4abcde (0.8)	4.5abcde (1.0)	5.4AB
	15	7.3a (1.1)	6.9ab (1.7)	6.5abcd (1.3)	5.2abcde (1.3)	6.4A
	17	7.7a (1.3)	7.4a (1.3)	6.4abc (1.0)	5.4abcde (1.0)	6.7A
Effect of Treatment		5.5X	5.0XY	4.3Y	5.3XY	
Flavour						
Green flavour (Treat*Day p 0.0018)	0	5.7a (1.9)	6.0ab (1.6)	3.3abcde (1.6)	2.4abcde (1.0)	4.3A

Characteristic	Storage Time (days)	Uncoated	Kafirin + PG Coated	Kafirin + PG + GDL Coated	Eat-ripe control[4]	Effect of Storage Time
	3	4.7abcde (2.6)	5.3abc (1.7)	4.9abcd (2.1)	1.4de (0.5)	4.1A
	6	1.4de (0.5)	2.9abcde (2.0)	5.1abcd (2.0)	1.7cde (0.6)	2.8AB
	10	1.6cde (0.5)	1.6cde (0.5)	1.8bcde (0.8)	1.8bcde (0.5)	1.7B
	13	UF 1.0e	1.9abcde (1.1)	3.1abcde (1.9)	1.9abcde (0.9)	2.0B
	15	UF 1.0e	UF 1.0e	1.5cde (0.8)	2.0abcde (0.6)	1.4B
	17	UF 1.0e	UF 1.0e	1.8bcde (1.1)	1.9abcde (0.8)	1.4B
Effect of Treatment		2.3XY	2.8XY	3.1X	1.8Y	
Avocado-like flavour (flavour intensity) (Treat*Day p <0.0001)	0	2.7d (1.4)	3.1bcd (1.2)	4.0bcd (1.3)	5.3bcd (0.6)	3.8D
	3	3.7bcd (1.8)	2.9cd (1.2)	3.1bcd (1.2)	5.8abcd (0.8)	3.9D
	6	5.6abcd (1.0)	4.6bcd (1.5)	2.9cd (1.1)	5.7abcd (0.8)	4.7CD
	10	5.9abcd (0.8)	5.9abcd (1.2)	5.4bcd (1.0)	5.8abcd (1.1)	5.8BC
	13	UF 9.0a	6.3abc (0.5)	4.6bcd (1.2)	5.6abcd (0.5)	6.4AB
	15	UF 9.0a	UF 9.0a	5.9abcd (2.0)	6.3abc (1.3)	7.7A
	17	UF 9.0a	UF 9.0a	6.5ab (1.2)	6.4ab (1.5)	7.7A
Effect of Treatment		6.4X	5.8X	4.6Y	5.8X	
Bitter aftertaste (Treat*Day p 0.1969)	0	4.1a (1.8)	3.8a (1.8)	2.4a (1.3)	1.6a (0.7)	3.0A
	3	2.9a (1.4)	3.3a (1.3)	3.1a (1.6)	1.6a (1.0)	2.7AB

Characteristic	Storage Time (days)	Uncoated	Kafirin + PG Coated	Kafirin + PG + GDL Coated	Eat-ripe control[4]	Effect of Storage Time
	6	1.6a (0.8)	1.6a (0.5)	3.0a (1.4)	1.1a (0.4)	1.8ABC
	10	1.9a 1.0)	1.3a (0.5)	2.0a (1.6)	2.8a (1.3)	2.0ABC
	13	UF 1.0a	1.3a (0.5)	2.2a (1.5)	1.4a (0.8)	1.5BC
	15	UF 1.0a	UF 1.0a	1.6a (0.7)	1.4a (0.5)	1.3BC
	17	UF 1.0a	UF 1.0a	2.7a (2.2)	1.4a (0.7)	1.5BC
Effect of Treatment		1.9X	1.9X	2.4X	1.6X	
Texture						
Creaminess (Treat*Day p <0.0001)	0	1.9ef (1.7)	1.8ef (1.1)	3.4bcdef (1.5)	5.0bcdef (0.8)	3.0D
	3	2.7cdef (1.9)	1.5f (0.9)	2.3def (1.6	6.0abcd (0.8)	3.1D
	6	6.2abc (0.9)	4.1bcdef (2.2)	1.6ef (0.8)	5.2bcde (1.0)	4.3CD
	10	6.1abc (1.0)	5.9abcd (0.9)	4.4bcdef (1.4)	5.0bcdef (0.7)	5.4BC
	13	UF 9.0a	6.6ab (1.1)	3.3bcdef (1.9)	5.1bcdef (0.6)	6.0AB
	15	UF 9.0a	UF 9.0a	5.3abcde (1.6)	5.1bcdef (1.0)	7.1A
	17	UF 9.0a	UF 9.0a	4.5bcdef (1.6)	5.1bcdef (0.8)	6.9A
Effect of Treatment		6.3X	5.4XY	5.2Y	3.5Z	
Softness (Treat*Day p <0.0001)	0	2.1fg (1.7)	2.5efg (1.3)	3.9bcdefg (1.5)	4.9bcdefg (1.1)	3.5D
	3	2.9cdefg (1.9)	1.8g (0.8)	2.8defg (1.5)	6.2abc (0.8)	3.4D
	6	6.6ab (0.8)	4.6bcdefg (1.7)	1.9g (1.0)	5.3bcdef (1.1)	4.6CD
	10	6.4ab (0.7)	5.9abcd (0.7)	4.9bcdefg (0.9)	5.6bcde (0.6)	5.7BC
	13	UF 9.0a	6.6ab (0.5)	4.8bcdefg (1.4)	4.7bcdefg (0.9)	6.3AB
	15	UF 9.0a	UF 9.0a	5.7abcde (1.3)	5.6bcde	7.3A

Characteristic	Storage Time (days)	Uncoated	Kafirin + PG Coated	Kafirin + PG + GDL Coated	Eat-ripe control[4]	Effect of Storage Time
					(1.3)	
	17	UF 9.0a	UF 9.0a	6.3ab (1.3)	5.3bcdefa (1.0)	7.4A
Effect of Treatment		6.4X	5.6XY	4.4Z	5.4Y	
Oiliness (fatty) (Treat*Day p 0.0048)	0	4.2ab (1.2)	4.4a (0.9)	3.0abcde (1.2)	2.0abcde (0.6)	3.4A
	3	3.3abcde (1.3)	4.1abc (0.9)	3.4abcde (0.9)	1.6cde (0.5)	3.1AB
	6	1.4de (0.5)	2.6abcde (1.3)	3.6abcd (1.3)	1.5de (0.7)	2.3BC
	10	1.7bcde (0.7)	1.6cde (0.8)	1.9abcde (0.7)	1.4de (00.6)	1.7C
	13	UF 1.0e	1.4de (0.5)	2.3abcde (1.1)	1.9abcde (0.8)	1.7C
	15	UF 1.0e	UF 1.0e	1.9abcde (0.8)	1.8bcde (0.6)	1.4C
	17	UF 1.0e	UF 1.0e	1.7bcde (0.9)	1.7bcde (0.8)	1.4C
Effect of Treatment		1.9XY	2.3XY	2.5X	1.7B	

[1] Mean values with different lower case letter in a group differ significantly from each other (Treatment*Day p values stated under descriptor)

[2]Mean values with different upper case letter in a column differ significantly from each other ($p<0.05$)

[3]Mean values with different upper case letter in a row differ significantly from each other ($p<0.05$)

Standard deviation values in parenthesis, n=8

[4]Prepared by ripening green, physiologically mature avocados for 8 days at 18 °C.

UF-Unfit for consumption. For statistical analysis scored as highest or lowest value as indicated

Figure 1A

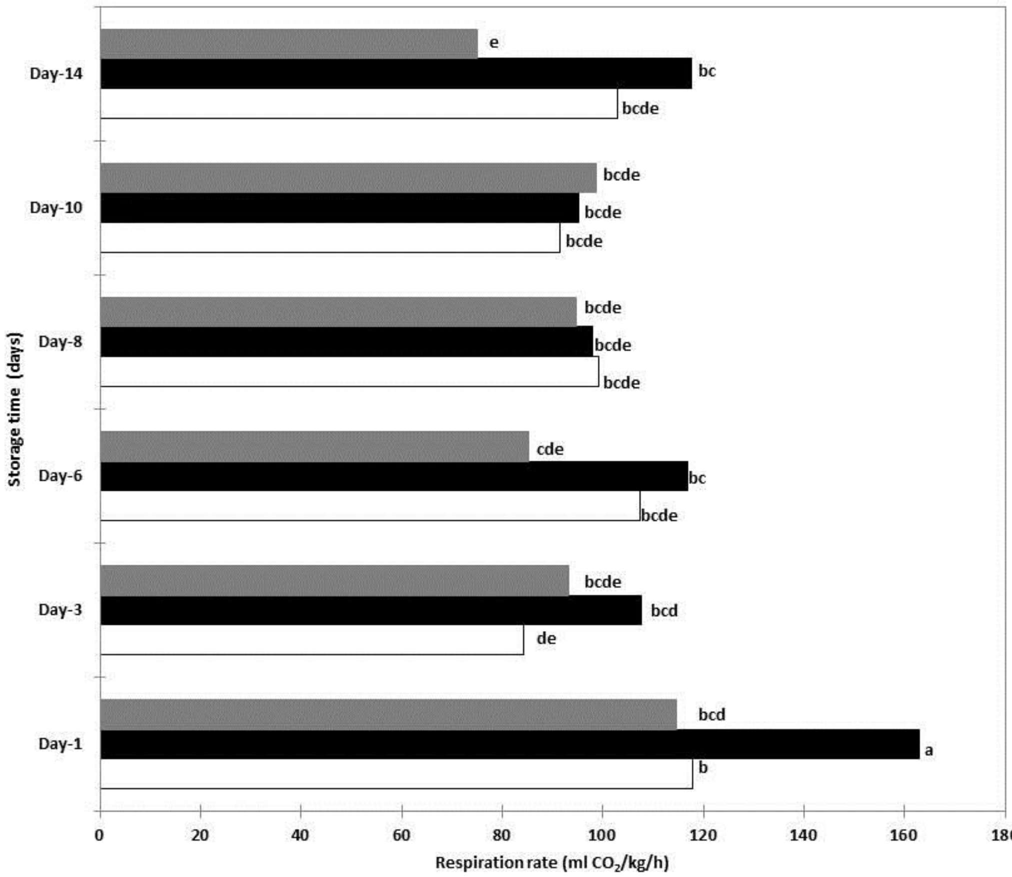

Day-14
e
bc
bcde
Day-10
bcde
bcde
bcde
Day-8
bcde
bcde
bcde
Day-6
cde
bc
bcde
Day-3
bcde
bcd
de
Day-1
bcd
a
b
Storage time (days)
0
20
40
60
80
100
120
140
160
180
Respiration rate (ml CO_2/kg/h)

Figure
1B

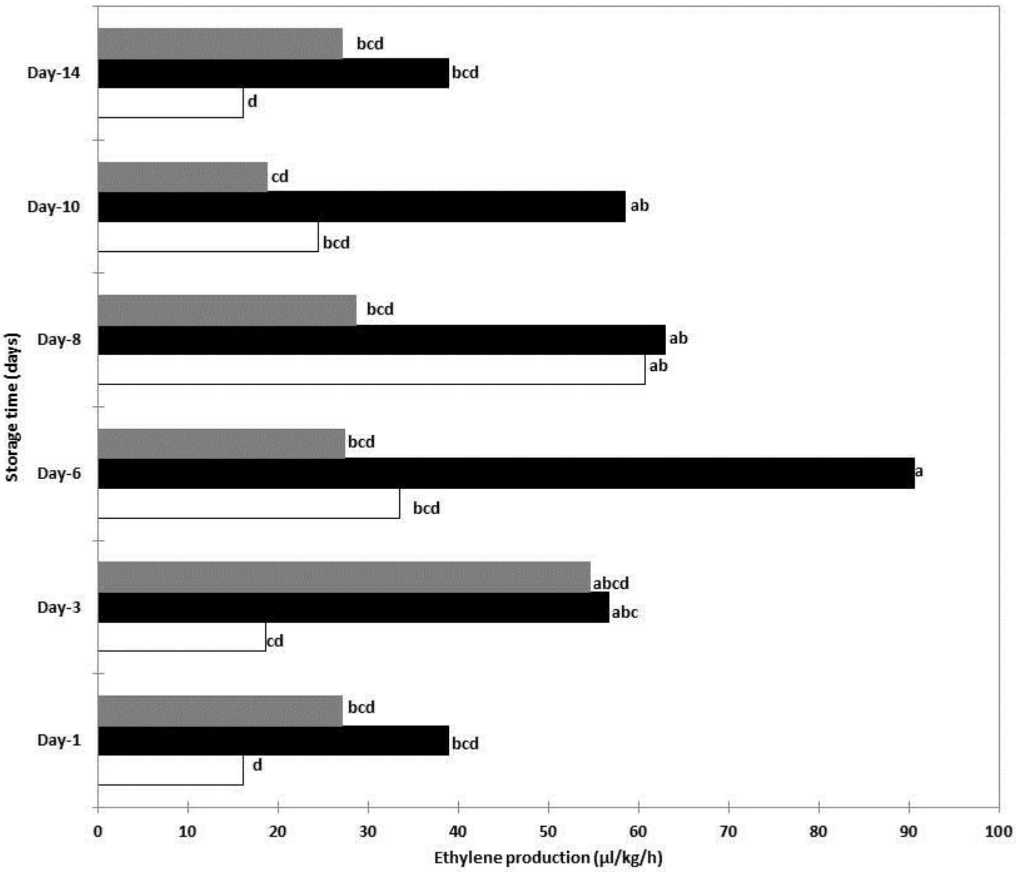

Day-14
Day-10
Day-8
Day-6
Day-3
Day-1
bcd
bcd
d
cd
ab
bcd
bcd
ab
ab
bcd
a
bcd
abcd
abc
cd
bcd
bcd
d
Storage time (days)
0
10
20
30
40
50
60
70
80
90
100
Ethylene production (µl/kg/h)

Figure 2

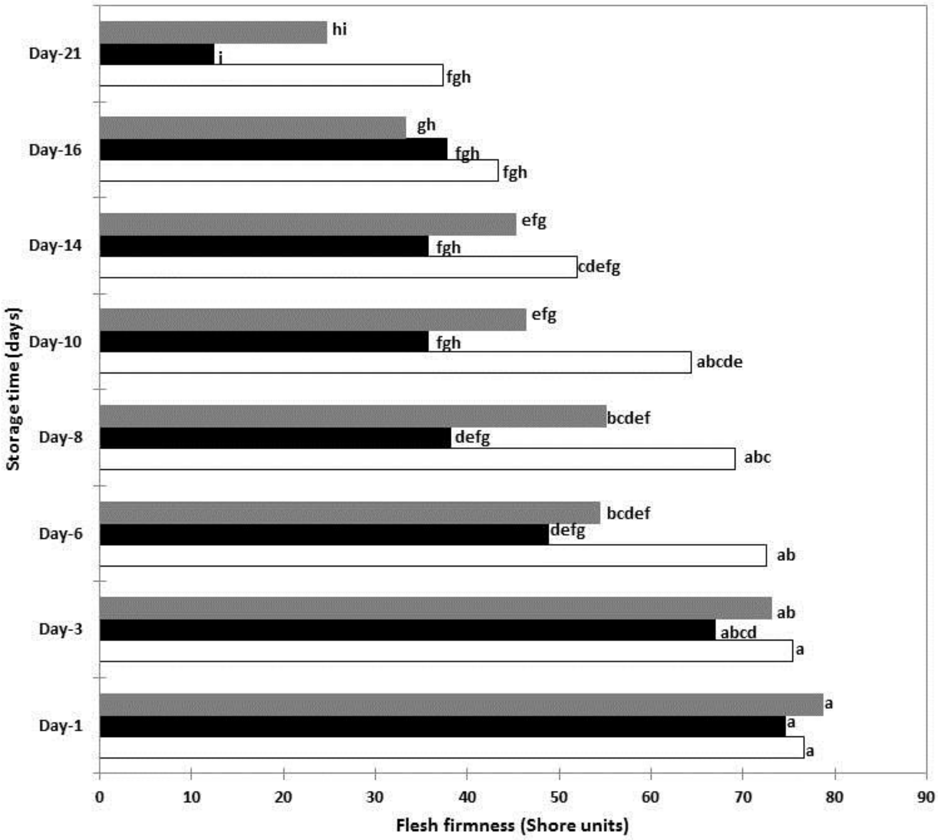
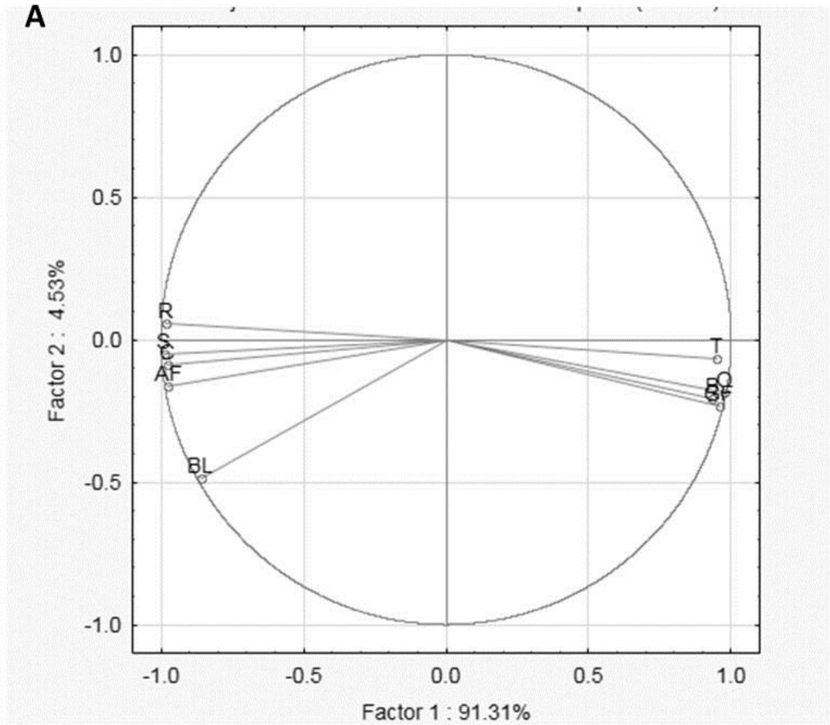
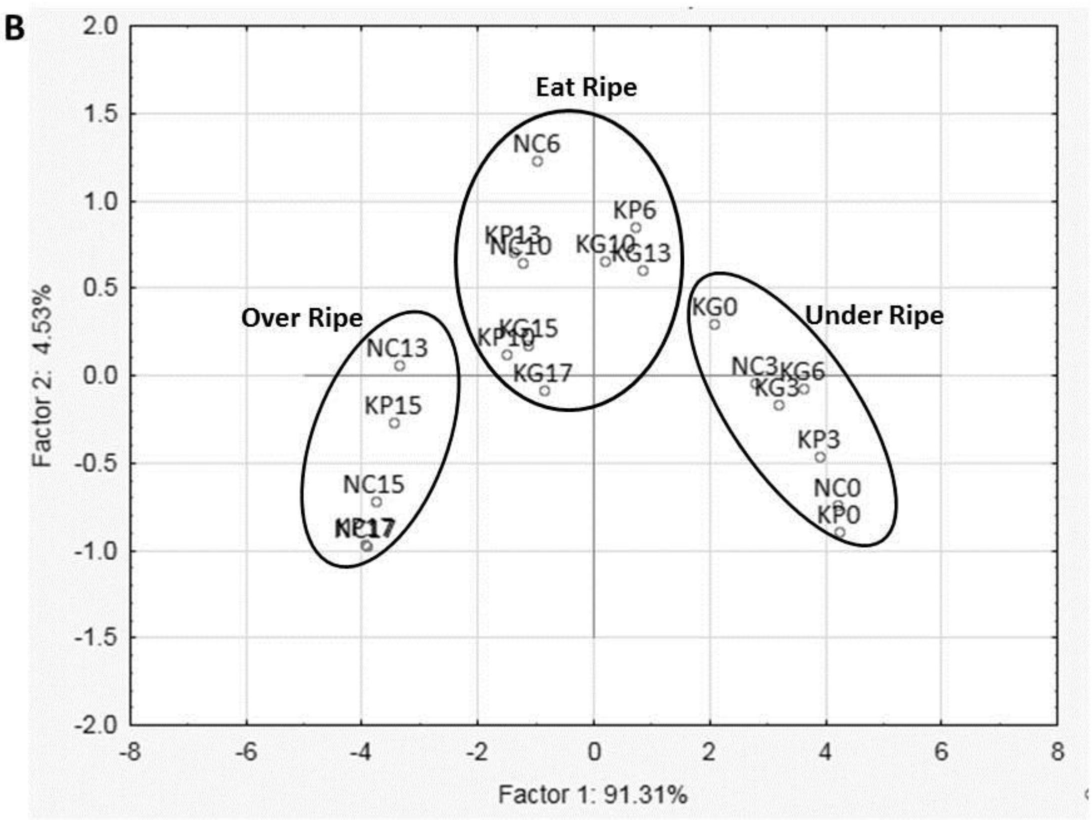

Day-21
hi
i
fgh
Day-16
gh
fgh
fgh
Day-14
efg
fgh
cdefg
Day-10
efg
fgh
abcde
Day-8
bcdef
defg
abc
Day-6
bcdef
defg
ab
Day-3
ab
abcd
a
Day-1
a
a
a
Storage time (days)
0
10
20
30
40
50
60
70
80
90
Flesh firmness (Shore units)

Figure 3


A
1.0
0.5
0.0
-0.5
-1.0
Factor 2 : 4.53%
R
S
C
AF
BL
T
Q
B
BF
-1.0
-0.5
0.0
0.5
1.0
Factor 1 : 91.31%


B
Eat Ripe
Over Ripe
Under Ripe
NC6
KP6
KP13
NC10
KG10
KG13
KG0
KG15
KP10
KG17
NC3
KG6
KG3
NC13
KP15
KP3
NC15
NC0
KP0
KP17
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Factor 2: 4.53%
-8
-6
-4
-2
0
2
4
6
8
Factor 1: 91.31%