Research Article

Received: 6 March 2011 Revised: 20 October 2011 Accepted: 21 December 2011 Published online in Wiley Online Library: 24 February 2012

(wileyonlinelibrary.com) DOI 10.1002/jsfa.5612

Effects of high-pressure argon and nitrogen treatments on respiration, browning and antioxidant potential of minimally processed pineapples during shelf life

Zhi-shuang Wu,[a] Min Zhang[a*] and Shao-jin Wang[b]

Abstract

BACKGROUND: High-pressure (HP) inert gas processing causes inert gas and water molecules to form clathrate hydrates that restrict intracellular water activity and enzymatic reactions. This technique can be used to preserve fruits and vegetables. In this study, minimally processed (MP) pineapples were treated with HP (∼10 MPa) argon (Ar) and nitrogen (N) for 20 min. The effects of these treatments on respiration, browning and antioxidant potential of MP pineapples were investigated after cutting and during 20 days of storage at 4 °C.

RESULTS: Lower respiration rate and ethylene production were found in HP Ar- and HP N-treated samples compared with control samples. HP Ar and HP N treatments effectively reduced browning and loss of total phenols and ascorbic acid and maintained antioxidant capacity of MP pineapples. They did not cause a significant decline in tissue firmness or increase in juice leakage. HP Ar treatments had greater effects than HP N treatments on reduction of respiration rate and ethylene production and maintenance of phenolic compounds and DPPH$^•$ and ABTS$^{•+}$ radical-scavenging activities.

CONCLUSION: Both HP Ar and HP N processing had beneficial effects on MP pineapples throughout 20 days of storage at 4 °C.
© 2012 Society of Chemical Industry

Keywords: minimally processed pineapples; argon; nitrogen; high pressure; gas hydrate

INTRODUCTION

Minimally processed (MP) fruits represent one of the most rapidly expanding segments of the lightly treated refrigerated food market owing to their increased functionality.[1] Minimal processing offers consumers highly nutritious, convenient and healthful fruits while maintaining freshness of the non-processed products.[2] However, MP fruits are perishable and have shorter a shelf life than whole produce, because ethylene production, respiratory activity, enzymatic and non-enzymatic browning and nutrient release from cells are stimulated by plant injuries.[3–6] Pineapple is a very popular fruit throughout the world for its nutritive and health-promoting properties.[7,8] MP pineapple has a commercial advantage in terms of weight reduction for transport, since bulky inedible crown and peel tissues are removed,[9] and MP product is the main form at retail. However, its shelf life is very limited (∼2–3 days) because of quality loss, including pulp browning, accumulation of liquid in the packaging, off-flavours and microbial growth.[4,10,11]

Argon and nitrogen as major components of the atmosphere in modified atmosphere packaging (MAP) have been reported to reduce microbial growth and improve quality retention of fresh produce.[12–14] Argon is reported to be biochemically active, probably owing to its enhanced solubility in water compared with nitrogen and its possible interference with enzymatic oxygen receptor sites.[15] A comparative study has been carried out on the effects of nitrogen and argon on the activities of tyrosinase and malic dehydrogenase, which are specific key enzymes related to browning of fresh fruits and vegetables and respiratory metabolism.[16] It was found that both nitrogen and argon reduced tyrosinase and malic dehydrogenase activities, with argon having a more significant effect than nitrogen. A gaseous inhibitor of the enzymes related to browning and respiration could have an important role in maintaining the quality of fresh fruits and vegetables as a replacement for some chemical treatments with their potential health risks.[16]

When certain gases such as neon, argon, krypton, xenon, nitrogen and oxygen are in contact with water under favourable temperature and pressure conditions, they can form ice-like crystals called clathrate hydrates or gas hydrates in which the gas molecules are trapped within a framework of cages of water molecules stabilised by physical bonding via van der Waals forces.[17–19] These gas hydrates are stabilised relative to the structure of pure water ice and can exist at temperatures well above

* Correspondence to: Min Zhang, School of Food Science and Technology, Jiangnan University, 214122 Wuxi, Jiangsu, China. E-mail: min@jiangnan.edu.cn

a School of Food Science and Technology, Jiangnan University, 214122 Wuxi, Jiangsu, China

b Department of Biological Systems Engineering, Washington State University, Pullman, WA 99164-6120, USA

 © 2012 Society of Chemical Industry

0 °C.[14,20,21] At 0 °C, argon and nitrogen clathrate hydrates can form and remain stable at more than 8.7 and 14.3 MPa respectively.[22] The gas hydrate structures are identified as I, II and the recently determined structure H.[19] Argon and nitrogen form structure II hydrates.[23] Four conditions must be met simultaneously within one region in order for a gas hydrate to be formed: presence of gas, water, high pressure and low temperature.[22] Behnke[24] suggested that high-pressure inert gases inhibit tyrosinase in non-fluid (e.g. gelatin) systems by decreasing oxygen availability rather than by physically altering the enzyme. Fujii *et al*.[25] reported that argon addition accelerated the inactivation of *Bacillus cereus* spores at 20 °C under a pressure of 600 MPa owing to argon clathrate hydrate formation. Oshita *et al*.[26] found that pressurised xenon treatments were efficient in maintaining the quality of fresh-cut carnation and broccoli owing to xenon clathrate hydrate formation. Clathrate hydration restrains the activity of intracellular water and inhibits enzymatic reactions, so vegetable metabolism is retarded. Zhang *et al*.[14] reported that the shelf life of asparagus spears could be extended to 12 days at 4 °C by treatment with mixtures of compressed (1.1 MPa absolute) argon and xenon (2 : 9 v/v) under partial pressure. They also found that many micropores appeared in the asparagus microstructure after the treatment process, with argon and xenon remaining in the structure as micropores, suggesting a positive connection with keeping asparagus spears fresh during the whole storage time.[14] It is desirable to use a pressurised low-cost inert gas such as argon or nitrogen to preserve MP fruits.

There have been few reports dealing with the application of high-pressure (HP) argon (Ar) or nitrogen (N) treatment to preserve MP fruits. The aim of this study was to investigate the effects of HP Ar and HP N treatments on respiration, browning and antioxidant potential of MP pineapples during 20 days of storage at 4 °C.

MATERIALS AND METHODS

Raw material and sample preparation

Pineapples (*Ananas comosus* L.) harvested in Hainan (China) were supplied by a local distributor and transferred to the laboratory and stored at 10 ± 1 °C overnight before processing.

Fruits were sorted to eliminate damaged or defective specimens. The shell colour stage was that where several to most of the shell eyes were partially filled with yellow colour, all of them surrounded by green.[27] Pineapple crown leaves were removed and fruits were washed in an aqueous solution of 200 μL L^{-1} sodium hypochlorite at 4 °C for 5 min, rinsed with tap water and drained on a clean paper towel. The fruits were peeled manually with a sharp knife and then cut into 2 cm thick wedges (∼15 g each). The knife and cutting board were sanitised with 200 μL L^{-1} sodium hypochlorite solution for 3 min prior to use.

Treatments included six groups: (1) control (not treated); (2) normal atmospheric-pressure Ar treatment (NAP Ar); (3) normal atmospheric-pressure N_2 treatment (NAP N); (4) high-pressure air treatment (HP air); (5) high-pressure Ar treatment (HP Ar); (6) high-pressure N_2 treatment (HP N).

Samples (∼90 g per tray) from each treatment were placed on polypropylene trays, which were then thermosealed using a packaging machine (BG-2, Wenzhou Chunlai Packaging Machinery Co., Zhejiang, China). The O_2 and CO_2 permeabilities of the sealing film (Su Zhou Deep Breaths Preservation Technology Ltd, Jiangsu, China) were 110 and 500 $cm^3\ m^{-2}\ day^{-1}\ bar^{-1}$ respectively at 23 °C and 0% relative humidity. Samples were stored in darkness at 4 °C for 20 days. They were analysed just before packaging (day 0) and after storage for 2, 5, 8, 11, 14, 17 and 20 days. Approximately 900 g of MP pineapples for each treatment were used for day 0 evaluations and 70 trays per sample were prepared, resulting in a total of 420 trays. Ten trays per sample were randomly taken and analysed at each storage time (2, 5, 8, 11, 14, 17 and 20 days). The whole process was conducted in triplicate as replications.

Experimental apparatus and procedure

The high-pressure gas treatment apparatus was designed by Jiangnan University (Jiangsu, China) and manufactured by Huaan Scientific Research Instrument Co. Ltd (Jiangsu, China). The apparatus had an operating temperature range from 0 to 50 °C at a maximum working pressure of 30 MPa. A diagram of the high-pressure gas treatment system is shown in Fig. 1. The primary components of the apparatus were a 500 mL stainless steel treatment vessel, a plunger pump, a vacuum pump and a thermally controlled bath. The sample treatment vessel had two circular viewing windows made of Plexiglas on the front and back and was submerged in a water bath to ensure the desired operating temperature. The water in the bath was recirculated through a plastic tube to a small thermally controlled bath, where the temperature of the water was stabilised by cooling via a refrigerator or heating via a heater. A plunger pump with a maximum pressure of 32 MPa was used to pressurise the vessel. A pressure transducer was fixed in the vessel lid to monitor the vessel pressure. All temperature and pressure data were displayed on a control panel. All parts of the system exposed to high pressure were made of stainless steel. The vessel had gas-tight connections to the gas inlet and outlet. The vessel lid could be sealed by screwed flanges and neoprene O-ring gaskets during high-pressure gas processing. A vacuum pump (2XZ-4, Huangyan Qiujing Vacuum Pump Factory, Zhejiang, China) was connected to the vessel for evacuating the air in the vessel and building the vacuum state of the vessel. Commercially available Ar and N_2 of 99.7% purity were purchased from Wuxi Xinnan Gas Co. (Jiangsu, China). Gas was injected into the plunger of the pressuriser from a gas cylinder and then entered the pressure vessel. The on–off valve on the feed line between the pump and the vessel was turned off after the required pressure level had been reached, then the pressure was held for the required treatment time. At the end of the experiments the system could be easily depressurised by opening the on–off valve on the vessel outlet line.

The pressure vessel was rinsed and sanitised with 200 μL L^{-1} sodium hypochlorite solution. Pineapple wedges were placed in the treatment vessel. The vessel containing pineapples wedges was sealed and vacuumised, then flushed with 99.7% pure Ar or N_2. For normal atmospheric-pressure Ar or N_2 treatment, pineapple wedges were kept in the vessel under normal atmospheric pressure for 20 min at 4 °C. For high-pressure Ar or N_2 treatment, samples were pressurised by the plunger pump to 10 MPa. That pressure was held for 20 min at 4 °C. For high-pressure air treatment, pineapples wedges were placed in the treatment vessel. The vessel was sealed and pressurised to 10 MPa. That pressure was held for 20 min at 4 °C. The time required for pressurisation of the vessel was about 5 min. The parameters of the operating pressure and time were selected on the basis of preliminary tests (results not shown). Then depressurisation was performed by opening the pressure relief valve at the gas outlet on the pressure vessel, which took only a few seconds. After treatment the pineapple wedges were immediately packaged in a room at 4 °C and stored at 4 °C for later analyses.

© 2012 Society of Chemical Industry

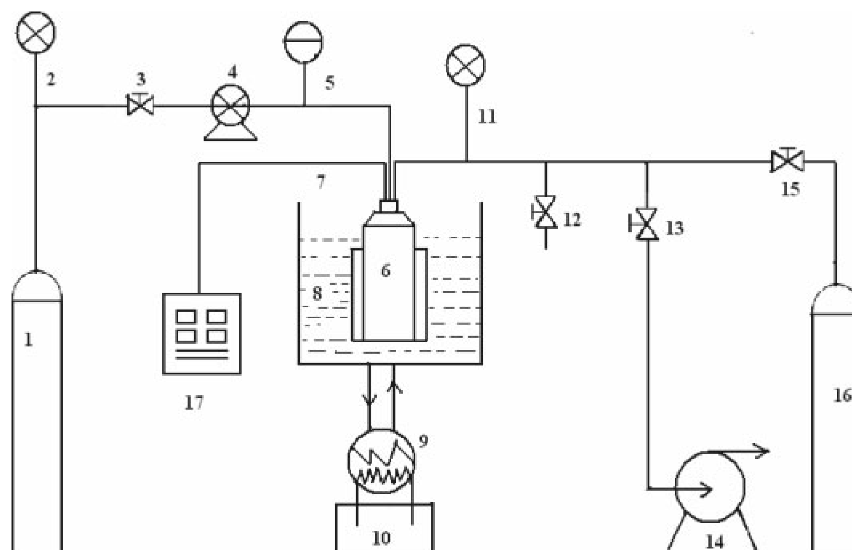

Figure 1. Schematic diagram of high-pressure gas-processing equipment: 1, gas cylinder; 2, pressure gauge; 3, on–off valve; 4, plunger pump; 5, pressure transducer; 6, high-pressure vessel; 7, thermocouples; 8, water bath container; 9, thermostatic bath; 10, refrigerant compressor; 11, pressure gauge; 12, relief valve; 13, on–off valve; 14, vacuum pump; 15, vent valve; 16, exhaust gas cylinder; 17, display panel.

Determination of respiration rate and ethylene production

Respiration is a basic physiological process in all living tissues. A higher respiration rate means faster overall metabolism and deterioration. It is known that wounding tissue induces elevated respiration.[28] About 200 g of fresh-cut pineapple was placed in a 1.5 L glass jar, sealed using a cap with a rubber septum and incubated at 4 °C for 1 h. A 1 mL headspace gas sample was taken with a gas-tight syringe through the septum, and CO_2 analysis was conducted using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector. A capillary column (i.d. 0.32 mm) was used with helium as carrier gas at a flow rate of 30 mL min^{-1} and the column temperature maintained at 55 °C. Respiration rate was calculated as mg CO_2 kg^{-1} h^{-1}.

Ethylene concentration was determined using the same gas chromatograph equipped with a flame ionisation detector and the same column as for CO_2 determination. Gas flows for N_2, H_2 and air were 47, 47 and 400 mL min^{-1} respectively and the column temperature was maintained at 50 °C. Ethylene production was calculated as µL kg^{-1} h^{-1}.

Browning evaluation

Measurement of colour

Tristimulus reflectance colorimetry was used to assess the extent of browning of pineapple wedges.[29,30] Colour was measured with a Minolta CR-400 colorimeter (Konica Minolta, Tokyo, Japan) according to the CIELAB colour parameters L^* (lightness), a^* (green chromaticity) and b^* (yellow chromaticity). The instrument was set up for illuminant D 65 and 10° observer angle and calibrated with a standard white calibration plate before measurement. Ten pineapple wedges were analysed for each treatment at each sampling time. Thus the reported values are the mean and standard deviation of 30 determinations. Numerical values of L^*, b^* and ΔE were considered for the evaluation of colour modification of fresh-cut pineapples. The value ΔE defines the magnitude of total colour difference and is expressed by the equation

$$\Delta E = [(L_t^* - L_{\text{initial}}^*)^2 + (a_t^* - a_{\text{initial}}^*)^2 + (b_t^* - b_{\text{initial}}^*)^2]^{1/2}$$

where L_{initial}^*, a_{initial}^* and b_{initial}^* are the readings for fresh pineapple wedges without any treatment and L_t^*, a_t^* and b_t^* are the readings at storage time *t* after pineapple wedge treatment.

Assay of enzyme activities

The extraction and activity determination of phenylalanine ammonia-lyase (PAL) were carried out according to the method of Zhou *et al.*[31] A 10 g pineapple sample was homogenised in 15 mL of 0.1 mol L^{-1} borate buffer (pH 8.8) with 5 mmol L^{-1} β-mercaptoethanol, 2 mmol L^{-1} ethylene diamine tetraacetic acid and 10 g L^{-1} polyvinyl polypyrrolidone at 4 °C for 1 h. The homogenate was then filtered through Whatman no. 1 filter paper and centrifuged at 15 000 × *g* for 15 min. To the supernatant (0.25 mL) was added 2.75 mL of 60 mmol L^{-1} L-phenylalanine in 0.1 mol L^{-1} borate buffer (pH 8.8). The substrate was incubated at 40 °C for 1 h and the reaction was stopped by adding 0.1 mL of 6 mol L^{-1} HCl. The increase in absorbance at 290 nm due to the formation of *trans*-cinnamate was measured in a UV–visible spectrophotometer (Precision Science Instrument Co., Ltd, Shanghai, China). One unit of enzyme activity was defined as the amount that caused an increase of 0.001 absorbance units per hour. Results were expressed as units h^{-1} mg^{-1} protein.

The assay of polyphenol oxidase (PPO) followed the method of Soares *et al.*[32] with some modification. A 10 g pineapple sample was homogenised in 25 mL of 0.05 mol L^{-1} phosphate buffer (pH 7) and the homogenate was filtered through Whatman no. 1 filter paper. After centrifugation at 9000 × *g* for 10 min at 4 °C the clear supernatant was collected as the enzyme extract. The enzyme extract (0.5 mL) was added to a mixture of 3.6 mL of 0.1 mol L^{-1} phosphate buffer (pH 7) and 0.1 mL of 10 mmol L^{-1} catechol as substrate and incubated at 30 °C for 30 min. The reaction was stopped by the addition of 0.8 mL of 2 mol L^{-1} perchloric acid. PPO activity was measured in the UV–visible spectrophotometer at 395 nm. One unit of enzyme activity was defined as the amount that caused an increase of 0.001 absorbance units per hour. Results were expressed as units h^{-1} mg^{-1} protein.

Peroxidase (POD) activity was measured by the procedure of Oms-Oliu *et al.*[2] POD was extracted from 50 g of pineapple

 © 2012 Society of Chemical Industry

sample by homogenisation in 100 mL of 0.2 mol L^{-1} sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 6000 × *g* for 15 min at 4 °C. The assay mixture consisted of 0.02 mol L^{-1} Na_2HPO_4/0.08 mol L^{-1} NaH_2PO_4 buffer (pH 6), 20 mmol L^{-1} guaiacol, 4 mmol L^{-1} H_2O_2 and enzyme extract (150 μL) in a final volume of 3 mL. The change in absorbance at 470 nm and 25 °C due to oxidation of guaiacol was recorded in the UV–visible spectrophotometer. One unit of enzyme activity was defined as the amount that caused an increase of 0.001 absorbance units per hour. Results were expressed as units h^{-1} mg^{-1} protein.

Soluble protein content in the crude enzyme of triplicate extractions was determined with bovine serum albumin as standard.[33] The absorbance at 595 nm was evaluated by graphic interpolation on a calibration curve.

Antioxidant potential

Fruits and vegetables have been associated with the prevention of degenerative diseases such as cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction, cataracts and cardiovascular diseases.[34,35] These protective effects are considered in large part to be related to various antioxidants they contain. The antioxidants might confer these health-protective benefits[36,37] by alleviating oxidative stress, i.e. preventing free radicals from damaging proteins, DNA and lipids.[38] Moreover, the antioxidant status of a fruit is related to its shelf life and may provide a useful indicator of the resulting overall slice quality. When the postharvest oxidative stress exceeds the natural antioxidant system's capacity, protection against active oxygen species (AOS) declines, resulting in AOS-induced injury translated into disorders such as browning, microbial contamination and poor sensory quality.[39] In MP fruits the tissues are primarily submitted to oxidative stress, which presumably causes membrane damage and alters the composition and content of antioxidant compounds, resulting in changes in the total antioxidant activity of the tissues.[40] Thus for MP fruits it is important to assess the effects of processing and storage on their antioxidant potential.

Total phenolic compound evaluation

The extraction of total phenolic compounds followed the method reported by Alothman *et al*.[41] and the total phenolic content in the extract was determined according to the Folin–Ciocalteu procedure. [42] A 5 g sample of fresh-cut pineapple was crushed and homogenised in 50 mL of 700 mL L^{-1} methanol. The mixture was centrifuged at 3000 × *g* for 10 min at 4 °C and the clear supernatant was collected. Then 1 mL of extract was transferred to a test tube and 1.5 mL of Folin–Ciocalteu reagent was added and mixed thoroughly for 6 min. Thereafter, 6 mL of 100 g L^{-1} Na_2CO_3 was added and the mixture was held for 2 h. The absorbance at 765 nm was measured in the UV–visible spectrophotometer. The concentration of total phenolic compounds was determined by comparison with the absorbance of gallic acid at different concentrations as standard. Results were expressed as mg gallic acid kg^{-1} sample.

Ascorbic acid evaluation

Ascorbic acid was analysed according to the dichlorophenol/indophenol titrimetric method.[43] A 10 g sample of fresh-cut pineapple was homogenised in 100 mL of 30 g L^{-1} metaphosphoric acid. A 10 mL aliquot of the filtrate was titrated with dye until the distinct rose pink colour persisted for 15–20 s. Results were expressed as mg kg^{-1} sample.

DPPH free radical-scavenging assay

Since most natural antioxidants and phytochemicals are multifunctional compounds, several methods covering various oxidation conditions should be tested to evaluate their antioxidant properties.[44] This explains why the use of multiple techniques to measure the antioxidant capacity of natural products has become a common feature in recent publications.[45] In our experiment the total antioxidant capacity of fresh-cut pineapples during shelf life was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric-reducing/antioxidant power (FRAP) methods and the results were correlated with total phenol and ascorbic acid contents to evaluate the overall antioxidant potential of MP pineapples.

The determination of the DPPH free radical ($DPPH^•$)-scavenging effect was carried out according to the method of Alothman *et al*.[41] Each fruit sample was centrifuged at 22 100 × *g* for 15 min at 4 °C and filtered through Whatman no. 1 filter paper. A 0.01 mL aliquot of the supernatant was mixed with 3.9 mL of 0.025 g L^{-1} methanolic DPPH and 0.090 mL of distilled water. The homogenate was vigorously shaken and kept in darkness for 30 min. The absorbance of the sample at 515 nm was measured in the UV–visible spectrophotometer against a blank of methanol without DPPH. Results were expressed as % inhibition of $DPPH^•$ according to the equation

$$\%\ \text{inhibition of DPPH}^\bullet = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100$$

where $Abs_{control}$ is the absorbance of DPPH solution without any extract.

ABTS radical cation decolourisation assay

The ABTS assay was performed according to Re *et al*.[46] ABTS radical cation ($ABTS^{•+}$) was generated by mixing ABTS (7 mmol L^{-1} final concentration in 25 mL) with potassium persulfate (2.45 mmol L^{-1} final concentration in 25 mL) and keeping the mixture in the dark at room temperature for 12–16 h (the reagent was stable for up to 2 days). A 3 g pineapple sample was homogenised in 9 mL of distilled water containing ascorbic acid (5 g L^{-1}), cysteine (0.5 g L^{-1}), citric acid (0.5 g L^{-1}) and oxalic acid (0.5 g L^{-1}). The homogenate was centrifuged at 15 000 × *g* for 15 min. The supernatant was used for antioxidant activity measurement. The ABTS solution was diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. After addition of 2.95 mL of diluted ABTS solution to 5 μL of extract or Trolox standard in ethanol and mixing for 6 min, the absorbance was measured at 30 °C. Solvent blanks were run in each assay. The % inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and Trolox for standard reference data. Total antioxidant activity was expressed as mmol Trolox equivalent antioxidant capacity (TEAC) kg^{-1} fresh weight (FW).

FRAP assay

The FRAP assay was based on the method proposed by Benzie and Strain[47] with slight modifications. Fresh FRAP reagent was prewarmed at 37 °C and prepared daily by mixing 2.5 mL of 10 mmol L^{-1} 2,4,6-tris(1-pyridyl)-5-triazine (TPTZ) solution in 40 mmol L^{-1} HCl with 2.5 mL of 20 mmol L^{-1} $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 mol L^{-1} acetate buffer (pH 3.6). A 3 mL aliquot of FRAP reagent was added to a test tube and a blank absorbance reading at 593 nm was taken in the UV–visible spectrophotometer.

A 100 µL aliquot of sample extract and 300 µL of distilled water were then added to the test tube. After addition of the sample to the FRAP reagent and 90 min of incubation at 37 °C in a water bath a second absorbance reading at 593 nm was taken. The change in absorbance after 90 min from the initial blank reading was compared with a standard curve. The calibration curve was prepared using aqueous solutions of $FeSO_4{\cdot}7H_2O$ (200, 400, 600, 800 and 1000 µmol L^{-1}). FRAP values were expressed as mmol Fe^{2+} kg^{-1} FW.

Texture analysis

Texture analysis was performed at 20 ± 2 °C about 30 min after removing samples from storage at 4 °C. A TA-XT2 texture analyser (Stable Micro Systems Ltd, Godalming, UK) equipped with a 5 kg load cell was employed. A 2 mm diameter rod was used to penetrate the pineapple wedge sample at a test speed of 0.5 mm s^{-1}. The maximum penetration force was measured and taken as firmness (N). Three trays were used at each sampling time to perform the analyses, and five wedges for each replicate were randomly withdrawn to carry out repetitions.

Juice leakage measurement

Juice leakage from pineapple wedges was measured according to the method of Montero-Calderón *et al.*[27] by tilting the packages at an angle of 20° for 5 min and recovering accumulated liquid with a calibrated syringe. Results were expressed as mL kg^{-1} FW.

Statistical analysis

All analyses were replicated in triplicate at each sampling time. All data were subjected to analysis of variance using SAS (SAS Institute, Cary, NC, USA). The significance of differences between means was determined by Duncan's multiple range test at a significance level of $P = 0.05$. Values were expressed as mean of all replicate determinations ± standard deviation.

RESULTS AND DISCUSSION

Respiration rate and ethylene production

The respiration rate of all MP pineapples during shelf life at 4 °C was rather low until 11 days (Fig. 2). From 14 days a sharp increase in the respiration rate of samples untreated and treated with HP air, NAP N and NAP Ar was detected. The results agreed with those observed by Marrero and Kader,[10] who reported that the respiration rate of fresh-cut pineapple was significantly affected by storage temperature and that a marked increase occurred after 12 days at 5 °C, which was followed by visual signs of microbial spoilage indicating the end of post-cutting life of MP pineapples. For samples treated with HP N or HP Ar, this stage was extended to 15 days, since a lower respiration rate was observed throughout the storage period compared with samples untreated and treated with HP air, NAP N or NAP Ar (Fig. 2). HP air- and HP Ar-treated samples showed the highest and lowest respiration rates respectively throughout the storage time. There was no difference in the respiration rate of untreated, NAP N- and NAP Ar-treated samples. The results demonstrated that both HP Ar and HP N treatments could lower the respiration rate of pineapple wedges during storage at 4 °C and that HP Ar treatment was significantly more efficient than HP N treatment ($P < 0.05$). Similar results were obtained by Zhang *et al.*[14] for asparagus spears with compressed (1.1 MPa absolute) Ar and Xe treatment and by Zhan and Zhang[48] for cucumber with compressed Xe treatment.

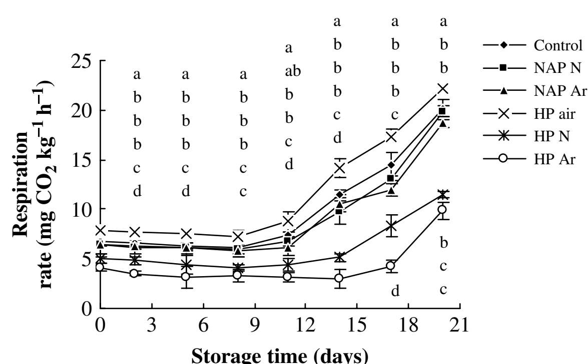

Figure 2. Changes (mean ± standard deviation) in respiration rate of MP pineapples of six treatments during 20 days of storage at 4 °C: control, untreated pineapple wedges; NAP N, pineapple wedges treated with atmospheric-pressure nitrogen for 20 min; NAP Ar, pineapple wedges treated with atmospheric-pressure argon for 20 min; HP air, pineapple wedges treated with high-pressure (10 MPa) air for 20 min; HP N, pineapple wedges treated with high-pressure (10 MPa) nitrogen for 20 min; HP Ar, pineapple wedges treated with high-pressure (10 MPa) argon for 20 min. For each storage time, means with the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

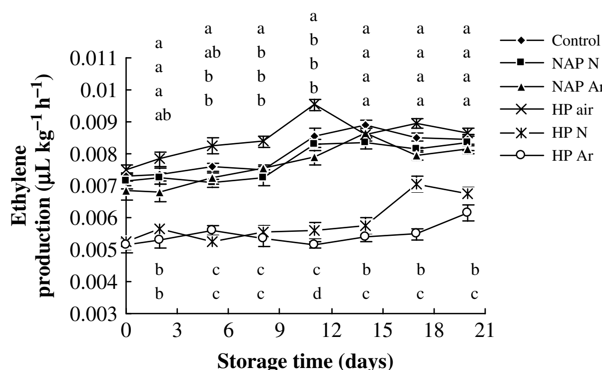

Figure 3. Changes (mean ± standard deviation) in ethylene production of MP pineapples of six treatments (see Fig. 2) during 20 days of storage at 4 °C. For each storage time, means with the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

Figure 3 shows the effects of different treatments on ethylene production of MP pineapples stored at 4 °C. Ethylene production of all samples maintained a stable pattern until a sharp increase occurred at 11 days. The peak in ethylene production of samples untreated and treated with HP air, HP N and HP Ar was detected after 14, 11, 17 and 20 days at 4 °C, with values of 0.0089, 0.0095, 0.0071 and 0.0061 µL kg^{-1} h^{-1} respectively. HP Ar- and HP N-treated samples showed less ethylene production than untreated samples, with HP Ar treatment resulting in the least ethylene production throughout storage. The results indicated that both HP Ar and HP N treatments reduced ethylene production and that HP Ar treatment had a significantly greater effect than HP N treatment. No difference was observed in ethylene production and peak time of untreated and NAP N- or NAP Ar-treated samples, which indicated that NAP N and NAP Ar treatments had no inhibitory effect on ethylene production of MP pineapples during cold storage.

The inhibitory effect of HP N or HP Ar treatment on the respiration rate and ethylene production of MP pineapples may be due to gas hydrate formation and residual N_2 or Ar in

© 2012 Society of Chemical Industry

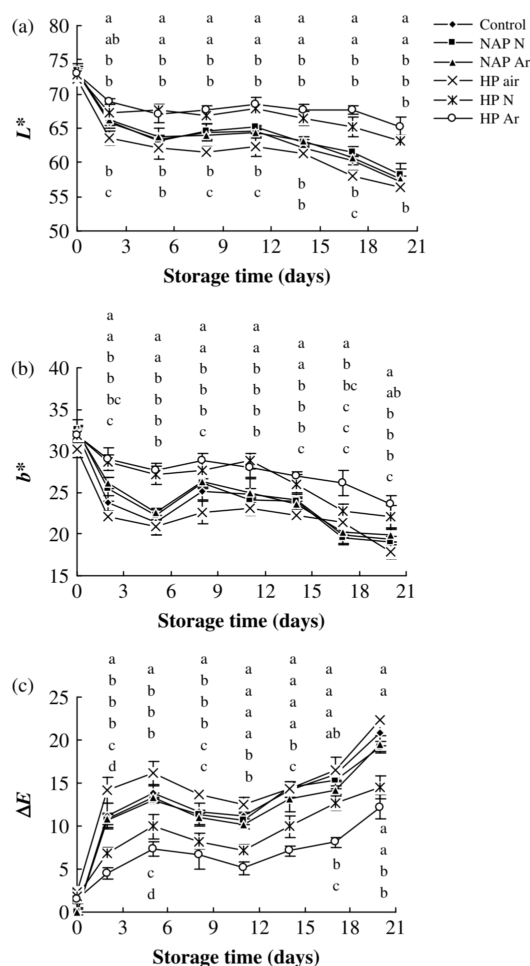

Figure 4. Changes (mean ± standard deviation) in colour parameters (a) L^* (lightness), (b) b^* (yellow chromaticity) and (c) ΔE (index of colour change) of MP pineapples of six treatments (see Fig. 2) during 20 days of storage at 4 °C. In each part, for each storage time, means with the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

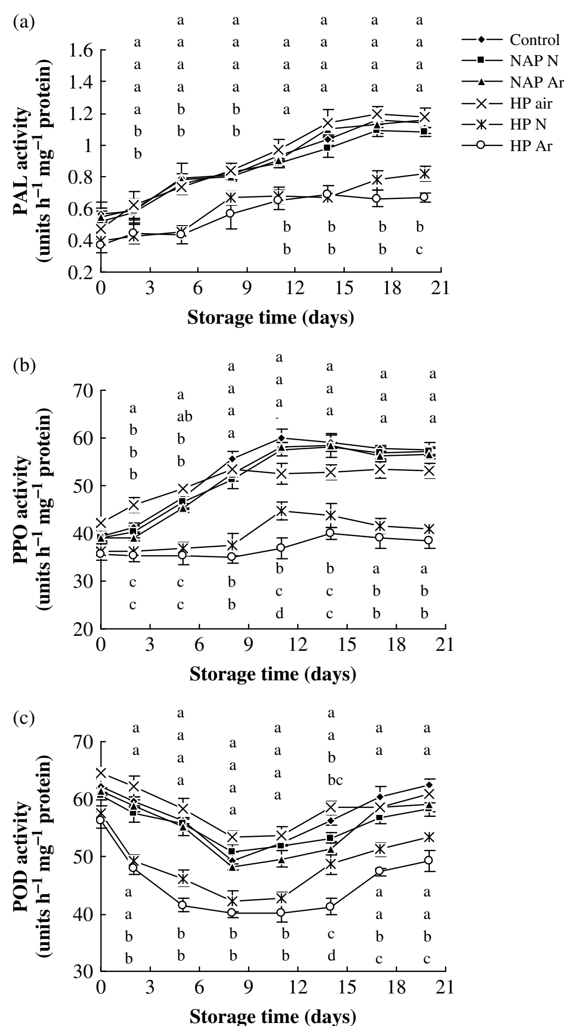

Figure 5. Changes (mean ± standard deviation) in (a) PAL, (b) PPO and (c) POD activities of MP pineapples of six treatments (see Fig. 2) during 20 days of storage at 4 °C. In each part, for each storage time, means with the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

micropores of pineapple wedges, which could restrain the activity of intracellular water and enzymes in the fruits and slow down the metabolism.[14,48] Another reason could be that HP Ar and HP N treatments rendered the MP pineapple tissues anaerobic and thereby reduced aerobic respiration and ethylene formation (which relies on oxygen). Further studies are needed to validate this hypothesis.

Browning evaluation

Changes in the colour parameters L^*, b^* and ΔE are shown in Fig. 4. In general, the colour of all samples became progressively browner and less pure yellow than that of recently cut fresh pineapples. A sharp decrease in L^* and b^* values and a marked increase in ΔE values were observed after 5 days at 4 °C. This phenomenon may be the result of phenolic oxidation, which is catalysed by PPO enzymes to form coloured melanins,[9] and elevated PPO activity could be induced by minimal processing.[49] After 5 days of shelf life, samples generally showed a slight increase in L^* and b^* values and decrease in ΔE values, which could be due to the surface dehydration of pineapple wedges that precedes the incidence of tissue senescence.[30] MP pineapples treated with HP Ar and HP N had higher L^* and b^* values and lower ΔE values than samples untreated and treated with HP air, NAP N or NAP Ar throughout the storage time. This suggested that HP Ar and HP N treatments were efficient in preventing pineapple wedge surfaces from browning at 4 °C. There was no difference between HP Ar and HP N treatments in colour retention of MP pineapples over the storage time.

PAL, PPO and POD play an important role in the browning process of many fruits and vegetables. PAL is a key enzyme of polyphenol synthesis and acts on the conversion of L-phenylalanine to *trans*-cinnamic acid in the phenylpropanoid pathway.[50] Browning reactions are generally assumed to be a direct consequence of PPO and POD actions on polyphenols to form quinones, which ultimately polymerise to produce the browning appearance of MP fruit and vegetable products.[51] It is well known that wounding induces increased enzyme activity. The effects of different treatments on PAL, PPO and POD activities of MP pineapple wedges are presented in Fig. 5. These activities showed different tendencies individually, with different rates of change over the storage time. PAL activity increased continuously until the end of storage (Fig. 5(a)), in agreement with the results

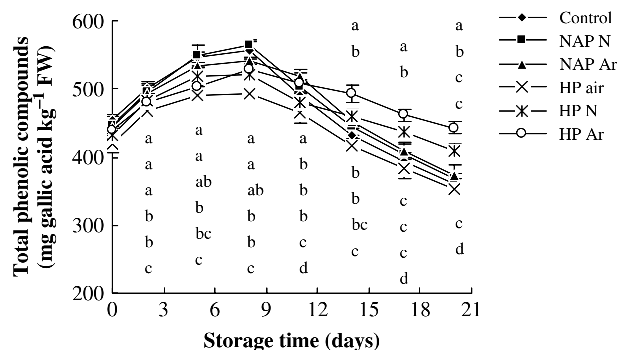

Figure 6. Changes (mean ± standard deviation) in total phenolic compound content of MP pineapples of six treatments (see Fig. 2) during 20 days of storage at 4 °C. For each storage time, means with the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

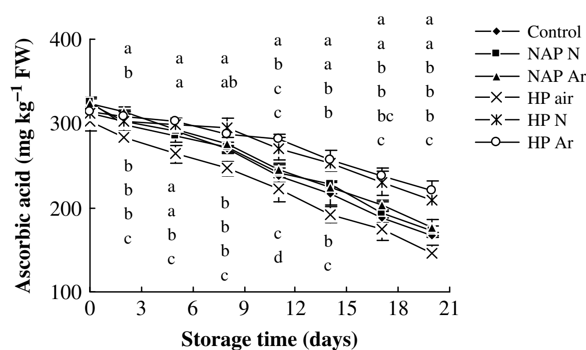

Figure 7. Changes (mean ± standard deviation) in ascorbic acid content of MP pineapples of six treatments (see Fig. 2) during 20 days of storage at 4 °C. For each storage time, means with the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

of Zhou *et al.*[31] for pineapple fruits stored at 6, 13 and 18 °C. Increases in PAL activity in several fruits and vegetables, including pineapple, are induced by cold temperatures.[52–55] PPO activity showed a clear increase from 5 to 11 days and then remained stable (Fig. 5(b)). POD activity decreased during the first 8 days of storage and then increased (Fig. 5(c)). HP Ar and HP N treatments inhibited PAL, PPO and POD activities of MP pineapples at 4 °C. Similar results were obtained by Zhan and Zhang[48] for cucumber with compressed Xe treatment. These results could possibly be attributed to two reasons. One is that HP Ar and HP N treatments may form gas hydrates in pineapple tissues, which will reduce the water activity in the fruit tissue and influence the protein structure of enzymes, so enzyme activity is restrained.[48] Zhang *et al.*[16] reported that Ar and N_2 can inhibit the activities of tyrosinase and malic dehydrogenase. Zhang *et al.*[14] reported that Ar and Xe remained in the structure as micropores after pressurised Ar and Xe treatments, suggesting a positive connection for keeping asparagus spears fresh. Another reason may be that residual Ar or N_2 in micropores of pineapple wedge tissues suppressed enzyme activity after HP Ar or HP N treatment.

Antioxidant potential

Total phenol and ascorbic acid contents

Polyphenolic compounds are very important fruit constituents because of their antioxidant activity in chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxyradicals.[56] The total phenol content of MP pineapples stored at 4 °C for 20 days is presented in Fig. 6. Total phenol content for all treatments increased during the first few days of storage, reached a maximum after 8 days and then decreased during the later period of storage, in agreement with the results of Zhu *et al.*[50] for peach slices. The accumulation of phenolic compounds during the first 8 days of storage may be promoted by PAL activity, which is induced by wounding in minimal processing and results in the production of major phenolic compounds and the synthesis of new polyphenolic substances.[57] After 8 days of storage, with increasing tissue senescence of pineapple wedges, total polyphenol content decreased with the oxidation of PPO in the presence of oxygen.

The total phenolic compound content in control (untreated) wedges and those treated with HP air, HP Ar and HP N prior to storage was 454, 420, 439 and 433 mg gallic acid kg^{-1} FW respectively (Fig. 6). However, after 20 days of storage the total phenol content in HP N- and HP Ar-treated samples was 409 and 443 mg gallic acid kg^{-1} FW respectively, higher than in untreated samples (362 mg gallic acid kg^{-1} FW) and HP air-treated samples (353 mg gallic acid kg^{-1} FW). There was no difference in the total phenol content of control and NAP N- or NAP Ar-treated samples throughout the storage time. HP Ar and HP N treatments were effective in retaining the total phenolic content of pineapple wedges at 4 °C, with HP Ar treatment showing a greater effect than HP N treatment. This result can be attributed to the inhibitory effect of HP Ar or HP N treatment on the activity of enzymes related to phenol degradation, thus reducing the loss of total phenols in pineapple wedges at 4 °C.

Ascorbic acid is one of the most effective antioxidants in fruits and vegetables since it suppresses free radicals via the formation of ascorbyl radicals.[58] Its enediol structure plays an effective role in scavenging free radicals.[59]

Figure 7 shows that ascorbic acid content decreased with increasing storage time for all samples, which is due to ascorbic acid degradation through oxidative processes.[60] During 20 days at 4 °C, there was a high reduction in ascorbic acid in wedges treated with HP air (∼52%), a moderate reduction in wedges untreated (∼48%) and treated with NAP N (∼47%) and NAP Ar (∼46%) and a low reduction in wedges treated with HP N (∼33%) and HP Ar (∼30%), thus demonstrating that HP N and HP Ar treatments were effective in reducing the loss of ascorbic acid in MP pineapples. This differs from the results of Zhang *et al.*,[14] who reported that compressed (1.1 MPa absolute) Ar and Xe treatment had no effect on maintaining the ascorbic acid content of asparagus spears. Further studies are needed to explain the phenomenon. The highest reduction in ascorbic acid content found in HP air wedges may be due to HP treatment and oxygen in the air accelerating the oxidative degradation of ascorbic acid. NAP N and NAP Ar treatments had no significant effect on retaining the ascorbic acid content of pineapples wedges.

Total antioxidant capacity

The DPPH•- and ABTS•+-scavenging activities of MP pineapples during cold storage are presented in Figs 8(a) and 8(b) respectively. The DPPH and ABTS assays showed the same trends in total antioxidant capacity (TAC) of samples, in agreement with the results of Leong and Shui[35] for pineapple. The correlation between the DPPH and ABTS methods may result partly from their similar mechanism and also because both antioxidants are soluble in aqueous/ethanolic systems.[35] For all samples, TAC determined by

© 2012 Society of Chemical Industry

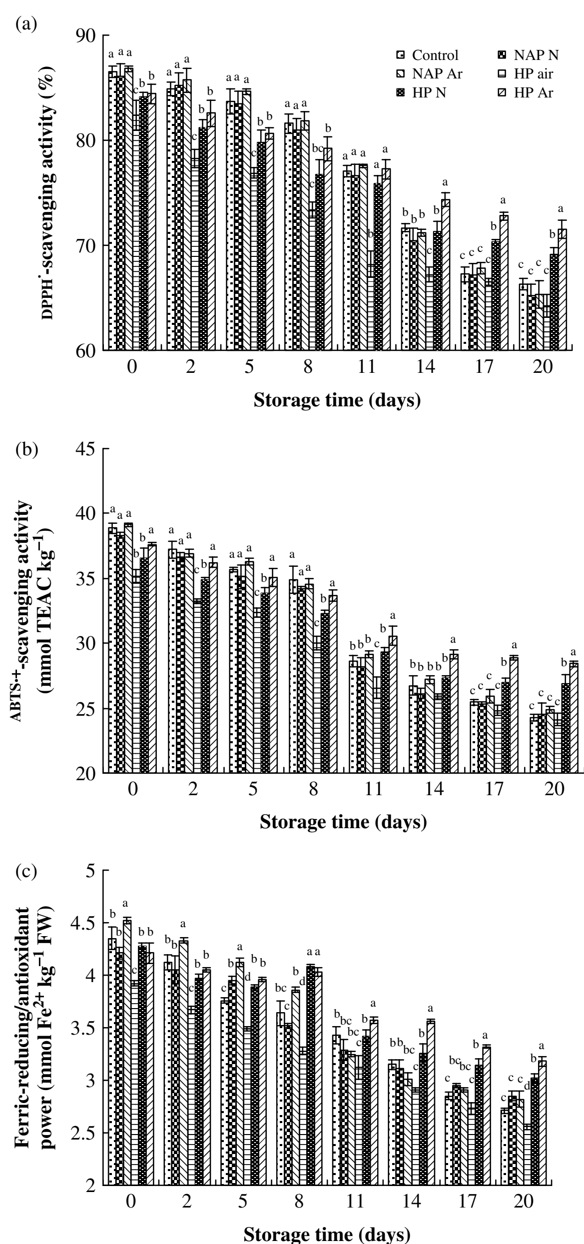

Figure 8. Changes (mean ± standard deviation) in antioxidant capacity of MP pineapples of six treatments (see Fig. 2) during 20 days of storage at 4 °C by (a) DPPH, (b) ABTS and (c) FRAP assays. In each part, for each storage time, means of bars with the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.

both methods decreased progressively over the storage time. After 8 days of storage the variation in TAC was relatively small, which could be related to the accumulation of phenolic compounds in fruit tissues (Fig. 6). A sharp variation in TAC of all samples was noticed after 8 days, which was more evident in samples untreated and treated with HP air and NAP N or NAP Ar, in correspondence with the rapid phenolic degradation (Fig. 6). However, during the first 8 days of storage, TAC did not show the same increasing trend as phenolic content. This suggested that phenols were the main antioxidants contributing to the TAC of MP pineapples determined by the DPPH and ABTS methods, but, besides phenolic compounds, other bioactive compounds such as ascorbic acid, thiols, carotenoids, anthocyanins, tocopherol and aromatic amino acids could be contributing to TAC. This implies a synergy of all antioxidants present, but further research is needed to validate this. Prior to storage, TAC of pineapple wedges treated with HP air, HP N and HP Ar was slightly less than that of untreated wedges (Figs 8(a) and 8(b)). However, from day 11, samples treated with HP Ar or HP N had higher TAC than samples untreated and treated with HP air, NAP N or NAP Ar. After 20 days at 4 °C the highest TAC was detected in HP Ar-treated samples (DPPH•- and ABTS•+-scavenging activities of 72% and 28 mmol TEAC kg^{-1} FW respectively), followed by HP N-treated samples (69% and 27 mmol TEAC kg^{-1} FW), control samples (66% and 24 mmol TEAC kg^{-1} FW) and samples treated with NAP N (65% and 25 mmol TEAC kg^{-1} FW) or NAP Ar (65% and 25 mmol TEAC kg^{-1} FW) and HP air (64% and 24 mmol TEAC kg^{-1} FW). The results indicated that HP Ar or HP N treatment could reduce the degradation of TAC of pineapple wedges during cold storage, probably due to Ar or N hydrate formation and residual gas in micropores of fruit tissues inhibiting the enzymes related to antioxidant degradation. HP Ar treatment was more effective than HP N treatment in the retention of TAC over the entire storage period.

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine complex (Fe^{3+}–TPTZ) and producing a coloured ferrous tripyridyltriazine complex (Fe^{2+}–TPTZ).[47] The FRAP values of MP pineapples during cold storage are shown in Fig. 8(c). The ferric-reducing power of all samples decreased with increasing storage time. The ferric-reducing power of samples untreated and treated with HP air decreased more rapidly from day 5 than that of samples treated with HP N and HP Ar, and this phenomenon could possibly be correlated with the loss of ascorbic acid content of MP pineapples (Fig. 7). After 20 days at 4 °C, samples treated with HP Ar or HP N were found to have higher ferric-reducing power than samples untreated and treated with NAP N or HP Ar and HP air. This suggested that HP Ar or HP N treatment could retard the decline in ferric-reducing power of MP pineapples during cold storage. No significant difference in ferric-reducing power was observed between samples treated with HP Ar and HP N.

Firmness and juice leakage

The changes in firmness and juice leakage of MP pineapples of different treatments during 20 days of storage at 4 °C are presented in Table 1. The firmness of pineapple wedges of all treatments remained unchanged over time (Table 1). This observation coincided with the appearance of the wedges, which kept their shape and size throughout the 20 days at 4 °C. Similarly, Gil *et al.*[61] found that the firmness (3 mm tip penetration test) of whole and MP pineapples of Tropical Gold cultivar did not change after 9 days of storage at 5 °C, and Montero-Calderón *et al.*[27] reported no differences in the texture profile analysis parameters of MP pineapples throughout 20 days of storage at 5 °C. The firmness of samples after HP treatments decreased slightly compared with that of samples without HP treatment, but no difference was observed in the firmness of those samples during the storage period (Table 1). The volume of juice that leaked from pineapple wedges of all treatments increased significantly over the storage time (Table 1). There was no difference in accumulated juice leakage inside the container between control and HP N- or HP Ar-treated samples. The results therefore showed that HP N and HP Ar treatments had no impact on tissue firmness and juice leakage of MP pineapples during 20 days at 4 °C.

Table 1. Changes in tissue firmness and juice leakage of MP pineapples of six treatments during 20 days of storage at 4 °C

Days	Control	NAP N	NAP Ar	HP air	HP N	HP Ar
Tissue firmness (N)						
0	23.46 ± 1.23a	22.25 ± 2.72a	21.51 ± 2.91a	18.42 ± 3.25ab	18.37 ± 5.14ab	19.25 ± 2.42a
2	21.35 ± 2.15a	20.54 ± 2.16a	19.37 ± 5.51a	18.23 ± 4.87ab	19.58 ± 3.73a	18.78 ± 5.26ab
5	22.81 ± 3.52a	21.39 ± 1.93a	20.31 ± 5.13a	20.34 ± 3.71a	17.28 ± 2.56b	18.46 ± 2.69ab
8	22.54 ± 1.93a	20.82 ± 3.74a	22.65 ± 4.25a	16.78 ± 2.41b	19.55 ± 4.33a	19.21 ± 4.53a
11	21.63 ± 2.74a	19.35 ± 5.58a	23.18 ± 3.17a	20.16 ± 2.96a	18.69 ± 5.22ab	20.19 ± 2.78a
14	20.45 ± 4.32a	23.15 ± 2.49a	22.12 ± 2.72a	18.51 ± 6.53ab	20.61 ± 3.18a	18.24 ± 1.94ab
17	19.37 ± 3.27a	18.72 ± 3.36ab	21.79 ± 2.53a	19.12 ± 4.32a	20.25 ± 3.78a	21.33 ± 5.36a
20	20.16 ± 2.53a	22.53 ± 2.25a	20.85 ± 4.86a	18.73 ± 1.93ab	19.12 ± 3.65a	20.13 ± 3.67a
Juice leakage (mL kg^{-1} FW)						
0	0	0	0	0	0	0
2	3.5 ± 0.5g	3.3 ± 0.6g	3.4 ± 0.4g	5.3 ± 0.3g	5.6 ± 0.4g	4.7 ± 0.5g
5	7.3 ± 0.8f	7.5 ± 0.5f	7.1 ± 0.5f	9.5 ± 1.4f	8.1 ± 0.5f	8.8 ± 1.2f
8	15.8 ± 0.5e	15.3 ± 1.1e	14.8 ± 1.7e	17.2 ± 1.8e	16.5 ± 1.5e	16.2 ± 1.3e
11	23.7 ± 1.3d	21.9 ± 1.8d	22.9 ± 1.8d	24.1 ± 1.5d	23.7 ± 2.1d	24.5 ± 2.5d
14	34.2 ± 2.5c	33.6 ± 2.1c	31.7 ± 1.5c	36.7 ± 2.3bc	35.2 ± 1.9c	34.3 ± 1.7c
17	42.1 ± 3.4b	40.8 ± 1.7b	39.4 ± 3.2b	41.9 ± 1.5b	42.8 ± 2.2b	41.6 ± 3.8b
20	48.5 ± 1.6a	43.7 ± 2.9b	45.6 ± 2.7ab	51.2 ± 3.6a	47.9 ± 2.8a	45.2 ± 2.3ab

Control, untreated pineapple wedges; NAP N, pineapple wedges treated with atmospheric-pressure nitrogen for 20 min; NAP Ar, pineapple wedges treated with atmospheric-pressure argon for 20 min; HP air, pineapple wedges treated with high-pressure (10 MPa) air for 20 min; HP N, pineapple wedges treated with high-pressure (10 MPa) nitrogen for 20 min; HP Ar, pineapple wedges treated with high-pressure (10 MPa) argon for 20 min. Data shown are mean ± standard deviation. For each parameter, different letters denote significant differences ($P < 0.05$) between means according to Duncan's multiple range test.

CONCLUSIONS

Both HP Ar and HP N treatments (10 MPa, 20 min) reduced the respiration rate and ethylene production in MP pineapples during 20 days of storage at 4 °C. They also inhibited the activity of PAL, PPO and POD enzymes and efficiently maintained surface brightness and good visual appearance of MP pineapples during shelf life. Beneficial effects of HP Ar and HP N treatments on TAC were expressed as reduced degradation of phenolic compounds and ascorbic acid, increased DPPH$^\bullet$- and ABTS$^{\bullet+}$-scavenging activities and higher ferric-reducing power. Moreover, HP Ar and HP N treatments did not influence tissue firmness and juice leakage of MP pineapples throughout 20 days at 4 °C.

The results of this study suggest that combining short-time HP application with Ar or N could be a promising method for preserving MP pineapples and probably other fruits. Further studies could include the effects of such treatments on microbial stability, sensory characteristics, etc. so as to provide a new technology for further extending the shelf life of MP products.

ACKNOWLEDGEMENT

The authors thank the National Natural Science Foundation of China for supporting this research under contract 30972058.

REFERENCES

1 Del Nobile MA, Conte A, Scrocco C and Brescia I, New strategies for minimally processed cactus pear packaging. *Innovat Food Sci Emerg Technol* **10**:356–362 (2009).

2 Oms-Oliu G, Odriozola-Serrano I, Soliva-Fortuny R and Martín-Belloso O, The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres. *Food Chem* **106**:1085–1092 (2008).

3 Wiley RC, Preservation methods for minimally processed refrigerated fruits and vegetables, in *Minimally Processed Refrigerated Fruits and Vegetables*, ed. by Wiley RC. Chapman and Hall, New York, NY, pp. 66–134 (1994).

4 Soliva-Fortuny RC and Martín-Belloso O, New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends Food Sci Technol* **14**:341–353 (2003).

5 Artés F, Gómez P, Aguayo E, Escalona V and Artés-Hernández F, Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharv Biol Technol* **51**:287–296 (2009).

6 Bico SLS, Raposo MFJ, Morais RMSC and Morais AMMB, Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh-cut banana. *Food Control* **20**:508–514 (2009).

7 Mortan JF, Pineapple, in *Fruits of Warm Climates*, ed. by Mortan JF. Florida Flair Books, Miami, FL, pp. 18–28 (1987).

8 Saxena S, Mishra BB, Chander R and Sharma A, Shelf stable intermediate moisture pineapple (*Ananas comosus*) slices using hurdle technology. *LWT – Food Sci Technol* **42**:1681–1687 (2009).

9 Budu AS and Joyce DC, Effect of 1-methylcyclopropene on the quality of minimally processed pineapple fruit. *Aust J Exp Agric* **43**:177–184 (2003).

10 Marrero A and Kader AA, Optimal temperature and modified atmosphere for keeping quality of fresh-cut pineapples. *Postharv Biol Technol* **39**:163–168 (2006).

11 Antoniolli LR, Benedetti BC, Sigrist JMM and Silveira NFA, Quality evaluation of fresh-cut 'Pérola' pineapple stored in controlled atmosphere. *Ciênc Technol Alim Camp* **27**:530–534 (2007).

12 Jamie P and Saltveit ME, Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen. *Postharv Biol Technol* **26**:113–116 (2002).

13 Nasar-Abbas SM, Plummer JA, Siddique KHM and White PF, Nitrogen retards and oxygen accelerates colour darkening in faba bean (*Vicia faba* L.) during storage. *Postharv Biol Technol* **47**:113–118 (2008).

14 Zhang M, Zhan ZG, Wang SJ and Tang JM, Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. *LWT – Food Sci Technol* **41**:686–691 (2008).

15 Spencer KC, The use of argon and other noble gases for the MAP of foods, in *Modified Atmosphere Packaging (MAP) and*

Related Technologies (Conference Proceedings) (September 1995), UK. Campden and Chorleywood Food Research Association, Chipping Campden, pp. 6–7 (1995).

16 Zhang DL, Quantick PC, Grigor JM and Wiktorowicz R, A comparative study of effects of nitrogen and argon on tyrosinase and malic dehydrogenase activities. *Food Chem* **72**:45–49 (2001).

17 Kvenvolden KA, Gas hydrates – geological perspective and global change. *Rev Geophys* **31**:173–187 (1993).

18 Bishnoi PR and Natarajan V, Formation and decomposition of gas hydrates. *Fluid Phase Equil* **00**:168–177 (1996).

19 Gbaruko BC, Igwe JC, Gbaruko PN and Nwokeoma RC, Gas hydrates and clathrates: flow assurance, environmental and economic perspectives and the Nigerian liquified natural gas project. *J Petrol Sci Eng* **56**:192–198 (2007).

20 Davidson DW, Clathrate hydrates, in *Water: a Comprehensive Treatise*, ed. by Frank F. Plenum, New York, NY, pp. 115–234 (1973).

21 Gaarder C and Englezos P, The use of clathrate hydrates for the concentration of mechanical pulp mill. *Nordic Pulp Pap Res J* **2**:, 144 110–113 (1995).

22 Makogon YF, Natural gas hydrates – a promising source of energy. *J Nat Gas Sci Eng* **2**:49–59 (2010).

23 Belosludov VR, Inerbaev TM, Belosludov RV, Kudoh J and Kawazoe Y, Absolute stability boundaries of clathrate hydrates of cubic structure. II. *J Supramol Chem* **2**:377–383 (2002).

24 Behnke EA, Enzyme-catalysed reactions as influenced by inert gases at high pressures. *J Food Sci* **34**:370–375 (1969).

25 Fujii K, Ohtani A, Watanabe J, Ohgoshi H, Fujii T and Honma K, High-pressure inactivation of *Bacillus cereus* spores in the presence of argon. *Int J Food Microbiol* **72**:239–242 (2002).

26 Oshita S, Seo Y and Kawagoe Y, Relaxation time of protons in intracellular water of broccoli. *Proc. CIGR Int. Symp.*, Kyoto, pp. 77–82 (2000).

27 Montero-Calderón M, Rojas-Graü MA and Martín-Belloso O, Effect of packaging conditions on quality and shelf-life of fresh-cut pineapple. *Postharv Biol Technol* **50**:182–189 (2008).

28 Chung HS and Moon KD, Browning characteristics of fresh-cut 'Tsugaru' apples as affected by pre-slicing storage atmospheres. *Food Chem* **114**:1433–1437 (2009).

29 Sapers GM and Douglas Jr FW, Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. *J Food Sci* **52**:1285–1261 (1987).

30 González-Aguilar GA, Ruiz-Cruz S, Cruz-Valenzuela R, Rodriguez-Félix A and Wang CY, Physiological and quality changes of fresh-cut pineapple treated with antibrowning agents. *LWT – Food Sci Technol* **37**:369–376 (2004).

31 Zhou YC, Dahler JM, Underhill SJR and Wills RBH, Enzymes associated with blackheart development in pineapple fruit. *Food Chem* **80**:565–572 (2003).

32 Soares AG, Trugo LC and Botrel N, Reduction of internal browning of pineapple fruit (*Ananas comosus* L.) by preharvest soil application of potassium. *Postharv Biol Technol* **35**:201–207 (2005).

33 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* **72**:248–254 (1976).

34 Liu RH, Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *Am J Clin Nutr* **78**:517S–520S (2003).

35 Leong LP and Shui G, An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem* **76**:69–75 (2002).

36 Lako J, Trenerry VC, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S and Premier R, Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem* **101**:1727–1741 (2007).

37 Naczk M and Shahidi F, Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J Pharmaceut Biomed Anal* **41**:1523–1542 (2006).

38 Shahidi F and Naczk M, *Antioxidant Properties of Food Phenolics*. CRC Press, Boca Raton, FL, pp. 403–437 (2004).

39 Aguayo E, Requejo-Jackman C, Stanley R and Woolf A, Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices. *Postharv Biol Technol* **57**:52–56 (2010).

40 Lana MM and Tijskens LMM, Effects of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chem* **97**:203–211 (2006).

41 Alothman M, Bhat R and Karim AA, Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chem* **115**:785–788 (2009).

42 Singleton VL and Rossi Jr JA, Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *Am J Enol Vitic* **16**:144–158 (1965).

43 AOAC, *Official Methods of Analysis* (16th edn). Association of Official Analytical Chemists, Arlington, VA (1997).

44 Frankel NE and Meyer SA, The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J Sci Food Agric* **80**:1925–1941 (2000).

45 Mathew S and Abraham TE, Studies on antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various *in vitro* models. *Food Chem* **94**:520–528 (2006).

46 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* **26**:1231–1237 (1999).

47 Benzie IFF and Strain JJ, The ferric reducing ability of plasma as a measure of 'antioxidant power', the FRAP assay. *Anal Biochem* **239**:70–76 (1996).

48 Zhan ZG and Zhang M, Effects of inert gases on enzyme activity and inspiration of cucumber. *J Food Biotechnol* **24**:16–18 (2005).

49 Rolle RS and Chism GW, Physiological consequences of minimally processed fruits and vegetables. *J Food Qual* **10**:157–177 (1987).

50 Zhu LQ, Zhou J, Zhu SH and Guo LH, Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. *Food Chem* **114**:174–179 (2009).

51 Chen Z, Zhu CH, Zhang Y, Niu DB and Du JH, Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L.). *Postharv Biol Technol* **58**:232–238 (2010).

52 Graham D and Paterson BD, Responses of plants to low, nonfreezing temperatures: proteins, metabolism and acclimation. *Annu Rev Plant Physiol* **33**:347–372 (1972).

53 Paull RE and Rohrbach KG, Symptom development of chilling injury in pineapple fruit (*Ananas comosus*). *J Am Soc Hort Sci* **110**:100–105 (1985).

54 Vukomanovic CR, Effect of ripening and low temperature on chemical composition and internal browning of pineapple. *Food Science Master Thesis*, ESAL, Lavras (1988).

55 Abreu CMP, Effect of polyethylene packing and refrigeration on internal browning and chemical composition during ripening of pineapple cv. Smooth Cayenne. *PhD Thesis*, UFLA, Lavras (1995).

56 Oliveir AC, Valentim IB, Silva CA, Bechara EJH, Barros MP, Mano CM, *et al*, Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. *Food Chem* **115**:469–475 (2009).

57 Caro AD, Piga A, Vacca V and Agabbio M, Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. *Food Chem* **84**:99–105 (2004).

58 Yamaguchi F, Yoshimura Y, Nakazawa H and Ariga A, Free radical scavenging activity of grape seed extract and antioxidants by electron spin resonance spectrometry in an H_2O_2/NaOH/DMSO system. *J Agric Food Chem* **47**:2544–2548 (1999).

59 Darkwa J, Mundoma C and Simoti RH, Antioxidant chemistry reactivity and oxidation of DL cysteine by some common oxidants. *J Chem Soc Faraday Trans* **94**:1971–1978 (1998).

60 Davey MW, Van Montagu M, Inzé D, Sanmartin M, Kanellis A and Smirnoff N, Plant L-ascorbic: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric* **80**:825–860 (2000).

61 Gil MI, Aguayo E and Kader AA, Quality changes and nutrient retention in fresh-cut *versus* whole fruits during storage. *J Agric Food Chem* **54**:4284–4296 (2006).

 © 2012 Society of Chemical Industry