INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Int. Agrophys., 2013, 27, 75-79
doi: 10.2478/v10247-012-0070-2

Mixed noble gas effect on cut green peppers**

L.V. Raymond[1], *M. Zhang*[1,2]*, *E. Karangwa*[1], *and M.J. Chesereka*[2]

[1]State Key Laboratory of Food Science and Technology, [2]School of Food Science and Technology
Jiangnan University, 214122Wuxi, Jiangsu, China

Received May 1, 2012; accepted August 8, 2012

A b s t r a c t. Increasing attempts at using gas which leads to hydrate formation as a preservative tool in fresh-cut fruits and vegetables have been reported. In this study, changes in some physical and biochemical properties of fresh-cut green peppers under compressed noble gas treatments were examined. Mixed argon-krypton and argon treatments were performed before cold storage at 5°C for 15 days. Mass loss and cell membrane permeability were found to be the lowest in mixed argon-krypton samples. Besides, a lower CO_2 concentration and vitamin C loss were detected in gas-treated samples compared to untreated samples (control). While the total phenol degradation was moderately reduced, the effect of the treatment on polyphenoloxidase activity was better at the beginning of the storage period. The minimum changes in quality observed in cut peppers resulted from both mixed and gas treatment alone.

K e y w o r d s: fresh produce, noble gas mixture, gas hydrates, cold storage, physical and biochemical properties

INTRODUCTION

One of the major advances reached to preserve and to prolong shelf life of fresh fruits and vegetables includes the use of noble gas (Zhang *et al*., 2001). When noble gas is compressed in water with appropriate conditions of noble gas, water, pressure and temperature, ice-like gas hydrates can be formed (Makogon, 2010). In some fruits and vegetables, argon, krypton and xenon were reported to form gas hydrates (Zhang *et al*., 2008); they can be formed by application of compressed gas which by its dissolution induces hydrophobic hydration resulting in formation of both structured water and gas hydrate. The use of a noble gas mixture was attempted in order to preserve foods. Under such treatments, restrained metabolism of vegetables resulted in extended shelf life observed during cold storage (Zhan *et al*., 2005; Zhang *et al*., 2008). Quality of a product is related to its metabolism. Once water molecules become more structured due to increased hydrogen bonds, viscosity also increases resulting in a reduced enzymatic reaction rate, as well as limited water evaporation. Fresh-cut fruits and vegetables are in general more susceptible to rapid deterioration, which is influenced by the preparation process and associated with a reduced shelf-life compared to whole fresh fruits and vegetables (Conesa *et al*., 2007). Pepper fruits (*Capsicum annuum* L.) are appreciated for their appearance, taste and nutrients as well as for their health benefits (Raffo *et al*., 2007). However, low resistance to physical and compositional changes occurs during storage period by means of water loss, decay development, chilling injury (Maalekuu *et al*., 2003; Raffo *et al*., 2007). Besides, changes in membrane and enzymatic and nonenzymatic oxidation alter cellular properties such as delocalization of cell components and ion leakage. Researches on the effect of application of noble gas as part of storage condition of fresh-cut products are acknowledged. In the present study, compressed noble gas, in a mixture or alone, was used to treat fresh-cut green peppers.

The purpose of this study is to evaluate the effect of mixed argon and krypton pretreatment on cold preservation of cut green peppers based on the respiration rate, enzyme activity, and cell membrane permeability during storage.

MATERIALS AND METHODS

Peppers, *Capsicum annuum* L. were harvested from a commercial farm in Wuxi, Jiangsu, China. Pepper slices obtained from washed edible portions of the fruits were subjected to treatments including:

*Corresponding author e-mail: min@jiangnan.edu.cn
**This work was supported by the China National Natural Science Foundation under contract No. 30972058, 2010-2013.

– control: untreated;
– compressed mixed argon and krypton at room temperature at 2 MPa, 2 h (mixed argon-krypton, MG);
– compressed argon gas at room temperature at 2 MPa, 2 h (argon, AG).

Samples (150 g) from the control, MG and AG treatments were placed in a glass container, tightly wrapped with polyethylene film (oxygen permeability 1.14 10^{-12} mol s^{-1} mm^{-2} kPa), carbon dioxide permeability 3.06 10^{-14} mol s^{-1} mm^{-2} kPa, and stored at 5°C for 15 days.

Compressed gas treatments were applied using a compressing machine HCYF-3 (Hunan Scientific Research Instrument, Jiangsu, China). The samples were loaded in the treatment vessel, tightly closed, and the gas was vacuum pumped to the vessel to the desired pressure and time.

Carbon dioxide (CO_2) changes in the container containing ambient air as initial atmosphere were analyzed using a Gas Analyzer Cyes-II (Jiading Federation Instrument, Shanghai, China) in gas samples withdrawn with a 20 ml syringe and injected into the instrument. The percentage of CO_2 accumulation was calculated taking into account the initial concentration.

Samples mass loss, expressed as % fresh basis (w.b.), was calculated taking into account the former mass.

In order to evaluate the sample membrane leakage, diced samples (10 g) were washed, 50 ml distilled water was added, incubated at 30°C, and electrical conductivity of the solution was measured at 10 min of incubation using a conductivity meter (DDS-11A, Shanghai Huaguang Co., Shanghai, China). Conductivity of the solution was measured again after immersion in boiling water for 15 min (after cooling at 30°C). Electrolyte leakage was then calculated from the electrical conductivity ratio of the pre- and post-heated solution (Zhang *et al.*, 2008).

The vitamin C content was determined by means of the iodine titration method (AOAC, 1999), according to a standard ascorbic acid solution (1 mg ml^{-1}). The results were expressed as mg 100 g^{-1} wet basis (w.b.).

Polyphenoloxidase (PPO, E.C. 1.14.18.1) activity was determined spectrophotometrically using catechol as a substrate at pH 7 with a UV-visible spectrophotometer (Precision Science Instrument Co., Ltd., Shanghai, China) set at 410 nm, for 200 s, 25°C (Arnnok *et al.*, 2010). One unit of PPO was defined as the amount of the enzyme present in the extract that resulted in an absorbance increase of 0.001 U min^{-1}. The activity was expressed in units of PPO per minute and gram (U min^{-1} g^{-1}) of wet basis (w.b.).

The total phenolic content (TPC) was quantified by the Folin-Ciocalteu technique (Singleton and Rossi, 1965) using an acetonic extract from powdered plant material. The mixture (extract, Folin-Ciocalteu reagent, sodium bicarbonate) was incubated at 45°C for 30 min and absorbance was measured at 760 nm with a UV-spectrophotometer (Precision Science Instrument, Shanghai, China). The results were expressed as mg gallic acid equivalents (GAE) g^{-1} dry basis (d.b.).

The analyses were replicated three times; the data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test ($p \leq 0.05$) using the SPSS 16 statistical software (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Detached from the original plant, fruits and vegetables continue respiration that is enhanced especially after cutting. As it can be seen in Table 1, the compressed gas treatments influenced the CO_2 production of the cut green peppers during the storage period. The MG and AG samples revealed the lowest CO_2 concentration ($p \leq 0.05$) when the data were compared with those of the control samples. A steady increase in gas production by the MG and AG samples was observed during the first five days, and their respective

Table 1. Parameters of cut green peppers as affected by different treatments

Storage time (days)	Control	MG	AG
	CO_2 concentration (%)		
3	1.43 ± 0.15^A	0.06 ± 0.06^B	0.20 ± 0.00^B
5	2.03 ± 0.11^A	0.33 ± 0.06^B	0.23 ± 0.06^B
10	2.70 ± 0.12^A	1.17 ± 0.06^C	1.53 ± 0.06^B
15	3.87 ± 0.06^A	2.43 ± 0.12^C	2.63 ± 0.12^B
	Mass loss (% w.b.)		
3	3.32 ± 0.51^A	1.88 ± 0.12^B	1.45 ± 0.22^B
5	4.27 ± 0.25^A	2.62 ± 0.23^B	2.99 ± 0.11^B
10	5.16 ± 0.19^A	3.81 ± 0.19^C	4.23 ± 0.13^B
15	7.45 ± 0.22^A	4.93 ± 0.22^C	5.72 ± 0.17^B
	TPC (mg GAE g^{-1} d.b.)		
0	2.88 ± 0.09^A	$2.88 \pm 0.0\ 9^A$	2.89 ± 0.09^A
3	2.75 ± 0.03^B	2.88 ± 0.04^A	2.85 ± 0.00^A
5	2.69 ± 0.00^C	2.82 ± 0.00^A	2.81 ± 0.01^B
10	2.68 ± 0.00^B	2.81 ± 0.00^A	2.81 ± 0.02^A
15	2.60 ± 0.05^B	2.78 ± 0.02^A	2.74 ± 0.00^A
	PPO (U min^{-1} g^{-1} w.b.)		
0	6.43 ± 0.15^A	6.43 ± 0.15^A	6.43 ± 0.15^A
3	6.87 ± 0.35^A	6.00 ± 0.46^B	6.05 ± 0.23^B
5	6.63 ± 0.15^A	5.96 ± 0.42^A	5.92 ± 0.40^A
10	5.73 ± 1.27^A	5.57 ± 0.29^A	5.77 ± 0.50^A
15	5.58 ± 0.08^A	5.17 ± 0.40^A	5.28 ± 0.47^A

Control – no treatment, MG – mixed gas, AG – argon gas, TPC – total phenolic content, PPO – polyphenoloxidase activity. The results are mean ± standard deviation (n = 3). Values with different superscript letters in a row are significantly different ($p \leq 0.05$).

maximum CO_2 concentration recorded at the end of the storage period (2.43% and 2.63% for MG and AG samples, respectively) was reached within ten days in the control samples, which was related to microbiological decay. While there was no clear distinction of CO_2 concentration between the MG and AG samples at the beginning, indicating that the mixed gas treatment did not affect the respiration rate, the MG samples were characterized with a lower respiratory rate than those treated with argon gas alone when the storage period was extended. Keeping product freshness can be done when respiration is restrained, which was exhibited by the gas-treated cut peppers, probably due to structurization of water molecules (Zhan *et al.*, 2005). The reduced respiration rate could be due to formation of gas hydrates increasing the number of hydrogen bonds and limiting water to evaporate through respiration. In previous studies, the respiration rate of cucumber or pineapple slices was also maintained lower than that of control during the storage period when they were treated with a mixture of noble gases (Wu *et al.*, 2012a; Zhan *et al.*, 2005). The current result indicates similarly that compressed noble gas treatments, mixed or alone, effectively reduced the respiration rate of the cut peppers.

In fresh products, preventing the moisture loss can lengthen the shelf life. The percentage of mass loss observed for the differently treated samples is presented in Table 1. The control samples exhibited a consistently higher level of mass loss in comparison to both gas-treated samples. On the other hand, from the tenth day of storage, significantly lower mass loss ($p \leq 0.05$) was observed for the MG samples compared to the AG samples. The present maximum mass loss of the MG samples (4.93%) was in the range of permissible mass loss (5%) reported in the literature for pepper fruits (Sakaldas and Kaynas, 2010; Zhang *et al.*, 2012). The fact that the cut peppers exposed to the gas treatment preserved their mass can be due to minimum changes, allowing better, fresh appearance. Moreover, the activity of restricted water mole- cules confined by hydrogen bonds during gas hydrate for- mation can decrease the loss of water and consequently can maintain the lower rate of mass loss.

During storage of fruits and vegetables, membrane permeability increases, and the outward spreading of ions in the cell can thus be used to characterize the cell membrane permeability changes. Figure 1a shows the effect of the different treatments on the permeability changes of the cut green peppers. An increment in the electro leakage ratio with increasing storage time was observed, which is related to quality degradation. However, the gas treatments were able to constrain the increased permeability, compared with the control samples, which presented significantly higher permeability ($p \leq 0.05$). The final percentage of permeability was in the range of 22.47 to 41.42%, the samples exposed to mixed gas and argon gas alone treatments presented the lowest values. Comparison of the data of both compressed gas-treated samples demonstrated a significant difference ($p \leq 0.05$) in the electro-leakage ratio between the MG samples and AG samples from the fifth day, the results showed similarity to the results of mass loss. The relatively lower permeability of the gas-treated samples indicated minimum changes in the cell membrane; thus, they can keep better quality. In a previous study, exposing cucumber to a treatment with a noble gas mixture was found to reduce the cell membrane permeability, and electro-leakage underwent a steady increase (Zhan *et al.*, 2005). In measuring the membrane permeability of asparagus spears during cold storage, the increase in mineral ion spreading was characterized by a lower rate (Zhang *et al.*, 2008).

One of the most effective antioxidant in fruit and vegetable is vitamin C, which plays an important role in scavenging of free radicals. The vitamin C content decreased significantly ($p \leq 0.05$) in all the samples during storage (Fig. 1b). Higher loss was detected in the control samples, while a progressive decrease occurred in the gas-treated samples during the first five days, and the vitamin C content remained higher in the treated samples towards the end of the storage period. In a previous study, it was observed that vitamin C in peppers was also influenced by treatment and was less affected by pressure treatment than heat treatment (Castro *et al.*, 2008). In the present study, vitamin C loss was noted with increasing storage time; however, the gas treatments were able to reduce the vitamin C degradation. It was found that 89.25 and 86.74% of the initial vitamin C content was kept in the MG and AG samples, respectively, by the end of storage, with the highest content observed in samples

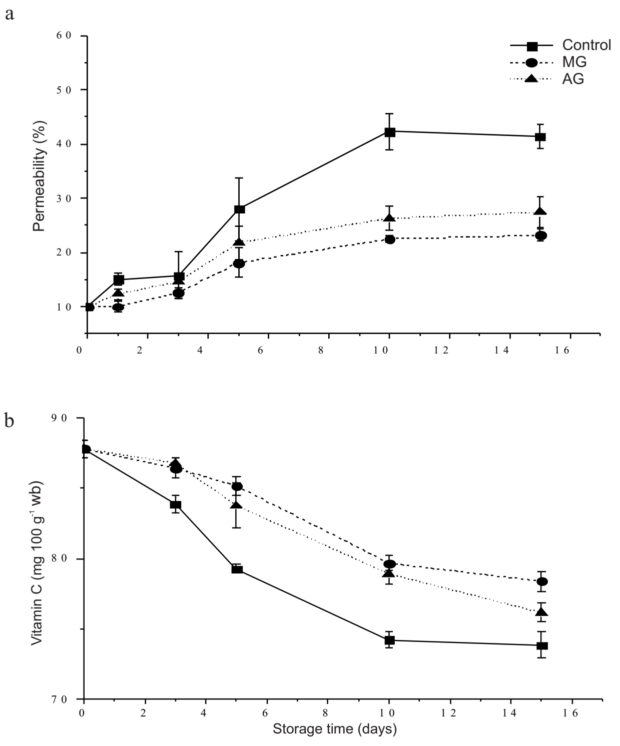

Fig. 1. Changes of: a – cell membrane permeability, b – vitamin C content of the cut peppers under the different treatments.

exposed to the mixed-gas treatment. The oxidation process can be reduced with oxygen receptor sites of enzymes (Spencer and Humphreys, 2003) inhibited by gas dissolution in water, allowing lower loss of vitamin C. On the other hand, the fact that hydrogen bonds increase through gas hydrate formation bound intracellular water; thus, the lower water loss can partially explain the moderate vitamin degradation rate.

The cut surface browning of fruits and vegetables during storage may be due to dehydratation or loss of pigments or due to enzymatic activities. As presented in Table 1, PPO activity of the cut peppers treated under gas treatments showed a progressive decrease; it increased on the third day followed by its decrease in the control samples, which can be related to tissue senescence. No significant effect of MG and AG on the observed PPO activity was observed compared to the control samples during the storage time. However, on the third day of storage, both treatments showed a significant difference compared to the control samples, where the lowest activity indicated reduced activity after both gas treatments. Therefore, the observed effect of gas hydrates has a better impact on the PPO activity during the early storage period. The inhibition of PPO, like other oxidases involved in several metabolic changes and reactions responsible for tissue browning can be achieved by the application of noble gas (Spencer and Humphreys, 2003). On the other hand, limited oxygen availability can influence the enzyme reaction rate, as well as dissolution of the gas than can induce a restrained metabolism rate. Additionally, the fact that PPO increased initially in the control samples can be partially explained by the sensitivity of enzyme due to decompartmentalization of cell components, mixing phenolics substrates with enzymes, and being more exposed to oxygen after cutting.

The inhibition of free radical reactions by antioxidants may protect cells against oxidative damage. Found in fruits and vegetables, phenolic compounds play an important antioxidant role (de Oliveira *et al*., 2009). Table 1 summarizes the total phenol content (TPC) of the cut green peppers as affected by the three treatments. The TPC of the gas-treated samples were consistently higher ($p \leq 0.05$) than that of the control. Marginal changes in the TPC were found during the first three days of storage of the MG and AG samples compared to the control. A significant effect of MG and AG on TPC appeared on the fifth day. However, there was no clear difference in the following decrease in TPC between the MG and AG samples. In measurements of the TPC, a drop of 4.38% of the initial content was observed on the third day in the control samples compared to 0.09 and 0.88% for the MG and AG samples respectively. When phenolics are freed during cutting, they can involve browning reaction once in contact with enzymes, which can partially explain the drop in TPC in the control samples. Besides, the decrease in the TPC can be related to tissue senescence and oxidation of PPO, but the treatments with argon gas and mixed gas were found to be effective in retaining the total phenolic content of the cut green peppers. Similarly, a moderate decrease in the TPC of fresh-cut apples or mushrooms occurred under noble gas treatment compared to the control (Lagnika *et al*., 2011; Wu *et al*., 2012b). It was reported that noble gas can inhibit some enzymes and influence their reaction rates (Spencer and Humphreys, 2003). The activity of enzymes related to phenol degradation could have been inhibited by the gas treatment, thus reducing the loss of total phenols.

CONCLUSIONS

1. The application of compressed noble gas treatments induced moderate changes during cold storage (5°C) of cut green peppers for 15 days.

2. It was found that in the mixed gas treated samples, mass loss and cell membrane permeability underwent minimum changes among all the samples. Compared to the compressed argon treatment alone, the mixture of gas treatment resulted in a similar outcome for the vitamin C content, total phenol content, and PPO activity.

3. In this study, both gas treatments allowed maintenance of better quality compared to the untreated cut peppers. Although the high cost of some noble gases may limit their application, further studies are needed in order to develop practical preservative tools for cut fruits and vegetables.

REFERENCES

AOAC, **1999**. Official Methods of Analysis. Gaithersburg, MD, USA.

Arnnok P., Ruangviriyachai C., Mahachai R., Techawongstien S., and Chanthai S., 2010. Optimization and determination of polyphenol oxidase and peroxidase activities in hot pepper (*Capsicum annuum* L.) pericarp. Int. Food Res. J., 17, 385-392.

Castro S.M., Saraiva J.A., Lopes-da-Silva J.A., Delgadillo I., Loey A.V., Smout C., and Hendrickx M., 2008. Effect of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits (*Capsicum annuum* L.). Food Chem., 107, 1436-1449.

Conesa A., Verlinden B.E., Artés-Hernández F., Nicolai B., and Artés F., 2007. Respiration rates of fresh-cut bell peppers under supertamospheric and low oxygen with or without high carbon dioxide. Postharvest Biol. Technol., 45, 81-88.

de Oliveira A.C., Valentim I.B., Silva C.A., Bechara E.J.H., Barros M.P.D., Mano C.M., and Goulart M.O.F., 2009. Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. Food Chem., 115, 469-475.

Lagnika C., Zhang M., and Wang S., 2011. Effect of high argon pressure and modified atmosphere packaging on the white mushroom (*Agaricus bisporus*) physico-chemical and microbiological properties. J. Food Nutr. Res., 50, 167-176.

Maalekuu K., Elkind Y., Tuvia-Alkalai S., Shalom Y., and Fallik E., 2003. Quality evaluation of three sweet pepper cultivars after prolonged storage. Adv. Hort. Sci., 17, 187-191.

Makogon Y.F., 2010. Natural gas hydrates - a promising source of energy. J. Natural Gas Sci. Eng., 2, 49-59.

Raffo A., Baiamonte I., Nardo N., and Paoletti F., 2007. Internal quality and antioxidants content of cold-stored red sweet peppers as affected by polyethylene bag packaging and hot water treatment. European Food Res. Technol., 225, 395-405.

Sakaldas M. and Kaynas K., 2010. Biochemical and quality parameters changes of green sweet bell peppers as affected by different postharvest treatments. African J. Biotechnol., 9, 8174-8181.

Singleton V.L. and Rossi J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enology Viticulture, 16, 144-158.

Spencer K.C. and Humphreys D.J., 2003. Argon packaging and processing preserves and enhances flavor, freshness, and shelf life of foods. Proc. ACS Symp. Series Freshness and Shelf Life of Foods, October 10, Middlesex, UK.

Zhan Z.G., Zhang M., and An J.-S., 2005. Effects of mixture of argon and xenon treatments on preservation of asparagus spear. J. Food Sci. Biotechnol., 24, 69-72.

Wu Z.S., Zhang M., and Adhikari B., 2012a. Application of high pressure argon treatment to maintain quality of fresh-cut pineapples during cold storage. J. Food Eng., 110, 395-404.

Wu Z.S., Zhang M., and Wang S., 2012b. Effects of high pressure argon treatments on the quality of fresh-cut apples at cold storage. Food Control, 23, 120-127.

Zhan Z.G., Zhang M., and An J.-S., 2005. Effects of mixture of argon and xenon treatments on preservation of asparagus spear. J. Food Sci. Biotechnol., 24, 69-72.

Zhang D.L., Quantick P.C., Grigor J.M., Wiktorowicz R., and Irven J., 2001. A comparative study of effects of nitrogen and argon on tyrosinase and malic dehydrogenase activities. Food Chem., 72, 45-49.

Zhang M., Zhan Z.G., Wang S.J., and Tang J.M., 2008. Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. LWT - Food Sci. Technol., 41, 686-691.

Zhang Peng, Zhang Min, Wang Shaojin, and Wu Zhishuang, 2012. Effect of 1-MCP treatment on post-harvest quality of green asparagus during cold storage. Int. Agrophys., 26, 407-411.