グルタチオン,アスコルビン酸,色素等を考慮したブロッコリーの最適包装設計

誌名	園藝學會雜誌
ISSN	00137626
著者名	石川,豊 Wessling,C. 平田,孝 長谷川,美典
発行元	園藝學會
巻/号	67巻3号
掲載ページ	p. 367-371
発行年月	1998年5月

園学雑. (J. Japan. Soc. Hort. Sci.) 67(3): 367-371. 1998.

Optimum Broccoli Packaging Conditions to Preserve Glutathione, Ascorbic Acid, and Pigments

Yutaka Ishikawa[1], Carolina Wessling[2], Takashi Hirata[3]
and Yoshinori Hasegawa[4]
[1]*National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Kannondai, Tsukuba, Ibaraki 305-8642*
[2]*Department of Applied Nutrition and Food Chemistry, University of Lund, Lund, Sweden*
[3]*Faculty of Agriculture, Kyoto University, Oiwake-cho, Kitashirakawa, Sakyo-ku, Kyoto 606-8224*
[4]*Shikoku National Agricultural Experiment Station, Ministry of Agriculture, Forestry and Fisheries, Ikuno-cho, Zentsuji, Kagawa 765-8508*

Summary

Changes in chlorophyll, carotenoid, ascorbic acid, and glutathione levels were determined during broccoli storage in 9 controlled atmospheres ranging from 0 to 10% O_2 and 2 to 20% CO_2 concentrations. In low O_2 concentration, the level of glutathione decreased, whereas in high O_2 and low CO_2 concentrations, the level of chlorophyll and ascorbic acid decreased significantly. A mixture of 2% O_2 and 4 to 10% CO_2 was found to be optimum for simultaneous maintenance of pigments, ascorbic acid, and glutathione. Respiration models were developed as functions of storage time and O_2 and CO_2 concentrations using a multiple regression analysis based on these gas compositions. When the modified atmosphere gas environment inside a broccoli package was simulated, an optimum gas condition of 2% O_2 and 5% CO_2 was derived using a film having a 1,000 ml/day/atm O_2 transmission rate. Simulation result agreed well with experimental data.

Key Words: broccoli, modified atmosphere packaging, glutathione.

Introduction

Modified atmosphere packaging (MAP) is used to maintain the quality of fresh fruits and vegetables (Kader et al., 1989). Proper packaging films and optimized packaging design can favorably alter the gas composition inside the package. Low O_2 and elevated CO_2 lower the respiration and transpiration rates of fresh produce and extend shelflife (Kader, 1986; Brecht, 1980). The most common storage problems with broccoli are the yellowing of florets, the development of undesirable odors, stem toughening, and mold (Kasmire et al., 1974; Lipton and Harris, 1974). Color (Makhlouf et al., 1989; Wang, 1979) and chemical components, such as ascorbic acid and chlorophyll (Perrin and Gaye, 1986; Wills et al., 1984) are measured to determine the quality of fresh produce.

Fruits and vegetables contain many nutrient that contribute to maintaining human homeostasis (Tateishi et al., 1980; Iwata, 1980). Glutathione is such nutrient found in many vegetables (Nakagawa et al., 1986); its level is a factor in controlling maximum phytochelatin synthesis (Fujita and Izumi, 1990), and is related to the seasonal growth pattern of deciduous trees (Schneider et al., 1994; Schupp et al., 1991). Information on glutathione in harvested plants is scarce, however.

Hirata et al. (1995) observed that glutathione was significantly decreased by low concentrations of oxygen but not by high concentrations of carbon dioxide in a controlled atmosphere. Gas conditions suitable for maintaining glutathione differed from those favoring ascorbic acid and chlorophyll.

We determined the optimum gas conditions for maintaining glutathione, ascorbic acid, and chlorophyll by using 9 different oxygen and carbon dioxide concentrations. Earlier studies attempted to simulate the gas composition inside the package by computer (Ishikawa et al., 1992; Sato et al., 1993), whereas our packaging design creates a suitable gas composition in MAP by computer simulation to maintain glutathione, ascorbic acid, and pigments.

Materials and Methods

Broccoli

Heads of *'Ryokurei'* broccoli (*Brassica oleracea* L. var. *italica* cv. Ryokurei) were obtained from a local market in Tsuchiura. Broccoli heads were held at 15 °C and 90% RH before packaging and storage. Heads were trimmed to about 200 g, packaged, and stored within 2 hours after arrival at the laboratory.

Received; April 14, 1997. Accepted; July 9, 1997.

Table 1. Oxygen, carbon dioxide and nitrogen concentrations within chambers for controlled atmosphere storage.

Chamber	Gas concentration (%)[z]		
	O_2	CO_2	N_2
1	0 (0)	20 (21.30 ± 0.02)	78 (78.70 ± 0.02)
2	1 (0.93 ± 0.03)	4 (3.77 ± 0.16)	95 (95.29 ± 0.13)
3	1 (1.21 ± 0.08)	10 (9.98 ± 0.08)	89 (88.80 ± 0.01)
4	2 (2.07 ± 0.17)	4 (3.99 ± 0.04)	94 (93.94 ± 0.18)
5	2 (2.20 ± 0.14)	10 (9.89 ± 0.08)	88 (87.90 ± 0.12)
6	4 (3.75 ± 0.06)	2 (1.99 ± 0.01)	94 (94.27 ± 0.05)
7	4 (4.15 ± 0.20)	4 (4.06 ± 0.03)	92 (91.79 ± 0.23)
8	7 (6.90 ± 0.09)	2 (1.98 ± 0.01)	91 (91.12 ± 0.08)
9	10 (9.85 ± 0.06)	2 (2.13 ± 0.10)	88 (88.02 ± 0.05)

[z] Programmed concentration (actual concentration, mean ± SD)

Table 2. Changes in glutathione, chlorophyll, carotenoid and ascorbic acid in broccoli within chambers in initial and 6 days after storage.

Chamber	Glutathione (mg/100 g fw)	Chlorophyll (mg/100 g fw)	β-carotene (mg/100 g fw)	Ascorbic acid (mg/100 g fw)
1	$38.0^{z} \Rightarrow 21.6^{y}$	50.0⇒52.6	1.95⇒1.93	87.0 ⇒ 44.5
2	38.0 ⇒ 29.2	50.0⇒46.4	1.95⇒1.70	87.0 ⇒ 61.9
3	38.0 ⇒ 32.7	50.0⇒42.6	1.95⇒1.65	87.0 ⇒ 50.1
4	49.0 ⇒ 92.6	46.2⇒45.7	1.61⇒1.32	97.1 ⇒ 67.2
5	49.0 ⇒ 71.9	46.2⇒39.1	1.61⇒1.24	97.1 ⇒ 69.1
6	53.4 ⇒102.8	37.7⇒37.2	1.07⇒2.06	116.0 ⇒ 45.3
7	53.4 ⇒111.2	37.7⇒38.7	1.07⇒1.74	116.0 ⇒ 38.9
8	53.4 ⇒129.2	37.7⇒27.6	1.07⇒2.05	116.0 ⇒ 24.1
9	53.4 ⇒155.3	50.0⇒20.3	2.12⇒0.87	102.7 ⇒ 10.6

[z] Initial
[y] After 6 days

Table 3. Respiration models for predicting changes in in-package gas composition.

Model	Equation
1 = O_2 consumption rate	$RO_2 = 1.98 \times 10^4 \times [O_2] - 2.79 \times 10^3 \times [CO_2]^{1/3} + 1.63 \times 10^3$ r = 0.956
2 = CO_2 evolution rate	$RCO_2 = -2.18 \times 10^3 \times T^{1/3} - 8.40 \times 10^3 \times [CO_2]^2 + 3.07 \times 10^2 \times T - 6.33 \times 10^3 \times T \cdot [O_2] + 3.53 \times 10^3$ r = 0.721

Controlled atmosphere conditions

Broccoli heads were placed in chambers (volume : 30 *l*) in a dark room at 15 °C and 90% RH and stored for a maximum of 8 days. Oxygen, nitrogen, and carbon dioxide were mixed by a gas blender (Kofloc Gas Blender GB-3BS) and fed into chambers through a water tank for humidification at 400 ml/min, which is sufficient to maintain a constant gas composition (Table 1). Broccoli packaged in perforated (6 mm diam. × 8) polyethylene film was stored as a control.

Respiration models

Respiration models for predicting changes in gas composition in packages were constructed as proposed by Sato et al. (1993). Samples of fresh broccoli were packaged in different polymeric films (oxygen permeability : 1,900-7,640 ml/m^2 · day · atm at 15 °C) and stored at 15 °C for 8 days. Gas compositions in packages were regularly monitored by a gas chromatograph according to Hirata et al. (1993). Respiration rates were calculated from continuous changes in gas composition in packages, surface area, initial package void volume, and film gas transmission rate. Respiration models were developed based on O_2 and CO_2 concentrations and storage time, by using multiple regression analysis.

Modified atmosphere conditions

Broccoli, weighing 200 g, was placed in a polymeric pouch and kept in a dark room at 15 °C and 90% RH for 7 to 8 days. Six types of film were used for modified atmosphere packaging (MAP) (Table 4). The film gas transmission rate was determined, using a Gasperm-100 (Nihon Bunko Inc., Tokyo, Japan). Broccoli, packaged in perforated (6 mm diam.×8) polyethylene films, was stored as a control sample.

Chemical analysis

Broccoli florets were cut and homogenized with ice to minimize changes in glutathione, ascorbic acid, and pigments during pretreatment. Samples of 2.0 g for chlorophylls and carotenoids, 0.5 g for ascorbic acid, and 1.0 g for glutathione-related compounds were placed in individual test tubes, extracted, and the respective compounds measured using HPLC as reported by Hirata et al. (1995). In this experiment, duplicate samples were analyzed for glutathione, ascorbic acid, and pigments.

Results and Discussion

Changes in compounds under controlled conditions

Broccoli was stored (Table 1), and changes in glutathione, ascorbic acid, and pigments concentrations at the initial day and 6 days after storage were determined (Table 2). In chambers 1, 2, and 3, which maintained low O_2 levels, chlorophyll, carotenoid, and ascorbic acid were maintained at relatively high levels during storage, but glutathione decreased to a low level. Inversely, in chambers 6, 7, 8, and 9, which maintained high O_2 and low CO_2 levels, glutathione increased gradually, but chlorophyll, carotenoid, and ascorbic acid decreased rapidly. Optimum gas conditions under which glutathione did not decrease and chlorophyll, carotenoid, and ascorbic acid retained at least half of their initial content were in chambers 4 (O_2 : 2%, CO_2 : 4%) and 5 (O_2 : 2%, CO_2 : 10%). Thus, an O_2 concentration of 2% and a CO_2 concentration of 4-10% were found to be optimum conditions for maintaining glutathione, ascorbic acid, and pigments in broccoli.

Glutathione content tends to increase during storage except at low oxygen concentrations. Rennenberg

(1984) suggested that glutathione mediats the response of plant cells to biological stress. Hirata et al. (1995) surmised that glutathione increases in harvested broccoli were caused by mechanical stress such as handling and trimming during packaging and storage. Iwahashi et al. (1996) reported that detached *Brassica* leaves with stems kept in 3 mM reduced glutathione, showed increased intracellular glutathione levels, and decreased chlorophyll content. These results suggest that degradation of chlorophyll in detached *Brassica* leaves was greatly influenced by the intracellular glutathione concentration. Our data reveal that increased glutathione and decreased chlorophyll in broccoli during storage agreed with the finding of Iwahashi (1996). Further study will be required, however, to clarify the reasons for this glutathione increase during storage.

Respiration Models

Equilibrium was observed in low O_2 and high CO_2 concentrations in packaging with a low gas transmission rate, and the opposite trend was observed in packaging with a high gas transmission rate (Fig. 1). Three different O_2 and CO_2 concentration curves covered the possible gas concentration range of MAP for fresh produce. This data was used for respiration models. Multiple regression analysis was conducted to determine respiration models (Table 3), the O_2 consumption rate (RO_2), and CO_2 evolution rate (RCO_2) were presented as functions of oxygen concentration ($[O_2]$), carbon dioxide concentration ($[CO_2]$), and storage time($[T]$), where $[O_2]$ and $[CO_2]$ are in fractions and T in days.

In general, the O_2 transmission rate is 3-5 times that of N_2 and the CO_2 rate is 3-5 times that of O_2. We simulated in-package gas concentration changes, assuming that the $CO_2 : O_2 : N_2$ gas transmission ratio was 9 : 3 : 1. When gas concentrations were simulated at a O_2 transmission rate of 1,000 ml/day/atm, a steady equilibrium of 2% O_2 and 5% CO_2 concentrations was attained (Fig. 2). Using polymeric films for a lower O_2 transmission rate, the gas condition in pouches changed to low O_2 and high CO_2 concentrations, decreasing glutathione content. On the other hand, when high permeability film was used, high O_2 and low CO_2 concentrations were not effective as MA packaging, therefore, ascorbic acid and chlorophyll decreased.

A pouch, that has an O_2 transmission rate of 1,000 ml/day/atm corresponds to a LDPE film (29 μm) of 0.175 m^2, was calculated to have an O_2 transmission rate of 5,700 ml/m^2 · day · atm at 15 °C. To create suitable MAP for fresh broccoli, Sato et al. (1993) used LDPE film (17.5 μm thick, 0.124 m^2 surface area), Miyazaki (1985) tried a LDPE film (30.0 μm thick,

Table 4. Film type, thickness, size and gas transmission rate of polymeric film used to verify packaging conditions calculated by computer simulation.

	Film	Thickness (μ m)	Gas transmission rate (cc/m^2 · day · atm)			Surface area (m^2)
			N_2	O_2	CO_2	
a	OPP[z]	24	250	1,900	6,900	0.0972
b	OPP	24	250	1,900	6,900	0.1998
c	LDPE[y]	29	1,100	5,700	28,500	0.0972
d	LDPE	29	1,100	5,700	28,500	0.1998
e	LDPE	18	2,150	7,640	33,900	0.1040
f	LDPE	18	2,150	7,640	33,900	0.2170

[z] Oriented polypropylene
[y] Low density polyethylene

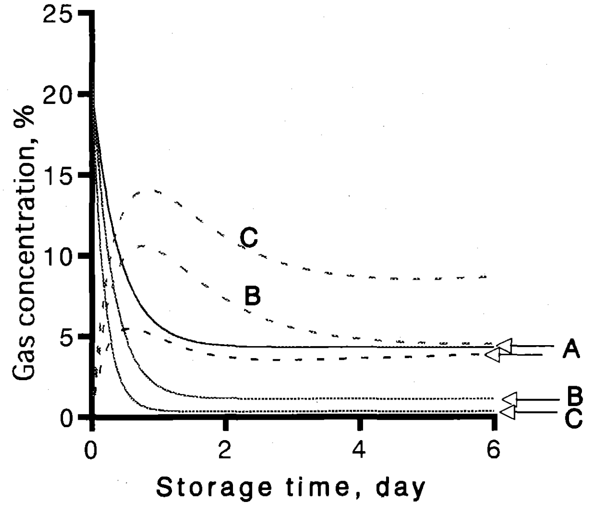

Fig. 1. Changes in gas concentration in three pouch types for packaging fresh broccoli for constructing respiration models proposed by Sato et al. (1993).
—— Oxygen, ------ Carbon dioxide
A : LDPE (18 μm, 27×40 cm) B : LDPE (18 μm, 20×26 cm) C : LDPE (29 μm, 20×24 cm)

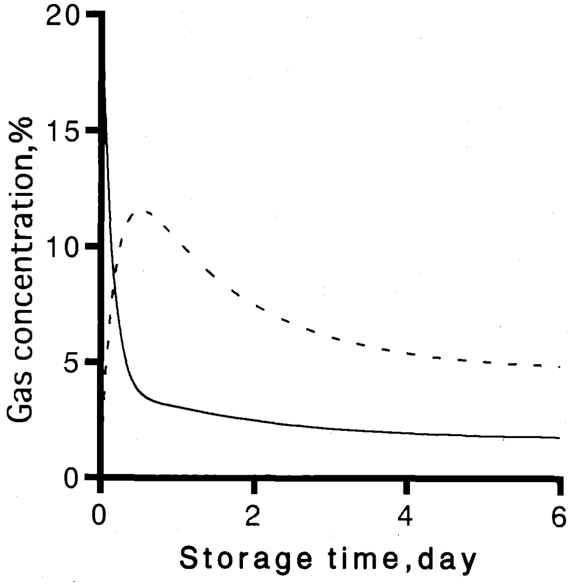

Fig. 2. Computer simulation of gas concentration in polymeric film, assuming oxygen transmission rate of 1,000 ml/day/atm.
—— Oxygen, ------ Carbon dioxide

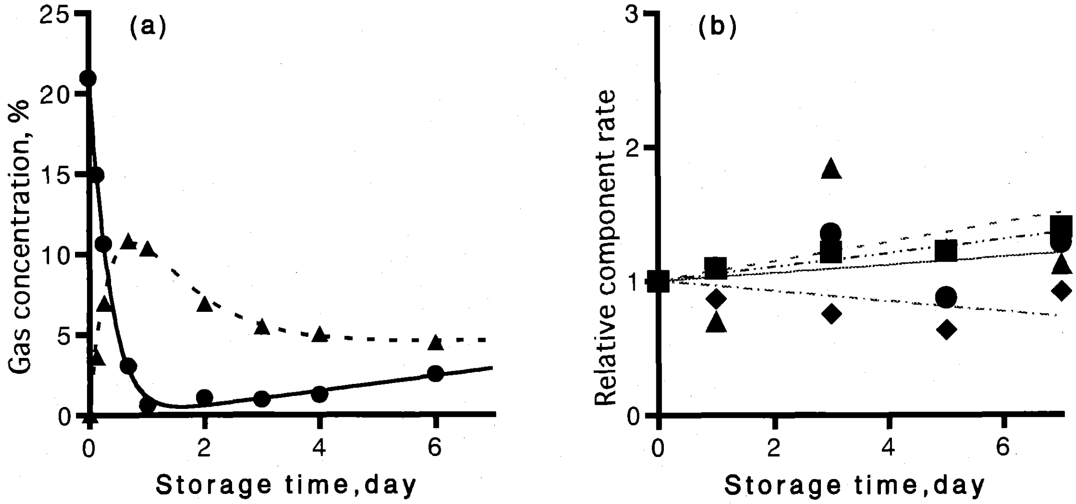

Fig.3. (a) Changes in oxygen and carbon dioxide concentrations in packege in Table 4 (d)
—— Oxygen, ······ Carbon dioxide
(b) Changes in chlorophyll, carotenoid, ascorbic acid and glutathione content in packege in Table 4 (d)
—●— Chlorophyll, --■-- Carotenoid, --◆-- Ascorbic acid
--▲-- Glutathione

0.175 m^2 surface area) at 15 °C. Their pouches had O_2 transmission rates from 950 to 960 ml/day/atm. These data support the use of computer simulation for designing suitable MA conditions.

Verification

Respiration models predicted that, in a pouch with an O_2 transmission rate of 1,000 ml/day/atm, O_2 and CO_2 concentrations would be equilibrated at 2% and 5%. This MAP also maintained chlorophyll, ascorbic acid, carotenoid, and glutathione in fresh broccoli. This prediction was verified, using the pouch in Table 4(d) which had O_2 transmission rate (O_2 transmission rate × surface area) of 1,140, closest to 1,000 ml/day/atm. Changes in O_2 and CO_2 concentration during broccoli storage in MAP (Fig. 3(a)) were rapid, reaching concentrations of 2 and 5%. Experimental data agreed well with simulation results (Fig. 2).

Experimental gas concentrations reached equilibrium two days after the start of storage, earlier than predicted by computer, probably due to the difference in the initial void volumes of packages and the CO_2 transmission rate of the film. Differences in time until equilibrium was reached were very slight, and were found to not affect changes in chlorophyll, carotenoid, ascorbic acid, and glutathione in broccoli.

Changes in chlorophyll, carotenoid, ascorbic acid, and glutathione (Fig. 3(b)) during MAP storage suggest that the MAP condition (Table 4(d)) was suitable for maintaining fresh broccoli components. It was also found that low gas permeability film created anaerobic conditions in packages and decreased glutathione. In contrast, high gas permeability film did not lower the O_2 concentration, so chlorophyll, ascorbic acid, and carotenoid decreased.

Fresh produce has many important components, e.g., nutritional, hedomic, and functional. The dependence of these components on the gas environment differ, suitable MAP conditions depend on the components to be maintained and differ between vegetables and fruits (Dilley, 1978). The results of our study indicate that an optimum packaging condition maintains essential components and desirable gas concentrations for storage under controlled atmosphere. Our data helped to design respiration models and simulate gas concentrations.

Literature Cited

Brecht, P. E. 1980. Prevention of ripening in fruits by use of controlled atmospheres. Food Technol. 34(3) : 45-50.

Dilley, D. R. 1978. Approaches to maintenance of postharvest integrity. J. Food Biochem. 2 : 235-242.

Fujita, M. and K. Izumi. 1990. Induction of phytochelatin and variation of glutathione level in fruits and callus cultures of pumpkin by treatment with heavy metals. J. Food Hygienic Soc. Japan. 31(5) : 404-408.

Hirata, T., T. Nishiyama, H. Sato, T. Shiina and T. Ishitani. 1993. A fast gas chromatographic method for the separation of nitrogen, oxygen, carbon dioxide, and argon and its application to inpackage modified atmosphere. J. Packaging Sci. and Technol. 2 : 15-23.

Hirata, T., A. Nakatani, Y. Ishikawa, C. Yamada and S. Katsuura. 1995. Changes in chlorophylls, carotenoids, ascorbic acid and glutathiones of broccoli during storage in modified atmosphere packaging. J. Jap. Soc. Food Sci. and Technol. 42 : 996-1002.

Ishikawa, Y., H. Sato, T. Ishitani and T. Hirata. 1992. Evaluation of broccoli respiration rate in modified atmosphere packaging. J. Packaging Sci. and Technol. 1 : 143-153.

Iwahashi, Y., H. Koizumi and H. Hosoda. 1996. Relationship between glutathione and dark-induced senescence of Brassica campestris. Biosci. Biotech. Biochem. 60(1) : 65-68.

Iwata S. 1980. Glutathione in relation to senile cataract. Metab. and Disease. 17(12) : 2173-2184.

Kader, A. A. 1986. Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. Food Technol. 40(5) : 99-104.

Kader, A. A., D. Zagory and E. L. Kerbel. 1989. Modified atmosphere packaging of fruits and vegetables. Critical Review in Food Sci. and Nutrition 28(1) : 1-30.

Kasmire, R. F., A. A. Kader and J. A. Klaustermeyer. 1974. Influence of aeration rate and atmospheric composition during simulated transit on visual quality and offodor production by broccoli. HortSci. 9 : 228-229.

Lipton, W. J. and C. M. Harris. 1974. Controlled atmosphere effects on the market quality of stored broccoli (*Brassica oleracea* L. Italica group). J. Amer. Soc. Hort. Sci. 99 : 200-205.

Makhlouf, J., F. Castaigne, J. Arul, C. Willemot and A. Gosselin. 1989. Long-term storage of broccoli under controlled atmosphere. HortSci. 24(4) : 637-639.

Miyazaki, T. 1985. Keeping qualities of broccoli by seal-packaging with plastic bags and ethylene removing agent. Japan Packaging Res. 6(1) : 1-6.

Nakagawa, K., M. Ikeuchi and Y. Tsugita. 1986. Glutathione content of vegetables. J. Food Hygienic Soc. Japan. 27(4) : 425-427.

Perrin, P. W. and M. M. Gaye. 1986. Effects of simulated retail display and overnight storage treatments on quality maintenance in fresh broccoli. J. Food Sci. 51 : 146-149.

Rennenberg, H. 1984. The fate of excess sulfur in higher plants (Essential nutrients, nutritional status). Annu. Rev. Plant Physiol. 35 : 121-153.

Sato, H., Y. Ishikawa and T. Hirata. 1993. Respiration model for broccoli packaged in polymeric films. J. Packaging Sci. and Technol. 2 : 25-33.

Schneider, A., J. Kreuzwieser, R. Schupp, J. J. Sauter and H. Rennenberg. 1994. Thiol and amino acid composition of the xylem sap of poplar trees. Can. J. Bot. 72 : 347-351.

Schupp, R., V. Glavac and H. Rennenberg. 1991. Thiol composition of xylem sap of beech trees. Phytochemistry 30 : 1415-1418.

Tateishi, N, T. Higashi and Y. Sakamoto. 1980. Nutritional significance of hepatic glutathione as a reservoir of cysteine. Metab. and Disease. 17(12) : 2163-2172.

Wang, C. Y. 1979. Effect of short-term high CO_2 treatment on the market quality of stored broccoli. J. Food Sci. 44 : 1478-1482.

Wills, R. B. H., P. Wimalasiri and H. Greenfield. 1984. Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity. J. Agric. Food Chem. 32 : 836-838.

グルタチオン，アスコルビン酸，色素等を考慮したブロッコリーの最適包装設計

石川　豊[1]・Carolina Wessling[2]・平田　孝[3]・長谷川美典[4]

[1]農林水産省食品総合研究所　305-8642　茨城県つくば市観音台

[2]Department of Applied Nutrition and Food Chemistry, University of Lund, Lund, Sweden

[3]京都大学農学部　606-8224　京都府京都市左京区北白川追分町

[4]農林水産省四国農業試験場　765-8508　香川県善通寺市生野町

摘　要

ブロッコリーを9種類のガス環境条件下で貯蔵し，グルタチオン，クロロフィル，アスコルビン酸，β-カロテン等成分変化を測定した．低酸素，高二酸化炭素条件ではグルタチオンの減少が見られ，高酸素，低二酸化炭素条件ではクロロフィル，アスコルビン酸等の減少が見られた．最もバランス良く各種成分保持が可能であったのは，酸素2%，二酸化炭素4～10%の条件で貯蔵した試料であった．ガス濃度，貯蔵時間等の関数である呼吸モデルを作成し，コンピュータシミュレーションを行い，包装内適正ガス組成を得るために必要な条件を検討した．袋の酸素透過量を1000 ml/day/atm程度にすることにより袋内酸素濃度2%，二酸化炭素濃度5%を達成することができ，最適なMA効果が得られると予測することができた．種々のガス透過率のフィルムを使い実証試験を行った結果，酸素透過量1000 ml/day/atm程度のフィルムで最も品質保持効果が高いことを確認することができた．