Active Packaging for Inhibition of Yellowing in Broccoli

Part 1 : PE-bags with Ethylene Removers Stored at $5^{0}C\pm2^{0}C$

Part 2 : PE-bags with Ethylene Removers stored at $10^{0}C\pm2^{0}C$

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I. Introduction

The flowers of the broccoli head continue to develop after harvest and the commodity becomes unsalable when the flowers begin to open or turn yellow. The yellowing of broccoli occurs in three days at room temperature and is the main problem in its prepackaging (Salunkhe & Desai, 1984).

Reduction of green pigmentation and consequently the predominance of yellow pigments is a normal process in ripening or senescence of many fruits and vegetable ; such changes can be accelerated by ethylene (Garcia & Barret, 2002).

Broccoli emits a significant amount of ethylene in storage. The production of ethylene and the subsequent yellowing are important factors in the post-harvest handling of broccoli (Salunkhe & Desai, 1984).

The refrigeration of packaged broccoli reduces the production of ethylene considerably (Salunkhe & Desai, 1984). Thompson & Kelly (1975) vide Salunkhe & Desai (1984) stated that packaging of broccoli did not eliminate the need for refrigeration, but accentuated the need for refrigeration to keep the produce constantly cold. Broccoli is generally hydro-cooled to 4.4^{o}C and then packed with ice in the crate and stored in refrigerators for later sale (Salunkhe & Desai, 1984).

According to Reid (1992), a number of techniques have been developed to protect sensitive commodities from the effect of ethylene. Selection of the appropriate method depends on the commodity and the handling techniques used in its marketing.

Removing of ethylene from the atmosphere around the commodity is the preferable method of preventing deterioration of ethylene-sensitive produce. The main methods are : 1). eliminating sources of ethylene such as avoiding the use of internal combustion engine vehicles, or fitting them with engine exhausts with catalytic convertors, and removal of overripe or rotting produce, 2). good ventilation, 3). chemical removal using potassium-permanganate, ultraviolet lamps, activated or brominated charcoal, catalytic oxidizers or bacterial systems, 4). hypobaric storage (Reid, 1992).

Potassium-permanganate oxidizes ethylene to CO_2 and H_2O. The requirements of such techniques are a high surface area coated with the permanganate and ready permeability to gases. Many porous materials have been used to manufacture permanganate absorbers, such as vermiculite, pumice and brick. Activated or brominated charcoal can absorb ethylene from the air. This method is confined to laboratory use because potassium permanganate absorbers are cheaper and widely available (Reid, 1992).

The effect of ethylene could also be inhibited by reducing spiration rate, production of ethylene and other metabolic processes through controlled atmosphere or modified atmosphere packaging. For example, bananas transported in polyethylene-lined boxes containing K-permanganate absorbers at 15-25^oC arrive in better condition due to the effect of accumulated CO_2 produced by the fruit on preventing the action of ethylene (Reid, 1992).

The objective of this experiment was to find a feasible method to delay yellowing of broccoli curds using low temperature storage respectively at $5^0C \pm 2^0C$ (Part 1), and $10^0C \pm 2^0C$ (Part 2), modified atmosphere packaging in PE-bags with and without holes and chemicals to remove ethylene from the atmosphere within the package by potassium-permanganate and active coal.

II. Time and Place of Research

The first part of the experiment was conducted at the Food Process Engineering and the Post-harvest Technology Laboratories of The Faculty of Agriculture Industrial Technology of The Padjadjaran University at Jatinangor, West-Java from August 9 up to August 23, 2007 at $5^0C \pm 2^0C$, followed by removal from cold storage and further observation for 2 successive days at ambient temperature.

The second part of the experiment was carried out at the same laboratories at $10^0C \pm 2^0C$, from August 26th up to September 10th, 2007.

III. Materials and Methods

<u>Materials :</u>

Broccoli (*Brassica oleracea* gp Italica) cv Luky (From the Bejo Company, the Netherlands) harvested at two months after planting and possessing curds of 15 cm diameter, dark green in color, and compact was used. Others materials employed were PE bags (45 cm x 30 cm) with a film thickness of 50µm, polypropylene sachets (15 cm x 7 cm) for the ethylene removers, respectively potassium-permanganate (pp) and active coal (ac) of technical grade.

Equipments

Film-bag sealer, paper perforator, electric balance, Canon digital camera, sling hygrometer, sensory evaluation lab-equipments and a sensory panel consisting of trained panelists.

Methods

A descriptive experimental method in triplicates, followed by regression analyses consisting of two variables (dependent and independent variables) was employed. Four broccoli curds were put into a perforated/un-perforated PE-bag, together with/without a sachet containing ethylene removing substance/substances (Fig. 1) according to treatment given. Either 10 g potassium-permanganate or active coal and a mixture of 5 g potassium-permanganate and 5 g of active coal were used.

a. Broccoli cv Luky   b. Sachet Containing Ethylene Remover   c. Sample of Broccoli

Fig 1. Sample of Package Holding Broccoli Curd and Sachet Containing Ethylene Remover

The treatments in Part (1) of the experiment were :

A. PE bag without holes
B. PE bag without holes and an active coal sachet (10 g)
C. PE bag without holes and a potassium-permanganate sachet (10 g)
D. PE bag without holes and a sachet containing potassium-permanganate (5 g) and active coal (5 g)
E. PE bag with 4 holes (control)
F. PE bag with 4 holes and an active coal sachet (10 g)

G. PE bag with 4 holes and a potassium-permanganate (10 g)
H. PE bag with 4 holes and a sachet containing potassium-permanganate (5 g) and active coal (5 g)

The treatments in Part (2) of the experiment were:

A. PE bag without holes
B. PE bag without holes and a K-permanganate sachet (10 g)
C. PE bag without holes and an active coal sachet (10 g)
D. PE bag without holes and a sachet containing K-permanganate (5 g) and active coal (5 g)
E. PE bag with 4 holes

The procedure of sample preparation for the Part (1) and (2) experiments is shown in Fig. 2.

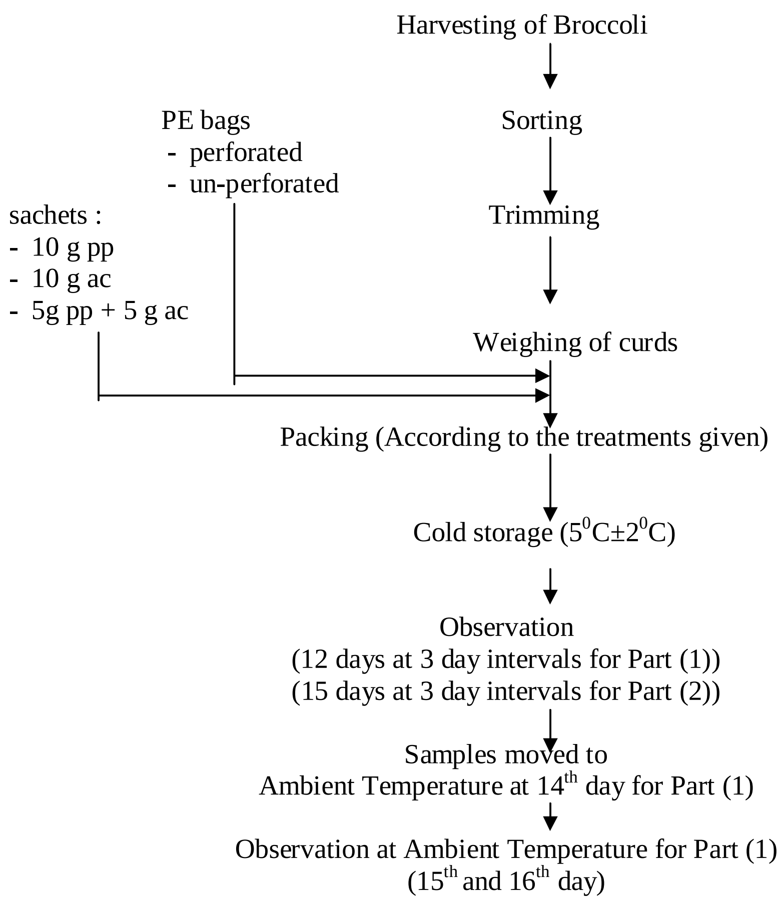


Fig 2. Sample Preparation

The samples of the Part (1) experiment were then stacked in columns and stored at $5^0C \pm 2^0C$ for 12 days and then removed to ambient temperature for 3days. The samples of the Part (2) experiment were stored at $10^0C \pm 2^0C$ for 15 days. The experimental lay out is presented in Fig. 3.

Replicate I	Replicate II
A	B
D	F
C	G
E	H
G	C
H	D
F	A
B	E

a. Part 1.

- **($5^0C \pm 2^0C$) for 12 days**
- **23^o-27^oC for3 days**

Replicate I	Replicate II
B	A
D	C
C	B
A	E
E	D

b. Part 2.

- **($10^0C \pm 2^0C$) for 15 days**

Fig. 3. Experiment lay-out: a. Part 1; b. Part 2

Observation were carried out at three day intervals, consisting of :

1. Curd surface color by the CIE-lab method
2. Sensory test using a panel of experienced panelists to evaluate :
   a. Discoloration, Wilting and Decay. A 9 point-scale, respectively 1 (none), 3 (slight), 5 (moderate), 7 (severe) and 9 (extreme) was employed
   b. Overall visual quality, which were rated also on a 9 point-scale respectively, 1 (extremely poor), 3 (poor), 5 (fair), 7 (good) and 9 (excellent).
3. Incidence of soft rot (% no of samples)
4. Weight loss (% of initial weight)

Considering no yellowing of curds were detected, the cold storage experiment was terminated at day 12. The samples were then transferred to ambient temperature on the 14^{th} day; and storage continued for 3 more days. Observation as the above was continued respectively on the 15^{th} and 16^{th} day. Due to these results the Part (2) experiment was stored at $10^0C \pm 2^0C$.

IV. Results and Discussion

Part 1. of the Experiment : Storage at $5^0C \pm 2^0C$ for 12 days and at ambient temperature for 3 days.

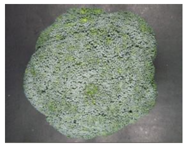
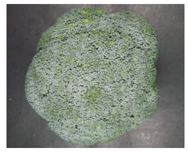
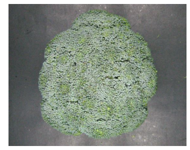
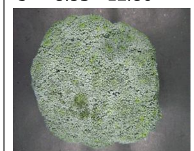
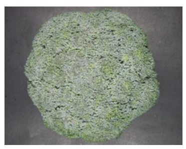
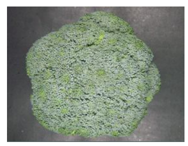
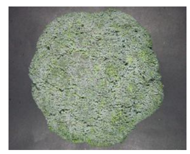
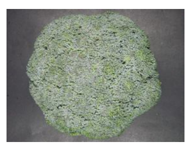
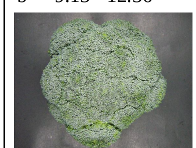
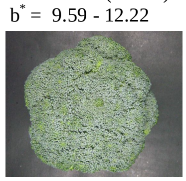
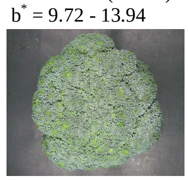
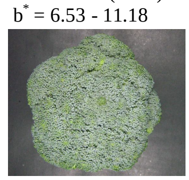
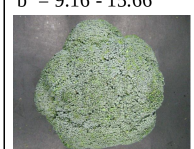
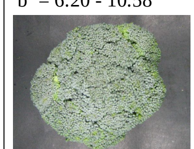
4.1. Cold Storage (day 0-12)

4.1.1. Curd Surface Color

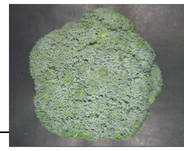
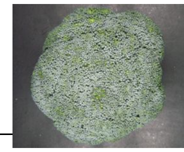
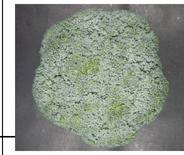
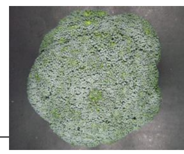
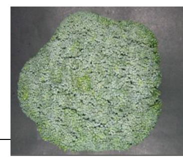
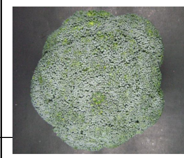
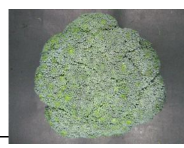
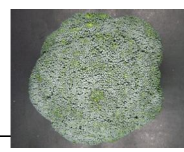
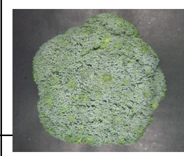
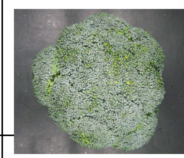
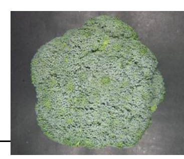
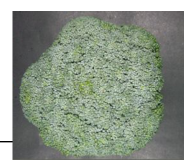
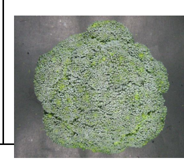
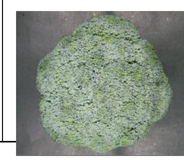
Curd surface color evaluated by the CIE-lab method is presented in Table 1.

Table 1. Broccoli Curd Surface Color Changes during Cold Storage at 3.8^{0}C

Replication 1

Treatment	Storage (Days)				
	0	**3**	**6**	**9**	**12**
A	L^* = 62.13 - 65.56 a^* = -6.55 - (-9.90) b^* = 9.36 - 12.56 	L^* = 63.54 - 67.64 a^* = -6.70 - (-10.00) b^* = 9.59 - 13.17 	L^* = 61.18 - 68.49 a^* = -6.40 - (-11.33) b^* = 7.16 - 13.06 	L^* = 61.03 - 66.92 a^* = -7.83 - (-10.70) b^* = 10.87 - 15.23 	L^* = 61.12 - 69.19 a^* = -6.90 - (-11.30) b^* = 8.93 - 12.86 
B	L^* = 63.01 - 66.10 a^* = -6.34 - (-9.99) b^* = 9.02 - 10.99 	L^* = 62.54 - 65.22 a^* = -6.17 - (-8.37) b^* = 8.97 - 13.17 	L^* = 61.58 - 66.38 a^* = -9.34 - (-11.67) b^* = 10.81 - 15.42 	L^* =64.78 - 70.48 a^* = -8.53 - (-10.28) b^* = 10.73 - 12.54 	L^* = 60.36 - 66.20 a^* = -6.80 - (-10.96) b^* = 8.43 - 14.11 
C	L^* = 63.00 - 67.82 a^* = -6.96 - (-8.98) b^* = 9.72 - 13.54 	L^* = 62.00 - 66.88 a^* = -6.85 - 10.12 b^* = 7.74 - 12.54 	L^* = 62.57 - 68.22 a^* = -6.94 - (-8.61) b^* = 6.53 - 10.62 	L^* = 59.93 - 66.27 a^* = -7.81 - (-8.95) b^* = 9.13 - 12.36 	L^* = 63.62 - 70.67 a^* = -7.99 - (-10.13) b^* = 9.62 - 11.21 
D	L^* = 62.94 - 65.98 a^* = -8.99 - (-10.16) b^* = 9.59 - 12.22 	L^* = 63.16 - 66.31 a^* = -9.04 - (-10.31) b^* = 9.72 - 13.94 	L^* = 61.96 - 65.09 a^* = -4.49 - (-8.26) b^* = 6.53 - 11.18 	L^* = 60.31 - 68.42 a^* = -8.35 - (-11.57) b^* = 9.16 - 15.66 	L^* = 65.37 - 69.96 a^* = -5.08 - (-8.41) b^* = 6.20 - 10.58 

Replication 2

Treatment	**Storage (Days)**				
	0	**3**	**6**	**9**	**12**
A	L^* = 62.94 - 67.43 a^* = -6.89 - (-9.83) b^* = 8.69 - 12.05 	L^* = 61.43 - 64.71 a^* = -5.97 - (-9.77) b^* = 9.00 - 11.60 	L^* = 61.09 - 65.09 a^* = -4.49 - (8.26) b^* = 6.47 - 11.18 	L^* = 62.65 - 68.00 a^* = -7.62 - (9.39) b^* = 9.49 - 11.50 	L^* = 64.20 - 68.76 a^* = -7.10 - (12.01) b^* = 8.37 - 15.62 
B	L^* = 60.06 - 66.36 a^* = -6.34 - (-11.43) b^* = 6.87 - 13.71 	L^* = 62.95 - 65.98 a^* = -7.77 - (-11.48) b^* = 8.80 - 17.21 	L^* = 58.62 - 67.47 a^* = -7.13 - (-10.96) b^* = 8.37 - 14.03 	L^* = 59.95 - 65.07 a^* = -6.19 - (12.12) b^* = 9.68 - 17.17 	L^* = 62.46 - 68.48 a^* = -5.70 - (-10.43) b^* = 6.82 - 13.83 
C	L^* = 60.18 - 69.29 a^* = -6.36 - (-10.30) b^* = 6.90 - 13.71 	L^* = 61.62 - 65.32 a^* = -6.73 - (-10.80) b^* = 8.98 - 16.21 	L^* = 61.90 - 69.72 a^* = -7.75 - (-10.71) b^* = 8.83 - 12.75 	L^* = 60.23 - 66.42 a^* = -7.58 - (9.44) b^* = 8.02 - 14.10 	L^* = 65.56 - 69.92 a^* = -8.28 - (-11.28) b^* = 9.20 - 16.12 
D	L^* = 60.18 - 69.29 a^* = -6.36 - (-10.30) b^* = 62.29 - 65.34 	L^* = 62.34 - 67.78 a^* = -6.95 - (-9.86) b^* = 8.35 - 14.11 	L^* = 60.61 - 66.33 a^* = -7.50 - (-11.89) b^* = 8.31 - 14.46 	L^* = 59.35 - 65.87 a^* = -7.05 - (-9.25) b^* = 8.72 - 13.06 	L^* = 62.65 - 67.66 a^* = -8.50 - (-10.57) b^* = 9.11 - 14.13 

Curd surface color during the entire 12 day cold storage at 3.8^0C did not show any change. This might be related to the various treatments given, respectively:

a. Low-temperature storage (3.8^0C)

   Lougheed, Murr and Toivonen (1987) said that the many effects of ethylene upon vegetables in storage are reduced by low temperatures.

b. Modified Atmosphere Packaging

Barth et al., (1993) reported that retention of green color was attained by modified atmosphere packaging and storage at 10^0C. These authors reported that within 48 hours CO_2 concentration in broccoli packages reached equilibrium at 8% and oxygen content at 10%. Lougheed et al., (1987) stated that retention of green color in vegetable stored in controlled atmosphere was due to competitive inhibition of ethylene action by CO_2 and reduced ethylene synthesis at low oxygen partial pressures.

c. Chemical Removal of Ethylene

The low concentration of ethylene within the packages maybe oxidized to CO_2 and H_2O by potassium-permanganate and/or absorbed by the active coal included within each package of broccoli (Reid, 1992).

d. The Cultivar Luky (From the Bejo Company, The Netherlands)

Although the production of ethylene by edible floral parts, such as broccoli and cauliflower maybe quit high, some cultivars may produce variable amounts of this substance. Moreover, there may also be a genetic control of chlorophyll loss in harvested vegetables, for example some cabbage cultivars are able to retain their green color longer, although it is not related to endogenous rates of ethylene production (Lougheed, et al., 1987). In this experiment the broccoli cultivar used was Luky; which is greener in color than other cultivars and likely more resistent to yellowing / degreening.

Due to these results, the experiment at low temperature storage was terminated at day 12; and the same experiment was then continued at ambient temperature for 3 more days starting from day 14.

4.1.2. Changes in Sensory Score Scores during Cold Storage at 3.8^{o}C

Changes in sensory discoloration, wilting, decay and overall quality during cold storage are presented in Table 2, 3, 4, and 5.

Table 2. Changes in Discoloration Sensory Score during Cold Storage at 3.8° C

Treatment	Discoloration Score*)**) on Day				
	0	3	6	9	12
A	1	1	1	1	1
B	1	1	1	1	1
C	1	1	1	1	1
D	1	1	1	1	1
E	1	1	1	1	1
F	1	1	1	1	1
G	1	1	1	1	1
H	1	1	1	1	1

***)** Average of 2 replications
****)** Sensory score scale :

1. none
3. slight
5. moderate
7. severe
9. extreme

Table 3. Changes in Wilting Sensory Score during Cold Storage at 3.8°C

Treatment	Wilting Sensory Score*)**) on Day				
	0	3	6	9	12
A	1	1	1	1	1-3
B	1	1	1	1	1-3
C	1	1	1	1	1-3
D	1	1	1	1-3	1-3
E	1	1	1	1-3	1-3
F	1	1	1	1-3	1-3
G	1	1	1	1	1
H	1	1	1	1	1

***)** Average of 2 replications
****)** Sensory score scale :

1. none
3. slight
5. moderate
7. severe
9. extreme

Table 4. Changes in Decay Sensory Score during Cold Storage at 3.8°C

Treatment	Decay Score*)**) on Day				
	0	3	6	9	12
A	1	1	1	1	1
B	1	1	1	1	1
C	1	1	1	1	1
D	1	1	1	1	1
E	1	1	1	1	1
F	1	1	1	1	1
G	1	1	1	1	1
H	1	1	1	1	1

***)** Average of 2 replications
****)** Sensory score scale :
1. none 7. severe
3. slight 9. extreme
5. moderate

Table 5. Changes in Overall Quality Sensory Score during Cold Storage at 3.8°C

Treatment	Overall Visual Quality *)**) on Day				
	0	3	6	9	12
A	9	9	9	9	9
B	9	9	9	9	9
C	9	9	9	9	9
D	9	9	9	9	9
E	9	9	9	9	9
F	9	9	9	9	9
G	9	9	9	9	9
H	9	9	9	9	9

***)** Average of 2 replications
****)** Sensory score scale :
1. extremely poor 7. good
3. poor 9. excellent
5. fair

Practically no changes in discoloration, decay and overall quality sensory scores were observed during the 12 day cold storage, except for wilting score which showed some slight changes on day 9 and 12. The reasons for these results were already discussed in point 4.1.1 – 4.1.3.

4.1.3. Incidence of Soft Rot

Incidence of soft rot during the 12 day low temperature storage is presented in Table 6.

Table 6. Incidence of Soft Rot during Cold Storage (3.8°C)

Treatment	Incidence of Soft Rot *) (% no of samples) on Day				
	0	**3**	**6**	**9**	**12**
A	0	0	0	0	0
B	0	0	0	0	0
C	0	0	0	0	0
D	0	0	0	0	0
E	0	0	0	0	0
F	0	0	0	0	0
G	0	0	0	0	0
H	0	0	0	0	0

***)** Average of 2 replications

No Soft rot was observed during the cold storage period. Broccoli is often infected by gray mold rot (Botrytis cinerea), downy mildew (Peronospora parastica), and black rot (Xanthomonas campestris) (Salunkhe and Desai, 1984). This experiment was conducted in the dry monsoon of the year 2007, and in West Java the current dry monsoon is more severe than normal; this might be the reason there is no incidence of rot observed.

4.1.4. Weight Loss

Result of regression analyses showed there is a very close linear relationship between weight loss and storage time in all treatments. Regression curves are presented in Figure 4 while regression equations and coefficients of determination and coefficients of correlation are presented in Table 7. Coefficients of determination (R^2) ranged between 0.901 – 0.971, showing a very close fit. Temperature of the cold storage was 3.8^{0}C.

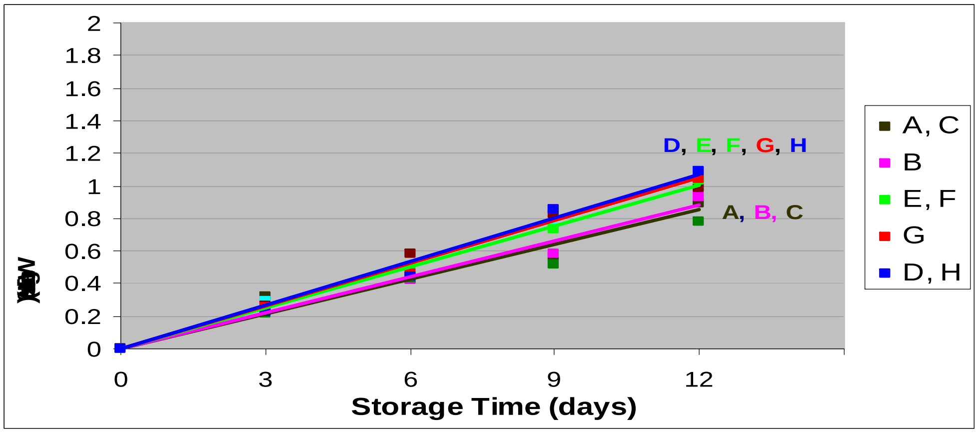


Figure 4. Regression Curves of Weight Loss during Cold Storage at 3.8°C

Table 7. Regression Equations of Weight Loss (% Initial Weight) during Cold Storage at 3.8°C

Treatment	Regression Equation	R^2	r
A	Y = 0.034 + 0.067 X	0.901	0.949
B	Y = 0.009 + 0.072 X	0.940	0.969
C	Y = 0.017 + 0.061 X	0.904	0.951
D	Y =-0.003 + 0.084 X	0.958	0.979
E	Y = 0.020 + 0.085 X	0.962	0.981
F	Y =-0.013 + 0.087 X	0.956	0.978
G	Y =-0.015 + 0.089 X	0.971	0.985
H	Y =-0.021 + 0.086 X	0.916	0.957

At the end of the 12 day cold storage at 3.8^{0}C , weight loss in the perforated bags (E to H) ranged between 0.99% - 1.09% as compared to 0.78% - 1.04% in the un-perforated ones (A to D). The perforations were designed to maintain RH in the bags at about 90% - 95%, but they also allow some air movement, including moisture vapor, into the environment. However, RH in the cold storage during this experiment was 94% - 99%, which is slightly higher than is recommended for vegetable storage and transport, respectively 90% - 98% (Kader, 1992), causing some moisture vapor loss into the surrounding atmosphere from the perforations. Moisture vapor loss also occurred due to the permeability of the LDPE film to moisture vapor, which at 23^{0}C, 85% RH is about 3000 ml.m^{-2}. per 24 h (Fellows, 2000). Consequently, weight loss values for both perforated and un-perforated packs were close to one another.

The chemicals to remove ethylene in the bags, respectively potassium-permanganate, active coal and their combination, did not seem to affect weight loss. The perforations do exert greater effect than these substances on weight loss.

4.2. Ambient Temperature Storage (day 14-16)

4.2.1. Weight Loss

Average weight loss during the 3 day room-temperature storage is presented in Table 8.

Table 8. Average Weight Loss at Day 15 and 16 at Ambient Temperature (23^o-27^oC)

Treatment	Weight Loss (% by initial weight)*) on Day	
	15	16
A	0.56	0.85
B	0.36	0.65
C	0.51	0.89
D	0.21	0.86
E	0.45	0.99
F	0.49	1.06
G	0.43	0.78
H	0.60	0.71

*)Average of 2 replicates

Average weight loss on day 15 and 16 were higher in the perforated poly-bags than in the un-perforated ones. This is possibly due to the free movement of air from the packages into the environment, resulting greater evaporation of moisture from the commodity.

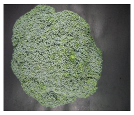
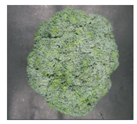
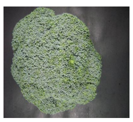
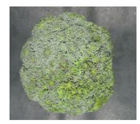
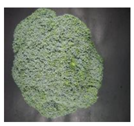
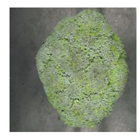
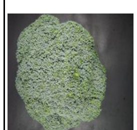
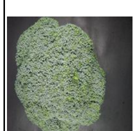
The ethylene removing chemicals within the poly-bags, however, did not show consistent effects on average weight loss.

4.2.2. Curd Surface Color

Curd surface color changes during the 3 day storage at ambient temperature (23^0-27^0C) is presented in Table 9.

Table. 9 Curds Surface Color Changes during Ambient Storage (23°-27°C)

Replicate 1

Treat-ment	Day 14		Day 15		Day 16	
A		L^* = 65.82 - 66.53 a^* = -7.5 - (-8.05) b^* = 8.74 - 10.22		L^* = 55.03 - 61.58 a^* = -7.16 - (-9.68) b^* = 9.36 - 14.35		L^* = 60.15 - 62.33 a^* = -6.2 - (-7.6) b^* = 12.25 - 13.80
B		L^* = 54.90 - 61.31 a^* = -7.85 - (-10.22) b^* = 11.25 - 13.54		L^* = 60.27 - 62.52 a^* = -6.41 - (-7.33) b^* = 12.71 - 13.46		L^* = 58.84 - 63.96 a^* = - 10.32 - (-12.87) b^* = 13.78 - 18.55
C		L^* = 55.04 - 61.16 a^* = -7.14 - (-10.26) b^* = 9.35 - 13.47		L^* = 60.81 - 68.01 a^* = -7.8 - (-9.51) b^* = 10.84 - 13.41		L^* = 62.52 - 65.58 a^* = - 6.41 - (-10.42) b^* = 12.71 - 19.84
D		L^* = 64 - 59.02 a^* = -7.65 - (-9.42) b^* = 10.77 - 13.89		L^* = 64.64 - 68.4 a^* = -7.71 - (-9.27) b^* = 10.37 - 14.23		L^* = 62.04 - 62.89 a^* = -6.41 - (-10.42) b^* = 12.71 - 19.84
E		L^* = 59.05 - 61.01 a^* = -7.31 - (-8.45) b^* = 10.01 - 11.56		L^* = 67.18 - 69.76 a^* = -9.13 - (-9.63) b^* = 11.62 - 11.70		L^* = 62.44 - 66.94 a^* = - 10.75 - (-11.45) b^* = 20.09 - 22.41
F		L^* = 59.02 - 59.06 a^* = -7.65 - (-9.42) b^* = 10.77 - 13.89		L^* = 65.27 - 68.4 a^* = -8.84 - (-9.88) b^* = 10.25 - 12.28		L^* = 61.56 - 64.70 a^* = - 11.38 - (-12.50) b^* = 19.3 - 21.52
G		L^* = 59.65 - 61.25 a^* = -7.31 - (-8.45) b^* = 10.01 - 11.56		L^* = 62.50 - 64.69 a^* = -7.46 - (-8.29) b^* = 9.49 - 10.65		L^* = 62.93 - 64.09 a^* = -9.7 - (-10.85) b^* = 18.88 - 23.17
H		L^* = 61.05 - 64.74 a^* = -7.56 - (-10.15) b^* = 9.35 - 10.46		L^* = 55.89 - 60.60 a^* = -7.5 - (-9.83) b^* = 10.51 - 14.24		L^* = 64.57 - 65.78 a^* = -12.75 - (-12.88) b^* = 20.65 - 23.13

Curd surface color on day 15 in the un-perforated bags as well as the perforated ones did not change much when compared to day 14. However on day 16 both showed significant yellowing. Yellowing of curds were more severe in the perforated poly-bags (A to H) than in the un-perforated ones (A-D).

Less severe yellowing in the un-perforated bags might be related to the modified atmosphere packaging. Salunkhe and Desai (1984) reported that modified atmospheres containing 10%, 5%, 2.5% or 1% oxygen flushed over broccoli at 23.9^{0}C for 3 days, significantly reduced respiration rate and completely stopped yellowing of the curd. In this experiment yellowing still occurred, but was less intense. No different effect on yellowing could be detected among the different ethylene removing substances used.

The more severe yellowing in the perforated bags could be caused by the room temperature of 23^0-27^{0}C. Ambient temperature increased both rate of respiration and other metabolic processes, including ethylene synthesis. These resulted in rapid loss of chlorophyll and the green color of the curd (Reid, 1992). Moreover, normal O_2 and CO_2 concentrations within the perforated package promote ethylene production (Reid, 1992), while normal CO_2 concentration accelerated ethylene induced chlorophyll degradation and thus yellowing of the curd (Barth et al., 1993).

4.2.3. Incidence of Soft rot

Average incidence of soft rot (% no. of samples) during the 3 day room temperature storage is presented in Table 10.

Table 10. Incidence of soft rot (% no. of samples) during Room Temperature Storage (23^{0}C-27^{0}C)

Treatment	Average Incidence of Soft Rot *) (% no. of samples) on Day		
	14	15	16
A	0	0	0
B	0	0	0
C	0	0	0
D	0	0	0
E	0	0	0
F	0	0	0
G	0	0	0
H	0	0	0

***)**Average of 2 replications

No soft rot incidence was observed during the 3 day room temperature storage, which might be related to the low mold and mildew contamination during the dry monsoon and the low RH of 50-63% in the storage room at the time of the experiment.

4.2.4. Sensory Score Changes

Average sensory score changes for discoloration, wilting, decay and overall quality at room temperature storage is presented in Table 11, 12, 13, and 14.

Table 11. Average Discoloration Sensory Score during Room Storage (23^o-27^oC)

Treatment	Discoloration Score *)**) on Day		
	14	15	16
A	1	1	1
B	1	1	1
C	1	1	1
D	1	1	1
E	1	1-3	1-3
F	1	1-3	1-3
G	1	1-3	1-3
H	1	1-3	1-3

***)** Average of 2 replications
****)** Sensory score scale :
1. none 7. severe
3. slight 9. extreme
5. moderate

Table 12. Average Sensory Wilting Score during Room Temperature Storage (23^0-27^0C)

Treatment	Average Wilting Score *)**) on Day		
	14	15	16
A	1	1	1
B	1	1	1
C	1	1	1
D	1	1	1
E	1	1	1
F	1	1	1
G	1	1	1
H	1	1	1

***)**Average of 2 replications
****)**Sensory score scale :
1. none 7. severe
3. slight 9. extreme
5. moderate

Table 13. Average Sensory Decay Score during Room Temperature Storage (23^o-27^oC)

Treatment	Average Decay Score*)**) on Day		
	14	**15**	**16**
A	1	1	1
B	1	1	1
C	1	1	1
D	1	1	1
E	1	1	1
F	1	1	1
G	1	1	1
H	1	1	1

***)**Average of 2 replications
****)**Sensory score scale :

1. none 7. severe
3. slight 9. extreme
5. moderate

Table 14. Overall Quality Sensory Score during Room Temperature Storage (23^o-27^oC)

Treatment	Overall Quality Score*)**) on Day		
	14	**15**	**16**
A	9	9	9
B	9	9	9
C	9	9	9
D	9	9	9
E	9	7-9	7-9
F	9	7-9	7-9
G	9	7-9	7-9
H	9	7-9	7-9

***)**Average of 2 replications
****)**Sensory score scale :

1. extremely poor 7. good
3. poor 9. excellent
5. fair

Average discoloration sensory scores (Table 11) showed that broccoli curds in the un-perforated bags (A to D) on both day 15 and 16 were not discolored (score 1), but those in the perforated bags (E to H) showed some yellowing (score 1-3). This may be due to:

a) The normal room temperature of 23^o-27^oC increased respiration rate and other metabolic processes including ethylene production (Salunkhe and Desai, 1984).

b) The normal O_2 and CO_2 concentration within the package due to the perforations. Normal O_2 concentration may cause ethylene production to go back to normal (Reid, 1992) while the normal CO_2 concentration of the atmosphere promoted chlorophyll degradation and thus increased yellowing (Bartth et al., 1993).

Average wilting sensory scores (Table 12) revealed that no wilting was observed on day 15 and 16 in all treatments. The PE-bags with and without perforations probably provided sufficient barrier to moisture loss in all treatments.

Similar results were also observed in decay sensory scores (Table 13). The low mold and mildew contamination rate in the dry monsoon, the low RH in the storage room (RH 50-63 %) and the very short observation period (3 days) were probably the reason.

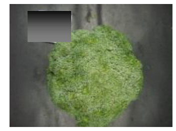
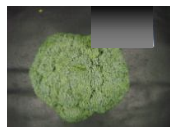
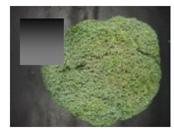
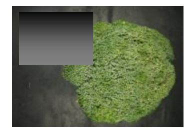
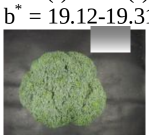
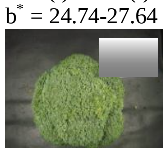
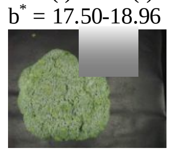
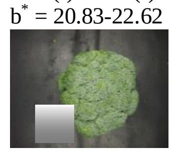
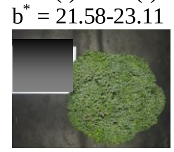
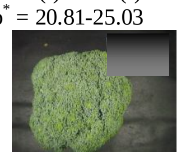
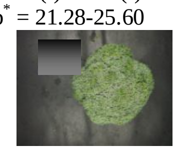
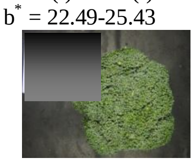
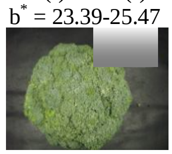
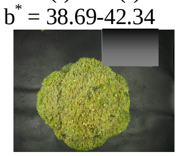
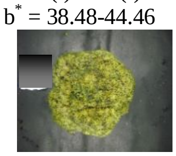
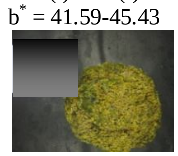
The overall quality sensory scores (Table 14) on day 15 and 16 gave excellent scores (score 9) for the un-perforated packs (A to D) and good scores for the perforated ones (E to H). Scores were affected primarily by the yellowing of the curd (Table 11).

4.3 Part 2 of the Experiment : storage at 10^0C±2^0C for 15 days.

4.3.1. Curd Surface Color

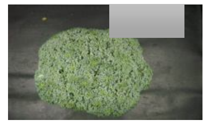
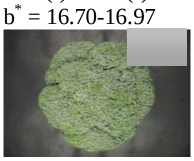
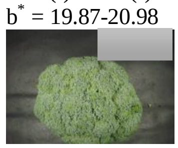
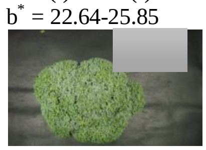
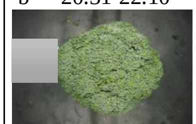
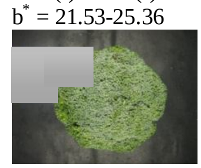
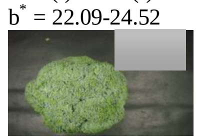
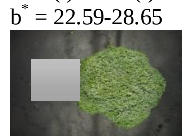
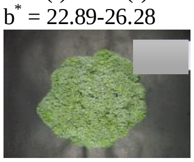
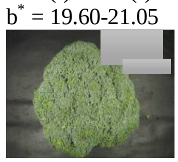
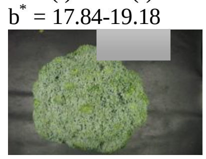
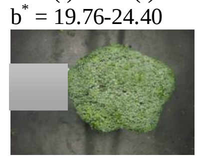
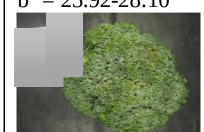
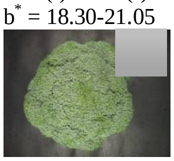
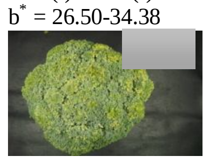
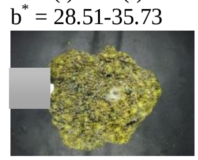
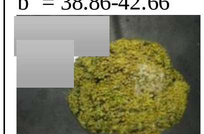
Curd surface color evaluated by the CIE-lab method is presented in Table 15.

Table 15 Broccoli Curd Surface Color during Cold Storage at $10^0C\pm2^0C$ Replicate I

T	Storage (Days)				
	3	6	9	12	15
A	L^* = 54.71-62.51 a^* = (-)13.50-(-)11.59 b^* = 18.82-21.76 	L^* = 53.36-61.78 a^* = (-)13.27-(-)13.15 b^* = 21.82-24.48 	L^* = 53.83-58.82 a^* = (-)13.02-(-)11.76 b^* = 21.46-22.93 	L^* = 50.20-50.89 a^* = (-)16.35-(-)14.20 b^* = 27.66-29.48 	L^* = 50.75-60.20 a^* = (-)14.69-(-)12.99 b^* = 24.88-31.32 
B	L^* = 53.24-58.52 a^* = (-)12.00-(-)11.12 b^* = 17.04-18.66 	L^* = 53.92-59.27 a^* = (-)11.71-(-)10.75 b^* = 18.60-19.32 	L^* = 58.34-29.80 a^* = (-)12.71-(-)12.12 b^* = 20.04-21.92 	L^* = 54.50-59.01 a^* = (-)11.81-(-)11.62 b^* = 20.31-26.20 	L^* = 53.47-55.88 a^* = (-)15.57-(-)13.78 b^* = 25.22-29.53 
C	L^* = 57.14-62.15 a^* = (-)12.66-(-)12.13 b^* = 19.12-19.31 	L^* = 55.00-57.28 a^* = (-)14.51-(-)13.66 b^* = 24.74-27.64 	L^* = 57.54-59.18 a^* = (-)11.37-(-)10.44 b^* = 17.50-18.96 	L^* = 58.66-63.85 a^* = (-)13.74-(-)12.58 b^* = 20.83-22.62 	L^* = 47.62-50.51 a^* = (-)13.32-(-)12.08 b^* = 21.58-23.11 
D	L^* = 56.57-61.99 a^* = (-)15.05-(-)13.27 b^* = 23.56-25.68 	L^* = 52.76-57.17 a^* = (-)12.59-(-)10.54 b^* = 19.24-22.32 	L^* = 54.56-60.38 a^* = (-)14.84-(-)12.31 b^* = 20.81-25.03 	L^* = 53.18-58.77 a^* = (-)13.68-(-)11.71 b^* = 21.28-25.60 	L^* = 49.64-49.93 a^* = (-)13.79-(-)12.90 b^* = 22.49-25.43 
E	L^* = 56.08-62.55 a^* = (-)10.78(-)10.48 b^* = 17.98-20.33 	L^* = 52.58-60.41 a^* = (-)13.52-(-)13.09 b^* = 23.39-25.47 	L^* = 59.29-64.67 a^* = (-)9.65-(-)6.75 b^* = 38.69-42.34 	L^* = 55.56-63.96 a^* = (-)8.56-(-)7.94 b^* = 38.48-44.46 	``L^* = 48.78-54.99 a^* = (-)4.27-(-)2.12 b^* = 41.59-45.43 

*) Power Failure on day 12, 15 hours; temperature with up to 22^0C

Replicate 2

Perlakuan	Lama Penyimpanan (Hari)				
	3	6	9	12	15
A	L^* = 53.35-65.55 a^* = (-)12.90-(-)11.26 b^* = 18.76-23.64 	L^* = 54.18-60.73 a^* = (-)11.20-(-)10.38 b^* = 17.93-18.53 	L^* = 57.28-61.47 a^* = (-)12.40-(-)11.13 b^* = 18.27-19.57 	L^* = 54.50-57.44 a^* = (-)15.74-(-)14.21 b^* = 23.80-25.30 	L^* = 50.84-53.38 a^* = (-)12.40-(-)10.82 b^* = 22.75-23.61 
B	L^* = 56.43-60.77 a^* = (-)10.14-(-)9.38 b^* = 16.70-16.97 	L^* = 51.94-63.16 a^* = (-)12.31-(-)12.01 b^* = 19.87-20.98 	L^* = 53.35-60.44 a^* = (-)15.31-(-)13.81 b^* = 22.64-25.85 	L^* = 56.98-60.89 a^* = (-)13.08-(-)11.64 b^* = 20.31-22.10 	L^* = 59.95-62.58 a^* = (-)13.10-(-)12.38 b^* = 21.53-25.36 
C	L^* = 55.20-65.36 a^* = (-)12.03-(-)11.43 b^* = 18.55-19.76 	L^* = 54.83-58.81 a^* = (-)16.54-(-)14.69 b^* = 27.22-29.39 	L^* = 55.00-64.69 a^* = (-)13.93-(-)13.36 b^* = 22.09-24.52 	L^* = 53.70-55.74 a^* = (-)13.83-(-)13.14 b^* = 22.59-28.65 	L^* = 50.74-55.28 a^* = (-)14.49-(-)11.04 b^* = 18.93-25.91 
D	L^* = 57.96-63.04 a^* = (-)15.24-(-)13.77 b^* = 22.89-26.28 	L^* = 54.08-57.42 a^* = (-)12.21-(-)10.99 b^* = 19.60-21.05 	L^* = 57.70-60.24 a^* = (-)11.63-(-)11.10 b^* = 17.84-19.18 	L^* = 52.59-57.39 a^* = (-)14.28-(-)12.57 b^* = 19.76-24.40 	L^* = 47.95-53.95 a^* = (-)13.87-(-)13.12 b^* = 25.92-28.10 
E	L^* = 52.80-58.91 a^* = (-)12.47-(-)10.32 b^* = 18.30-21.05 	L^* = 48.41-61.45 a^* = (-)13.71-(-)12.40 b^* = 22.66-31.14 	L^* = 56.84-64.21 a^* = (-)13.04-(-)12.84 b^* = 26.50-34.38 	L^* = 53.24-57.14 a^* = (-)5.15-(-)3.33 b^* = 28.51-35.73 	L^* = 47.24-54.93 a^* = (-)3.94-(-)1.43 b^* = 38.86-42.66 

*) Power Failure on day 12, 15 hours; temperature with up to 22^{0}C

Photos in Table 15 show that for treatment A, B, C and D green color of the broccoli curds could be maintained for 15 days, although some slight yellowing was evident in treatment A on day 12 and 15. On day 12 there was power failure causing a rise in cold storage temperature to 22^0C. Treatment E, however, started to turn yellowish after day 9, while on day 12 was already yellow and on day 15 was brownish yellow. This might be related to Breene's (1976) statement that quality of fresh produce could be maintained at higher storage temperature in controlled or modified atmospheres.

Evidently modified atmosphere within the package in treatment A (0 holes) was able to inhibit yellowing considerably when compared to E (4 holes). Modified atmosphere plus ethylene removers in treatment B, C and D were able to inhibit yellowing to a greater extent than MAP only (A). Furthermore, active coal (C) seemed to be best in preventing yellowing of the curds, followed by the mixture of K-Permanganate and active coal, and finally K-Permanganate; however the difference in yellowing among the three ethylene removers was very small.

4.3.2. Changes in Sensory Scores for Discoloration, Wilting, Decay and Overall Quality during Cold Storage at $10^oC \pm 2^oC$

Changes in sensory discoloration, wilting, decay and overall quality during cold storage are presented in Table 16, 17, 18, and 19. Regression equations for all parameters were undefined

Table 16. Discoloration Score during Cold Storage at $10^oC \pm 2^oC$

Treatment	Discoloration Score*)**) on Day					
	0	**3**	**6**	**9**	**12**	**15**
A	1	1	1	3	1-5	1-3
B	1	1	1-3	1-3	1-5	3-5
C	1	1	1	1	1	1-3
D	1	1	1-3	1-3	1-3	1-3
E	1	1	1-5	5-7	9	7-9

***)** Average of 2 replications
****)** Sensory score scale :
1. none 7. severe
3. slight 9. extreme
5. moderate

Table 16 shows that:

a. The unperforated bag (A) gave better results than the perforated one (E) which was the worst among the 5 treatments

b. Inspite of power failure on day 12, treatment C (0 holes + ac) was best among the 5 treatments tested because during the first 12 days there was no yellowing observed, but on day 15 slight yellowing was evident. Treatment B (0 holes + pp) gave similar results as treatment D (0 holes + mixture pp + ac) for 9 days i.e. no discoloration on day 3 followed by some slight yellowing on day 6 and 9. However, on day 12 and 15, treatment D (0 holes + pp + ac) gave better results i.e. discoloration was stable at 1-3 (none-slight); but B (0 holes + pp) showed increase of yellowing from slight to moderate (3-5).

Table 17. Wilting Sensory Score during Cold Storage at 10^{o}C ± 2^{o}C

Treatment	Wilting Sensory Score*)**) on Day					
	0	3	6	9	12	15
A	1	1	1	1-3	5	3-7
B	1	1	1	1-3	1-3	5
C	1	1	1	1-3	3-5	3
D	1	1	1	1-3	1-5	1-5
E	1	1	1	5	3	3-5

***)** Average of 2 replication
****)** Sensory score scale :
1. none 7. severe
3. slight 9. extreme
5. moderate

Table 17 shows that:

a. All treatments tested were able to prevent wilting up to 6 days
b. On day 9, treatments A to D (no holes) was better than E (with 4 holes)
c. Among the ethylene removers active coal showed larger wilting scores than the two others starting from day 12 onwards. This could be because active coal absorbs not only ethylene but also other gases, such as water vapor; on other hand K-permanganate works on just ethylene, i.e. as ethylene oxidizer.

Table 18. Decay Sensory Score during Cold Storage at 10^oC ± 2^oC

Treatment	Wilting Sensory Score*)**) on Day					
	0	3	6	9	12	15
A	1	1	1	1	7	7
B	1	1	1	1	5	7
C	1	1	1	1	5-7	7
D	1	1	1	1	5-7	7
E	1	1	1	1	7-9	7-9

***)** Average of 2 replication
****)** Sensory score scale :
1. none 7. severe
3. slight 9. extreme
5. moderate

Table 18 shows that all treatments were able to inhibit decay score up to day 9. however, on day 12 and 15 decay scores soared from none to severe/extreme in treatment A and E, while in treatment B, C, and D decay score ranged between moderate and severe. The results on day 12 and 15 are biased due to power failure on day 12, causing temperature to rise to 22^{0}C. Pantastico et al., (1975) reported that in tropical climates CA-storage and use of PE-bags without refrigeration cause fast deterioration of fresh produce as a result of heat and CO_2 built-up within the package.

Table 19. Overall Quality Score during Cold Storage at 10^oC ± 2^oC

Treatment	Overall Quality Score*)**) on Day					
	0	3	6	9	12	15
A	9	9	9	7	5	5
B	9	9	9	9	7	5
C	9	9	9	9	7	7
D	9	9	9	9	7	7
E	9	9	9	5	1	1

***)** Average of 2 replications
****)** Sensory score scale :
1. extremely poor 7. good
3. poor 9. excellent
5. fair

Table 19 shows that:

a. All treatments could maintain overall quality score of curds in excellent condition for 6 days.
b. On day 9-15 overall quality score of treatment E (with holes) declined rapidly and became poor to extremely poor, treatment A (without holes) also declined but at less severe level, respectively good to fair. The treatments with ethylene removers were all higher in overall quality score, with C and D about equal i.e excellent to good and B excellent to fair. Thus, for storage at $10^0C\pm2^0C$ MAP with active coal as ethylene remover (C) was the best.

4.3.3. Incidence of Soft Rot

Incidence of soft rot during the 15 day low temperature storage is presented in Table 20. Regression equations however were undefined.

Table 20. Incidence of Soft Rot at Cold Storage at $10^0C\pm2^0C$

Treatment	Incidence of Soft Rot *) (% no of samples) on Day					
	0	3	6	9	12**	15**
A	0	0	0	0	100	100
B	0	0	0	0	100	100
C	0	0	0	0	100	100
D	0	0	0	0	100	100
E	0	0	0	0	100	100

***)** Average of 2 replications
**) Power failure overnight (± 15 hours)

All treatment were free of soft-rot up to day 9, but soared up to 100% on day 12 and 15. On day 12, power failure occurred causing a rise in temperature to 22^0C, which triggered growth of fungus.

4.3.4. Weight Loss

Weight Loss could not be observed longer than day 9 because on day 12 all treatments showed extensive soft rot of the curd’s stems. This could be the result of power failure on day 12. Result of regression analyses showed there is linear relationship between weight loss and storage time in treatment A and D, but none exist in B, C and E. Regression curves are presented in Figure 5 while regression and equations, coefficients of determination and coefficients of correlation are presented in Table 21. Coefficient of Determination (R^2) for A and D ranged between 0.826-0.876 showing sufficiently good fit.

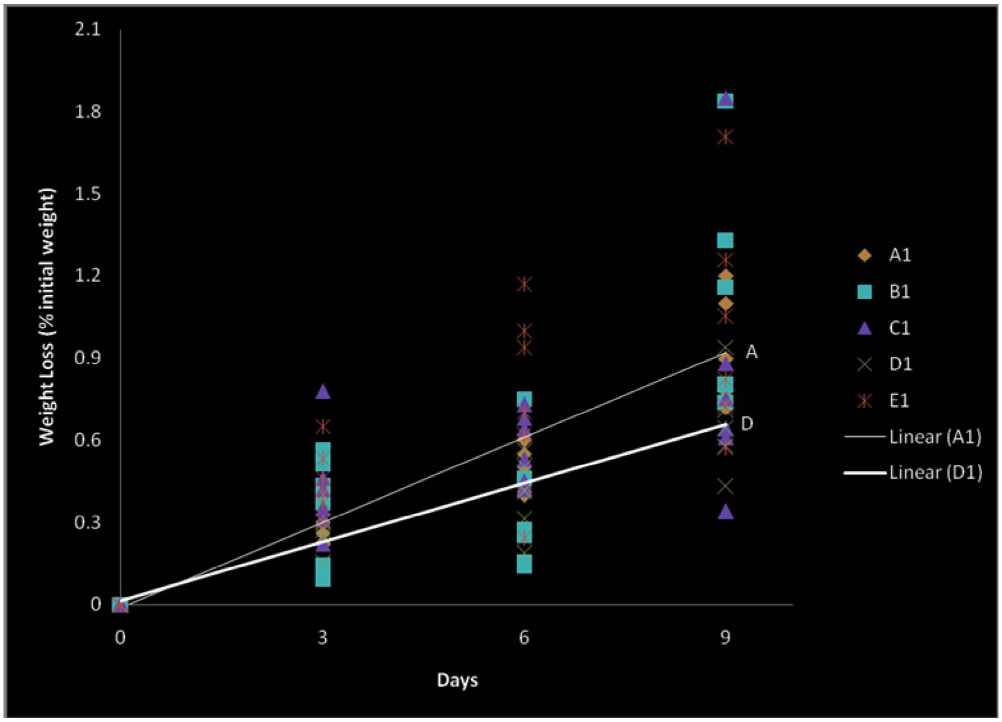


Figure 5. Regression Curves of Weight Loss during Cold Storage at $10^0C\pm2^0C$

Table 21. Regression Equations of Weight Loss (% Initial Weight) during Cold Storage at $10^0C\pm2^0C$

Treatment	Regression Equation	R^2	r
A	Y = 0.103x-0.014	0.876	
B	Undefined	-	-
C	Undefined	-	-
D	Y =0.071x+0.012	0.826	
E	Undefined	-	-

V. Conclusion

Part (1) : Storage at 3.8^{0}C

1. Cold Storage at 3.8^{0}C for 12 days of broccoli packed in perforated and un-perforated poly-bags and each provided with sachet containing K-permanganate, active coal or K-permanganate and active coal, gave excellent results, in terms of :
   a. Curd surface color: Dark Green (No discoloration/yellowing)
   b. Incidence of soft rot : 0% (none)
   c. Sensory score
      - Discoloration : 1 (None)
      - Decay sensory score : 1-3 (None to slight)
      - Wilting sensory score : 1-3 (None to slight)
      - Overall quality sensory score : 9-7 (Extremely good to good)
   d. Weight loss : low (0.78% – 1.09%)
2. Room Temperature storage (23^0-27^{0}C) for 3 days showed that :
   a. Slight curd surface yellowing occurred in both perforated and un-perforated poly-bags, with slightly more severe yellowing in the former
   b. No incidence of soft rot
   c. Sensory scores for discoloration, wilting, and decay were lower and overall quality was higher in the un-perforated bags than in the perforated ones
   d. The ethylene removing chemicals respectively K-permanganate, active coal and their mixture did not affect yellowing of curd, incidence of soft rot and sensory scores

Part 2 : Storage at 10^{0}C±2^{0}C.

1. Curd Surface Color
   a) Un-perforated bags inhibited yellowing better
      - Un-perforated bags : slight yellowing on day 12 and 15
      - Perforated bags : - yellowing on day 9 and become more severe on day 12 and 15
        - worst among the treatments

b) MAP + ethylene removers better than MAP alone
   - Active coal : best, no yellowing on day 15
   - Active coal and permanganate : second best, very slight yellowing on day 9, 12 and 15
   - K-Permanganate : Third best, slight yellowing on day 9, 12 and 15

2. Sensory Scores
   a. Discoloration score : - Perforated package worst, on day 6 : 1-5
      - Un-perforated + ac best, on day 15 : 1-3
   b. Wilting score : - Wilting evident on day 6
      - Un-perforated bags better, on day 9 : 1-3
      - Active coal higher, wilting score on day 12 : 3-5
   c. Decay score : - No decay up to day 9
      - Power failure on day 12 increase decay score : 5-7-9
   d. Overall quality score : - Un-perforated + ac : best
      - Un-perforated better than perforated
      - Difference among ethylene removers : very small
3. Incidence of Soft Rot Score
   - Soft rot score up to day 9 : none
   - On day 12-15 due to power failure : 100 % soft rot
4. Weight Loss
   - Observation limited to day 9 due to soft rot of stems
   - Weight loss : 0.67 – 1.17 % (initial weight) on day 9

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