

DEVELOPMENT OF IN-TRANSIT RIPENING TECHNOLOGY FOR DELIVERING

READY-TO-EAT PEARS

By

CHENYI XU

A thesis submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Food Science

Written under the direction of

Kit L. Yam

And approved by

---

---

---

New Brunswick, New Jersey

May, 2017

# **ABSTRACT OF THE THESIS**

DEVELOPMENT OF IN-TRANSIT RIPENING TECHNOLOGY

FOR DELIVERING READY-TO-EAT PEARS

By CHENYI XU

Thesis Director:

Kit L. Yam

Non-availability of year-around fully-ripened ready-to-eat pears at retail level has become one of major factors that limit fresh pear consumption in U.S. market in recent years. The objective of this project is to test the feasibility of developing a ripening technology that can have pear fully-ripened when they are delivered to distribution center. Ripening is triggered by ethylene encapsulated in  $\alpha$ -cyclodextrin, packaged in sachet and control released by moisture from respiration of pears, under refrigeration temperature during transportation.

The objective is composed of three sub-objectives: (1) Ethylene encapsulation in  $\alpha$ -cyclodextrin. (2) Release study of sachet (PVA film and Tyvek® paper) packaged encapsulated complex with RH as trigger (70%, 80% and 90%). (3) Biological evaluation

of efficacy of ethylene released from sachet system.

Ethylene encapsulation was controlled by encapsulation duration time and headspace ethylene concentration. Under  $1 \times 10^6$  ppm headspace concentration, ethylene was encapsulated in  $\alpha$ -cyclodextrin with 2.1% inclusion ratio within 20hrs. Headspace ethylene concentration and duration time did not change inclusion ratio of encapsulated complex, remaining around 2.1%. However, higher headspace ethylene concentration and longer duration time would increase yield of encapsulated complex.

PVA film and Tyvek® paper were used as sachet materials to control release ethylene from encapsulated complex at 4°C with RH as trigger. Release of ethylene from Tyvek® sachet system was faster than from PVA sachet system. Under 90% RH, it took around 72hrs to completely release ethylene from PVA sachet system and 48hrs to reach complete release from Tyvek® sachet system.

Unripened pears were used to evaluate biological efficacy of ethylene released from encapsulated complex packaged in Tyvek® sachet. After 10 days of ethylene treatment from sachet system along with stored at 4°C, firmness of pears decreased to 4.2 lbf, within the range of firmness of pears with good eating quality, from 18 lbf. While the firmness of those pears without ethylene treatment had only decreased to 10.3 lbf, which were still unripened.

In conclusion, it is technically feasible to develop an in-transit ripening technology to

provide ready-to-eat pears when they arrive at the distribution center. RH from the respiration of pears was able to trigger the release of ethylene to induce their ripening along with low temperature during transportation.

## **Acknowledgement**

I would like to give deepest appreciation to my advisor, Dr. Yam, who is not only a research advisor but also shares wisdom about life with me. I still remember the phone call between us when I was trying to apply for the admission of master degree of Food Science Department at Rutgers three years ago. He told me to have a big picture in my mind of what I want to achieve and always keep thinking. What I learned from him is not only science and research knowledge, but also philosophy about life which I will encounter and use in my continuing whole life. He trained us to take turns to be chair and co-chair to take notes of every group meeting in lab. He taught us to have “Ha Ha” moment when giving a presentation. All these will become priceless treasure in my life.

I would like to give my sincere appreciation to Dr. Mir, who offered me all the materials and resources. It is Dr. Mir who brought me from the environment of university to industry. He showed me the real industry and business, which is full of challenges and surprises.

I would also like to show my thankfulness to Dr. Xi Chen, my lab mate. We have been worked together for more than two years and the cooperation between us was perfect. He devoted himself on working and the way he works always encourages me to try our best and keep moving.

In addition, I want to thank all my lab mates and visitors. We had an unforgettable memory in Dr. Yam’s lab, room 413.

Finally, I would like to thank my parents and husband. They supported me for my study not only financially but also mentally. I really appreciate them for their understanding. They always offer me a hug when I encounter difficulties and this energizes me to work harder to payoff.

## Table of Contents

ABSTRACT OF THE THESIS .....	ii
Acknowledgement .....	v
Table of Contents .....	vii
Lists of Tables .....	x
Lists of Illustrations .....	xi
1 Introduction.....	1
1.1 Roles of ethylene in fruit ripening .....	1
1.2 Chilling need for pear ripening .....	2
1.3 Problem of ready-to-eat pears .....	4
1.4 In-transit ripening technology to solve problem .....	5
2 Technique Background .....	7
2.1 Temperature management and ethylene for pear ripening.....	7
2.2 Cyclodextrin.....	8
2.3 Sachet material.....	12
3 Objective .....	15
4 Materials and Methods .....	17
4.1 Materials .....	17
4.2 Ethylene encapsulation in $\alpha$ -cyclodextrin.....	17

4.3 Ethylene quantification .....	21
4.4 Release study of ethylene from sachet system.....	23
4.4.1 Sachet preparation .....	23
4.4.2 Simulated RH conditions.....	24
4.4.3 Water vapor transmission rate of sachet materials .....	25
4.4.4 PVA permeability to ethylene .....	26
4.4.5 Release of ethylene from sachet system.....	27
4.5 Biological evaluation of efficacy of ethylene released from sachet system ....	28
5 Results and Discussion .....	30
5.1 Standard curve for ethylene quantification .....	30
5.2 Ethylene encapsulation .....	31
5.2.1 Inclusion ratio and yield .....	31
5.2.2 Encapsulation process .....	33
5.2.3 Influence of headspace ethylene concentration and duration time on inclusion ratio.....	36
5.2.4 Influence of headspace ethylene concentration and duration time on yield	38
5.3 Ethylene release process .....	40
5.4 Water vapor transmission rate of PVA film and Tyvek® paper .....	42
5.5 Release profile of encapsulated complex.....	45



5.6 PVA and Tyvek® paper permeability of ethylene.....	46
5.7 Release profile of sachet system .....	49
5.8 Biological evaluation of efficacy of ethylene released from sachet system ....	52
5.9 Overall analysis.....	54
6 Conclusion .....	57
7 Future Work .....	58
8 References.....	60

## **Lists of Tables**

Table 2.1 Appropriate number of days needed to induce ripening capacity of pears .	7
Table 2.2 Characteristic of cyclodextrins.....	11
Table 4.1 Materials and suppliers .....	17
Table 4.2 GC method condition .....	22
Table 4.3 Equilibrium relative humidity and saturated salt solutions under 5°C .....	24
Table 5.1 Water vapor transmission rate of PVA film and Tyvek® paper .....	43
Table 5.2 Sachet permeability of ethylene and PVA film thickness .....	48
Table 5.3 Firmness of pears both before and after ethylene treatment .....	54

## Lists of Illustrations

Figure 1.1 Ethylene molecule structure .....	1
Figure 1.2 Ethylene biosynthesis during pear ripening .....	3
Figure 1.3 In-transit system .....	5
Figure 2.1 Chemical structure of cyclodextrin molecule.....	9
Figure 2.2 3-D molecular model of ethylene .....	11
Figure 2.3 Molecular structure of encapsulated complex .....	12
Figure 2.4 Chemical structure of PVA .....	13
Figure 2.5 Tyvek® paper structure .....	14
Figure 3.1 In-transit system and its components .....	15
Figure 4.1 Setup of ethylene encapsulation in $\alpha$ -cyclodextrin .....	18
Figure 4.2 Formation of encapsulated complex.....	19
Figure 4.3 Setup of drying process after encapsulation.....	20
Figure 4.4 Ethylene- $\alpha$ -cyclodextrin encapsulation complex .....	21
Figure 4.5 Gas chromatography with FID detector .....	22
Figure 4.6 Ethylene headspace sample .....	23
Figure 4.7 PVA sachet packaged encapsulated complex .....	24
Figure 4.8 Setup of gravimetric method .....	26
Figure 4.9 Setup of experiment for testing PVA permeability to ethylene .....	27

Figure 4.10 Setup of release of ethylene from sachet system.....	28
Figure 5.1 Relationship between PA and ethylene concentration .....	31
Figure 5.2 Encapsulation process .....	35
Figure 5.3 Relationship between headspace ethylene concentration and inclusion ratio and duration time .....	37
Figure 5.4 Relationship between headspace ethylene concentrations and encapsulation duration time and yield .....	39
Figure 5.5 Ethylene release process triggered by RH.....	42
Figure 5.6 Relationship between the weight of jar and time .....	43
Figure 5.7 Pears that before treatment and after 10 days ethylene treatment..	53
Figure 5.8 In-transit system and its components .....	55

# 1 Introduction

## 1.1 Roles of ethylene in fruit ripening

Ethylene (Figure 1.1), a small hydrocarbon gas with formula  $C_2H_4$ , is called natural fruit ripening hormone. It can induce those genes encoding for cell wall modifying enzymes, therefore softening texture and triggering ripening of climactic fruits and vegetables (Fischer and Bennett 1991). Ripening of fruits is associated with changes of color, alteration of sugar metabolism, softening and changes in texture, synthesis of aroma volatiles and changes on nutritional status (Jermyn and Isherwood 1956, Ben-Arie, Kislev et al. 1979, Abeles, Morgan et al. 2012). Some fruits, like apple, avocado, banana, mango, tomato and pear, are climacteric fruits. They will produce ethylene during ripening process with preceding respiratory climacteric and it is naturally occurring (Burg and Burg 1965, Lelièvre, Latchè et al. 1997, Hiwasa, Nakano et al. 2004). Non-climacteric fruit, like lemon, orange, pineapple and strawberry, do not exhibit a burst but a decline of ethylene production (Brady 1987, Seymour, Taylor et al. 2012).

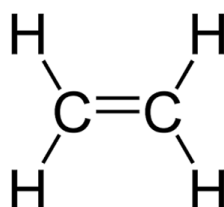


Figure 1.1 Ethylene molecule structure

## 1.2 Chilling need for pear ripening

Pear, unlike other climacteric fruits, even harvest at right maturity or left on tree to ripen, possess resistance to ripening and needs to go through a long-time chilling process (usually lasts from 20 to 60 days at 0°C) and/or external exposure of ethylene to trigger the following ripening process (Murayama, Takahashi et al. 1998, Hernández-Pérez, Carrillo-López et al. 2005). Without chilling process and/or exposure of external ethylene, the texture of pear will breakdown and become coarse and mealy which is unsatisfied by customers (Westwood and HO 1968).

Pear needs to be stored at low temperature between -1 to 0°C for a long time, around 20 to 60 days, in order to trigger ethylene biosynthesis during subsequent ripening at room temperature. The time required for normal ripening varies among different pear varieties. Bartlett needs about 21 days, Bosc needs about 14 days and D'Anjou needs about 60 days (Silos-Espino, Fabian-Morales et al. 2003, Villalobos-Acuña and Mitcham 2008, Sugar, Mitcham et al. 2009).

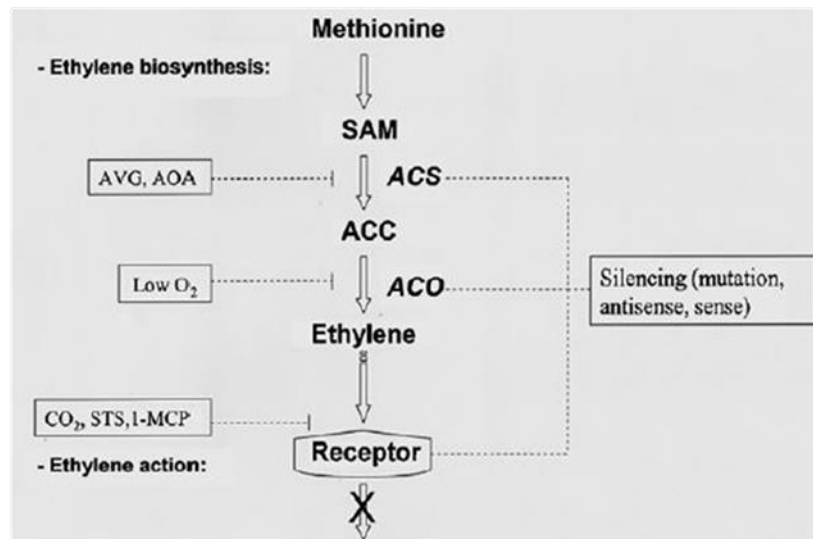


Figure1.2 Ethylene biosynthesis during pear ripening

Figure 1.2 shows the main compounds and enzymes related to ethylene biosynthesis during ripening of pears (Itai, Kawata et al. 1999, Fonseca, Hackler et al. 2004, Fonseca, Monteiro et al. 2005). Methionine, a kind of amino acid is first converted to S-adenosyl-methionine (SAM), and then converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and further oxidized to ethylene with the help of ACC oxidase (ACO) (Wang, Li et al. 2002). Before functioning, ethylene molecules need to be bind to ethylene receptor in order to trigger the following ripening (Lacey and Binder 2014). Low temperature tends to increase the activity of these two enzymes (ACS and ACO), as a result of this, the content of ACC will increase. Therefore, chilling process has an effect to promote biosynthesis of ethylene and then trigger the ripening of pears by increasing enzyme activity (Yang and Hoffman 1984).

### 1.3 Problem of ready-to-eat pears

Pear needs to go through a long time chilling period in order to trigger the ripening capacity. However, the demand of pear consumption in U.S. market in recent years has increased because people are encouraged to eat more fruits and vegetables which is a more healthy eating habit instead of fried foods (Gibson, Wardle et al. 1998, O'Neil, Nicklas et al. 2014). The unbalance between long-time chilling period and increasing fresh pear demand may cause inconsistent fruit quality at supermarket level. Some pears may not become fully ripened when displayed at supermarket and consumers have to wait for a few days to get them reaching preferred stage of ripeness. Thereby, it is a problem of non-availability of year-round fully ripened ready-to-eat pears at retail level.

Research shows that sales will increase by 20% if fully ripened pears are sold at market. Proper ripening either through chilling process or external ethylene helps to develop better taste and texture thereby improving sales and consumption of pears. For example, fully ripened Bartlett pears sell 3 times more than unripened ones and D'Anjou pears ripened with ethylene are sold 16% more than untreated ones. Therefore, Pear Bureau is looking for ways to supply year-round fully-ripened pears to increase the consumption of pear in both U.S. market and internationally. Controlled atmosphere technology in combination with 1-MCP application can extend the shelf life of pears but it is a challenge to ripen pear after 1-MCP application (Baritelle, Hyde et al. 2001, Argenta, Fan et al. 2003, Lee, Beaudry et al. 2006, Seglie,



Devecchi et al. 2013). It is recommended that pears are ripened with external ethylene when arriving at supermarket before displayed at retail. However, it is quite difficult to conduct this due to the lack of cold chain and infrastructure for ripening.

#### 1.4 In-transit ripening technology to solve problem

To solve the problem of non-availability of year-round fully ripened ready-to-eat pears at retail level, an efficient in-transit ripening technology was proposed here by combining low temperature management and external ethylene treatment, where ripening of pears was triggered by ethylene under refrigeration temperature ( $4^{\circ}\text{C}$ ) during transportation. Therefore, pears can become ready-to-eat when arriving at distribution center. Figure 1.3 shows the in-transit system.



Figure 1.3 In-transit system



A major component of this technology was ethylene encapsulation system. Ethylene was encapsulated in cyclodextrin and packaged in PVA film or Tyvek® paper sachet, which can release the appropriate amount of ethylene at a controlled rate with RH,

produced by respiration of pear, as a trigger to further activate the ripening of pear during transportation.

The whole ripening process happens in the truck during transportation, thus, it can reduce traditional long time chilling treatment and thereby save time. With the combination of proper temperature management and external ethylene treatment, the system would not only trigger the ripening of pears but also get the pears to the right eating quality level when reaching supermarket. In addition, the sachet package is more simple and convenient to use compared to using compressed gas, catalytic converters, power or specialized training.

## 2 Technique Background

### 2.1 Temperature management and ethylene for pear ripening

The combination of temperature management and external ethylene treatment were used to obtain good eating quality of pears and meet the market time requirement at the same time. Table 2.1 shows the appropriate number of days needed to induce ripening capacity of different kinds of pears (harvested at earliest maturity, no pre-cooling before conditioning) (Maxie, Mitchell et al. 1974, Agar, Biasi et al. 2000, Sugar, Mitcham et al. 2009).

Table 2.1 Appropriate number of days needed to induce ripening capacity of pears

Variety	No ethylene			After 24hr in ethylene at 20°C			After 48hr in ethylene at 20°C		
	0°C	5°C	10°C	0°C	5°C	10°C	0°C	5°C	10°C
Bosc	15	9	5	0	0	0	0	0	0
Comice	30	19	12	15	7	3	7	2	2
Anjou	>60	35	17	44	18	<10	38	12	<10

After 48hrs of ethylene treatment at 20°C, it only needs 18 days at 5°C to trigger ripening capacity of Anjou pear compared to those without ethylene treatment, it needs around 35 days at 5°C to trigger ripening capacity. Controlling the storage temperature at 10°C tends to reduce the conditioning time from more than 60 days to

17 days compared to store at 0°C for Anjou pear. Therefore, the combination of low temperature management and external ethylene treatment would reduce the long time chilling period and be the solution to solve the problem of non-availability of year-round fully-ripened ready-to-eat pears at retail level.

## **2.2 Cyclodextrin**

A major component in this technology is ethylene encapsulation system. It is quite dangerous and inconvenient to use compressed gas as an external ethylene source. Thus, certain protective and storage technology is quite important to overcome this limit. In this work, cyclodextrin is used as a storage carrier to encapsulate ethylene. Cyclodextrins are composed of 5 or more  $\alpha$ -D-glucopyranoside units linked together by  $\alpha$ -1,4 link to form a cone structure (Szejtli 2013). Figure 2.1 shows the structure of cyclodextrin molecule (Bender and Komiyama 2012). Typical cyclodextrins have a number of glucose monomers ranging from 6 to 8. If there are 6 glucose units, then it is  $\alpha$ -cyclodextrin. If it contains 7 sugar molecules in the ring, it is  $\beta$ -cyclodextrin. If it is made of 8 glucose units, it is called  $\gamma$ -cyclodextrin.

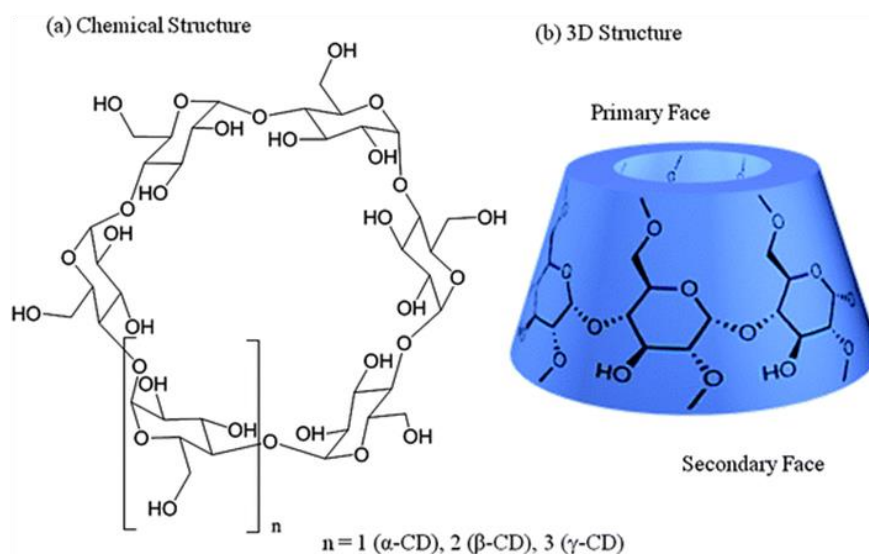


Figure 2.1 Chemical structure of cyclodextrin molecule

C-O skeleton groups of each glucose unit are linked together to form a toroidal structure and the hydroxyl groups of each glucose unit are towards the top and bottom of the whole cyclodextrin ring. The cyclodextrin ring is not like a symmetrical cyclinder but a toroid with smaller and larger openings, which is called primary face and secondary face. Due to those hydroxyl groups on the primary and secondary faces, the exterior of cyclodextrin is hydrophilic because of hydrogen bonds between hydroxyl groups and water molecules in the environment. In contrast, the inner cavity of cyclodextrin is considerably less hydrophilic than the exterior, though not hydrophobic (Szejtli 1998, Marques 2010, Trotta, Cavalli et al. 2011). Because of this specific structure, cyclodextrin can encapsulate hydrophobic molecules in its inner cavity, such as ethylene, to form encapsulated complex. When the encapsulated complex is in contact with water, water will serve as a plasticizer to make the ring structure loose. As a result of this, hydrophobic guest molecules can be released from

cyclodextrin ring. Due to this arrangement, cyclodextrin can trap hydrophobic ethylene guest molecule inside, serving as a delivering carrier and then control release it with RH as trigger (Duchene, Wouessidjewe et al. 1999, Ho 2013, Bazzano, Barolo et al. 2016).

The formation of encapsulated complex has a great influence on both physical and chemical properties of guest molecule. It can encapsulate hydrophobic guest molecule inside and increase its water solubility due to hydrophilic exterior. Thus, cyclodextrin has been used in many fields. For example, it can serve as powdered flavors and spices in food industry (Saenger 1980). In addition, it can be used for detergent and hair spray in cosmetics. And in pharmaceutical application, the encapsulated complex of cyclodextrin with hydrophobic guest molecule can work as delivering agent, penetrating body to release biologically active compounds under specific conditions (Uekama, Hirayama et al. 1998, Davis and Brewster 2004).

The principle governs encapsulation is size match (Del Valle 2004). Two criteria are used to determine the kind of cyclodextrin which ethylene can be encapsulated in: (1) molecule volume of ethylene must be smaller than the volume of inner cavity of cyclodextrin and (2) the dimension of ethylene guest molecule must be smaller than and also close to the dimension of inner cavity of cyclodextrin, so that ethylene can be tightly trapped in cyclodextrin.

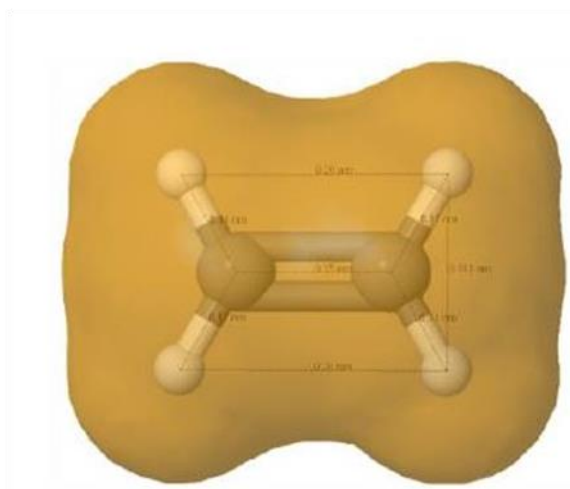


Figure 2.2 3-D molecular model of ethylene

Figure 2.2 shows the 3-D molecular model of ethylene. The molecular volume and dimension are calculated based on Van der Waals surface. The volume of ethylene molecule is  $64.63 \text{ \AA}^3$ , and dimension of ethylene molecule is  $4.9 \times 3.4 \times 3.9 \text{ \AA}$ .

Table 2.2 Characteristic of cyclodextrins

	$\alpha$ -cyclodextrin	$\beta$ -cyclodextrin	$\gamma$ -cyclodextrin
<b>Number of glucose units</b>	6	7	8
<b>Molecular weight</b>	972.86	1135.01	1297.15
<b>Water solubility(g/L)</b>	145	18.5	232
<b>Internal diameter(<math>\text{\AA}</math>)</b>	4.7-5.2	6.0-6.4	7.5-8.3
<b>Depth(<math>\text{\AA}</math>)</b>	6.7	7.0	7.0

Table 2.2 shows the internal diameter of three typical cyclodextrins,  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrin. The volume of ethylene molecule is smaller than the internal cavity

volume of both  $\alpha$ -,  $\beta$ - and  $\gamma$ - cyclodextrin. In terms of dimension, one side of ethylene molecule ( $3.4 \times 3.9 \text{ \AA}$ ) is a little bit smaller than the internal diameter of  $\alpha$ -cyclodextrin ( $4.7\text{-}5.2 \text{ \AA}$ ). On the other hand, the internal diameter of  $\beta$ - and  $\gamma$ -cyclodextrin is much larger than dimension of ethylene molecule. Therefore, ethylene is geometrically compatible with  $\alpha$ -cyclodextrin. Because the dimension of ethylene is close to the internal diameter of  $\alpha$ -cyclodextrin, so that ethylene can be tightly bounded around the area of cavity opening and only one molecule of ethylene can be encapsulated in one molecule of  $\alpha$ -cyclodextrin, showed by Figure 2.3.

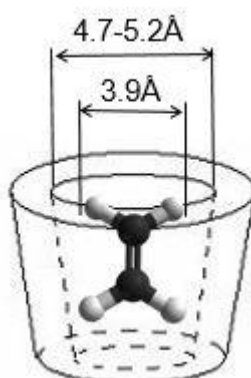


Figure 2.3 Molecular structure of encapsulated complex

Since ethylene is geometrically compatible with  $\alpha$ -cyclodextrin, it was used in this work as a carrier to control release ethylene with RH as trigger.

## 2.3 Sachet material

PVA (polyvinyl alcohol) film and Tyvek<sup>®</sup> paper were used as sachet material to control release ethylene with RH as trigger.



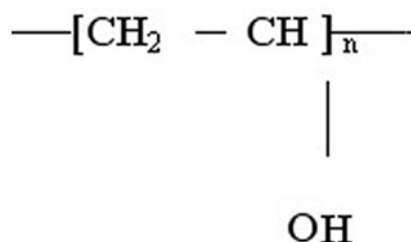


Figure 2.4 Chemical structure of PVA

PVA (polyvinyl alcohol) is a water-soluble synthetic polymer with idealized formula  $[\text{CH}_2\text{CH}(\text{OH})]_n$ . It is white (colorless) and odorless and mainly used in papermaking, textiles and coatings. Figure 2.4 shows the structure of PVA. It has hydroxyl group on each monomer unit, As a result of this, the solubility of PVA is very high compared to other polymers because of the hydrogen bonds between hydroxyl group and water molecule. Besides high solubility, PVA has excellent film forming, emulsifying and adhesive properties. It has high resistance to oil, grease and solvents. Thus, PVA film was used in this work as sachet material to package encapsulated complex in order to control release ethylene with RH as trigger since PVA film has very low barrier to water molecules.

Tyvek® paper is a spun-bonded synthetic material made of high-density polyethylene (HDPE) fiber and the name is registered trademark of DuPont. The polymer HDPE itself has low water permeability and it is liquid water resistant. However, Tyvek® paper is made by combining continuous HDPE fibers into a sheet through a process using heat and pressure, picture shown by Figure 2.5.

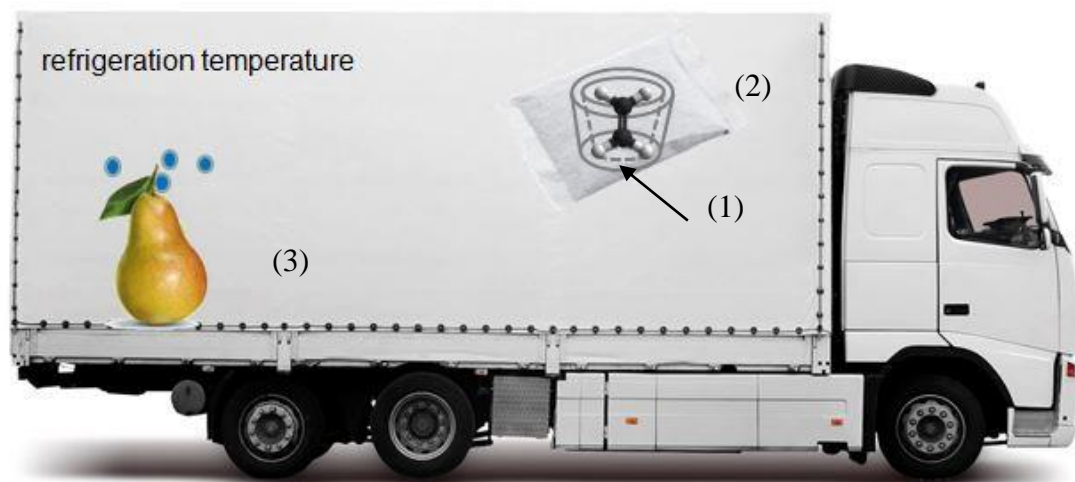


Figure 2.5 Tyvek® paper structure

There are lots of small holes between fibers compared to polymer film and water vapor molecules can easily pass through those holes but liquid water cannot. Meanwhile, it is difficult to tear but easy to be cut with a knife. Due to this special property, Tyvek® paper is useful in many applications. It is mainly used as housewrap to protect building during construction. In this work, Tyvek® paper was used as sachet material to package encapsulated complex because water vapor can easily pass through it to trigger the release of ethylene from  $\alpha$ -cyclodextrin.

### 3 Objective

The objective of this work is to prove the concept of a ripening technology that can provide fully-ripened and ready-to-eat pears when they arrive at the distribution center. In this technology, ripening is triggered by ethylene encapsulated in  $\alpha$ -cyclodextrin, packaged in sachet and control released by RH, under refrigeration temperature during transportation. This objective is composed of three sub-objectives, shown in figure below:



- (1): Ethylene encapsulation
- (2): Release of ethylene from sachet
- (3): Ripening of pears triggered by ethylene released from sachet system

Figure 3.1 In-transit system and its components

(1) Ethylene encapsulation in  $\alpha$ -cyclodextrin to get as high inclusion ratio as possible.

Inclusion ratio is the weight of ethylene been encapsulated in  $\alpha$ -cyclodextrin over the weight of total encapsulated complex gotten, shown as equation below.

$$\text{inclusion ratio} = \frac{\text{weight of ethylene been encapsulated (g)}}{\text{weight of total encapsualted complex (g)}} \times 100\%$$

The higher the inclusion ratio, the more ethylene is encapsulated in  $\alpha$ -cyclodextrin.

Thus, less  $\alpha$ -cyclodextrin can be used to encapsulate the same amount of ethylene, in order to reach same target ethylene concentration to trigger ripening of pears. This will reduce the cost of material since  $\alpha$ -cyclodextrin is quite expensive.

(2) Release study of sachet (PVA film and Tyvek® paper) packaged encapsulated complex with RH as trigger (70%, 80% and 90%). Different RH conditions simulate moisture coming from respiration of pears, which can trigger the release of ethylene from  $\alpha$ -cyclodextrin with different package by different rate.

(3) Biological evaluation of efficacy of ethylene released from sachet system. This was to test whether the RH coming from respiration of pears can the release of ethylene from sachet system and the efficacy of ethylene to trigger the ripening of pear.

## 4 Materials and Methods

### 4.1 Materials

Table 4.1 shows all the materials and their suppliers.

Table 4.1 Materials and suppliers

Chemical	Supplier
Ethylene	Matheson
$\alpha$ -cyclodextrin	TCI
PVA film	Acello
Tyvek® paper	DuPont USA
Potassium Iodide	Sigma-Aldrich
Sodium Nitrate	Sigma-Aldrich
Potassium Chloride	Sigma-Aldrich

### 4.2 Ethylene encapsulation in $\alpha$ -cyclodextrin

Ethylene was encapsulated in  $\alpha$ -cyclodextrin using the method described in literature (Ho, Joyce et al. 2011, Bazzano, Barolo et al. 2016). There are two factors affecting encapsulation, encapsulation duration time and headspace ethylene concentration (Ho, Joyce et al. 2011, Ho 2013). Experiments were designed to get as high inclusion ratio as possible based on encapsulation duration time and headspace ethylene concentration.



Figure 4.1 Setup of ethylene encapsulation in  $\alpha$ -cyclodextrin

Figure 4.1 shows the setup of ethylene encapsulation in  $\alpha$ -cyclodextrin. One gram of  $\alpha$ -cyclodextrin was dissolved in 11.5ml DI water inside a glass jar, which is the highest solubility. After that, the jar was tightly closed with lid and connected to vacuum pump to remove the headspace air inside. Then the jar was connected to ethylene gas tank and the inlet valve on the top of lid was slowly opened for ethylene gas to go inside. The amount of ethylene gas went inside the jar controlled the headspace ethylene concentration of encapsulation. Three different headspace ethylene concentrations were used to test the effect on encapsulation, which is  $0.5 \times 10^6$ ,  $0.75 \times 10^6$  and  $1 \times 10^6$  ppm. The glass jar was then placed on a stir plate set for 300 rpm for 2, 5, 8, 14, 16, 18 and 20hrs as different encapsulation duration time. During encapsulation, white powder was separated out from solution, which was

ethylene- $\alpha$ -cyclodextrin encapsulation complex, shown as Figure 4.2.



Figure 4.2 Formation of encapsulated complex

After encapsulation, the solution was filtered and dried to get encapsulated complex.

Figure 4.3 shows the setup of drying process of encapsulated complex.



Figure 4.3 Setup of drying process after encapsulation

A filter flask was connected to vacuum pump to separate encapsulated complex from solution quickly. Once the jar been opened, all the solution including white powder was quickly dumped onto filter paper. During filtration, ethanol was used to rinse the solution in order to increase its evaporation. After the filtration, the encapsulated complex would be on the top of filter paper and water would be in the filter flask. The filter paper was then taken out and put in 50°C oven to be further dried until the weight did not decrease. Encapsulation complex was scratched from the filter paper and stored in a plastic test tube in desiccator. Figure 4.4 shows ethylene- $\alpha$ -cyclodextrin encapsulated complex.



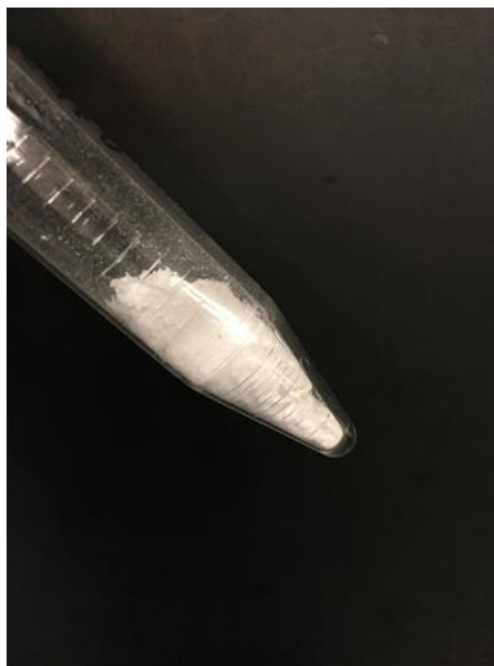


Figure 4.4 Ethylene- $\alpha$ -cyclodextrin encapsulation complex

### 4.3 Ethylene quantification

Gas chromatography with FID detector, shown in Figure 4.5, was used to quantify the inclusion ratio of ethylene in encapsulated complex.



Figure 4.5 Gas chromatography with FID detector

Table 4.2 shows the detailed GC method conditions.

Table 4.2 GC method condition

GC type: Hewlett packed 5890

Column: 6m long, 2mm i.d. stainless steel column packed with activated alumina

Injection system

Injector mode: splitless injection

Injector volume: 0.25ml

Detector: flame ionization detector

Temperature

Injection port: 120°C

Detector: 180°C

Oven program: 90°C, hold

Retention time: 0.85-0.95min

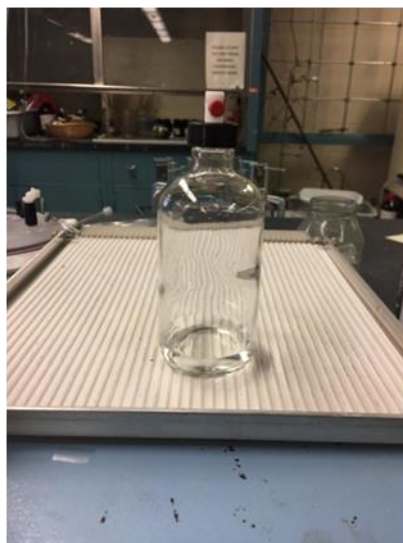


Figure 4.6 Ethylene headspace sample

The quantification was performed by dissolving the encapsulated complex in 20ml DI water inside a 250ml closed jar and shaking for 30mins on a shaker to completely release ethylene into the headspace, shown as Figure 4.6.

#### **4. 4 Release study of ethylene from sachet system**

Release study of ethylene was conducted from PVA film and Tyvek® paper sachet system under different RH condition (70%, 80% and 90%) at 4°C.

##### **4.4.1 Sachet preparation**

PVA film and Tyvek® paper were used as sachet materials to pack encapsulated complex in order to control release ethylene with RH as trigger.

PVA film and Tyvek® paper were cut to 2cm×4cm pieces and then sealed to 2cm×2cm sachets by impulse heat sealer using 0.7s heating and 0.5s cooling time.

Then 0.012g ethylene- $\alpha$ -cyclodextrin encapsulated complex was placed in each sachet, shown as Figure 4.7.



Figure 4.7 PVA sachet packaged encapsulated complex

#### 4.4.2 Simulated RH conditions

Different RH conditions (70%, 80% and 90% RH) created by saturated salt solution were used to simulate moisture coming from respiration of pears to trigger the control release of ethylene with different rate.

Table 4.3 Equilibrium relative humidity and saturated salt solutions under 5 °C

Chemical	Relative humidity (%)
Magnesium chloride	33.60 $\pm$ 0.28
Sodium iodide	42.42 $\pm$ 0.99
Potassium carbonate	43.13 $\pm$ 0.50

---

<b>Magnesium nitrate</b>	58.86±0.43
<b>Sodium bromide</b>	63.51±0.72
<b>Potassium iodide</b>	73.30±0.34
<b>Sodium nitrate</b>	78.57±0.52
<b>Sodium chloride</b>	75.65±0.27
<b>Potassium chloride</b>	87.67±0.45

---

Table 4.3 shows the equilibrium relative humidity and saturated salt solutions under 5°C (Greenspan 1977). Since the temperature of 4°C was used to trigger the ripening of pears along with external ethylene treatment, potassium iodide, sodium nitrate and potassium chloride were used to give simulated 70%, 80% and 90% RH creating by respiration of pears respectively.

#### **4.4.3 Water vapor transmission rate of sachet materials**

Water vapor transmission rate (WVTR) of PVA film and Tyvek® paper were tested by weight method under different RH condition (70%, 80% and 90%) at 4°C to evaluate the rate of permeation of water vapor molecules through sachet materials, which has some effect on release of ethylene from sachet system.

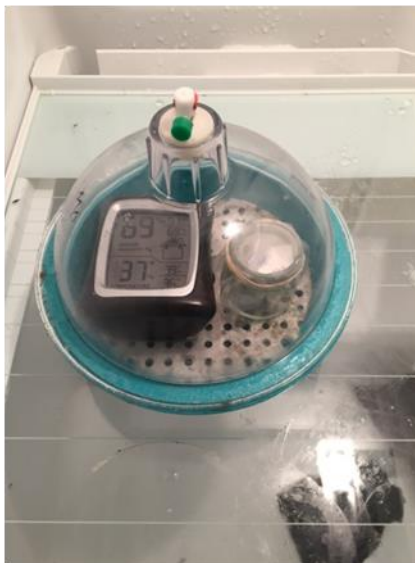


Figure 4.8 Setup of gravimetric method

Figure 4.8 shows the setup of gravimetric method (cup method). Ten grams of desiccant were placed in a small jar, covered by PVA film or Tyvek® paper tightly using rubber bands to make sure there is no leak between film and jar. Then the jar was placed in a closed container with different saturated salt solution inside at 4°C, which can give 70%, 80% and 90% RH respectively. The weight of the jar was recorded every 30mins.

#### 4.4.4 PVA permeability to ethylene

PVA permeability to ethylene were tested under different RH condition (70%, 80% and 90%) at 4°C to evaluate the rate of permeation of ethylene molecules through sachet materials, which also has some effect on release of ethylene from sachet system.



Figure 4.9 Setup of experiment for testing PVA permeability to ethylene

Figure 4.9 shows the setup of experiment for testing PVA permeability to ethylene under different RH condition at 4°C. Saturated salt solution was placed in a plate in a chamber to create simulated RH condition. PVA film was sealed to a large bag and a stainless steel frame was placed in the bag on the plate on top of solution without in contact with it. The whole system was placed in fridge set at 4°C for 15mins to reach equilibrium. After 15mins, the bag was tightened using rubber bands and 20ml ethylene gas was injected into the bag with a 20ml syringe from the septa stick on the bag. Then the chamber was tightly close and 0.25ml headspace gas was withdrawn from the bag every 15mins to test the concentration of ethylene gas in it by GC.

#### **4.4.5 Release of ethylene from sachet system**

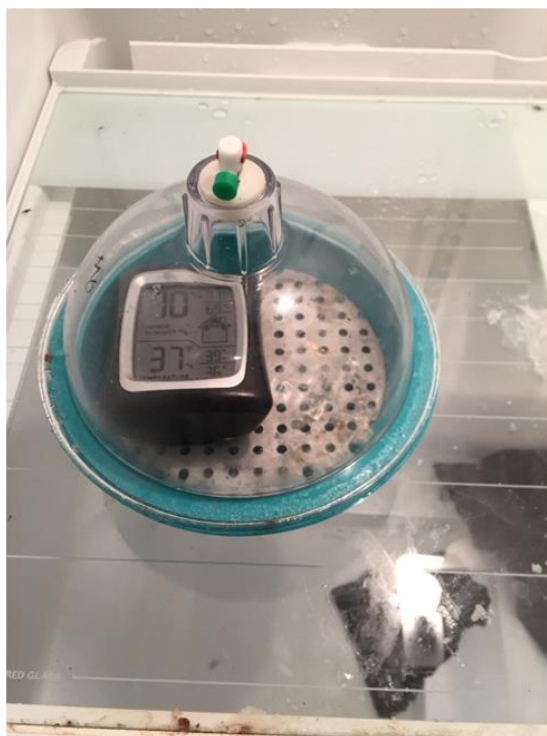


Figure 4.10 Setup of release of ethylene from sachet system

Release of ethylene from sachet (PVA film and Tyvek® paper) packaged encapsulated complex were conducted under different RH condition (70%, 80% and 90%) at 4°C.

Figure 4.10 shows the setup of release of ethylene from sachet system. 100ml saturated salt solution was placed at the bottom of chamber and a stainless steel plate with lots of small holes was placed on the liquid level of solution so that moisture can go through those holes and reach equilibrium RH condition in the chamber. Sachet packaged encapsulated complex was then placed on the plate. Thus, moisture can go through sachet material and release ethylene from it into the headspace of chamber. Gas sample was withdrawn from the headspace of chamber to test the ethylene concentration every 4hrs using GC.

#### **4.5 Biological evaluation of efficacy of ethylene released**



### **from sachet system**

A box of unripened pears was get from market to evaluate the efficacy of ethylene released from sachet system. Those pears were divided into two groups. One was control group, stored at 4°C for 10days. The other one was treated group, stored in a 0.3m<sup>3</sup> chamber with 2.5g encapsulated complex packaged in Tyvek® paper inside at 4°C for 10 days, which can give around 150 ppm ethylene in headspace, the optimum ethylene concentration to trigger the ripening of pear. The firmness of pears both before and after the experiment was tested to evaluate the ripening level of pears.

## **5 Results and Discussion**

### **5.1 Standard curve for ethylene quantification**

Calibration curve was used to quantify ethylene inclusion ratio in encapsulated complex and it was produced based on pure ethylene gas from gas tank. The retention time of ethylene under this GC condition was around 8.5 to 9.5 min. According to the principle of GC, peak area of a compound was proportional to the concentration of this compound in headspace. Therefore, a linear equation could be derived from the relationship between peak area (PA) and ethylene concentration in the headspace, which was ethylene calibration curve. 25, 50, 100, 125 and 250 $\mu$ m of ethylene gas from gas tank was injected into 250ml bottles with lids respectively and shaking for 30mins to reach equilibrium in order to get 100, 200, 400, 500 and 1000 ppm of ethylene in the headspace. After injection of the headspace, peak areas were recorded and Figure 5.1 shows the relationship of PA and ethylene concentration.

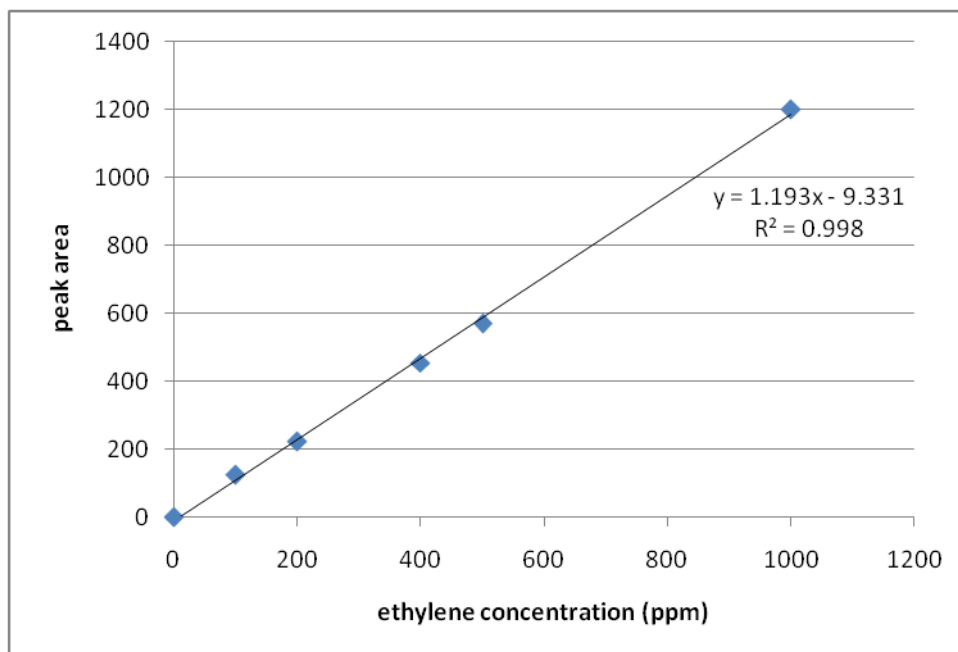


Figure 5.1 Relationship between PA and ethylene concentration

According to Figure 5.1, the relationship between PA and ethylene concentration was linear described by the equation below with  $R^2=0.998$ .

$$PA = 1.193X - 9.331$$

Where X= ethylene concentration in the headspace, ppm

## 5.2 Ethylene encapsulation

### 5.2.1 Inclusion ratio and yield

Ethylene encapsulation was evaluated by on two facts, inclusion ratio and yield, with two independent variables, headspace ethylene concentration and encapsulation duration time.

Inclusion ratio is important for ethylene encapsulation since higher inclusion ratio means more ethylene been encapsulated in  $\alpha$ -cyclodextrin, which can reduce its cost.

Inclusion ratio is described in following equation.

$$\text{inclusion ratio} = \frac{\text{weight of ethylene been encapsulated (g)}}{\text{weight of total encapsualted complex (g)}} \times 100\%$$

The relationship between ethylene weight and concentration can be described in equation below based on the ideal gas law.

$$M = \frac{\frac{101325 \times C(\text{ethylene})}{10^6} \times V \times 10^{-3} \times 28.05}{8.314 \times 298.15}$$

Where

M= weight of ethylene, g

101325= atmosphere pressure, Pa

C (ethylene) = concentration of ethylene in headspace, ppm

V = volume of headspace, L

28.05= molecular weight of ethylene, g/mol

8.314= gas constant, J/(mol K)

298.15= room temperature at 25 °C, K

The headspace volume of the jar used to measure ethylene concentration is 230ml.

Thus, the inclusion ratio of encapsulation complex is described as following equation.

$$\text{inclusion ratio} = \frac{\frac{101325 \times C(\text{ethylene})}{10^6} \times 0.23 \times 10^{-3} \times 28.05}{8.314 \times 298.15 \times W} \times 100\%$$

Where C (ethylene) = concentration of ethylene in headspace, ppm

W= weight of total encapsulation complex, g

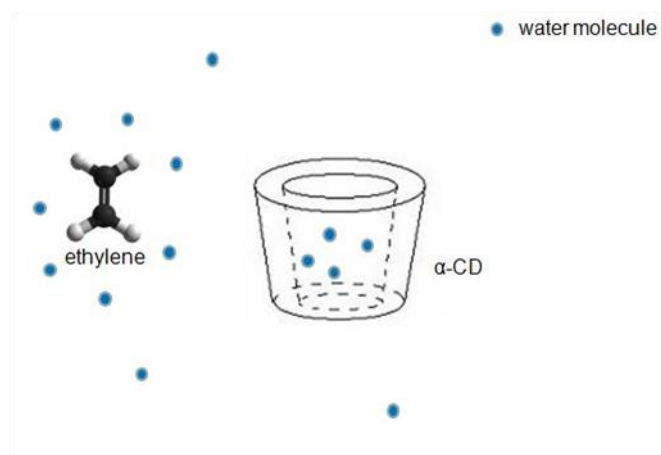
Besides inclusion ratio, another essential factor in evaluating encapsulation is yield, shown in equation below.

$$yield = \frac{\text{weight of encapsulated complex (g)}}{\text{weight of } \alpha - CD \text{ been used (g)}} \times 100\%$$

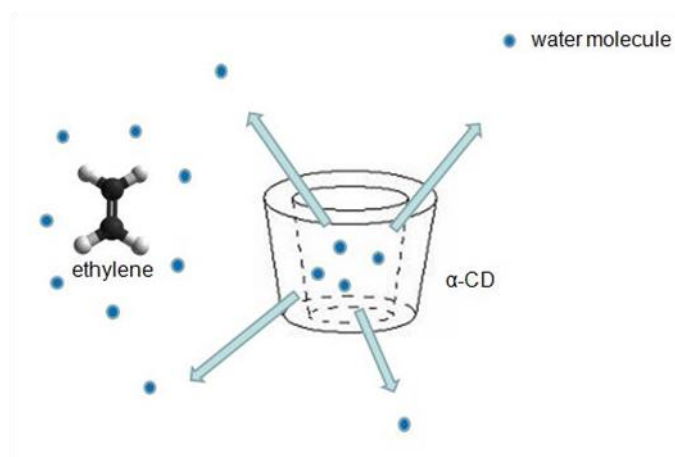
Higher yield means more encapsulated complex can be get from the same amount of  $\alpha$ -cyclodextrin, which will also save the high cost of  $\alpha$ -cyclodextrin.

### 5.2.2 Encapsulation process

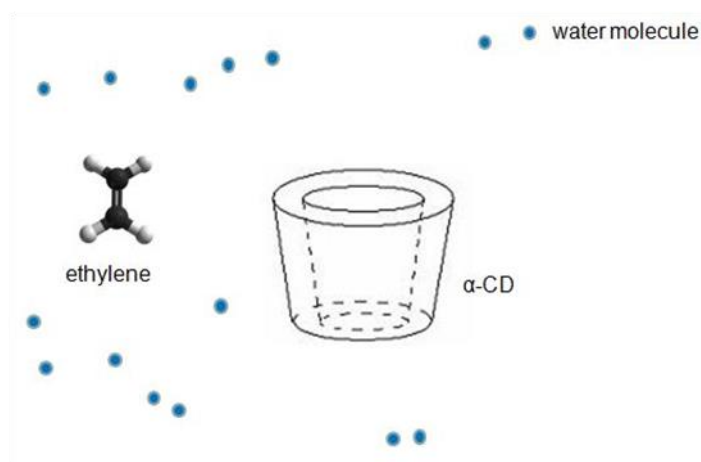
Figure 5.2 (a)→(b)→(c)→(d) described the mechanism of encapsulation process.



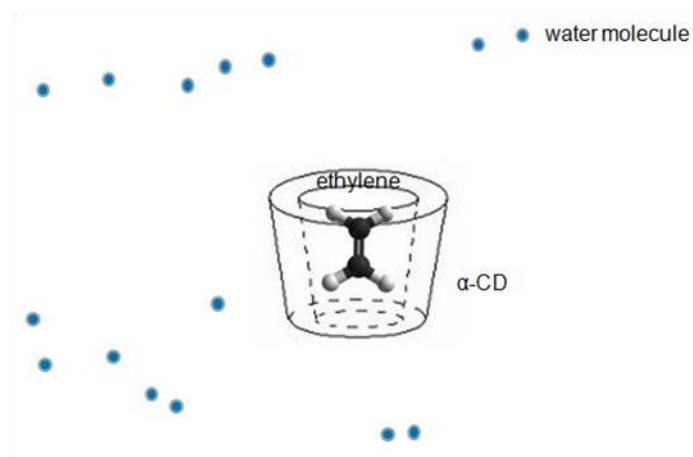
(a)



(b)



(c)



(d)

Figure 5.2 Encapsulation process

When  $\alpha$ -cyclodextrin was completely dissolve in water, becoming solution, water molecules would full of both inside the inner cavity of  $\alpha$ -cyclodextrin and in the outside water environment, shown as Figure 5.2(a). When the solution was in contact with hydrophobic ethylene gas molecules, those water molecules that stay inside the inner cavity tend to go out to the aqua environment, shown by Figure 5.2(b). Therefore, the number of hydrogen bonds between water molecules increased as

water molecules returned back to the environment. The attraction interaction between those water molecules surrounding ethylene molecules and in the solution increased at the same time. As a result of this, the repulsive interaction between hydrophobic ethylene molecules and hydrophilic water molecules reduced, shown in Figure 5.2(c). Thus, hydrophobic ethylene molecules would go into hydrophobic inner cavity of  $\alpha$ -cyclodextrin and the hydrophilic exterior kept ethylene molecules staying inside, shown as Figure 5.2(d). Those  $\alpha$ -cyclodextrin that had encapsulated ethylene inside would precipitate and went through further drying process to get ethylene- $\alpha$ -cyclodextrin encapsulated complex.

When encapsulated complex was in contact with water, water molecules would serve as a plasticizer to make the structure of  $\alpha$ -cyclodextrin become loose, therefore, ethylene would be released from it with RH as a trigger.

### **5.2.3 Influence of headspace ethylene concentration and duration time on inclusion ratio**

Ethylene encapsulation was conducted based on two independent variables, headspace ethylene concentration and duration time. Figure 5.3 shows the relationship between headspace ethylene concentration and duration time and inclusion ratio. X axis is encapsulation duration time, Y axis presents inclusion ratio of encapsulated complex and different color of lines means different headspace ethylene concentration.



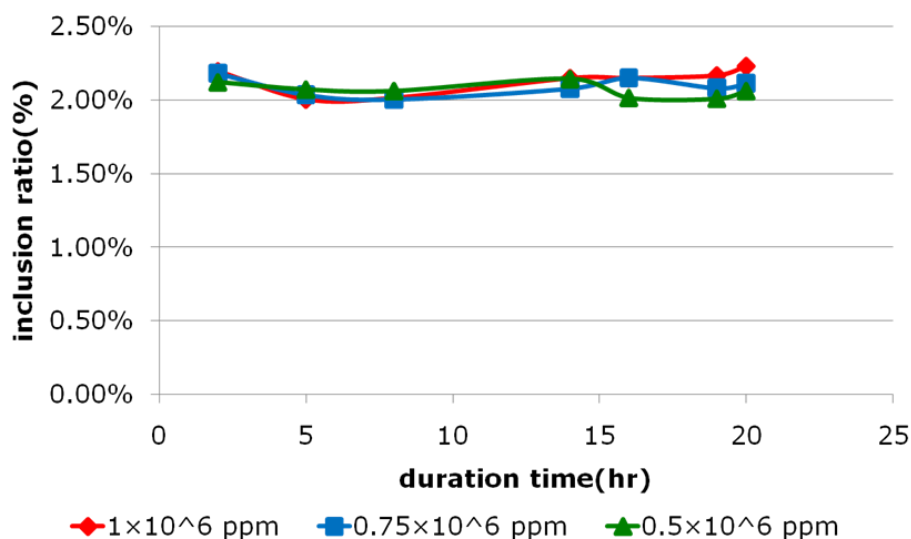


Figure 5.3 Relationship between headspace ethylene concentration and inclusion ratio and duration time

According to Figure 5.3, the inclusion ratio of encapsulation complex remained almost same, around 2.1%, under three different headspace ethylene concentrations. That's because one molecule of  $\alpha$ -cyclodextrin can only encapsulate one molecule of ethylene, only those  $\alpha$ -cyclodextrin that have already trapped ethylene inside can precipitate out from the solution. Therefore, the encapsulated complex always had one molecule of ethylene in each  $\alpha$ -cyclodextrin, resulting same inclusion ratio. Since one molecule of  $\alpha$ -cyclodextrin can only encapsulate one molecule of ethylene, the theoretical inclusion ratio should be shown as equation below

$$\text{theoretical inclusion ratio: } \frac{28.05}{972.86 + 28.05} \times 100\% = 2.80\%$$

Where

28.05 = molecular weight of ethylene, g/mol

972.86 = molecular weight of  $\alpha$ -cyclodextrin, g/mol

The experiment data of inclusion ratio we got was around 2.1%, which is compatible

with theoretical inclusion ratio. The loss of inclusion ratio may come from following steps during encapsulation.

- (1) During the encapsulation, not all of the  $\alpha$ -cyclodextrin can trap ethylene molecule inside, some  $\alpha$ -cyclodextrin may linked together without ethylene been encapsulated inside and then precipitate.
- (2) During the drying process, some ethylene molecules may come out of  $\alpha$ -cyclodextrin once the encapsulation glass jar has been opened.

In terms of duration time, inclusion ratio also remained almost same at around 2.1% with it increasing. No matter how long the encapsulation happened, only one molecule of ethylene can be encapsulated in one molecule of  $\alpha$ -cyclodextrin and those  $\alpha$ -cyclodextrins with ethylene molecule inside can precipitate. Thereby, duration time did not change the inclusion ratio of encapsulation.

#### **5.2.4 Influence of headspace ethylene concentration and duration time on yield**

Figure 5.4 shows the relationship between headspace ethylene concentration and encapsulation duration time and yield of encapsulated complex. X axis is encapsulation duration time, Y axis means yield of encapsulated complex and different color of lines representative different headspace ethylene concentration.

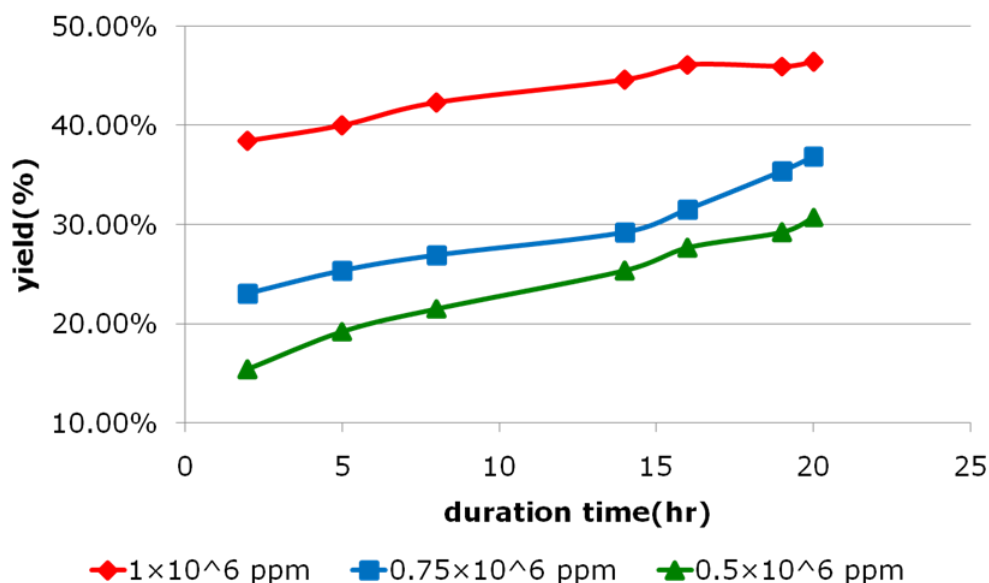


Figure 5.4 Relationship between headspace ethylene concentrations and encapsulation duration time and yield

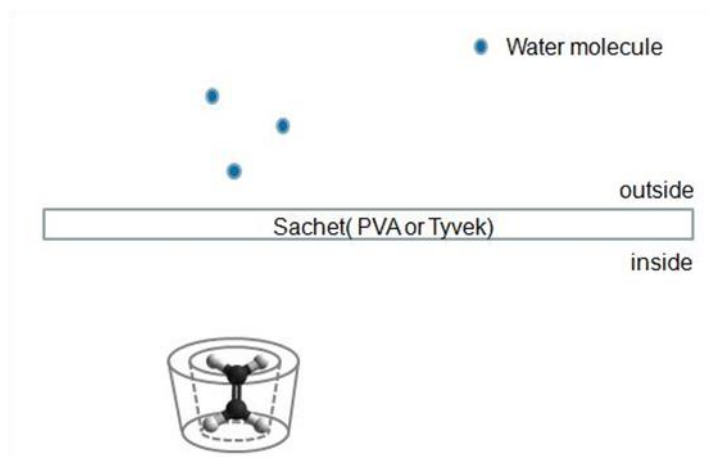
According to Figure 5.4, yield of encapsulated complex increased with the increase of duration time under all three different headspace ethylene concentrations. Under  $0.5 \times 10^6$  ppm headspace ethylene concentration, the yield of encapsulated complex went from 15.38% to 19.23%, 21.54%, 25.38%, 27.69%, 29.23% and 30.77% within 2, 5, 8, 14, 16, 19 and 20 hrs. Under  $0.75 \times 10^6$  ppm headspace ethylene concentration, the yield of encapsulated complex went from 23.08% to 25.38%, 26.92%, 29.23%, 31.54%, 35.38% and 36.92% within 2, 5, 8, 14, 16, 19 and 20 hrs. Under  $1 \times 10^6$  ppm headspace ethylene concentration, the yield of encapsulated complex went from 38.46% to 40.00%, 42.31%, 44.62%, 46.15%, 46.00% and 46.43% within 2, 5, 8, 14, 16, 19 and 20 hrs. With duration time increase, there would be more chances for  $\alpha$ -cyclodextrin molecules to in contact with ethylene molecules and thereby ethylene molecules can be encapsulated in more  $\alpha$ -cyclodextrin, as a result of such, yield

increased as more encapsulated complex formed.

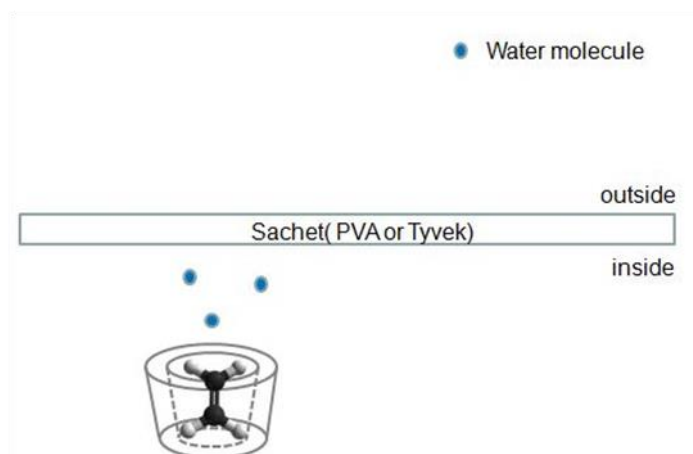
Higher headspace ethylene concentration can get higher yield of encapsulated complex. Under encapsulation duration time of 20hrs, the yield went from 30.77% to 36.92% and 46.43% when headspace ethylene concentration went from  $0.5 \times 10^6$  ppm to  $0.75 \times 10^6$  ppm and  $1 \times 10^6$  ppm. Higher headspace ethylene concentration means more ethylene molecules in the headspace, as a result of this, the chances of more ethylene molecules to be in contact with  $\alpha$ -cyclodextrin molecule increased at the same time. Therefore, more ethylene can be encapsulated in more  $\alpha$ -cyclodextrin molecules and only one molecule of ethylene would be encapsulated in one molecule of  $\alpha$ -cyclodextrin. Thus, more encapsulation complex would be formed and yield would increase.

### **5.3 Ethylene release process**

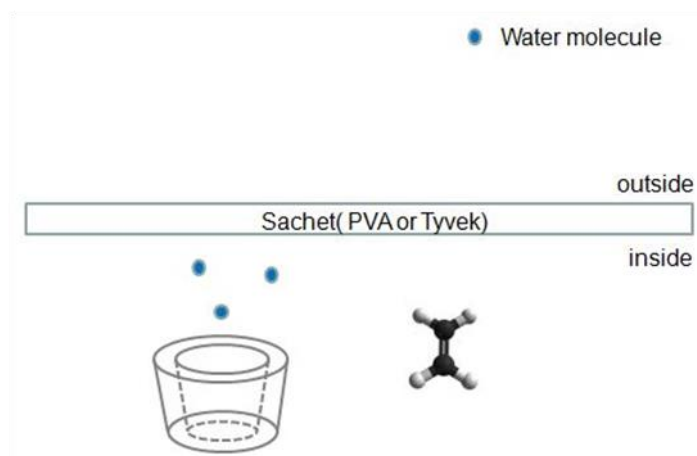
Figure 5.5(a)→(b)→(c)→(d) shows the ethylene release process triggered by RH.



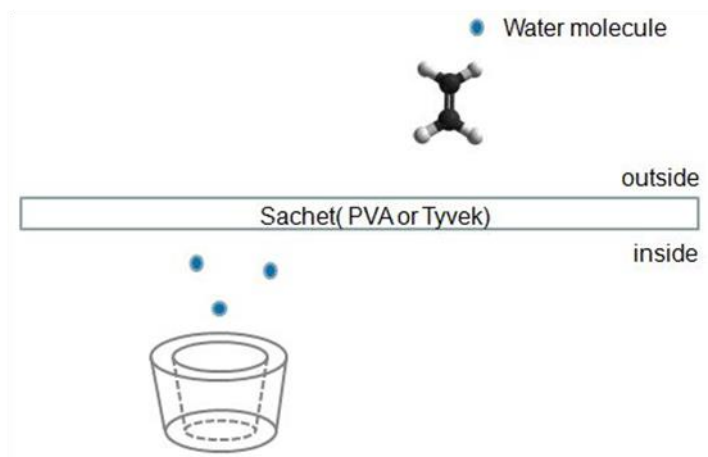
(a)



(b)



(c)



(d)

Figure 5.5 Ethylene release process triggered by RH

Water molecules coming from respiration of pears need to pass the sachet film from outside to inside, and then control release ethylene from  $\alpha$ -cyclodextrin inside the sachet. After that, ethylene molecules would go out of sachet film and into the environment to trigger the ripening of pears along with low temperature ( $4^{\circ}\text{C}$ ). Therefore, water vapor transmission rate of sachet film and their permeability of ethylene under different RH condition at  $4^{\circ}\text{C}$  are two important roles in controlling release rate of ethylene from sachet system.

#### **5.4 Water vapor transmission rate of PVA film and Tyvek® paper**

Water vapor transmission rate (WVTR) of PVA film and Tyvek® paper were measured using gravimetric method under different RH condition (70%, 80% and 90% RH) and the weights of glass jar were recorded as a function of time. Use PVA film under 90% RH as an example, the relationship between the weight of jar and time was

plotted in Figure 5.6.

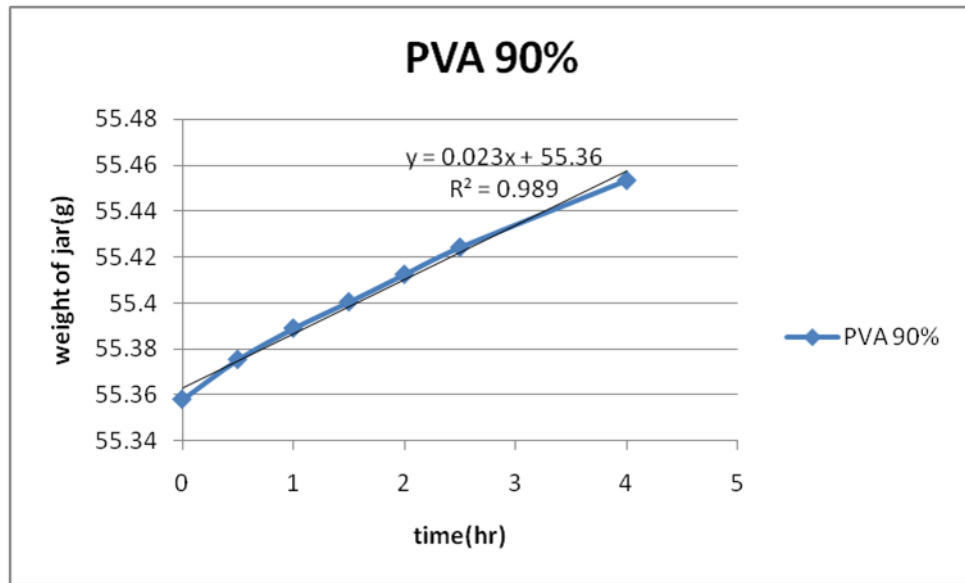


Figure 5.6 Relationship between the weight of jar and time

WVTR of PVA film under 90% RH at 4°C is described in equation below.

$$WVTR = \frac{Q}{A}$$

Where  $Q = \frac{\text{weight gain}}{\text{time}} = \text{slope of the line} = 0.023 \text{ g/hr}$

$A = \text{the area of PVA film} = \pi \times \left(\frac{1.30}{2}\right)^2 \div 100 = 0.013 \text{ } 100 \text{ in}^2$

$$WVTR = \frac{Q}{A} = \frac{0.023}{0.013} = 1.77 \frac{\text{g}}{100 \text{ in}^2 \text{ hr}}$$

Table 5.1 lists water vapor transmission rate of PVA film and Tyvek® paper under 70%, 80% and 90% RH at 4°C.

Table 5.1 Water vapor transmission rate of PVA film and Tyvek® paper

WVTR at 4°C ( $\frac{\text{g}}{100 \text{ in}^2 \text{ hr}}$ )		
RH	PVA film	Tyvek® paper
70%	0.69	1.00

80%	1.31	1.31
90%	1.77	1.46

For PVA film, its water vapor transmission rate at 4°C under 70%, 80% and 90%RH was 0.69, 1.31 and  $1.77 \frac{g}{100in^2hr}$  respectively. WVTR of PVA film increased with increasing RH. Permeation includes three steps, (1) adsorption of water molecules onto film, (2) diffusion of water molecules inside the film and (3) desorption of water molecules out of film. Under higher RH condition, more water molecules can be adsorbed into PVA film due to hydrogen bonds between hydroxyl groups on PVA and water molecules. Meanwhile, high moisture content would impair the structure of PVA film and make it loose. As a result of this, it was more quickly for water molecules to diffuse through PVA film. Therefore, WVTR of PVA film would increase with increasing RH.

For Tyvek® paper, its water vapor transmission rate at 4°C under 70%, 80% and 90% RH was 1.00, 1.31 and  $1.46 \frac{g}{100in^2hr}$  respectively. The water vapor transmission of Tyvek® paper was mainly physically passing through it, not like permeation for PVA film. Those big holes between Tyvek® fibers made water vapor molecules easily pass through them. Under higher RH condition, more water vapor molecules could pass through Tyvek® paper, thereby increasing its WVTR.

Under 70% RH at 4°C, WVTR of Tyvek® paper was higher than PVA film. It was more easily for water molecules to physically pass through Tyvek® fibers compared to chemically adsorb into PVA film under 70% RH where the structure of PVA film



had not been destructed by water vapor molecules since the size of holes between Tyvek® fibers were higher larger than that for PVA film. However, WVTR of PVA film was higher than Tyvek® paper under 90% RH at 4°C. The structure of PVA film had been highly impaired by water vapor molecules and the thickness of film had decreased a lot under 90% RH. As a result, it would be much easily for water vapor molecules to absorb and diffuse through PVA film than physically pass through Tyvek® paper.

## 5.5 Release profile of encapsulated complex

Figure 5.7 shows the release profile of ethylene- $\alpha$ -cyclodextrin encapsulated complex with 70%, 80% and 90% RH as trigger at 4°C.

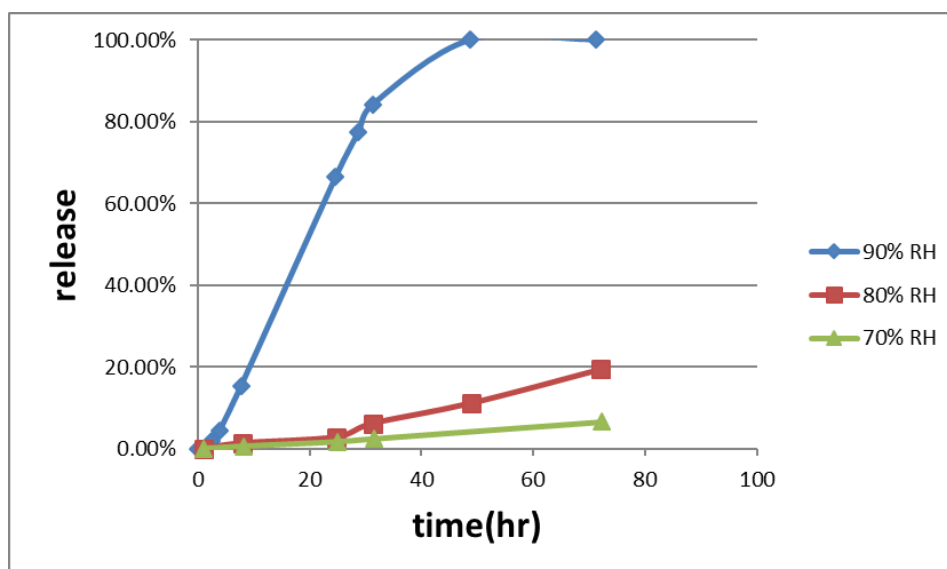


Figure 5.7 Release profile of encapsulated complex

With 90% RH as trigger, ethylene started to release from  $\alpha$ -cyclodextrin at around

0.9hrs with 2% release and reached completely release in around 48hrs. Under 80% RH condition, ethylene started to release at around 8hrs with 1.48% release and reached around 19.53% release within 72hrs and continued releasing. However, with 70% RH as trigger, there was only 6.61% release in 72hrs and continued releasing. Under High RH condition, more water vapor molecules would swell the structure of  $\alpha$ -cyclodextrin much quickly. After that, the inner cavity of  $\alpha$ -cyclodextrin would slightly open and ethylene could be released from it. Therefore, it was much easier for ethylene to be released from encapsulated complex with higher moisture content in the air.

## **5.6 PVA and Tyvek® paper permeability of ethylene**

PVA permeability of ethylene under 70%, 80% and 90% RH at 4°C was measured using method described in 4.4.4. Use 70% RH condition as an example, the relationship between ethylene concentration inside the bag and time was plotted as Figure 5.8.

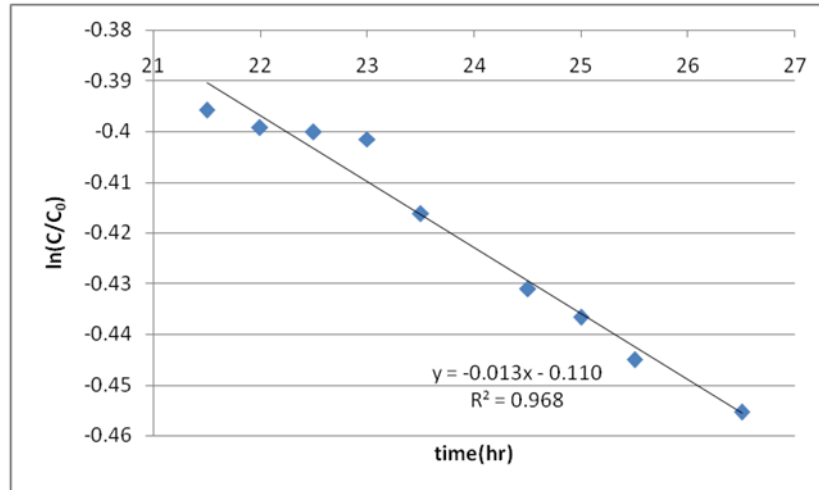


Figure 5.8 Relationship between ethylene concentration inside the bag and time

X axis is time and Y axis is  $\ln\left(\frac{C}{C_0}\right) = \ln\left(\frac{\text{ethylene concentration inside bag}}{\text{initial ethylene concentration inside bag}}\right)$ .

Equation below shows the relationship between PVA permeability of ethylene and ethylene concentration inside the bag.

$$C = C_0 e^{-\frac{\bar{P}A}{LV}t}$$

$$\Downarrow$$

$$\bar{P} = -\frac{\ln\left(\frac{C}{C_0}\right) \times L \times V \times 1000 \times 24}{A}$$

Where  $\bar{P}$  = PVA permeability of ethylene,  $\text{ccmilday}^{-1}100\text{in}^{-2}\text{atm}^{-1}$

$\ln\left(\frac{C}{C_0}\right) = \ln\left(\frac{\text{ethylene concentration inside bag}}{\text{initial ethylene concentration inside bag}}\right)$  = the slope of the line

L = thickness of PVA film, mil

V = volume of the bag, L

A = surface area of bag,  $100\text{in}^2$

Table 5.2 shows PVA film and Tyvek® paper permeability of ethylene and PVA film thickness under different 70%, 80% and 90% RH at 4°C. Tyvek® paper permeability

of ethylene was hard to get since it was difficult to seal Tyvek® paper into a bag.

Table 5.2 Sachet permeability of ethylene and PVA film thickness

<b>RH</b>	<b><math>\bar{P}</math> at 4°C</b>		<b>Thickness(mil)</b>
	<b>PVA film</b>	<b>Tyvek® paper</b>	
	<b>(<math>ccmilday^{-1}100in^{-2}atm^{-1}</math>)</b>		
70%	1038.192	Very fast	2.559
80%	1179.484	Very fast	2.362
90%	1351.493	Very fast	2.165

For PVA film, its permeability of ethylene increased and the film thickness decreased with increasing RH. Due to increasing RH, high moisture content would impair the structure the PVA film and made it loose, thereby ethylene molecule could pass through it more easily.

PVA (polyvinyl alcohol) is a water-soluble polymer, with hydroxyl groups on each monomer unit. Due to the hydrogen bonds between hydroxyl groups and water molecules, PVA is a hydrophilic film. However, ethylene molecule is hydrophobic molecule. As a result, there would be some repulsive interactions between PVA film and ethylene molecules which would affect its permeability of ethylene. However, Tyvek® paper is made of HDPE polymer and it is liquid water insoluble and hydrophobic. Thereby the attraction interaction between hydrophobic Tyvek® paper and ethylene molecules tended to promote its permeability of ethylene. In addition, those big holes between Tyvek® fibers offered more chances for ethylene molecules

to physically pass through it and there was much less barrier for ethylene molecules compared to repulsive interaction between ethylene and PVA film. Therefore, ethylene molecules could easily go through Tyvek® paper compared to PVA film and its permeability of ethylene was much higher than PVA film.

## 5.7 Release profile of sachet system

Figure 5.9 shows the release profile of PVA film sachet packaged ethylene- $\alpha$ -cyclodextrin encapsulated complex with 70%, 80% and 90% RH as trigger at 4°C.

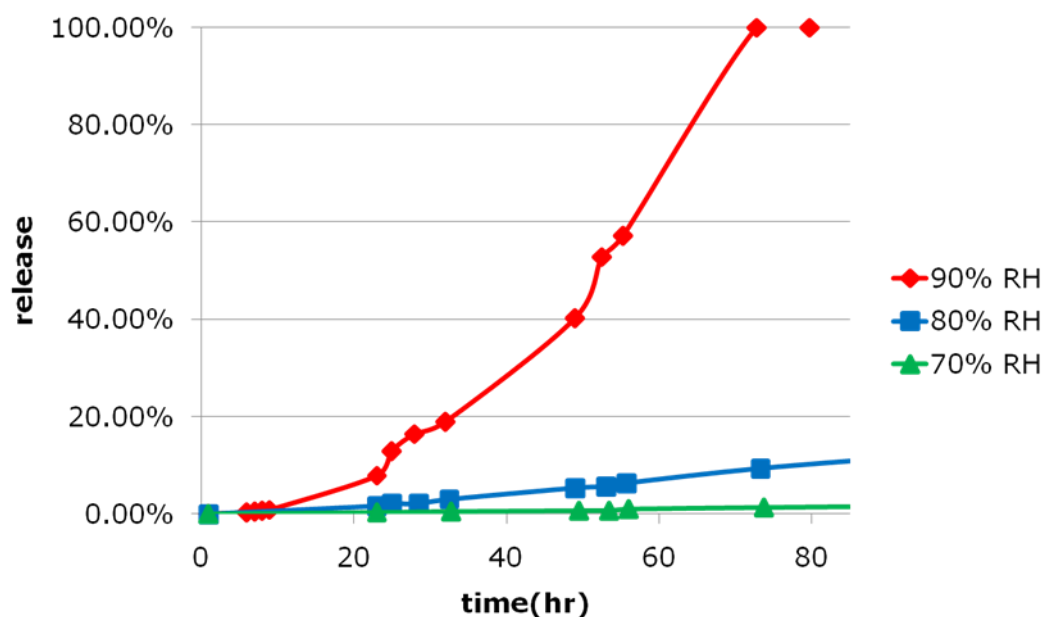


Figure 5.9 Release profile of PVA sachet system

With 90% RH as trigger, ethylene started to release from PVA sachet system at around 6hrs with 0.3% release and reached completely release in around 72hrs. Under 80%

RH condition, ethylene started to release at around 23hrs with 1.58% release and reached around 9.41% release within 72hrs and continued releasing. However, with 70% RH as trigger, there was only 1.29% release in 72hrs.

Higher RH would increase the release rate of ethylene from encapsulated complex and PVA sachet since both water vapor transmission rate of PVA and its permeability of ethylene at 4 °C would increase with increasing RH as water vapor molecules and ethylene molecules could pass through PVA film more quickly under higher RH condition. In addition, it was much easier to loosen  $\alpha$ -cyclodextrin structure and release ethylene from it with higher moisture content in the air.

Figure 5.10 shows the release profile of Tyvek® paper sachet packaged ethylene- $\alpha$ -cyclodextrin encapsulated complex with 70%, 80% and 90% RH as trigger at 4°C.

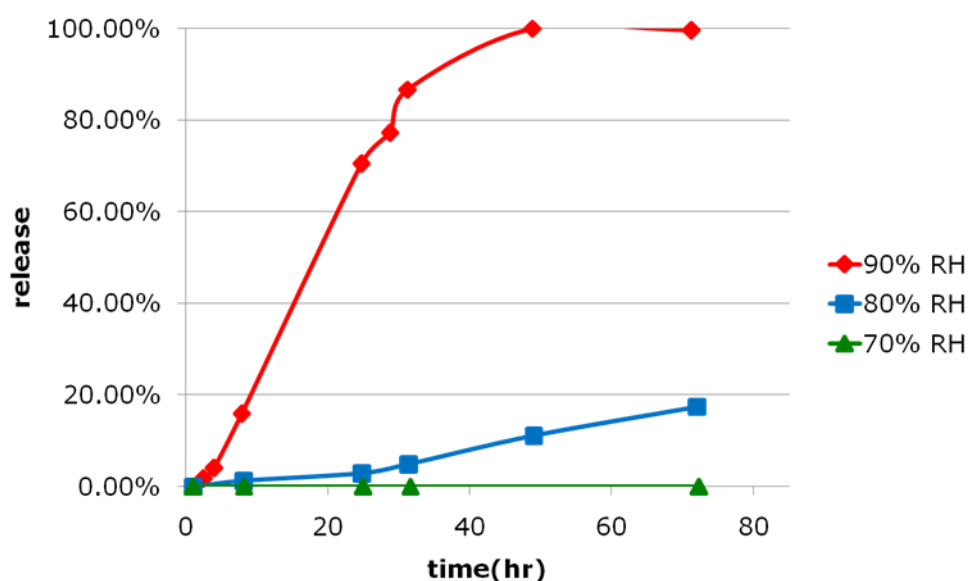


Figure 5.10 Release profile of Tyvek® paper sachet system

With 90% RH as trigger, ethylene started to release from Tyvek® paper system at around 2.5hrs with 1.7% release and reached completely release in around 48hrs.

Under 80% RH condition, ethylene started to release at around 8.1hrs with 1.24% release and reached around 17.44% release within 72hrs and kept releasing. However, with 70% RH as trigger, there was only 0.29% release in 73hrs.

Compared to the release profile of encapsulated complex, the release rate of ethylene from Tyvek® paper system was same as just from  $\alpha$ -cyclodextrin, both reaching completely release within 48hrs under 90% RH. Therefore, the release of ethylene from Tyvek® paper system was just the release from  $\alpha$ -cyclodextrin and Tyvek® paper didn't serve as a barrier for either water vapor molecules or ethylene molecules. The size of those big holes between Tyvek® paper fibers were much bigger than the

size of both water vapor molecules and ethylene molecules. Therefore, it was just a physically passing through not chemically permeation process and there was little barrier for those two molecules to pass through it. However, the permeation of water vapor and ethylene through PVA film was chemical interaction containing adsorption, diffusion and desorption steps, which was much more complicate than Tyvek® paper. Therefore, PVA sachet was a “barrier” for water vapor molecules and ethylene molecules compared to Tyvek® paper. As a result, the release of ethylene from Tyvek® sachet system was faster than from PVA sachet system under same RH condition at 4°C.

Higher RH would promote the release rate of ethylene from encapsulated complex and Tyvek® paper sachet. Since water vapor molecules could swell the structure of  $\alpha$ -cyclodextrin and then ethylene could be released from it, it was much easier to swell  $\alpha$ -cyclodextrin structure and more ethylene could be released from it under high RH condition.

## **5.8 Biological evaluation of efficacy of ethylene released from sachet system**

Figure 5.10 shows the color of pears that before treatment and after 10days ethylene treatment release from Tyvek® paper sachet system at 4°C. The picture on the top was pears before treatment and the one at bottom was pears after treatment. Left half of pears in second picture were control group, stored at 4°C without any ethylene treatment. Right half of pears in it were treated with external ethylene for 10 days at



4°C.

After ethylene treatment released from Tyvek® paper sachet system at 4°C, the color of pears became more yellow and ripen compared to control group that only stored at 4°C for 10 days.



Before



After

(a) Control

(B) Treated

Figure 5.7 Pears that before treatment and after 10 days ethylene treatment

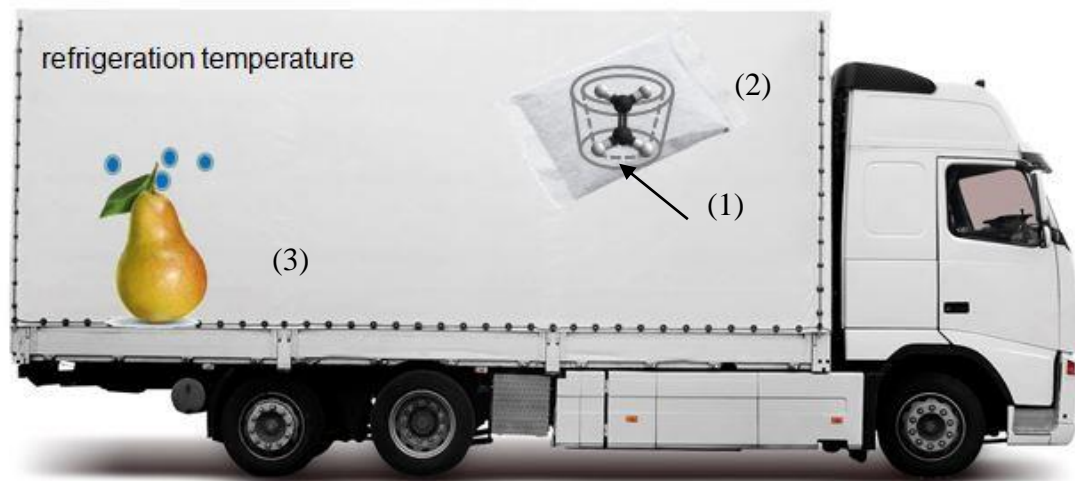
Table 5.3 shows the firmness of pears both before ethylene treatment and after treatment.

Table 5.3 Firmness of pears both before and after ethylene treatment

	<b>Firmness(lbf)</b>	
	<b>control</b>	<b>treated</b>
Before treatment	18	18
After treatment	10.3	4.2

Before treatment, the average firmness of pears was around 18lbf. After 10 days of ethylene treatment released from Tyvek® sachet system at 4°C, the average firmness of pears decreased to 4.2lbf while that of control group was 10.3lbf. The optimum range of firmness of pears that have best eating quality is around 3 to 5lbf. Therefore, those pears in treated group were fully-ripened and ready-to-eat and ethylene control released from encapsulated complex and sachet packaged system could trigger the ripening of pear and get it ready-to-eat at retail level.

## 5.9 Overall analysis



- (1): Ethylene encapsulation
- (2): Release of ethylene from sachet
- (3): Ripening of pears triggered by ethylene released from sachet system

Figure 5.8 In-transit system and its components

The overall results were analyzed based on three sub-objectives, described as Figure 3.1.

Sub-objective 1: ethylene could be encapsulated in  $\alpha$ -cyclodextrin with around 2.1% inclusion ratio and 46% yield under  $1 \times 10^6$  ppm headspace ethylene concentration within 20hrs, which is compatible with theoretical maximum inclusion ratio of 2.80% and high sufficient to reach the optimum ethylene concentration to trigger the ripening of pears. Headspace ethylene concentration and duration time did not change inclusion ratio of inclusion complex because only one molecule of ethylene could be encapsulated in one molecule of  $\alpha$ -cyclodextrin and then precipitate. However, higher headspace ethylene concentration and longer duration time were able to increase yield of encapsulated complex.

Sub-objective 2: ethylene could be released from encapsulated complex packaged in PVA film sachet and Tyvek® paper sachet at 4°C with RH as a trigger. Under 90% RH, it took around 72hrs to completely release ethylene from PVA sachet system and 48hrs to completely release from Tyvek® sachet system. The release of ethylene from Tyvek® sachet system was faster than from PVA sachet system under same RH condition at 4°C since those big holes between Tyvek® paper fibers made both water vapor molecules and ethylene molecules pass through it much more easily compared to permeation through PVA film. The release of ethylene from Tyvek® system was just from encapsulated complex since there was no barrier to pass through Tyvek® paper.

Sub-objective 3: RH coming from respiration of pears was sufficient to trigger the release of ethylene from encapsulated complex and Tyvek® sachet system, which was able to induce the ripening of pears in 10 days along with low temperature of 4°C. The firmness of pears that stored with Tyvek® sachet system at 4°C for 10 days went from 18 to 4.2lbf, within the optimum range of firmness for pears that have best eating quality, 3 to 5lbf.

## 6 Conclusion

It is technically feasible to develop an in-transit ripening technology to provide ready-to-eat pears when they arrive at the distribution center. RH from the respiration of pears was able to control the release of ethylene, which would further induce their ripening along with low temperature during transportation. Ethylene was encapsulated in  $\alpha$ -cyclodextrin with 2.1% inclusion ratio and then packaged in PVA and Tyvek® sachet to be control released by RH coming from respiration of pears, which could reach 150ppm in the truck, the optimum concentration to trigger the ripening of pear. Ethylene could be completely released from PVA sachet and Tyvek® sachet within 72hrs and 48hrs respectively under 90% RH, which may satisfy different transportation time requirements ranging from 7 to 10 days. This work supported the concept of in-transit ripening and further development may fulfill different target transportation requirements.

This technology can be applied to long-term transportation requirements, lasting several days or weeks, especially for oversea shipping. For short-term transportation requirements, it may not work since it takes several days to release ethylene. In addition, there's no incentive to ripen pears during transportation since they can be ripened at source.

## 7 Future Work

- (1) The biological evaluation was conducted under simulated transportation condition.

Therefore, it is essential to apply it to real transportation situation for further validation of this in-transit ripening technology.

- (2) In this work,  $\alpha$ -cyclodextrin was used to encapsulate ethylene and get 2.1% inclusion ratio. However,  $\alpha$ -cyclodextrin is quite expensive compared to other materials. Therefore, an alternative encapsulation agent that has lower price and higher encapsulation capacity is crucial for developing ethylene technology.

- (3) The optimum ethylene concentration to trigger the ripening of pear used to determine the amount of encapsulated complex packed in sachet was 150ppm, which is a constant concentration. However, it is possible that the concentration profile changing with time may be more efficient to trigger the ripening compared to constant concentration. Thereby it is essential to have an optimum concentration profile of ethylene ripening pears in order to further developing this technology.

- (4) Two kinds of sachet material were used to package encapsulation complex and control release ethylene. In addition to PVA film and Tyvek® paper, more materials can be tested as sachet film and control release ethylene with different release profiles under different RH condition, which will meet different transportation time requirements in order to get ready-to-eat pears when arriving

distribution center.

- (5) More extensive work on biological test can be done to evaluate the efficacy of ethylene release system. For example, sensory test may help to estimate the difference of taste between pears with and without ethylene release treatment, providing more basis for evaluating the efficacy of ethylene release system.
- (6) In order to further extend the shelf life of pear, it is possible to have 1-MCP treatment, which is a compound that will bind ethylene active sites and delay the ripening of fresh produce, after ethylene and low temperature treatment to prevent over-ripening and maintain freshness of pears.

## 8 References

- Abeles, F. B., et al. (2012). Ethylene in plant biology, Academic press.
- Agar, I. T., et al. (2000). "Cold Storage Duration Influences Ethylene Biosynthesis and Ripening of Bartlett Pears." HortScience **35**(4): 687-690.
- Argenta, L. C., et al. (2003). "Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit." Journal of agricultural and food chemistry **51**(13): 3858-3864.
- Baritelle, A. L., et al. (2001). "Using 1-MCP to inhibit the influence of ripening on impact properties of pear and apple tissue." Postharvest Biology and Technology **23**(2): 153-160.
- Bazzano, M., et al. (2016). "Controlled atmosphere in food packaging using ethylene-  $\alpha$ -cyclodextrin inclusion complexes dispersed in photocured acrylic films." Industrial & Engineering Chemistry Research **55**(3): 579-585.
- Ben-Arie, R., et al. (1979). "Ultrastructural changes in the cell walls of ripening apple and pear fruit." Plant Physiology **64**(2): 197-202.
- Bender, M. L. and M. Komiyama (2012). Cyclodextrin chemistry, Springer Science & Business Media.
- Brady, C. (1987). "Fruit ripening." Annual review of plant physiology **38**(1): 155-178.
- Burg, S. P. and E. A. Burg (1965). "Ethylene action and the ripening of fruits." Science **148**(3674): 1190-1196.
- Davis, M. E. and M. E. Brewster (2004). "Cyclodextrin-based pharmaceuticals: past, present and future." Nature Reviews Drug Discovery **3**(12): 1023-1035.
- Del Valle, E. M. (2004). "Cyclodextrins and their uses: a review." Process biochemistry **39**(9): 1033-1046.
- Duchene, D., et al. (1999). "Cyclodextrins and carrier systems." Journal of Controlled Release **62**(1): 263-268.
- Fischer, R. L. and A. B. Bennett (1991). "Role of cell wall hydrolases in fruit ripening." Annual review of plant biology **42**(1): 675-703.
- Fonseca, S., et al. (2004). "Monitoring gene expression along pear fruit development, ripening and senescence using cDNA microarrays." Plant Science **167**(3): 457-469.



Fonseca, S., et al. (2005). "Expression of genes encoding cell wall modifying enzymes is induced by cold storage and reflects changes in pear fruit texture." Journal of Experimental Botany **56**(418): 2029-2036.

Gibson, E., et al. (1998). "Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children." Appetite **31**(2): 205-228.

Greenspan, L. (1977). "Humidity fixed points of binary saturated aqueous solutions." Journal of research of the national bureau of standards **81**(1): 89-96.

Hernández-Pérez, T., et al. (2005). "Biochemical and nutritional characterization of three prickly pear species with different ripening behavior." Plant foods for human nutrition **60**(4): 195-200.

Hiwasa, K., et al. (2004). "European, Chinese and Japanese pear fruits exhibit differential softening characteristics during ripening." Journal of Experimental Botany **55**(406): 2281-2290.

Ho, B. T. (2013). "Production of ethylene powder by encapsulation of ethylene gas into  $\alpha$ -cyclodextrin and its application for the ripening of fruit."

Ho, B. T., et al. (2011). "Encapsulation of ethylene gas into  $\alpha$ -cyclodextrin and characterisation of the inclusion complexes." Food Chemistry **127**(2): 572-580.

Ho, B. T., et al. (2011). "Release kinetics of ethylene gas from ethylene- $\alpha$ -cyclodextrin inclusion complexes." Food Chemistry **129**(2): 259-266.

Itai, A., et al. (1999). "Identification of 1-aminocyclopropane-1-carboxylic acid synthase genes controlling the ethylene level of ripening fruit in Japanese pear (*Pyrus pyrifolia* Nakai)." Molecular and General Genetics MGG **261**(1): 42-49.

Jermyn, M. and F. Isherwood (1956). "Changes in the cell wall of the pear during ripening." Biochemical Journal **64**(1): 123.

Lacey, R. F. and B. M. Binder (2014). "How plants sense ethylene gas—The ethylene receptors." Journal of inorganic biochemistry **133**: 58-62.

Lee, Y. S., et al. (2006). "Development of a 1-Methylcyclopropene (1-MCP) Sachet Release System." Journal of food science **71**(1).

Lelièvre, J. M., et al. (1997). "Ethylene and fruit ripening." Physiologia plantarum **101**(4): 727-739.

Marques, H. M. C. (2010). "A review on cyclodextrin encapsulation of essential oils and volatiles."

Flavour and fragrance journal **25**(5): 313-326.

Maxie, E., et al. (1974). "Effect of elevated temperature on ripening of Bartlett pear, *Pyrus communis* L." Journal of the American Society for Horticultural Science.

Murayama, H., et al. (1998). "Cell wall changes in pear fruit softening on and off the tree." Postharvest Biology and Technology **14**(2): 143-149.

O'Neil, C., et al. (2014). "Fresh pear consumption is associated with a better nutrient intake profile, better diet quality, and lower risk of obesity in adults (19+ y): NHANES (2001-2010)(810.16)." The FASEB Journal **28**(1 Supplement): 810.816.

Saenger, W. (1980). "Cyclodextrin inclusion compounds in research and industry." Angewandte Chemie International Edition in English **19**(5): 344-362.

Seglie, L., et al. (2013). "β-Cyclodextrin-based nanosponges improve 1-MCP efficacy in extending the postharvest quality of cut flowers." Scientia Horticulturae **159**: 162-165.

Seymour, G. B., et al. (2012). Biochemistry of fruit ripening, Springer Science & Business Media.

Silos-Espino, H., et al. (2003). "Chemical and biochemical changes in prickly pears with different ripening behaviour." Molecular Nutrition & Food Research **47**(5): 334-338.

Sugar, D., et al. (2009). "Re-thinking the chill requirement for pear ripening." Washington State University-Tree Fruit Research and Extension Center: 1-5.

Szejtli, J. (1998). "Introduction and general overview of cyclodextrin chemistry." Chemical reviews **98**(5): 1743-1754.

Szejtli, J. (2013). Cyclodextrin technology, Springer Science & Business Media.

Trotta, F., et al. (2011). "Cyclodextrin nanosponges as effective gas carriers." Journal of inclusion phenomena and macrocyclic chemistry **71**(1-2): 189-194.

Uekama, K., et al. (1998). "Cyclodextrin drug carrier systems." Chemical reviews **98**(5): 2045-2076.

Villalobos-Acuña, M. and E. J. Mitcham (2008). "Ripening of European pears: the chilling dilemma." Postharvest Biology and Technology **49**(2): 187-200.

Wang, K. L.-C., et al. (2002). "Ethylene biosynthesis and signaling networks." The plant cell **14**(suppl 1): S131-S151.

Westwood, M. and B. HO (1968). Chilling requirements of dormant seeds of 14 pear species as related to their climatic adaptation. Proceedings of the American Society for Horticultural Science, AMER SOC HORTICULTURAL SCIENCE 701 NORTH SAINT ASAPH STREET, ALEXANDRIA, VA 22314-1998.

Yang, S. F. and N. E. Hoffman (1984). "Ethylene biosynthesis and its regulation in higher plants." Annual review of plant physiology **35**(1): 155-189.