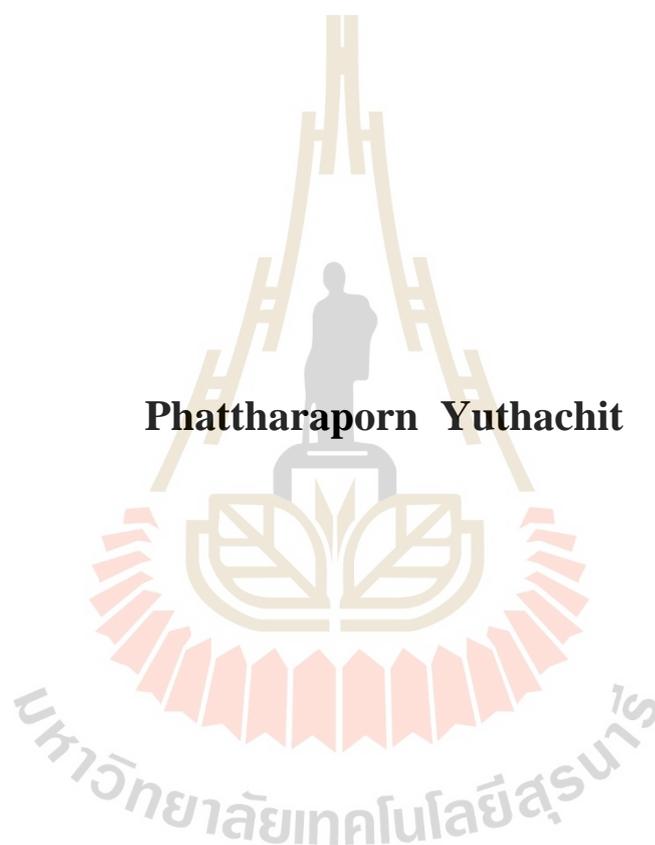


**POSTHARVEST CHANGES OF QUERCETIN IN  
THAI SHALLOTS (*Allium ascalonicum* Linn.)**



**A Thesis Submitted in Partial Fulfillment of the Requirements for the  
Degree of Doctor of Philosophy in Food Technology**

**Suranaree University of Technology**

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การเปลี่ยนแปลงหลังการเก็บเกี่ยวของเคอร์ซีทินในหอมแดงไทย  
(*Allium ascalonicum* Linn.)



นางสาวภัทรพร ยูธาชิต

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต  
สาขาวิชาเทคโนโลยีอาหาร  
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ปีการศึกษา 2556

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SHALLOTS (*Allium ascalonicum* Linn.)**

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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ดร.สุเวทย์ นิงสานนท์, 80 หน้า.

การศึกษานี้มุ่งประเมินการเปลี่ยนแปลงทางชีวเคมีหลังการเก็บเกี่ยวของหัวหอมแดง โดยมุ่งเน้นการกระจายตัวและการเปลี่ยนแปลงของเคอร์ซีตินและไกลโคไซด์ของเคอร์ซีติน ในชั้นเนื้อเยื่อและขนาดหัวที่แตกต่างกัน ภายใต้สภาวะการเก็บรักษาที่หลากหลาย การทดลองดำเนินการเป็นระยะเวลา 30 วัน โดยใช้การจัดการแบบแฟกทอเรียลระหว่างอุณหภูมิ (15°C และ 30°C) และความเข้มแสง (ความมืด, 9,000 ลักซ์ และ 18,000 ลักซ์) ผลการศึกษาพบว่า หัวขนาดใหญ่มีปริมาณสารแห้งเพิ่มขึ้นจากชั้นนอกสู่ชั้นใน ขณะที่หัวขนาดเล็กมีการสะสมสารแห้งและสารกลุ่มเคอร์ซีตินไกลโคไซด์ สูงในชั้นนอก ส่วนน้ำตาลรีดิคัลและกรดไพรูวิกซึ่งเป็นตัวบ่งชี้ความหวานและความฉุน นั้น พบมากที่สุดที่ชั้นนอกของหัวขนาดใหญ่ จากการวิเคราะห์เอนไซม์พบว่า เอนไซม์ฟลาโวนอลซินเทส และกลูโคซิลทรานสเฟอเรส มีระดับกิจกรรมต่ำ แต่เอนไซม์ Q4'G-กลูโคซิเดส มีระดับกิจกรรมสูงในชั้นนอกของหัวหอม ซึ่งบ่งชี้ว่ากระบวนการดี-กลูโคซิเดชัน เป็นกลไกหลักในการควบคุมการสะสมของสารฟลาโวนอล

ระหว่างการเก็บรักษาหัวหอมแดง พบว่าหัวหอมสูญเสียน้ำหนักอย่างต่อเนื่อง โดยเฉพาะภายใต้สภาวะอุณหภูมิสูงและแสงเข้ม การเก็บรักษาที่อุณหภูมิ 15°C ในความมืดช่วยลดการสูญเสีย น้ำและรักษาความเสถียรทางเคมีได้ดีที่สุด น้ำตาลรีดิคัลและสารกลุ่มเคอร์ซีตินไกลโคไซด์ มีแนวโน้มการเปลี่ยนแปลงสองช่วง โดยมีค่าสูงสุดในวันที่ 12 ก่อนที่จะลดลงเนื่องจากกระบวนการหายใจและการเสื่อมสลายของเนื้อเยื่อ ความเข้มข้นของเคอร์ซีตินอิสระสูงสุด (27 กรัม/กก. น้ำหนักแห้ง) เมื่อใช้สภาวะการเก็บที่ 15°C และ 18,000 ลักซ์ ในวันที่ 12 ซึ่งเป็นช่วงเวลาที่เหมาะสมที่สุดในการรักษาสารออกฤทธิ์ทางชีวภาพ

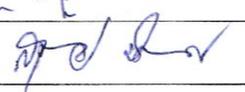
ผลการศึกษาแสดงให้เห็นชัดถึงอิทธิพลของขนาดหัว ชั้นเนื้อเยื่อ และสภาวะการเก็บรักษาต่อคุณภาพทางโภชนาการและสารพฤกษเคมีของหัวหอมแดง ซึ่งเป็นแนวทางที่สามารถนำไปใช้ประโยชน์ในการจัดการหลังการเก็บเกี่ยวและการคัดเลือกพันธุ์หอมแดงได้ต่อไป

สาขาวิชาเทคโนโลยีอาหาร

ปีการศึกษา 2556

ลายมือชื่อนักศึกษา

ลายมือชื่ออาจารย์ที่ปรึกษา

PHATTHARAPORN YUTHACHIT : POSTHARVEST CHANGES OF  
QUERCETIN IN THAI SHALLOTS (*Allium ascalonicum* Linn.). THESIS  
ADVISOR : ASST. PROF. SUWAYD NINGSANOND, Ph.D., 80 PP.

QUERCETIN/SHALLOT/FLOVONOL/STORAGE

This study examined the postharvest biochemical changes in Thai shallot bulbs (*Allium ascalonicum* L.), focusing on the distribution and transformation of quercetin and its glycosides across bulb layers and sizes under varying storage conditions. Two experiments were conducted over a 30-day period using factorial combinations of temperature (15°C and 30°C) and light intensity (darkness, 9,000 lux, and 18,000 lux). At harvest, large bulbs exhibited increasing dry matter from outer to inner layers, while small bulbs concentrated dry matter and quercetin glycosides in the outer tissues. Reducing sugars and pyruvic acid were highest in outer layers and larger bulbs, indicating stronger sweetness and pungency profiles.

Enzyme assays revealed low activity of flavonol synthase and glucosyltransferases, but high activity of Q4'G-glucosidase, suggesting that deglycosidation is the dominant pathway regulating flavonol accumulation. During storage, bulbs experienced progressive weight loss, accelerated under high temperature and light exposure. Storage at 15°C in darkness minimized moisture loss and preserved chemical stability. Reducing sugars and QG compounds followed biphasic trends, peaking at day 12 before declining due to respiration and tissue degradation. The highest concentration of free quercetin (27 g/kg DW) was observed under 15°C and 18,000 lux on day 12, indicating optimal conditions for bioactive compound retention. These findings highlight the influence of

bulb size, tissue layer, and storage environment on the nutritional and phytochemical quality of shallots, offering practical insights for postharvest handling and cultivar selection.



School of Food Technology

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Student's Signature P. Yuthachit

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## LIST OF ABBREVIATIONS

ACSOs	=	<i>S</i> -alk (en)ylcysteinesulfoxide
ANS	=	Anthocyanidin synthase
BHT	=	Butylatedhydroxytoluene
BSA	=	Bovine serum albumin
°C	=	Degree celsius
CHI	=	Chalcone isomerase
CHS	=	Chalcone synthase
DFR	=	Dihydroflavonol 4-reductase
DM	=	Dry matter
DMRT	=	Duncan's multiple range test
DNA	=	Deoxyribonucleic acid
DP	=	Degrees of polymerization
EDTA	=	Ethylenediaminetetraacetic acid
F3H	=	Flavanone 3-hydroxylase
F3'H	=	Flavanone 3'-hydroxylase
FLS	=	Flavonol synthase
FOS	=	Fructooligosaccharides
FW	=	Fresh weight
kg	=	Kilograms
kJ	=	Kilojoule
MCSO	=	<i>S</i> -methyl cysteinesulfoxide

**LIST OF ABBREVIATIONS (Continued)**

mg	=	Milligram ( $10^{-3}$ gram)
m <sup>2</sup>	=	Square meters
μg	=	Microgram ( $10^{-6}$ gram)
NSC	=	Non-Structural carbohydrates
PCSO	=	<i>S</i> -propyl cysteine sulfoxide
PeCSO	=	<i>S</i> -trans-prop-i-enylcysteine sulfoxide
PH	=	Hydrolysate prepared from pepsin
Q	=	Quercetin
Q3G	=	Quercetin-3- <i>O</i> -monoglucoside
QG	=	Quercetinglucoside
QDG	=	Quercetindiglucoside
QMG	=	Quercetinmonoglucoside
Q4'G	=	Quercetin 4'- <i>O</i> -βmonoglucoside
ROS	=	Reactive oxygen species
SSC	=	Soluble solids
TQC	=	Total quercetin compound
UV	=	Ultraviolet

# CHAPTER I

## INTRODUCTION

### 1.1 Introduction

Shallots (*Allium ascalonicum* Linn.) are categorized in the same genus (*Allium*) as onion and garlic of the Alliaceae family (Rubatzky and Yamaguchi, 1997). For many years, they have been considered separate species from onions and given the specific name *Allium ascalonicum*, but they are now considered to be onions and belong to the aggregatum group. This group differs from onions in that they multiply while still growing and produce many lateral bulbs (Thompson, 2003). Shallots have the origin in the Mediterranean area, the city of Ashkelon, today in southwestern Israel, and were discovered during the First Crusade.

Shallots grow spontaneously in Eastern Asia and the Middle East and are cultivated widely in Asia, including Indonesia, the Philippines, Thailand, Sri Lanka, and India (Răduică and Popescu, 2010), due to their distinct pungency and their use in traditional and folk medicines (Vu et al., 2013). Shallots are known in Thailand as “Hom-Dang” and are one of the most important economic vegetable crops with a great potential for mass production and export. Major shallot growing areas are the north and northeast regions, including Sri Sa Ket, Uttaradit, Phayao, Chiang Mai, and Lamphun provinces. The total growing areas are approximately 105,228 rai (ca 16,836 ha) with a total and average yields of approximately 228,221 tons and 2,194 kg/rai, respectively (Office of Agriculture Economics, 2012). Approximately 83% of the

production is consumed domestically, while the majority of the exporting portion (38,203 tons) is to Indonesia, Malaysia, and Singapore, with approximate sale values of 445 million baht (Office of Agriculture Economics, 2012).

Fresh bulb shallots contain diallyl trisulfide, flavonoids, glycosides, pectins, and glucokinin. In particular, flavonoids have been reported to exhibit antioxidative and free radical scavenging abilities. Habitual consumption of shallots helps decrease cholesterolemia, improve blood circulation, and decrease fat in the blood that causes hypertension and heart diseases. Also, it is reported that substances in shallots have antimicrobial properties (Prakash, Singh, and Upadhyay, 2007).

Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) possesses highly effective antioxidant properties among flavonol compounds belonging to a broad group of polyphenolic flavonoid substances. It plays an important role in the prevention of cancer (particularly colon cancer and prostate cancer), cardiovascular disease, inflammatory, arthritis, canker sores, respiratory diseases (asthma and bronchitis), platelet aggregation, fibromyalgia, eye disorder (cataracts and macular degeneration), aging, and viral activity against reverse transcriptase of HIV (Sharma and Gupta, 2010). Normally, the effectiveness of scavenging free radicals of any flavonoid depends on its chemical structure. It has been reported that quercetin possesses the most effective combination of all important chemical structures that can generate the maximal radical scavenging potential (Lugasi, Hóvári, Sági, and Bíró, 2003; Becker, Ntouma, and Skibsted, 2007). This combination contains three structural groups. The first group is the ortho-dihydroxy (catechol) structure in the B-ring, the obvious radical target site for all flavonoids with a saturated 2, 3-double bond (e.g., flavan-3-ols, flavanones, cyanidin chloride). The second group is the 2, 3-double bonds in

conjugation with a 4-oxo function, and the third group is the additional presence of both 3- and 5-OH groups in the C and A-ring. Quercetin aglycone (no sugar) exhibits higher effective scavenging activity for hydroxyl and peroxy radicals than for its glucosides (Hollman, Vries, Leeuwen, Mengelers, and Katan, 1995; Wach, Pyrzyńska, and Biesaga, 2007). Quercetin and quercetin-3-O-glycosides (Q3G) exhibit better antioxidant activity than butylated hydroxytoluene (BHT) in bulk fish oil and are more effective than  $\alpha$ -tocopherol in an emulsion system (Huber, Vasantha, Ruspasinghe, and Shahadi, 2009).

Plants of the Allium family are an important source of quercetin. The average quercetin is about 347 mg/kg in raw onions, which is 5-10 times higher than that found in other vegetables (Lachman et al., 2003). The amount of quercetin, including its glucosides in yellow and red onions and shallots, yields a high level of dietary flavonols, which is about 200-1,000 mg/kg (Rubatzky and Yamaguchi, 1997). Q4'G (4.93 $\pm$ 0.03  $\mu$ mol/g; 42%) and Q3, 4'G (4.93 $\pm$ 0.03  $\mu$ mol/g; 42%) are dominant in fresh shallots (Wiczkowski et al., 2008).

Quercetin is synthesized in plants starting from the amino acid phenylalanine. Phenylalanine is converted to 4-coumaroyl-CoA in a series of steps known as the general phenylpropanoid pathway. The metabolite 4-coumaroyl-CoA is added to three molecules of malonyl-CoA to form 4', 2', 4', 6'-tetrahydroxychalcone chalcone or naringenin chalcone) using chalcone synthase. Tetrahydroxy chalcone is then converted into naringenin using chalcone isomerase. Next, naringenin is converted into eriodictyol using flavanoid 3' hydroxylase. Eriodictyol is then converted into dihydroquercetin with flavanone 3-hydroxylase, which is then converted into quercetin using flavanol synthase (Yu, Matsuno, and Subramanian, 2006;

Chirumbolo, 2010). Quercetin biosynthesis increases greatly following leaf loss (early lifting). Quercetin content increases during late lifting (100% fallen leaves), whereas it is absent in the plants having erect leaves (Morgren, 2006).

Growth and postharvest conditions/treatments would impact shallot quality and its flavonol content. The formation of quercetin glucoside (QG) is normally dependent on the action of light, as it is induced by UV light (Bilyk, Cooper, and Saper, 1984; Parr and Bolwell, 2000; Treutter, 2005). QG has been shown to increase during curing (Downes, Chope, and Terry, 2009). Curing dries the thin outer layers of the bulb to form one or more complete outer skins which act as barriers against water loss and pathogen attack (O'Connor, 1979; Brewster, 2008). The onion bulbs treated with 40 kJ/m of UV-C radiation generate the highest level of total flavonols (26% increase), followed by bulbs treated with 20, 10, and 5 kJ/m<sup>2</sup> (Rodrigues, Pérez-Gregorio, García-Falcón, Simal-Gándara and Almeida, 2010). Both quercetin diglucoside (QDG) and quercetin monoglucoside (QMG) increase during field curing at 10- 25°C with 48-97% relative humidity for 10-14 days (Morgan, Olsson, and Gertsson, 2006). In addition, Q4´G and Q34´G in cured red onions at 20°C are found in greater concentrations than those cured at 24 and 28°C for six weeks. It is believed that curing temperature does not affect quercetin concentration (Olsson, Gustavsson, and Vågen, 2010).

As consumers are increasingly interested in functional foods, shallots become an exceptional dietary source of quercetin for these products. Currently, there is a lot of information regarding the effect of postharvest treatments on the changes of flavonol content or composition in onions under different environmental conditions. Most of the studies have been conducted using either light or temperature. However,

the combination of both effects used simultaneously on QG compounds has not been actively investigated.

## **1.2 Research objectives**

The objectives of this study are:

1. To investigate the types and contents of quercetin compounds, including the distribution of quercetin and its glucosides in different layers of shallot bulbs.
2. To evaluate the enzyme activities that participate in the synthesis of quercetin and its glucosides in layers of shallot bulbs.
3. To evaluate the impact of light and temperature on the types and content of quercetin and its glucosides during curing and storage.

## **1.3 Research hypotheses**

1. From the outer to the inner layer of shallot bulbs of different bulb sizes, the distribution and contents of various quercetin compounds are different.
2. Participating in quercetin biosynthesis, key enzymes are correlated with the amounts of quercetin and its glucosides generated.
3. Affected by light intensity and temperature, quercetin accumulation and distribution at different layers vary during curing and storage.

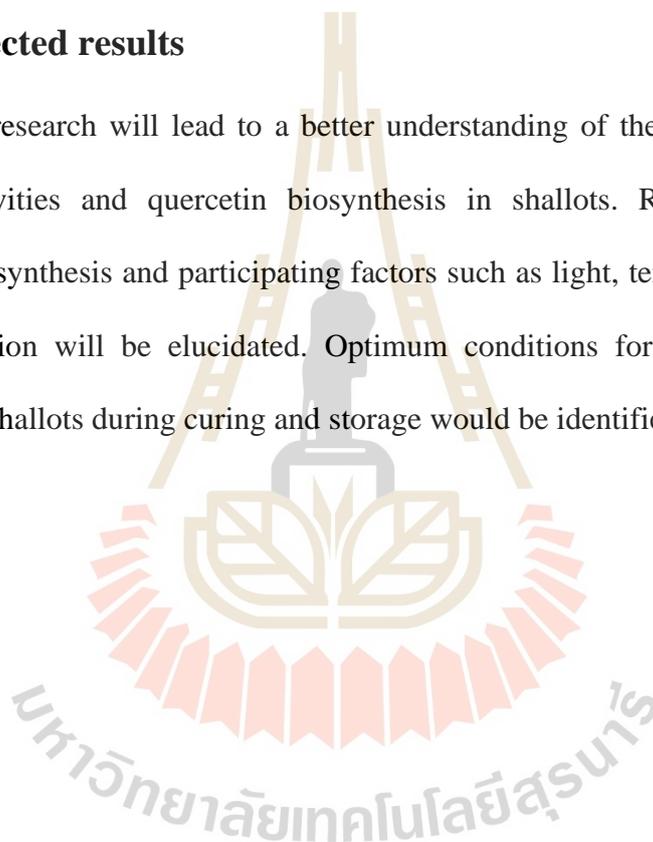
## **1.4 Scope of the study**

Contents of quercetin 3, 4' diglucoside, quercetin 4' monoglucoside, and quercetin aglucone, as well as enzyme activities relating to quercetin biosynthesis in the layers of shallot bulbs, are determined and compared. Shallots (*Allium*

*ascalonicum* L.) of the Sri Sa Ket cultivar, grown in the Daun Yai sub-district of Wang Hin district in Sri Sa Ket province, Thailand, are used to investigate effects of light irradiation and temperature on quercetin induction and enzyme activities during curing and storage. Mature bulbs are cured and stored at 15 and 30°C using visible light intensities of 0, 9,000, and 18,000 LUX for 30 days.

### **1.5 Expected results**

This research will lead to a better understanding of the relationship between enzyme activities and quercetin biosynthesis in shallots. Relationships between quercetin biosynthesis and participating factors such as light, temperature, and shallot bulb maturation will be elucidated. Optimum conditions for the accumulation of quercetin in shallots during curing and storage would be identified.



## CHAPTER II

### LITERATURE REVIEWS

#### 2.1 Shallot (*Allium ascalonicum* Linn.)

Shallots (*Allium ascalonicum* L. or *Allium cepa* L. var. *ascalonicum*) are perennial vegetable species related to the common onion (*Allium cepa* L.), which belongs to the Amaryllidaceae family. The term shallot is most commonly used for a specific variety of *A. cepa*, formerly classified as a distinct species, *A. ascalonicum* L. (Puizina, 2013). Botanically, it is very similar to the common onion (*Allium cepa* L.) in the morphological characters of inflorescence and flower, but the difference is found in a cluster of small bulbs attached at a common base rather than a single large bulb. Shallot bulbs are usually smaller in size and have a stronger smell than those of the single-headed onion bulbs (Rabinowitch and Kamenetsky, 2002). The bulbs are not encased by a sheath as in garlic. The name *Allium* is derived from the Celtic word “all” (pungent), whereas the name *ascalonicum* is derived from its original site of cultivation, Ashkelon, an ancient Philistine city in Israel (Fattorusso, Iorizzi, Lanzotti, and Tagliatela-Scafati, 2002).

There are about 500 species of shallots all over the world. The species vary in form and color depending on their sources. Shallots grow spontaneously in Eastern Asia and the Middle East. It can be grown in various climate zones ranging from Asia and Africa to northern Europe and North America (Francke and Klasa, 2009). Now, shallots are cultivated in a large area in Asia, such as Indonesia, the Philippines, Sri

Lanka, India, and Thailand. Shallot is the traditional allium produced in many tropical countries with seasonal climates. It grows and produces well near the equator in very humid climates and at low altitudes, whereas onion grows and produces well in the cool season (Currah, Cools, and Terry, 2012). Shallot is more widely cultivated and consumed in Southeast Asia than the common onion because of its shorter growth cycle, better tolerance to disease and drought stresses, and longer storage life. Besides the pungent flavor, the persistence of distinct flavor after cooking is also preferable (Currah, 2002; Woldetsadik, 2003; Phuong, Isshiki, and Tashiro, 2006). The previous study has reported that quite a few shallot strains have a close relationship found in Southeast Asia. These include one strain from Taiwan (Tainan), one from China (Kunmin), one from Malaysia (Kuala Lumpur), two from Thailand (Sri Sa ket and Chiang Mai), two from the Philippines (Quezon), six from Indonesia (Deli, Jakarta, Manado, Medan, and Pekanbaru), four from the North region (Laocai, Hanoi, Hochiminh, and Vinhphuc) and five strains from the South region (Camau and Hochiminh) of Vietnam. This investigation has confirmed that all strains are considered to be the true shallot (Phuong et al., 2006).

Shallot cultivars grown in Thailand are Sri Sa Ket, Bang Chang, White (local variety), and Chiang Mai. Bulbs of the Sri Sa Ket cultivar are round-shaped, reddish-purple-thick-skinned, strong, pungent, and sweet. There is a creamy-white, dull powder-like residue on the surface of the emerald-green leaf. Bang Chang cultivar is very similar in appearance to the Sri Sa Ket cultivar, but the skin bulb color is pale. Bulbs of white cultivars are round-shaped, white or yellowish-thin-skinned, not pungent or sweet. Compared to the emerald green leaf, there is a thicker creamy-white powder-like residue on the blue-green leaf. Bulbs of Chiang Mai cultivar are tapering-shaped, light-orange thin-skinned, less pungent, and sweet. Shallot bulbs are separated

into distinct petals without outer covering. A green leaf is a little creamy-white thin film (Department of Agricultural Extension, 2008).

## 2.2 Economic importance of the shallot crop

Shallots are one of the important alliaceous vegetables in world trading since they can be shipped during dormancy (Currah, Cools, and Terry, 2012). In Thailand, shallots are the highest mass-produced and important exported vegetable crop. The planting area of shallots in the last five years was 0.09 to 0.10 million rai (0.61 to 0.67 million hectares) (Table 2.1). Although the total planted area of shallots does not increase, their production increases every year (average yield of 0.20 million tons and 1,900 kg/rai).

**Table 2.1** Planting and harvest areas, the total and average productions, and the total quantity of shallots in Thailand from 2008 to 2012.

Year	Planting area (Rai)	Harvest area (Rai)	Yield (Kg/rai)	Production (Tons)	Domestic (Tons)	Imports (Tons)	Exports (Tons)
2008	106,561	106,053	1,881	199,435	208,815	31,850	21,944
2009	106,520	103,513	1,948	201,662	197,283	28,499	32,877
2010	103,144	98,033	1,843	180,696	171,005	15,428	25,120
2011	98,038	96,942	2,014	195,228	182,133	41,254	54,350
2012	105,228	104,004	2,194	228,221	250,127	59,494	38,203

Source: Office of Agriculture Economics, 2012.

In Thailand, major shallot growing areas are in the North and Northeast regions, particularly Sri Sa Ket, Uttaradit, Phayao, Chiang Mai, and Lamphun provinces. Sri Sa

Ket province is the largest planting area and the first area to produce shallots in the northeast region of Thailand. The shallot production of 77,594 tons (2,852 kg/rai) was harvested from a growing area of 27,340 rai, approximately 0.17 million ha (26% of the total harvesting area) in 2012. The exported portion was 17% of total production as fresh and dried shallots mainly to Indonesia, Malaysia, and Singapore, while about 83% of total production is not consumed domestically. Thus, 24% of total domestic consumption was imported. The volume of domestic consumption increased about 1.2-fold, the exports 1.7-fold, and the imports 1.9-fold between 2008 and 2012 (Office of Agriculture Economics, 2012).

The cost of shallot production during the last five years increased by 25%, rising from 8,644 baht per ton to 10,776 baht per ton (Table 2.2). Therefore, many farmers decided to plant shallots commercially whenever there was a high-profit potential.

**Table 2.2** Production cost and value of shallots in Thailand from 2008 to 2012.

<b>Year</b>	<b>Production cost (Baht/ton)</b>	<b>Farm price (Baht/ton)</b>	<b>Benefit price (Baht/ton)</b>	<b>Farm value (Million baht)</b>	<b>Exports value (Million baht)</b>
2008	8,644	15,530	6,886	3,043	158.26
2009	9,338	16,760	7,422	3,132	251.41
2010	9,858	13,680	3,822	3,028	204.73
2011	9,062	25,850	16,788	2,671	589.63
2012	10,776	22,150	11,374	5,900	445.57

Source: Office of Agriculture Economics, 2012.

### 2.3 Shallot production

Shallots can be grown using bulbs and seeds. Bulb growing is the preferred method for farmers because using seed growing results in low yields, with only one bulb produced. Due to heterozygosis, the crop is usually propagated using bulbs (Rubatzky and Yamaguchi, 1997). The suitable bulbs used for propagation are single complete large bulbs with firm flesh and are stored for 3-6 months. A mature shallot plant from bulb propagation can produce clusters of several bulb splits, having an average number of 6 bulbs per plant and a weight of 4-5 g/bulb. Bulbs of 200-300 kg/rai are used for the shallot production.

In Thailand, shallots can be grown for three crops in rotation all year round. Growing shallots in the rainy season can have two crops. The first crop is from May to June and is harvested between July and September. The second crop is from August to September and is harvested between October and November. In summer, shallots are planted between December and January and are harvested between February and March (Department of Agricultural Extension, 2008). The best growing season is winter, from November to February. During winter, shallots are highly developed, producing a lot of bulb splits and large bulbs under photoperiod (9-10 hours per day) (Kasikorn Research Centre, 2008).

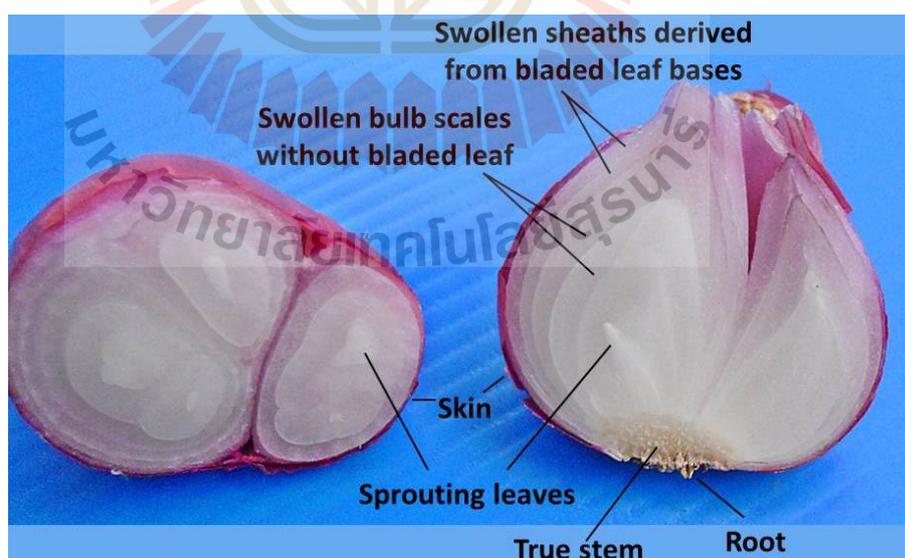
After planting, shallots are harvested between 90-110 days in the summer season and 45-60 days in the rainy season (Kasikorn Research Center, 2008). Shallots are lifted by hand when the foliage has fallen, and two-thirds of the plants become yellow (Currah, Cools, and Terry, 2012). They are usually field-cured on top of the beds for three to four hours before being taken to the storage site. After field curing, bulbs are cleaned, tied in bunches, and hung under ambient shed conditions to complete the

outer dry skin using air dry or forced air ventilation for 10–12 days (Department of Agricultural Extension, 2008). On a small scale, the bulbs are simply placed on a tray or in crates in a naturally well-ventilated shed or barn. The following data are suggested: one cubic meter of storage holds about 450 kg of bulbs, an airflow rate of 240 m<sup>3</sup> per hour and per m<sup>3</sup> of storage, appropriate air distribution channels, precise and reliable regulation of heating. The shallot's curing process is to prevent water loss from the fresh and obstruct pathogens from entering the bulb. Within the outer thin scales, chemical reactions take place to produce compounds that act as antifungal activity (Currah, Cools, and Terry, 2012). This activity also enhances the formation of well-colored intact outer skins (Rubatzky and Yamaguchi, 1997). Furthermore, shallot bulbs can be cured at a temperature between 16- 23°C within the relative humidity of 60-75% for 14-17 days. Shallot bulbs can be long-term stored for 8-10 months (0- 1.7°C, 60-70% RH) in net bags (Department of Agricultural Extension, 2008).

#### **2.4 The development of shallot bulbs**

A bulb of the *Allium* plant is an underground, modified stem that develops in some flowering plants. Its purpose is to store nutrients and water for the plant through the rainy or summer season. Shallot bulbs are formed from layers of leaves that have enlarged and united. Bulb formation in shallots occurs in response to long photoperiods and relatively high temperatures. The young shallot foliage responds to the photoperiodic stimulus, and the induction of bulb development does not involve cell division (Rabinowitch and Kamenetsky, 2002). The early stage of bulb formation in shallots is similar to onions, involving the swelling of leaf sheaths. During bulbing, the young developing leaves cease to form blades but develop into swollen, bladeless “bulb

layers” (Brewster, 2008). The outer layer of shallots is formed from the lower parts of leaf sheaths, which expand actively around the inflating fleshy scales during the bulbing process, and then dry out during bulb maturation and curing to produce one or more layers of dry papery layers. These dry or semi-dry layers tightly envelop and protect the bulbs during the dormant period (Currah, Cools, and Terry, 2012). Since leaf blades cease to form on the inner bulb layers, pseudostems become hollow. Also, the sheath tissues in the middle region of the neck soften lose turgidity, and ultimately cause the foliage to fall. This foliar fall-over is an indicator of bulb maturity (Brewster, 2008). A typical shallot bulb at maturity has one to three dry skins enclosing two swollen sheaths from blade leaves. These, in turn, enclose two or three swollen, bladeless bulb scales, and finally, at the center, are formed leaf initials with blades of doubled onions (Rabinowitch and Kamenetsky, 2002). These inner-bladed leaf initials emerge when the bulb sprouts, and hence are termed the “sprout leaves” (Figure 2.1).



**Figure 2.1** Horizontal and vertical cross-section of inner shallot bulb structure.

**Source:** Rabinowitch and Kamenetsky, 2002.

When onion plants are transferred from non-bulb-inducing conditions, reducing sugars, sucrose, and fructan concentrations increase in the sheath tissue and the leaf blades within 5-10 days. Approaching the end of bulbing and before harvesting, fructan concentrations decline, and fructose levels increase in storage layers, most rapidly in the outermost layers. Fructan concentrations increase in the bulb base plate as an intermediate storage source for carbohydrates to supply the developing sprout. Dry weights of storage layers decline, and base plate and sprout weights increase during storage, reflecting this translocation of carbohydrates. The sprout always contains enough carbohydrates for growth (50-60 mg/g dry weight, of which 30% is fructan). Decreases in water-soluble carbohydrates and fructan fractions are detected in the inner layer of leaves because they are utilized in the biosynthesis and respiration of sprout leaf growth (Brewster, 2008).

## **2.5 Chemical constituents and biological activity of shallot bulbs**

Shallot composition is variable and dependent on cultivar differences, plant nutrients, harvest maturity phase, irrigation schedule (rainfall), and the use of growth regulators and bulb section (Tendaj and Mysiak, 2010; Woldetsadik and Workneh, 2010; Beníteza et al., 2011).

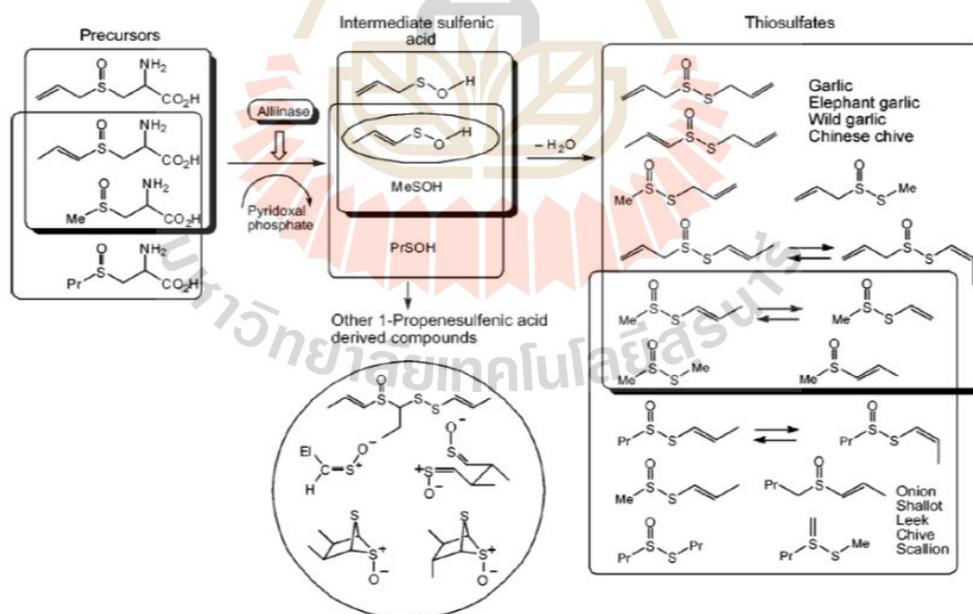
Water content in shallot bulbs is about 77%, which is lower than that of 90% in onion bulbs. The pH values of the solution in shallot bulbs and onion bulbs are similar at 6.4 and 6.2, respectively (Yin and Cheng, 1998). Dry matter content is an important quality parameter for the industry because it directly relates to the energy required for drying and to other quality attributes such as pungency, fructans, firmness, and storage life (Kahane et al., 2001; Beníteza et al., 2011). Shallot bulbs have higher levels of dry

matter than most onions. Contents of fructans, sugars, and sulfur compounds are 16-33% of dry matter compared to 7-15% in onions (Rabinowitch and Kamenetsky, 2002). On average, the fleshy layers of shallots grown in Poland and South Africa were found to be 16.7% and 17.7% dry matter (Tendaj and Mysiak, 2010; Woldetsadik and Workneh, 2010). In Australia, the dry matter of shallot was found to be as high as 28% (Muir et al., 2007) while the dry matter of shallot harvested in Indonesia showed the lowest level of 11% (Djali and Putri, 2013).

The major fraction of the dry matter of shallot bulbs is non-structural carbohydrates (NSC), consisting of 70-80% carbohydrates. The principal components of NSC are fructans, glucose, fructose, and sucrose. There is a 10-15% carbohydrate fraction in cell-wall components such as cellulose and pectin (Rabinowitch and Kamenetsky, 2002). In Poland, the total sugar content of shallot cultivars was 0.07 mg/g fresh weight (FW). The different shallot cultivars in Vietnam and the surrounding countries, including Indonesia, China, Malaysia, Philippines, Sri Lanka, and Thailand, had total sugar contents ranging between 137.83 and 477.4 mg/g FW. All cultivars showed wide content ranges of 33.3-83.5% (72.48-354.29 mg/g FW) fructan, 2.7-55.8% (6.38-171.26 mg/g FW) sucrose, and 0.1-23.4% (0.20-67.16 mg/g FW) fructose (Vu et al., 2013).

Many oriental dishes are flavored with shallot leaves, giving a fine tang to flavors, nuttier than an onion and distinct from garlic (Chomchalow, 2002). These flavors are created by the chemical transformation of a series of volatile sulfur compounds. They are generated by the cleavage of relatively stable, nonvolatile, odorless flavor precursors, *S*-alk(en)yl cysteine sulfoxide (ACSOs), by activities of alliinase (EC 4.4.1.4) and lachrymatory-factor synthase (Bhat, Desai, and Suleiman, 2010). The total content of ACSOs in shallots is 155.8 mg/100g FW, which consists of 1.1 mg/100g FW of *S*-allyl cysteine sulfoxide (ACSO, alliin), 41.1 mg/100g FW

of *S*-methyl cysteine sulfoxide (MCSO, methiin), 17.7 mg.100/g FW of *S*-propyl cysteine sulfoxide (PCSO, propiin), and 92.7 mg.100/g FW of *S*-trans-prop-1-enyl cysteine sulfoxide (PeCSO, isoalliin) (Rose, Whiteman, Moore and Zhu, 2005). The alliinase (*S*-alk(en)yl-L-cysteine sulfoxide lyase) is a pyridoxal 5'-phosphate dependent  $\alpha,\beta$ -eliminating lyase (Wang, Raghavan, and Ho, 2008). Upon tissue damage, the alliinase in the vacuole and the ACSOs in the cytoplasm come into contact and chemically interact to promote enzymatic hydrolysis of the ACSOs. As a result, pyruvate, ammonia, and thiosulfinates (or propane thial-*S*-oxide) are generated (Hounsome and Hounsome, 2011). An illustration of the formation pathways of thiosulfinates of *Allium* plants generated from the ACSOs via sulfenic acid intermediates is shown in Figure 2.2 (Wang, Raghavan and Ho, 2008).



**Figure 2.2** Formation of some flavor intermediates and primary flavor compounds in *Allium*.

**Source:** Wang, Raghavan and Ho, 2008.

After the enzyme reaction, sulfenic acid (a flavor intermediate) is generated. The sulfenic acid is very unstable and can further undergo condensation to form thiosulfinates. Thiosulfinates are unstable and converted into mono-, di-, and trisulfides and other odor compounds such as ajoene (Bianchini and Vainio, 2001). The content of diallyl sulfides in shallot water extract consists of 17  $\mu\text{g/g}$  diallyl sulfide, 46  $\mu\text{g/g}$  diallyl disulfide, and 13  $\mu\text{g/g}$  diallyl trisulfide (Yin, Hsu, and Chang, 2003).

The hydrolysis reaction catalyzed by alliinase completes in 6 min and produces thiopropanal *S*-oxide (tear-producing factor), pyruvic acid, ammonia, and many sulfur volatiles. Therefore, pyruvic acid is used as a measure of flavor strength. It is also widely used to determine pungency or flavor intensity in *Allium* plants (Wang, Raghavan, and Ho, 2008). Flavor compound accumulation is maximum in leaves at the bulb initiation period and then further increases in the bulb as the plant approaches maturity. In the final stage of bulbing, flavor intensity decreases as cells rapidly expand and dilution occurs. Flavor intensity is unequally distributed within the plant. In the bulb, flavor compounds are highest in the center layers at the base and lowest in the outside layers at the top (Randle, Kopsell, Kopsell, Snyder, and Torrance, 1998).

The total content of flavonoids is higher in shallots (20%) than in onions (8%) (Leelarungrayub, Rattanapanone, Chanarat, and Gebicki, 2006). Shallots contain high levels of dietary flavonols, quercetin, and quercetin glycosides, approximately 200-1,000 mg/kg (Rubatzky and Yamaguchi, 1997). The presence of flavone and polyphenolic derivatives such as quercetin, quercetin 4'-glucoside, quercetin 7, 4'-diglucoside, quercetin 3, 4'-diglucoside, and quercetin mono-D-glucose is found in shallot extracts (Leelarungrayub et al., 2006). In fresh shallot, more than 99% of total

quercetins ( $4.93 \pm 0.03 \mu\text{mol/g}$ ) are quercetin 3, 4'-*O*-bis- $\beta$ -glucoside (49.2%) and quercetin 4'-*O*-bis- $\beta$ -glucoside (49.2%) (Wiczowski et al., 2008).

From Thai food composition of edible shallot portions, there are 86.6% moisture, 10.4% carbohydrates (including soluble sugars), 1.9% proteins, 0.3% fats, 0.8% fibers, 0.04% phosphorus, 0.03% calcium, with traces of iron (Fe), carotene, thiamine, riboflavin, niacin, and ascorbic acid in shallot bubs (Health Department-Nutrition Division, 1987).

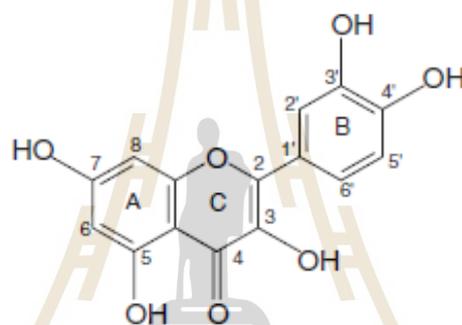
Previous studies have reported that feeding rabbits with freshly prepared shallot extract reduces the number of abnormal-shaped erythrocytes caused by high levels of cholesterol in the blood. Also, substances in shallots show antileukemia (Rabinowitch and Kamenetsky, 2002). Four food-borne bacteria, *Salmonella typhimurium* DT104, *E. coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*, and four nosocomial bacteria, methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, are inhibited by shallot oil (Yin et al., 2003). Shallots have shown antioxidant and free radical scavenging capacities against external and endogenous agents. Moreover, shallots decrease cholesterolemia, improve blood circulation, and decrease fat in blood vessels, causing hypertension and heart disease (Leelarungrayub, Rattanapanone, Chanarat, and Gebicki, 2006; Prakash et al., 2007).

## 2.6 Quercetin

### 2.6.1 Chemical structure and properties

Quercetin ( $\text{C}_{15}\text{H}_{10}\text{O}_7$ , 3, 3',4',5,7 -pentahydroxy-2-phenyl-chromen-4-one) is a member of naturally occurring polyphenolic compounds that share a common

flavone nucleus made up of two benzene rings linking through a heterocyclic pyrone one. It is one of the major dietary flavonoids belonging to a group of flavonols due to its hydroxylation at the 3-position of the C ring (Park, 2005; Materska, 2008; Chirumbolo, 2010). The quercetin structure has five hydroxyl groups at 3, 5, 7, 3' and 4' positions on the diphenylpropane (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>) skeleton (Figure 2.3). In addition, it contains the 2, 3 double bond and the 4-carbonyl group in the C ring (Rice-Evans, Miller and Paganga, 1996).

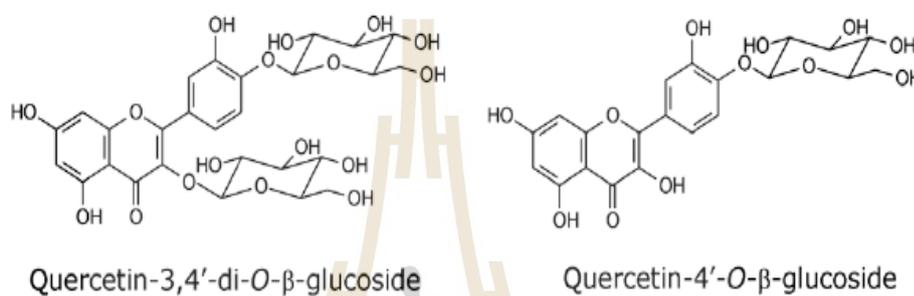


**Figure 2.3** Chemical structure of quercetin.

**Source:** Harwood et al., 2007.

Quercetin and glycosides occur mainly in leaves and in the outer parts of the plants. Glucosides have one or more sugar groups that bond to phenolic groups via glycosidic linkage. Among galactose, rhamnose, and xylose, glucose is the most common sugar (Hollman, De Vries, Van Leeuwen, Mengelers, and Katan, 1995; Wach, Pyrzyńska and Biesaga, 2007; Materska, 2008). The most commonly occurring flavonols are those with dihydroxylation in the 3' and 4' positions of the B-ring, and the preferred glycosylation site on the flavonoids is the 3-position. It has been reported that the hydroxyl group at the 3-position is required for the maximal radical

scavenging activity of flavonols (Kim, Jeong, and Jung, 2006). In general, the most common flavonoid glycosides are the 3-*O*- $\beta$ -glycosides of flavonols, but the two quercetin conjugated quercetin 3,4'-*O*- $\beta$ -glucoside and quercetin 4'-*O*- $\beta$ -glucoside (Figure 2.4) are the main flavonols from the approximately 20 detectable flavonols in onion species (Tsushida and Suzuki, 1996; Beesk et al., 2010).



**Figure 2.4** Chemical structures of the major quercetin glucosides in onion.

**Source:** Beesk et al., 2010.

Antioxidant properties are largely a function of quercetin structure, consisting of three criteria. First, an ortho-dihydroxy or catechol group in the B-ring confers higher stability to the radical form and participates in electron delocalization. Second, a 2, 3-double bonding conjugation with a 4-oxo function in the C ring is responsible for electron delocalization from the B ring. The antioxidant potency relates to structure in terms of electron delocalization of the aromatic nucleus. At the location where compounds react with free radicals, the phenoxyl radicals are stabilized by the resonance effect of the aromatic nucleus. Third, hydroxyl substitutions at positions 3 and 5 with 4-oxo function in A and C rings are required for maximal radical scavenging potential (Rice-Evans, Miller and Paganga, 1996; Burda and Oleszek, 2001; Harwood et al., 2007). In the case of quercetin derivatives, glycosylation at

C(4')-OH decreases the H-donating ability markedly, while C(3)-OH derivatives of quercetin show reducing potential comparable with that of the free aglycone. The lower antioxidant activity of quercetin glycosides is mainly due to the blocking of hydroxyl groups by sugars or alkoxy substituents (Materska, 2008; Santas, Carbo', Gordon, and Almajano, 2008).

The quercetin molecule is lipophilic, while its derivatives can be both lipo- and hydrophilic depending on the type of substituents in the molecule. Glycosylation of at least one hydroxyl group of quercetin derivatives results in increasing its hydrophilicity. This change from lipophilic to hydrophilic is very significant to plants for glycosidic derivatisation of quercetin, providing cytosol-soluble forms that are easily transported to various parts of the plant and stored in vacuoles (Materska, 2008).

### **2.6.2 Biological properties**

Quercetin and its derivatives have many biological activities including an anti-inflammatory effect, antiproliferative activity against human breast adenocarcinoma cell line (MCF-7), gastrointestinal tumor cells (Duo, Ying, Wang and Zhang, 2012; Tamura, Nakagawa, Tsushida, Hirayama and Itoh, 2007). Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals, bind transition metal ions, and inhibit oxidases. These properties of quercetin inhibit lipid peroxidation, creating deleterious effects throughout the body, such as cardiovascular and neurodegenerative diseases. These also reduce inflammation by scavenging free radicals and protect cells from oxidative stress induced by reactive nitrogen species, which play a key role in human cancer development. (Prakash, Singh and Upadhyay, 2007; Baghel, Shrivastava, Baghel, Agrawal and Rajput, 2012). Quercetin and quercetin-3-*O*-glycosides exhibit better antioxidant activity than butylated

hydroxytoluene (BHT) in bulk fish oil and are more effective than  $\alpha$ -tocopherol in an emulsion system (Huber, Vasantha, Ruspasinghe, and Shahadi, 2009)

Quercetin aglycone and glycosides are absorbed from the gastrointestinal tract to different extents. The absorption of quercetin glycosides depends on the position and nature of sugar substitutions. A lipophilic quercetin molecule can easily be absorbed by the stomach, while quercetin glycosides are partially deglycosylated and absorbed by the small intestine (Materska, 2008).  $\beta$ -glucosidases present in the cytosol of small intestine mucosa cells have a high affinity, particularly towards flavonol glycosides and prefer the sugar group at the 3-position (Day et al., 2000). Average diet supplies ranging from 15 to 40 mg of quercetin per day can be obtained from fruit and vegetable consumption (Lakhanpal and Rai, 2007). However, daily levels of quercetin as high as 200-500 mg are possibly attained by higher consumption of fruits and vegetables, especially quercetin-rich fruits and vegetables such as tomatoes, apples, and onions (Harwood et al., 2007).

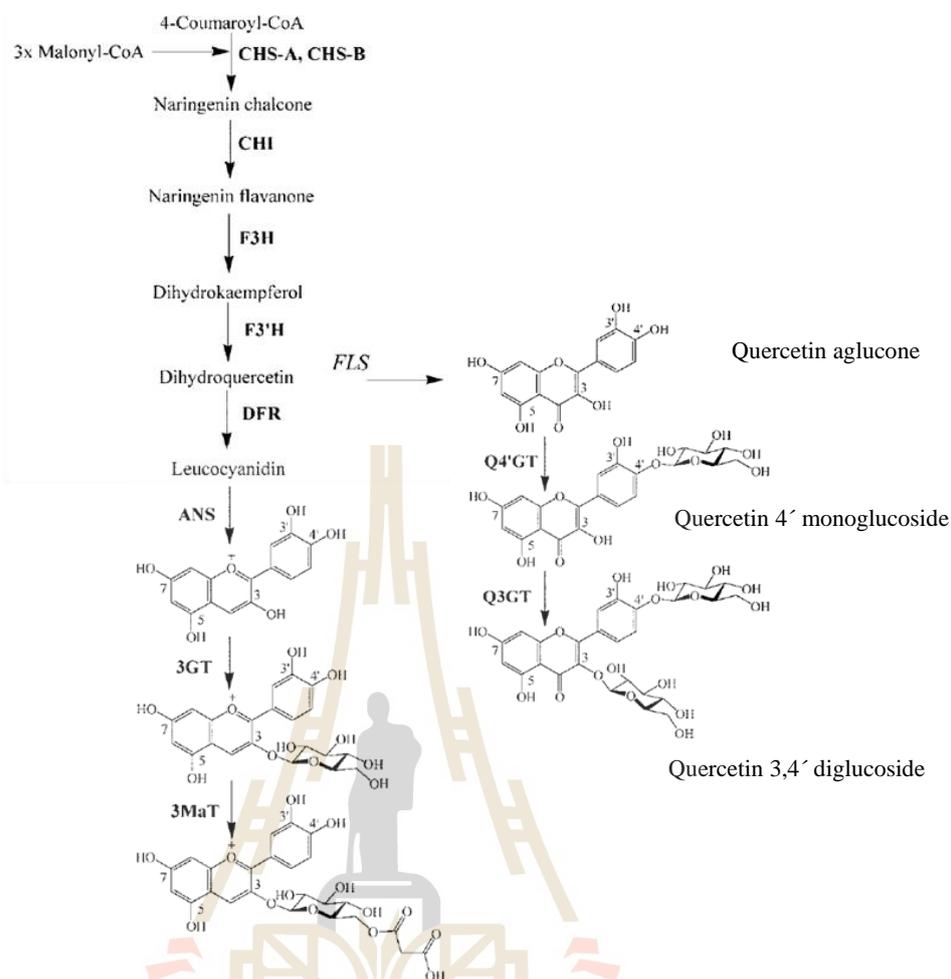
### **2.6.3 Biochemistry and key metabolic enzymes**

Flavonoids are naturally occurring compounds synthesized through the phenylpropanoid pathway, which represents the initial dedicated step in forming the phenylpropanoid structure. (Lister and Lancaster, 1996). In this biosynthetic pathway, phenylalanine, an amino acid, serves as the crucial precursor for all flavonoids and establishes the base of the flavonoid B-ring. Originating from the shikimate pathway within the plastids, phenylalanine is converted in a sequence of three steps into 4-coumaroyl-CoA. The initial stage of the phenylpropanoid pathway involves the enzyme phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), which catalyzes the conversion of phenylalanine into trans-cinnamic acid (Yu, Matsuno, and Subramanian, 2006; Ubi, 2007). PAL plays a pivotal role in regulating the flow of phenylpropanoids

in this pathway. (Chang, Luo, and He, 2009). The second step involves the addition of a hydroxyl group to the C4 position of cinnamic acid, utilizing molecular oxygen to form *trans*-4-coumaric acid through the enzyme cinnamate-4-hydroxylase (EC 1.14.13.11). In the third step, *p*-coumaric acid is converted into 4-coumaroyl-CoA, a precursor to flavonoids, by the 4-coumaroyl-CoA ligase (EC 6.2.1.12), facilitated by the addition of coenzyme A. (Lister and Lancaster, 1996; Yu, Matsuno and Subramanian, 2006).

In the biosynthesis of flavonoids (Figure 2.5), chalcone synthase (CHS; EC 5.5.1.6) is identified as the crucial enzyme for flavonoid synthesis, as it catalyzes the initial committed step in the flavonoid pathway. (Nugroho, Verberne and Verpoorte, 2002). CHS catalyzes the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA, resulting in one molecule of naringenin chalcone (chalcone or 4',2',4',6'-tetrahydrochalcone). The naringenin chalcone is the key intermediate to generate the C6-C3-C6 skeleton in the formation of flavonoids (Fang, Tang and Huang, 2013; To and Wang, 2006; Yu, Matsuno and Subramanian, 2006).

The next step, chalcone isomerase (CHI; EC 5.5.1.6), catalyzes the stereospecific cyclization of naringenin chalcone to naringenin flavanone. Consequently, flavanone-3-hydroxylase (F3H; EC 1.14.11.9) catalyzes the hydroxylation at C3 of flavanone to dihydrokaempferol. In addition, flavonoid 3'-hydroxylase (F3'H; EC 1.14.13.21) can convert dihydrokaempferol to dihydroquercetin which hydroxylates the C3' at the B-ring (Yu, Matsuno, and Subramanian, 2006).

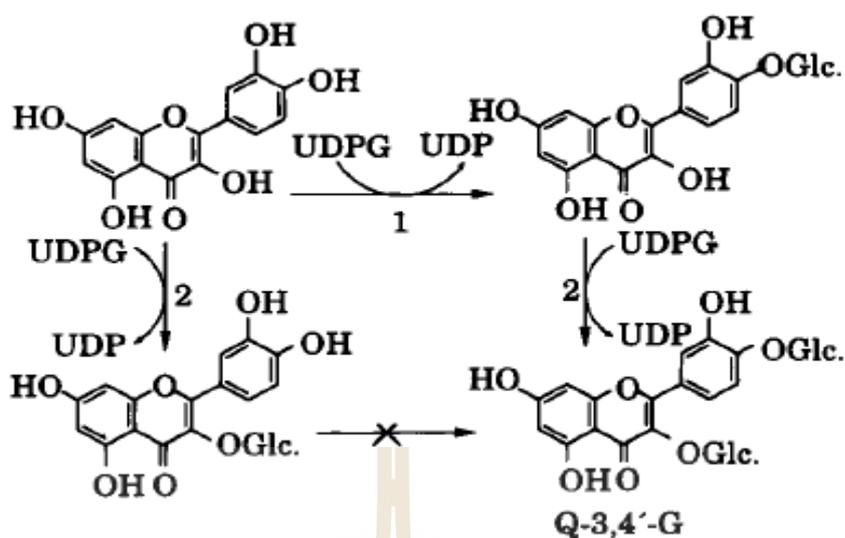


**Figure 2.5** Flavonoid biosynthesis pathway in *A. cepa*. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; 3GT, UDP glucose: flavonoid 3-O-glucosyltransferase; 3 MaT, malonylcoenzyme-A: anthocyanidin 3-O-glucoside-6''-O-malonyltransferase; FLS, flavonol synthase; Q4'GT, quercetin 4'-O-glucosyltransferase; Q3GT, quercetin 3-O-glucosyltransferase.

**Source:** Slimestad et al., 2007.

The biosynthesis of flavonols is one of the branches of the flavonoid pathway and one of the best-characterized aspects of the plant secondary metabolism (Fang, Tang, and Huang, 2013). Quercetin is a flavonol which is among the most abundant flavonoids in plants and the main flavonoid of pigmented layers of onions (Slimestad et al., 2007). Quercetin is synthesized through the flavonol branch (Li et al., 2012) and is usually found in the form of mono-, di-, or triglycosides (Zhou et al., 2013). Quercetin is formed from dihydroquercetin by introducing a double bond between C2 and C3 through the action of flavonol synthase (FLS; EC 1.14.11.23) which is the key enzyme in flavonol biosynthesis (Noda, Kanno, Kata, Kazuma and Zuzuki, 2004; Takahashi et al., 2007; Yu, Matsuno, and Subramanian, 2006).

Predominant among the glucosides are quercetin 4'-glucoside and quercetin 3,4'-diglucoside. These glucosides are mainly present in the abaxial (outward-facing) epidermis of the layers and not in mesophyll tissues (Hirota, Shimoda and Takahama, 1999; Yoo, Lee and Patil, 2013). Concentrations of two glucosides (Q4'G and Q3,4'G) and the concentration ratios of Q4'G per Q3,4'G increase from the interior to the exterior scales (Tsushida and Suzuki 1996). Quercetin 4'-glucoside is formed first, whereas the formation of quercetin 3,4'-diglucoside follows during storage, and their concentrations increase continuously (Slimestad, Fossen, and Vágen, 2007). The enzymes involved in the glucosidation of quercetin have been reported by Tsushida and Suzuki (1996). Quercetin-4'-*O*-glucosyltransferase catalyses the synthesis of quercetin 4'-*O*- $\beta$ -glucoside from quercetin. Since the quercetin-3-*O*-glucoside is not transglucosylated to 3,4'-di-*O*- $\beta$ -glucoside by this enzyme, 3,4'-*O*- $\beta$ -glucoside is likely to be synthesized from 4'-*O*- $\beta$ -glucoside in onion as shown in Figure 2.6.



**Figure 2.6** Proposed reactions involved in the formation of quercetin-3, 4'-di-O-glucoside.

1. quercetin-4'-O-glucosyltransferase ; 2. quercetin-3-O-glucosyltransferase.

**Source:** Tsushida and Suzuki, 1996.

The glucosyltransferase shows higher activities in layers 3 to 6 than in the outer layers. During the cell senescence and drying of onion outer layers at the curing stage, di- and mono-glucosides of quercetin are converted to free quercetin by the glucosidase enzyme, particularly in the almost dry area of the layers (Brewster, 2008). The quercetin-3-O- $\beta$ -glucosidase can convert quercetin 3,4'-diglucoside to quercetin 4'-glucoside and the quercetin-4'-O- $\beta$ -glucosidase converts quercetin 4'-glucoside to quercetin. Quercetin content is increased from the interior to the exterior layers, and a small amount of quercetin is also found in the abaxial epidermis (Hirota, Shimoda, and Takahama, 1999). The contents of quercetin 3,4'-di-O- $\beta$ -glucoside (Q3,4'G), quercetin 4'-O- $\beta$ -glucoside (Q4'G), and quercetin (Q) were higher in the outer layers than in the inner ones. In addition, higher ratios of Q3, 4'G per Q4'G in the outer layers indicate that the glucosidation is predominant in the outer layers. This results in

the accumulation of quercetin in the outer layers by glucosidase. The substrate changes in the ratio also seem to be due to the balance of activities of these glucosyltransferase and glucosidase (Tsushida and Suzuki, 1996).

Moreover, quercetin is oxidized by autooxidation, and cell-free extracts of drying areas (Takahama and Hirota, 2000). The hydrogen peroxide formed in the senescing cell is likely to be a factor favoring the peroxidase-dependent oxidation of quercetin to 3,4-dihydroxybenzoic acid (protocatechuic acid) and 2,4,6-trihydroxybenzoic acid. Protocatechuic acid has long been known to confer resistance to the onion smudge fungus (*Collectotrichum circinans*) and to be present in colored onion skins but not in white onion skins.

## **2.7 Factors affecting flavonol content in foods**

Interest in the role of antioxidants in human health leads to evaluate antioxidant properties of fruits and vegetables and to determine whether these properties can be maintained or improved through crop breeding, cultural practices, post-harvest storage and emergent technologies such as UV-C or ethylene treatments (Rodrigues, Pérez-Gregorio, García-Falcón, Simal-Gándara and Almeida, 2010). Shallot quality and flavonol content depend on many factors, including cultivar differences, preharvest conditions, harvesting and postharvest handling, food preparation, and processing.

### **2.7.1 Variation among species and cultivars**

There have been many studies on the qualitative genes affecting flavonol content in onions. These effects show changes in chromosomal DNA sequences resulting in modifications of gene control, enzyme synthesis, or enzyme function to modify a biosynthesis pathway and thereby the resulting plant (the phenotype). The

values reported for quercetin concentrations are highest in red cultivars, approximately 50% lower in the yellow cultivars, and 99.7% lower in white cultivars (Brewster, 2008). In Yellow onions, there is DNA elimination of the gene that transcribes the dihydroflavonol 4-reductase (DFR) enzyme. This mutation prevents the production of DFR and, therefore, blocks the pathway after dihydroquercetin, resulting in the accumulation of the yellow quercetin pigment without any red cyanidin (Kim, Binzel, Park, Yoo, and Pike, 2004). White-skinned onions lack the CHS enzyme to produce naringenin chalcone of the flavonol pathway (Kim, Jones, Yoo, and Pike, 2004). The amounts of quercetin in yellow and red onions are 1,187 and 1,917 mg/kg FW (Slimestad, Fossen, and Vagen, 2007). In France, shallot bulbs (*Allium ascalonicum* Hort.) are reported to have the highest quercetin 3, 4'-diglucoside content of 531-605 mg/kg FW. It is highly rich in two major quercetins, Q3,4'G and Q4'G, representing approximately 90% of the overall contents (Bonaccorsi, Caristi, Gargiulli, and Leuzzi, 2008).

### 2.7.2 Preharvest factors

Plant nutrients are known to affect the quality and storability of shallots. The range of 50-100 kg N.ha<sup>-1</sup> of nitrogen fertilization levels is a good compromise for yield, postharvest quality, and shelf life of shallot bulbs under ambient storage conditions (Woldetsadik and Workneh, 2010). Growing onion plants with inadequate nutrients is found to have lower yield, whereas a surplus of nitrogen shows no influence on the yield. The nitrogen fertilizer level does not affect quercetin glucoside content or composition either at the start of storage or during 5 months of cold storage (Morgren, Olesson, and Gertsson, 2006).

Ultraviolet (UV) radiation is considered to be particularly hazardous to living cells. The UV spectrum can be divided into three regions: UV-A (320-380 nm), UV-B

(280-325 nm), and UV-C (< 280 nm). The shorter the wavelength, the more damaging the radiation. Three cellular defense mechanisms have been identified: UV-B absorption by protective pigments, DNA repair, and antioxidants (Jordan, 2004). The accumulation of plant flavonoids is enhanced in response to the increased light exposure, especially UV-B rays. Greater concentrations of flavonols are found mainly in foods that are characterized by a large skin area per volume ratio, such as grape wines and cherry tomatoes (Aherne and O'Brien, 2002). Phenol content can be increased by being exposed to light or UV irradiation. Pinot Noir grapes on sun-exposed clusters had tenfold higher quercetin glucoside content compared with grapes from the shaded part of the cluster (González-Aguilar, Ayala-Zavala, Rosa, and Alvarez-Parrilla, 2010). It is reported that specific flavonols in petunia and *Arabidopsis* are synthesized with higher hydroxylation levels when exposed to UV. Because hydroxylation does not affect the UV-absorbing properties of these compounds but does affect their antioxidant capacity, it has been suggested that flavonols are likely to play uncharacterized roles in the UV stress response (Winkel-Shirley, 2002). Flavonols that occur in the upper epidermis of leaf tissues are consistent with their role in UV protection (Downey, Harvey, and Robinson, 2003). Quercetin and its glycosides are responsible for the prevention of UV radiation-induced damage to a variety of plants. Quercetin absorbs UV radiation with absorbance maxima in the UV-A ( $\lambda_{\text{max}} = 365 \text{ nm}$ ,  $\epsilon = 28,400 \text{ /M.cm}$ ) and UV-C ( $\lambda_{\text{max}} = 256 \text{ nm}$ ,  $\epsilon = 28,300 \text{ /M.cm}$ ) suggesting that one plausible photoprotective mechanism is direct absorption of UV radiation, thereby preventing the formation of ROS and direct DNA damage. The UV energy absorbed by quercetin is likely to be dissipated as heat, light, or through the decomposition of quercetin. The formation of excited-state keto and enol species from UV-A-treated quercetin-7-glucoside is a

mechanism for dissipation of absorbed UV energy and a contributing factor to presumed sun screening abilities of quercetin in plants (Fahlman and Krol, 2009). Onion contains large quantities of quercetin in its outer part but very little in the inner part. This is because UV rays penetrate only 100-200  $\mu\text{m}$  in plant tissues (Higashio, Hirokane, Sato, Tokuda, and Uragami, 2005).

Temperature also influences the composition of plant tissues during growth and development. Total available heat and the extent of low and high temperatures are the most important factors in determining growth rate and chemical composition of horticultural crops (Lee and Kader, 2000). In summer, flavonoid levels in these crops can be three to five times greater than in other seasons. Seasonal variations in quercetin content have also been observed in tomatoes and berries (Aherne and O'Brien, 2002). An increase in night temperature from 12 to 22°C, with the day temperature kept constant at 25°C, would result in significant increase in phenolic acid, flavonols, and anthocyanins (Wang and Zheng, 2001).

### **2.7.3 Harvesting factors**

Maturity is one of the major factors determining the compositional quality of fruits and vegetables (Lee and Kader, 2000). Quercetin is almost absent when all yellow onions have erect leaves (0% fallen leaves). Early lifting, 50% of the onions have fallen leaves, resulting in onions with better keeping quality and equal quercetin content compared with late lifting at 80% fallen leaves (Morgren, Olsson, and Gertsson, 2007). Shallots harvested at 75% top fall show better dry matter content of bulbs, reduced percent rot, sprouting, and weight loss, and improve marketability of bulbs (Woldetsadik and Workneh, 2010). The timing of harvesting strongly influences both yields and storability. Highest yields are achieved when plants remain intact until the leaves are completely dry. Late lifting (at 90% or more tops down) of European

storage-type onions shows an increase in sprouting and rooting, storage rots and weight loss, and high incidence of watery scale (Gubb and Mac Tavish, 2002).

#### 2.7.4 Postharvest factors

Fresh fruits and vegetables are subjected to continual changes after harvesting. Such changes cannot be stopped but can be delayed within certain limits by various postharvest procedures (Lee and Kader, 2000). Postharvest storage has an important impact on phenolic compounds and enzymes involved in phenolic metabolism, leading to changes (generally a decrease) in quality (Tomás-Barberán and Espín, 2001).

UV radiation is used in postharvest as a sanitizing treatment. It can induce biological stress in plants with the consequent production of stress-response compounds such as phenols, flavonoids, and phytoalexins (Schreiner and Huyskens-Keil, 2006; Rodrigues et al., 2010). The onion bulbs treated with 40 kJ/m<sup>2</sup> of UV-C radiation resulted in the highest level of total flavonols (about 26% increase), followed by bulbs treated with 20, 10, and 5 kJ/m<sup>2</sup> (Rodrigues et al., 2010). The control of postharvest diseases by the low UV dose of 1.2 kJ/m<sup>2</sup> in about 67 s likely involves two phenomena: a direct germicidal effect on the pathogen and indirect hermetic reactions that enhance the host disease resistance. The Q4'G content in the irradiated brown onion bulbs has increased approximately 2.5-fold as compared to that in the untreated control, with an increase in the free aglycone contents 4-5-fold (Rodov, Tietel, Vinokur, Horev and Eshel, 2010). The content of quercetin would be doubled in onion slices irradiated with a radiation dose of 184 μW/cm at 305 nm of UV-B lamp for 12 h (Higashio et al., 2005). The synthesis of Q3,4'G and Q4'G contents in the yellow onion leaves is enhanced by UV light with the peak emission at 254 nm (30 watts), to

a lesser extent, by visible light (34 watts) with a dose of  $100 \mu\text{mol}/\text{m}^2\cdot\text{s}$  (34 watts) (Yoo, Lee, and Patil, 2013).

The level of visible light (400-700 nm) to which experimental plants are exposed has been found to have a very great effect on UV-B injury (Zlatev, Lidon, and Kaimakanova, 2012). Flavonols and anthocyanins play an important role in protection against photodamage by visible light (Solovchenko and Merzlyak, 2008). Storing the second scales of onions in a dark room and exposing them to fluorescent light (3000 LUX) at  $25^\circ\text{C}$  for two photoperiods (24 and 72 h), the light exposed onion layers for 72 h show increases approximately 2.5-fold of total flavonoid content as compared to that in the untreated control and the treated at 24 h (Lee et al., 2008).

Quercetin content increases significantly less in dark environments compared to field curing, but some quercetin synthesis occurs regardless of light. Field curing, with or without foliage attached, does not affect quercetin content, indicating that no transportation of substances from the foliage to the scales occurred. (Morgren et al., 2006). Q4'G and Q3,4'G contents in cured red onions at  $20^\circ\text{C}$  are higher than in those cured at 24 and  $28^\circ\text{C}$  for six weeks. However, quercetin concentration would not be influenced by curing temperature. Onion curing at  $28^\circ\text{C}$  is likely to dry out the onion skin quicker than at  $20^\circ\text{C}$ ; therefore, a shorter time is sufficient for enzyme activity. This means, by the end of curing and storage, the dry weight of onion bulb flesh and skin cured at different temperatures was not significantly different. Therefore, skin water content could not explain differences in biochemical properties (Downes, Chope, and Terry, 2009).

The total quercetin content is reported to have a slight decrease during storage for 24 weeks at  $4^\circ\text{C}$  (Price, Bacon and Rhodes, 1997). During storage for 36 weeks, an increase of Q4'G and rutin contents in red and yellow onions has been observed,

especially at 22°C as compared with storage at low temperature (4°C). Accumulation of Q4'G during the storage period confirms flavonol interconversions and possible formation from Q3, 4'G (Lachman et al., 2003). Quercetin glycosides in cured red onions decrease by 70% (from 8.92 to 2.66 mg/g Q4'G and from 1.16 to 0.35 mg/g Q3,4'G) during storage at 1°C for seven months (Downes, Chope and Terry, 2009). Quercetin glycosides of onions are high and remain at a constant level during cold storage at 0- 1°C and 70–75% relative humidity for five months (Grzelak, Król, Adamicki, and Badelek, 2009).

### **2.7.5 Food preparation and processing**

The removal of outer onion scales, which constitute 27% of the total onion mass, during home cooking or industrial peeling of onions leads to losses of polyphenols (Grzelak et al., 2009). Quercetin monoglucoside has higher thermostability compared with quercetin diglucoside, due to the absence of the hydroxyl group at the C-3 position (Morgren, 2006). Roasting temperature (180 °C) leads to thermal-induced degradation of quercetin glycosides, from Q3,4'G to Q4'G. The mechanism is de-glycosylation (thermohydrolysis) to obtain the corresponding aglycone depending on roasting time and temperature (Rohn, Buchner, Driemel, Rauser, and Kroh, 2007). Domestic processing results in a loss of total flavonoids as follows (Lee et al., 2008): frying (32.8 %), sautéing (20.6 %), boiling in water with 3% salt (20%) and 1% salt (13.7%), steaming (5.7%), microwaving (4.4%), and baking (expecting 1.1% increase in total flavonoids).

## CHAPTER III

### MATERIALS AND METHODS

This research work was to study the distribution of chemical compositions and quercetin glucoside (QG) compounds of inner, middle, and outer layers of shallots with various bulb sizes. The activity enzymes involved in the metabolism of QG compounds was also assessed. Postharvest experiments were performed to investigate the effects of light intensity and temperature on changes in the shallot bulbs during storage.

#### 3.1 Plant materials

Shallots (*Allium ascalonicum* L.) of the Sri Saket cultivar were grown from bulbs in a farm located in a traditional shallot-growing region in Sri Saket province (Daun Yai sub-district of Wang Hin district) in Thailand (Figure 3.1a) on July 29, 2012 and harvested on September 13, 2012. When the neck tissues began to soften and collapse (Figure 3.1b), 47 days after being planted, the mature shallot bulbs were hand-harvested.



**Figure 3.1** Planting of shallot bulbs in a field in Sri Saket province.

### 3.2 Experimental design

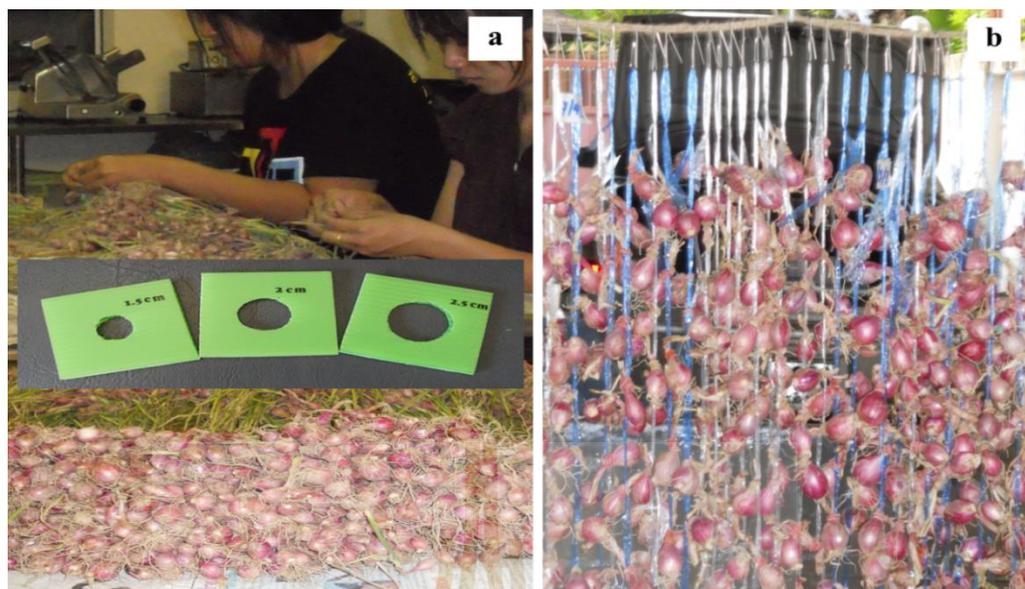
After being harvested, the shallot bulbs were divided for two experiments. A completely randomized design (CRD) with three replications was used to determine the content of dry matter, reducing sugars, and pyruvic acid of outer, middle, and inner layers of shallot bulbs. The type and content of QG compounds, together with the enzyme activity in each layer of the four shallot sizes, were compared. The shallots were size-graded into four categories based on diameter measured at the widest part of the bulb, or average bulb weight according to Thai agriculture standards (Table 3.1).

**Table 3.1** Size of a single bulb shallot.

Size	Number of Bulb/kg	Diameter (mm)	Average Bulb Weight (g)
1	<65	>30.0	>15
2	65-100	>25-30	>10-15
3	101-200	>20-25	>5-10
4	>200	10-20	<5

Source: Ministry of Agriculture and Cooperatives, 2008.

The second experiment was conducted to determine the effects of light intensity and temperature on changes in the content of chemical and QG compounds of shallot bulbs during storage. After being size-graded (Figure 3.2a),  $20 \pm 0.5$  mm diameter shallot bulbs (Size 4) were selected for this study. The leaves were removed from the bulbs, then twelve uniform bulbs were tied in a line with a plastic rope (Figure 3.2b).



**Figure 3.2** Size-grading (a) and bulb arrangement (b).

A factorial experiment in a Completely Randomized Design (CRD) with three replications was conducted to compare the chemical components and quercetin glycoside compounds among six treatments (Table 3.2). These treatments were factorial combinations of two factors, namely, light intensity (dark, 9000, and 18000 LUX) and storage temperature (15°C and 30°C). The effects of postharvest visible light on the chemical components and the changes in QG compounds in shallots, kept under continuous visible light from fluorescent lamps (589 mm long tube, 36 watts, 71 Lm/m, OSRAM), were studied. The lamps were set up in an incubator and a growth chamber at a controlled temperature. The intensity of the lamp's flux was measured by a Digital Light Meter (DIGICON LX-70, Norway). Temperature and relative humidity were recorded by a thermo-hygrometer (Testo 635, Germany). All treatments were randomly sampled at 0, 6, 12, 18, 24, and 30 days after storage. The samples were evaluated for weight loss, reducing sugar, and QG compounds.

**Table 3.2** Conditions of the different curing and storage treatments.

Treatment	Curing and storage conditions
1	15°C under darkness (15C-DK)
2	15°C under a light intensity of 9,000 LUX (15C-9 KLx)
3	15°C under a light intensity of 18,000 LUX (15C-18 KLx)
4	30°C under darkness (30C-DK)
5	30°C under a light intensity of 9,000 LUX (30C-9 KLx)
6	30°C under a light intensity of 18,000 LUX (30C-18 KLx)

### 3.3 Sample preparation for analysis

For all treatments, the stems and roots of forty shallot bulbs were removed, then the bulbs were cut vertically from the top to the basal bulbs. The outer skin of the edible bulbs was removed, and the layers of each shallot bulb were individually separated. Then, six layers of sorting from the outermost fresh layer inwards (Figure 3.4) were obtained.



**Figure 3.3** Vertical (a) and horizontal (b) cross-section of inner shallot bulb. Bulb layer assignment of shallots: 1=outermost, 2=second, 3=third, 4=forth, 5=fifth, and 6=innermost layers.

The six layers were divided into three groups: outer (1+2), middle (3+4), and inner (5+6). After separation, all three layer groups were shock-frozen in liquid nitrogen and packed into polyethylene zip bags before storing at  $-80^{\circ}\text{C}$  in the Ultra-Low Temperature Upright Freezers (Forma 900 Series, Thermo Scientific, USA).

### **3.4 Dry matter determination**

Fresh layers of shallot bulbs were hand-chopped and homogenized. Approximately 5 g of homogenized shallot samples from each treatment were placed in aluminum cups and then dried using a hot air oven at  $70^{\circ}\text{C}$  for 24 hours, followed by one hour at  $105^{\circ}\text{C}$  until constant weight. The dry matter content was calculated and expressed as mg/100 g wet weight basis (Olsson, Gustavsson, and Vågen, 2010).

### **3.5 Reducing sugar determination**

Reducing sugars were extracted from samples using the method of Galdón, Rodríguez, Rodríguez, and Romero (2009) with slight modifications. Frozen layers of shallot bulbs were hand-chopped and homogenized. Approximately 100-200 mg of the homogenized samples were weighed directly in 15 ml polyethylene tubes and mixed with 4 ml of 80% ethanol. Afterwards, the tubes were put into an ultrasonic cleaning bath (Model Transsonic Digital D-78224 Singen/Htw, Elma, Germany) operated at 40 KHz at room temperature for 30 min and then centrifuged for 10 min at 4000 rpm (Sorvall Legend Mach 1.6R, Thermo Fisher Scientific, Germany). The supernatant in the tubes was carefully separated to prevent contamination from the homogenized shallot layer. The supernatant was diluted in 10 ml of deionized water and transferred to an Eppendorf tube. The diluent was stored at  $-20^{\circ}\text{C}$  before analysis.

The content of reducing sugars was analyzed, following the Nelson-Somogyi method (Sadasivam and Manickam, 2005). The diluted sample of 0.2 ml was mixed with 0.5 ml of alkaline copper tartrate reagent and 0.3 ml of deionized water in a 15 ml tube. The tube was placed in boiling water for 20 min and cooled down in an ice bath. After cooling, 0.5 ml arsenomolybolic acid reagent was added into the tube. The solution in the tube was thoroughly mixed, and was rested for 30 min at ambient temperature. Then 2.5 ml deionized water was added before the absorbance was determined in a UV/VIS spectrophotometer (Model Libra S22, Biochrom Co., Ltd., UK) at 620 nm using deionized water as the blank. A standard curve was prepared using glucose and reducing sugars were calculated and expressed as mg/kg dry weight.

### **3.6 Pyruvic acid determination**

The content of pyruvic acid was measured as a pungency indicator and estimated using dinitrophenylhydrazine (DNPH). A sample of approximately 100-200 mg of the homogenized layers was weighed directly in 15 ml polyethylene tubes and mixed with 2 ml of deionized water. Afterwards, the tubes were put into an ultrasonic cleaning bath (Model Transsonic Digital D-78224 Singen/Htw, Elma, Germany) setting at 40 KHz for 30 min at room temperature and then transferred to a centrifuge (Sorvall Legend Mach 1.6R, Thermo Fisher Scientific, Germany) operated at 10,000xg for 10 min at 4°C. The supernatant was carefully recovered to prevent contamination from the homogenized layer pellets. The extracts were adjusted to 5 ml volumetric flask with deionized water and put into Eppendorf tubes and stored at -20°C until they were analyzed.

The method of Boyhan, Schmidt, Woods, Himelrick, and Randle (1999), with slight modifications using flat-bottomed-shaped microplates (96 wells), was employed to determine pyruvic acid. Sixty  $\mu\text{L}$  of standard solutions or sample supernatants was added individually to each well, followed by 30  $\mu\text{l}$  of 0.25 g/L 2, 4-DNPH in 1 M HCl. Then the microplates were covered, packed in polyethylene zip bags, and placed in a 37°C water bath (Julabo SW22, Julabo Labortechnik, Germany) for 10 min. After removing the microplates from the water bath, 100  $\mu\text{l}$  1.5 M NaOH was added to the well and mixed. The absorbance at 515 nm was measured using an Epoch monochromator microplate spectrophotometer (BioTek Instruments, Inc., USA). Pyruvic acid concentrations ( $\mu\text{mol/g}$  dry weight) in the samples were calculated using the standard curve of sodium pyruvate (0-10 mM) solutions.

### **3.7 Determination of Quercetin and Its Glucosides**

Quercetin and its glucosides were extracted according to the method of Lee et al. (2008) with slight modifications. Homogenized bulb layer samples of approximately 100-200 mg were weighed directly in 15 ml polyethylene tubes and mixed with 2 ml of 80% methanol in water acidulated with 0.1% formic acid. Subsequently, the tubes were put into an ultrasonic cleaning bath (Model Transsonic Digital D-78224 Singen/Htw, Elma, Germany), set at 40 KHz for 30 min at 40°C, and centrifuged (Sorvall Legend Mach 1.6R, Thermo Fisher Scientific, Germany) at 1500 $\times$ g for 10 min at 10°C. The supernatant was carefully recovered to prevent contamination by the homogenized layer of pureed pellets. The extracts were filled up to 5 ml with 80% methanol in water acidulated with 0.1% formic acid and stored at -40°C until they were analyzed.

The content of QG was determined according to the method of Downes, Chope, and Terry (2009) with slight modification. The extract was passed through a 0.45  $\mu\text{m}$  filter GHP before HPLC analysis. The extract (10  $\mu\text{L}$ ) was injected into an Agilent 1100 series HPLC (Hewlett-Packard GmbH, Waldbronn, Germany). Separation of quercetin and its glucosides was performed on an Agilent ZORBAX Eclipse XDB-C18 column, 150 $\times$ 4.6 mm internal diameter; 5  $\mu\text{m}$  particle diameter (Part no. 993967-902), with an Agilent ZORBAX Eclipse XDB guard column, 4.6 $\times$ 12.5 mm internal diameter; 5  $\mu\text{m}$  particle diameter (Part no.820950-925). The mobile phases consisted of HPLC-grade water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient involved a linear increase/decrease of solvent B: 5-10%, 5 min: 10-25%, 5 min: 25-85%, 6 min: 85-5%, 4 min and 5%, 5 min at a flow rate of 0.8 ml/min and a column temperature of 30°C. The absorbance was measured at the wavelength of 370 nm using an Agilent 1100 (DE30518297) photodiode array detector. The data were processed with an Agilent ChemStation software, and the content of QG compounds was calculated against authentic calibration standards (quercetin 3, 4'-diglucoside, quercetin 3-glucoside, quercetin 4'-glucoside and quercetin) and presented as mg/kg dry weight.

### **3.8 Enzyme and protein assay**

#### **3.8.1 Enzyme extraction**

Crude enzymes were extracted according to the method of Hirota, Shimoda, and Takahama (1999) with slight modifications. Frozen layers of shallot bulbs were ground in liquid nitrogen using a mortar and pestle. After grinding, approximately 100-200 mg samples were transferred into 15 ml polyethylene tubes,

then 4 ml 0.1 M sodium phosphate (pH 6.0) was added and homogenized by vortexing. The homogenate was centrifuged using a high-speed refrigerated centrifuge (Himac CR22GIII, Hitachi Koki Co., Ltd., Japan) at 12,000 rpm for 15 min at 4°C. The obtained supernatant was used as the crude enzyme solution. All of the procedures were carried out at 4°C.

### **3.8.2 The activity of flavonol synthase (FLS)**

FLS activity was determined according to the method of Wellman et al. (2002). FLS activity of the crude extracts was assayed at 37°C and pH 5.0. The assay mixture (360 µL total volume) contained 100 µM dihydroquercetin as a substrate, 83 µM 2-oxoglutarate, 42 µM ammonium iron (II) sulfate, 2.5 mM sodium ascorbate, and 2 mg/mL bovine catalase. The incubation was carried out in open vials after gentle shaking. The reaction linearity was assessed by proper choice of protein weight (4.5-22 µg) and incubation time (0.5-10 min). The reaction was stopped by the addition of 15 µL saturated aqueous EDTA solution. Subsequently, the supernatant was carefully recovered to prevent contamination with the homogenized scale puree pellet by a high-speed refrigerated centrifuge (Himac CR22GIII, Hitachi Koki Co., Ltd., Japan) at 20,000×g for 10 min at 4°C. The supernatant was then stored at -40°C until analysis. One milliliter of this solution was passed through a 0.45 µm filter GHP prior to HPLC analysis. Quercetin and its glucosides were separated and quantified in the same manner as 3.6. Enzyme activity was expressed as nmol/min/mg protein.

### **3.8.3 The activities of glucosyltransferases**

Quercetin 4'-glucosyltransferase transforms quercetin to quercetin 4'-glucoside (Q4'G), and Q4'G 3-glucosyltransferase transforms Q4'G to quercetin 3, 4'-diglucoside (Q3,4'G) (Tsushida and Suzuki, 1996). The activities of

glucosyltransferases were determined according to the method of Hirota, Shimoda, and Takahama (1999) with slight modifications. The reaction mixture contained 2  $\mu\text{l}$  of 9.2 mM quercetin 4'-glucoside (Q4'G) or 5 mM quercetin dissolved in methanol, 20  $\mu\text{l}$  of 40 mM UDP-glucose, 10  $\mu\text{l}$  of 0.1 M ascorbic acid, 20  $\mu\text{l}$  of the enzyme solution, and 58  $\mu\text{l}$  of 0.1 M sodium phosphate (pH 6.0 for measurement of Q4'G 3-glucosyltransferase) or 0.1 M Tricine-NaOH (pH 8.0 for measurement of quercetin 4'-glucosyltransferase). The reaction was carried out at 37°C for 10 min using a water bath (Julabo SW22, Julabo Labortechnik, Germany) and terminated by the addition of 100  $\mu\text{l}$  of methanol. Subsequently, the mixture was centrifuged (Sorvall Legend Mach 1.6R, Thermo Fisher Scientific, Germany) at 4000 $\times$ g for 5 min. QG compounds in the reaction mixture were quantified using an Agilent 1100 series HPLC system as described in 3.6. Enzyme activity was expressed as nmol/min/mg protein.

#### **3.8.4 The activities of the glucosidases**

Q3,4'G 3-glucosidase transforms Q3,4'G to Q4'G, and Q4'G glucosidase transforms Q4'G to quercetin (Tsushida and Suzuki, 1996). The activity of glucosidases was measured according to the method of Hirota, Shimoda, and Takahama (1999) with slight modifications. The reaction mixture of glucosidases consisted of 2  $\mu\text{l}$  9.2 mM Q4'G or 7.2 mM Q3,4'G and 18  $\mu\text{l}$  of 0.1 M ascorbic acid added to 80  $\mu\text{l}$  enzyme solution. The reaction took place a 37°C water bath (Julabo SW22, Julabo Labortechnik, Germany) for 1 h and was terminated by the addition of 100  $\mu\text{l}$  methanol. Subsequently, the mixture was centrifuged (Sorvall Legend Mach 1.6R, Thermo Fisher Scientific, Germany) at 4000 $\times$ g for 5 min. QG compounds in the reaction mixture were quantified using an Agilent 1100 series HPLC system as described in 3.6. Enzyme activity was expressed as nmol/min/mg protein.

### 3.8.5 Protein determination

Protein concentration was determined using the dye-binding method of Bradford (1976) as modified by Bollag, Rozycki, and Edelstein (1996). To a 96-well microtiter plate, 40  $\mu$ L of standard protein solution or sample protein solution was added, followed by 200  $\mu$ l Bradford reagent. Then the microtiter plate was covered with aluminium foil and packed in polyethylene zip bags. The mixture was incubated in a 25°C water bath (Julabo SW22, Julabo Labortechnik, Germany) for 10 min. The absorbance of the solution was measured at 595 nm using an Epoch monochromator microplate spectrophotometer (BioTek Instruments, Inc., USA). The concentration of the unknown samples was determined using a bovine serum albumin (BSA) standard curve. Protein concentration was expressed as mg/100 g dry weight.

### 3.9 Weight loss determination

The initial fresh weight ( $W_0$ ) was determined on day 0 , with follow-up measurements ( $W_t$ ) taken at predetermined intervals (days 6, 12, 18, 24, and 30). Weight loss was calculated using the formula:

$$\text{Weight loss (\%)} = [(W_0 - W_t)/W_0] \times 100$$

### 3.10 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SPSS 16.0 software. Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a significance level of  $p \leq 0.05$ .

## CHAPTER IV

### RESULTS AND DISCUSSION

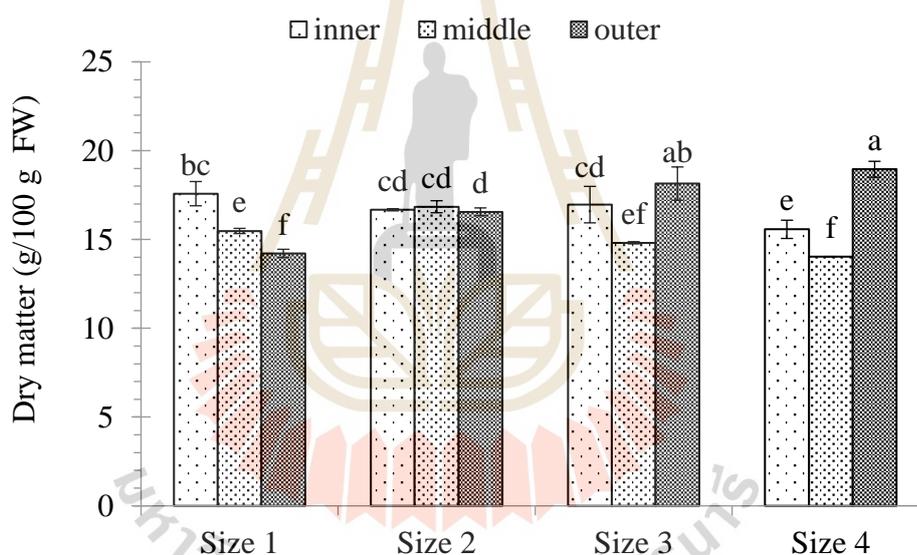
#### 4.1 Chemical Composition and QC Compounds of Shallot Bulbs

##### 4.1.1 Dry Matter

A typical shallot bulb contains several laterals in the axils of the inner leaves. All sets formed from a single propagule usually remain attached to the original basal plate, thereby forming a cluster of sets (Rabinowitch and Kamenetsky, 2002). These clusters may contain anywhere from a few bulbs up to as many as 15 bulbs, and the individual bulbs exhibit variability in shape and size (Rubatzky and Yamaguchi, 1997). In this study, four different shallot bulb sizes were investigated: large (Size 1), medium (Size 2), small (Size 3), and extra small (Size 4). Individual leaf bases were separated and numbered from 1 (the outermost and oldest fleshy base) to 6, the inner youngest base. These bases were then dissected into different tissue types: the outer layers (bases 1 and 2), the middle layers (bases 3 and 4), and the inner layers (bases 5 and 6).

The dry matter (DM) content of the three layers of different bulb sizes was determined, and the results are presented in Figure 4.1. A statistically significant difference ( $p < 0.05$ ) was observed in DM content among both the layers and the bulb sizes. In large bulbs (Size 1), DM content increased from 14.22 g/100 g on a dry weight basis (DW) in the outer layer to 17.58 g/100 g DW in the inner layer. In contrast, medium bulbs (Size 2) displayed no significant variation among layers, with

DM content ranging from 16.56 to 16.67 g/100 g DW ( $p \geq 0.05$ ). For small size (Size 3) and extra small bulbs (Size 4), the outer layer exhibited the highest DM content at 18.15 and 18.95 g/100 g DW, respectively, while the middle layer recorded the lowest values at 14.81 and 14.04 g/100 g DW, respectively. These findings suggested that the variance of DM content is more pronounced in the outer layer than in the middle or inner layers. Moreover, the DM content in the outer layer decreased with increasing bulb sizes, whereas the DM content of the middle layer and the inner layers increased with larger bulb sizes.



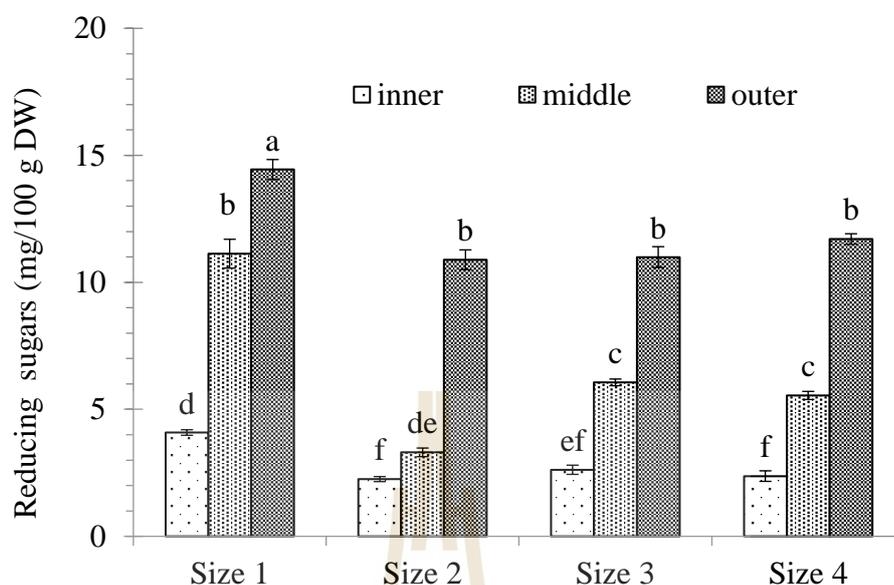
**Figure 4.1** Content of dry matter in different layers and sizes of shallot bulbs. Error bars denote standard deviation. Bars marked with different letters indicate statistically significant differences at  $p < 0.05$ .

Generally, cells in the outer layer exhibit more advanced aging than those in the inner layer (Cheng et al., 2013). In shallots, the outer layer serves as the base for several outer leaves that have lost their blades. Bulb formation in early developmental

stages involves swelling of the leaf sheaths, with the outermost one to three sheaths developing into thin, dry protective skins (Brewster, 2008). Woldetsadik and Workneh (2010) reported that photosynthate is translocated from the foliage leaf blades into storage leaves, after which the foliage becomes senescent upon bulb maturity. During bulb development, carbohydrates stored in the outermost leaf bases are reallocated to the inner developing layers. The DM in shallots comprised approximately 70-80% non-structural carbohydrates (NSC), primarily including fructans, glucose, fructose, and sucrose. The middle layers present leaf bases that never developed blade structures but later formed swollen sheaths (Rabinowitch and Kamenetsky, 2002; Brewster, 2008). The inner layer contains a few sprout leaves at the center that may develop into full leaves under favorable conditions (Rabinowitch and Kamenetsky, 2002). In the present study, the outer layer of large bulbs exhibited lower DM content compared to that of the other sizes, suggesting that large bulbs had reached a higher level of maturity.

#### **4.1.2 Distribution of Reducing Sugars**

The content of reducing sugars in freshly harvested shallot bulbs was significantly influenced ( $p < 0.05$ ) by both bulb size and layer position. Regarding the bulb size, the outer layer consistently exhibited the highest levels of reducing sugars, with glucose measured at 14.44 mg per 100 g DW. Moreover, the reducing sugar content gradually decreased from the outer to the inner layers across all bulb sizes (see Figure 4.2). A similar distribution pattern was observed in onions; for instance, Jaime et al. (2000) reported that reducing sugars decreased from the outer to the inner fleshy layers, while, Benítez et al. (2011) noted that the outermost two layers contained higher levels of fructose and glucose, resulting in a greater overall content of NSC).



**Figure 4.2** The content of reducing sugars in each layer and the size of the shallot bulbs. Error bars represent standard deviation. Bars with different letters indicate statistically significant differences at  $p < 0.05$ .

**Table 4.1** The measured values of reducing sugars (mg/100 g DW) are summarized below.

Layer	Size 1	Size 2	Size 3	Size 4
Inner	4.09	2.25	2.62	2.37
Middle	11.13	3.31	6.06	5.55
Outer	14.44	10.89	10.99	11.71

Notably, while the outer layers may exhibit lower concentrations of certain NSC components compared to the inner layers, an increasing gradient of sucrose from the outer to the inner flesh has been documented in onions. Darbyshire and Henry (1978)

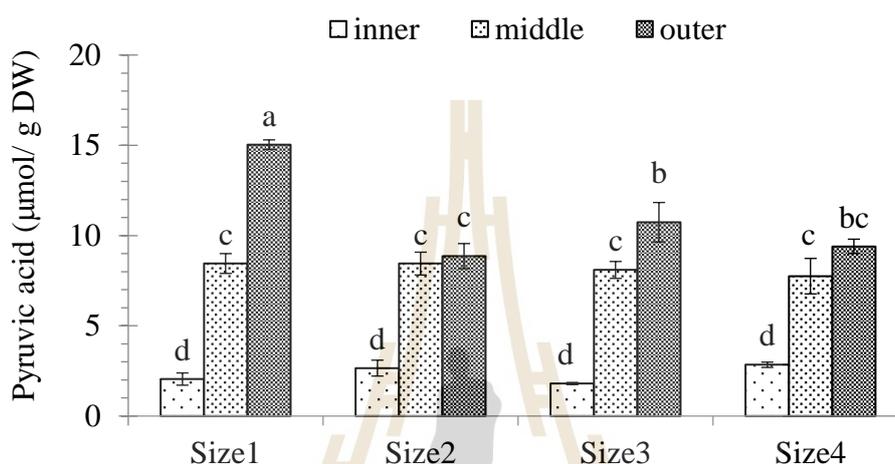
confirmed that the proportion of fructans within total NSC increases from the outer leaf base to the inner leaf base, likely driven by the predominant activities of enzymes such as SST (sucrose: sucrose fructosyltransferase) and FFT (fructose: fructose fructosyltransferase) in the inner layers. Additionally, fructan hydrolysis mediated by fructan hydrolase was a prominent enzyme activity in the outer leaves (Jaime et al., 2000), indicating that in fully mature onions, the majority of reducing sugars are concentrated in the older tissues.

#### **4.1.3 Distribution of Pungency**

Pungency in shallots at harvest was evaluated by measuring the content of enzymatically produced pyruvic acid. Both bulb sizes and tissue layers significantly affected ( $p < 0.05$ ) the pyruvic acid levels. In all bulb sizes, a clear decreasing trend in pyruvic acid content was observed from the outer to the inner layers (Figure 4.3). For large bulbs (Size 1), the outer layer exhibited the highest level of pyruvic acid content at 15.03  $\mu\text{mole/g DW}$ , whereas the middle and inner layers had lower values of 8.45 and 2.05  $\mu\text{mole/g DW}$ , respectively. No significant difference in pyruvic acid content was observed among different sizes within the middle and inner layers; overall, the middle layer consistently displayed higher values (7.75 - 8.45  $\mu\text{mole/g DW}$ ) than the innermost layers (1.80 to 2.84  $\mu\text{mole/g DW}$ ).

The present study demonstrated that, across all sizes, pyruvic acid content decreases from the outer to the inner layers. These findings contrast with those of Slimestad and Vågen (2009), who observed that in onion cultivars such as Red Baron and Hytech, pyruvate concentration was highest in the inner layer. Moreover, Abayomi and Terry (2008) reported a decrease in pyruvate concentration from the outer scales to the center tissues in the onion cultivar Renate, while the onion cultivar SupaSweet exhibited the opposite trend. Such variations in flavor concentration and

composition may be attributed to ontogenetic factors as well as inherent differences in tissue types (Lancaster, McCallion, and Shaw, 1986). In this investigation, large shallot bulbs were found to possess higher pyruvic acid content than smaller sizes, with the majority of flavor components concentrated in the mature, aged layers.

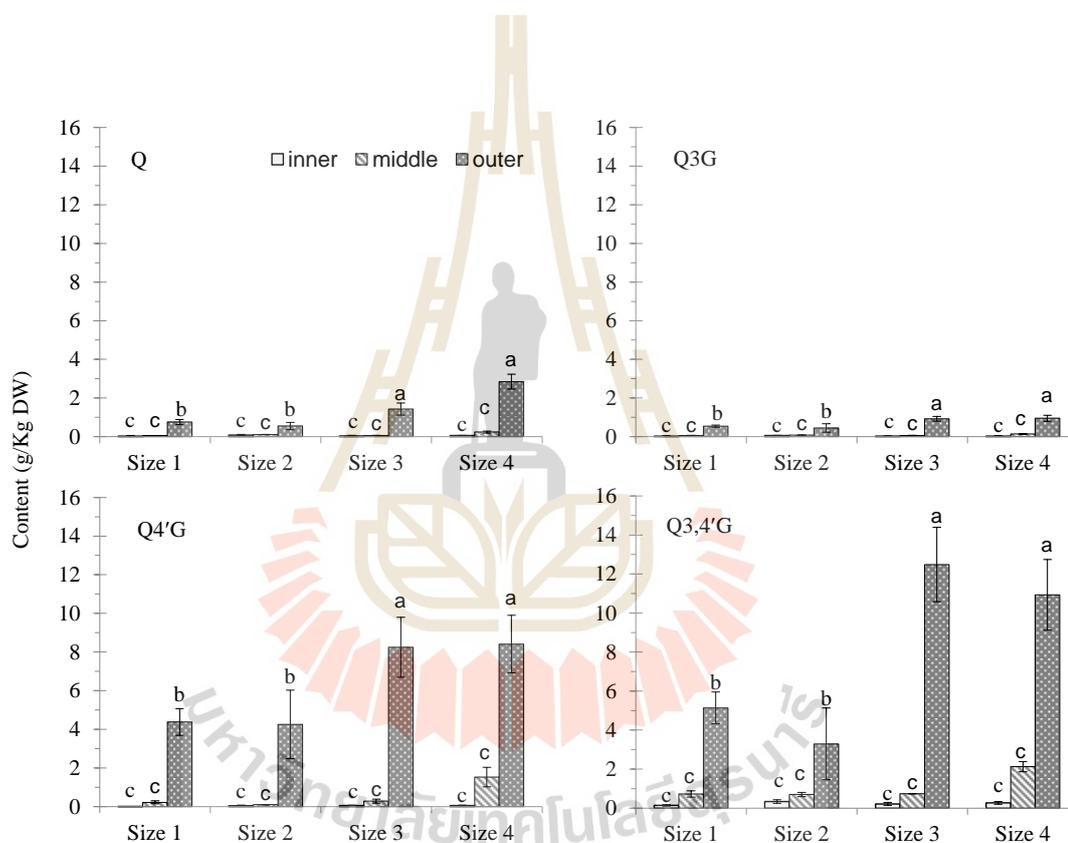


**Figure 4.3** Contents of pyruvic acid in different layers and sizes of shallot bulbs. Error bars denote standard deviation. Bars marked with different letters indicate statistically significant differences at  $p < 0.05$ .

#### 4.1.4 Distribution of Quercetin Glucoside Compounds

Four quercetin glucoside (QG) derivatives, namely, Q3,4'G, Q3G, Q4'G, and free quercetin, were quantified in shallot bulbs to assess the impact of bulb size and tissue layer at harvest. The QG contents differed significantly ( $p < 0.05$ ) among various sections of the bulb (Figure 4.4). In general, QG content increased as the sizes decreased from the large (Size 1) to the extra small (Size 4). Small (Size 3) and extra small bulbs exhibited higher QG levels than their larger counterparts, and in every size category, the outer layer consistently showed the highest QG content. Particularly,

Q3,4'G and Q4'G were the major components and varied markedly between the outer and the inner layers. In the outer layer, Q3,4'G content was notably high in small and extra small bulbs at 12.49 g/kg DW and 10.93 g/kg DW, respectively, while only minor amounts were detected in large and medium bulbs (5.14 g/kg DW and 3.29 g/kg DW, respectively). In contrast, the middle and inner layers contained only trace amounts of Q3,4'G (ranging from 0.14 g/kg DW and 2.12 g/kg DW).



**Figure 4.4** Content of quercetin (Q), quercetin 3-glucoside (Q3G), quercetin 4'-glucoside (Q4'G) and quercetin 3, 4'-diglucoside (Q3,4'G) in different layers and sizes of shallot bulbs. Error bars denote standard deviation. Bars marked with the different letters indicate statistically significant differences at  $p < 0.05$ .

A similar trend was observed for Q4'G content: the outer layer of small and extra small bulbs exhibited the highest levels (approximately 8.41 g/kg DW and 8.24 g/kg DW, respectively), whereas the middle and inner layers had considerably lower values (ranging from 0.22-1.53 g/kg DW and 0.04-0.06 g/kg DW (respectively)). Only trace amounts of Q3G were detected, with the highest in the outer layers of small and extra small bulbs (approximately 0.93 g/kg DW and 0.95 g/kg DW, respectively), significantly higher than those in larger bulbs ( $p < 0.05$ ). In addition, a significant amount of free quercetin was found in the outer layers of small and extra small shallots (approximately 1.43 g/kg DW and 2.85 g/kg DW, respectively), whereas large and medium bulbs exhibited considerably lower levels (approximately 0.76 g/kg DW and 0.55 g/kg DW, respectively).

These results concur with the findings of Pérez-Gregorio et al. (2010), which reported that small onions contain higher flavonol levels than large onions. This would probably be due to the expansion of the cells during plant organs (Brewster, 2008). The development of plant organs typically occurs in three stages: (1) cell division in the meristematic region, (2) irreversible cell expansion accompanied by vacuolation and water uptake, and (3) cell maturation during which cell walls stiffen and thicken, thereby arresting further enlargement (Kutschera, 2000). Small shallots, being in the stage of cell expansion, exhibit less cell swelling than large shallots that have reached later stages of cell expansion. Consequently, the large bulbs show relatively lower QG contents due to cell elongation under turgor pressure (Kutschera, 2000).

Overall, the highest QG levels were consistently observed in the outer layer across all bulb sizes, with a significant decrease ( $p < 0.05$ ) in QG content from the outer to the inner layers. This pattern agrees with previous studies (Slimestad and Vågen, 2009; Beesk et al., 2010; Pérez-Gregorio et al., 2010; Yoo et al., 2013). It has been proposed that flavonol content increases as cell age; since cells in the outer layers are more mature, and

in any given layer, the upper cells are older than the lower ones (Pérez-Gregorio et al., 2010). This likely explains the higher QG content observed in the outer layers. Furthermore, higher activity of phenylalanine ammonia-lyase in the outer layers may catalyze the synthesis of flavonol glucosides (Hirota et al., 1999). Notably, QG was localized exclusively in the abaxial epidermis cells, with negligible accumulation in the mesophyll tissue, and its concentration decreased toward the inner layers.

Among the QG derivatives, Q3,4'G and Q4'G were predominant, followed by free quercetin and Q3G. These findings are in agreement with Poblocka-Olechi et al. (2016). Previous research has reported an increase in QG concentrations during the final week of bulb growth (Morgren et al., 2007); typically, Q4'G is formed first, followed by the formation and continuous increase of Q3,4'G storage (Slimestad et al., 2007). During cell senescence, di- and mono-glucosides of quercetin are converted into free quercetin (Brewster, 2008). The present results suggest notable conversion from quercetin diglucoside (QDG) to quercetin monoglucoside (QMG) in the outer layer, possibly due to degradation or translocation during tissue drying and aging. Thus, the highest QG content was observed in small bulbs, which also tended to accumulate higher levels in the outer layers.

#### **4.1.5 Enzyme Activities in QG Changes**

Crude enzyme extracts from shallot bulbs of varying sizes and tissue layers were analyzed in this study. The activities of enzymes, including flavanol synthase (FLS), Q4'-glucosyltransferase, Q4'G3-glucosyltransferase, Q3,4'G3-glucosidase, and Q4'G-glucosidase, were determined for each bulb size and layer (see Table 2). No FLS activity (responsible for converting dihydroquercetin [DHQ] into quercetin) was detected in any layer. Moreover, the activity of FLS did not correlate with the quercetin levels observed across different layers and sizes, suggesting that the increase in quercetin is

not a result of de novo synthesis; this finding corroborates previous studies (Tsushida and Suzuki, 1996; Hirota et al., 1999; Takahama and Hirota, 2000; Yoo et al., 2013).

**Table 4.2** Enzyme activities in shallots with different bulb sizes and layers.

Enzyme	Substrate	Product	Size	Activity ( $\mu\text{mole/mg protein/min}$ )		
				inner	middle	outer
FLS	DHQ	Q	1	nd	nd	nd
			2	nd	nd	nd
			3	nd	nd	nd
			4	nd	nd	nd
Q4'- glucosyltransferase	Q	Q4'G	1	nd	nd	nd
			2	nd	nd	nd
			3	nd	nd	0.04 $\pm$ 0.04
			4	nd	nd	0.27 $\pm$ 0.07
Q4'G3- glucosyltransferase	Q4'G	Q3,4'G	1	nd	nd	nd
			2	nd	nd	nd
			3	nd	nd	nd
			4	nd	nd	nd
Q3,4'G 3- glucosidase	Q3,4'G	Q4'G	1	nd	nd	0.01 $\pm$ 0.00
			2	nd	nd	0.02 $\pm$ 0.01
			3	nd	nd	0.22 $\pm$ 0.06
			4	nd	nd	0.88 $\pm$ 0.23
Q4'G glucosidase	Q4'G	Q	1	nd	0.13 $\pm$ 0.05	0.14 $\pm$ 0.07
			2	nd	0.15 $\pm$ 0.02	0.16 $\pm$ 0.03
			3	0.07 $\pm$ 0.02	0.19 $\pm$ 0.02	0.67 $\pm$ 0.11
			4	0.30 $\pm$ 0.01	0.34 $\pm$ 0.01	0.91 $\pm$ 0.17

nd = not detected. Values are expressed as mean  $\pm$  standard deviation (n = 3).

Q4'-glucosyltransferase catalyzes the conversion of quercetin into Q4'G, whereas Q4'G3-glucosyltransferase converts Q4'G into Q3,4'G. Both enzymes facilitate the glucosidation of quercetin (Tsushida and Suzuki, 1996). In this study, Q4'-glucosyltransferase activity was detected at low levels in the outer layer of small (Size 3) and the extra small (Size 4) — specifically, 0.04  $\mu\text{mole/mg protein/min}$  and 0.27  $\mu\text{mole/mg protein/min}$  (respectively), — while Q3,4'-glucosyltransferase activity was undetectable in all layers. These observations align with the findings of Takahama and Hirota (2000), who reported a decrease in Q4'-glucosyltransferase activity from the fleshy to dried sections of onion bulbs, as well as the absence of Q4'G3-glucosyltransferase activity in other parts. Similar enzyme activity patterns have been documented in the inner scales of onion bulbs (Tsushida and Suzuki, 1996; Hirota et al., 1999).

These results indicate that the activities of Q4'-glucosyltransferase and Q4'G3-glucosyltransferase are not directly correlated with the respective levels of Q4'G and Q3,4'G, even though high concentrations of these compounds are present in the outer layer across all bulb sizes. Hence, the accumulation of Q4'G and Q3,4'G in the different layers of shallots does not appear to be driven by glucosyltransferase activity. It has been reported that Q4'G and Q3,4'G concentrations increase during the growing and maturation of onions in the field (Morgren et al., 2007).

Q3,4'G3-glucosidase catalyzes the conversion of Q3,4'G to Q4'G. In the outer layer, its activity decreased from large (Size 1) to extra small bulbs (Size 4), with activities ranging from 0.01 to 0.88  $\mu\text{mole/mg protein/min}$ , and was undetectable in the other layers. In contrast, Q4'G-glucosidase-which catalyzes the conversion of Q4'G to quercetin - exhibited considerably higher activity in the outer layer, particularly in the extra small bulbs, where activity reached approximately 0.91  $\mu\text{mole/mg}$

protein/min. These findings show that Q4'-glucosidase activity is higher than Q3,4'G 3-glucosidase activity, especially in the outer layer across all sizes. The predominant activities of both enzymes in the outer layer correspond with the observed levels of Q4'G and free quercetin, suggesting that the accumulation of these compounds is attributed to deglycosidation. These results are consistent with previous reports (Tsushida and Suzuki, 1996; Hirota et al., 1999; Takahama and Hirota, 2000).

Previous studies have also reported that the highest activities of glucosyltransferase and glucosidase occur in the abaxial epidermis, which is enriched with free quercetin, QMG, and QDG (Hirota et al., 1999). In onions, the concentration of flavonols along with their related enzymes varies from the bottom to the top, with quercetin concentration increasing toward the top despite lower glucosidase and transferase activities (Takahama and Hirota, 2000). In the present study, it was observed that the levels of quercetin and its glucosides increased from the inner to the outer layers of shallot bulbs and decreased with a reduction in bulb size. In addition, the accumulation of quercetin and its glucosides appears to be positively correlated with glucosidase activity.

#### **4.2 Postharvest during curing and storage**

The effects of temperature and light intensity on the physiological and biochemical quality of shallot bulbs during a 30-day postharvest storage period were investigated in this study. All shallot samples were stored in a controlled environment chamber (growth chamber) under six distinct storage conditions: 15°C in darkness (15C-DK), 15°C under 9,000 lux (15C-9KLx), 15°C under 18,000 lux (15C-18KLx), 30°C in darkness (30C-DK), 30°C under 9,000 lux (30C-9KLx) and 30°C under

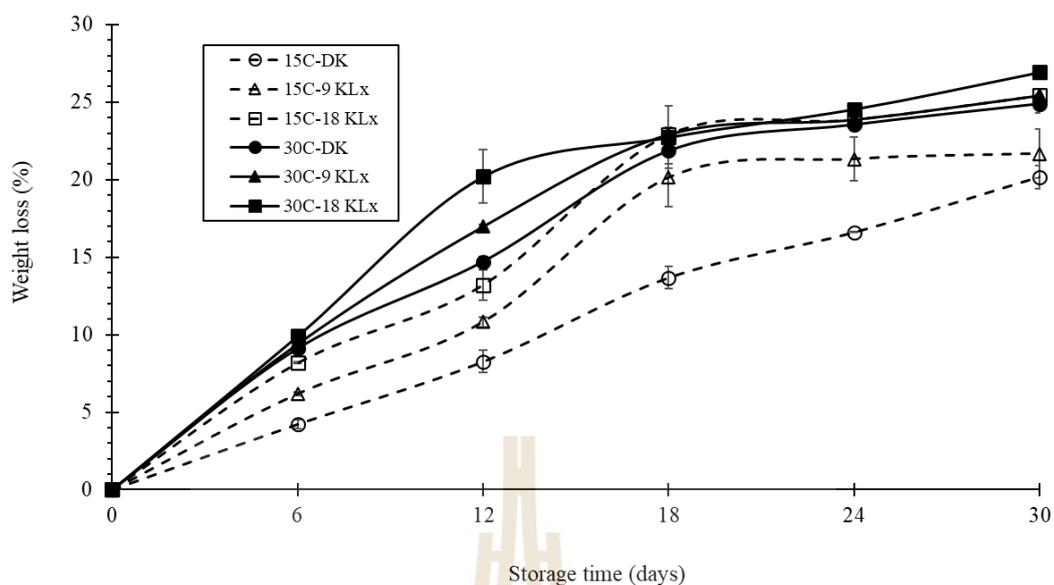
18,000 lux (30C-18KLx). These conditions were selected to represent typical curing and storage environments used in commercial shallot handling. Temperature and relative humidity (RH) varied with light intensity were monitored throughout the storage period and summarized in Table 1. As light intensity increased, a corresponding rise in temperature and decline in RH were observed. This indicates that light exposure contributes to thermal buildup and moisture depletion in the storage environment.

**Table 4.3** Actual Temperature and Humidity Across Storage Treatments.

Treatment	T <sub>mean</sub> (°C)	T <sub>max</sub> (°C)	T <sub>min</sub> (°C)	RH <sub>mean</sub> (%)	RH <sub>max</sub> (%)	RH <sub>min</sub> (%)
15C-DK	15.5	17.4	14.0	71.5	73.1	70.5
15C-9 KLx	17.5	18.2	15.6	70.7	75.8	67.5
15C-18 KLx	20.3	22.3	16.3	54.5	59.0	54.2
30C-DK	28.5	28.7	27.5	65.0	66.4	61.4
30C-9 KLx	30.1	30.7	29.2	56.5	62.8	52.2
30C-18 KLx	31.6	30.3	32.8	56.7	58.7	49.1

#### 4.2.1 Effect of storage conditions on weight loss

Weight loss is a critical indicator of postharvest deterioration, reflecting both water loss and metabolic activity. Shallot bulbs exhibited continuous moisture loss throughout the 30-day storage period, with temperature emerging as the dominant factor influencing the rate and extent of weight loss. At 30°C, rapid moisture loss occurred within the first 12 days, as shown in Figure 4.5.



**Figure 4.5** Weight loss patterns of shallot bulbs during 30-day storage under different temperatures and light conditions.

Shallot bulbs exhibited continuous moisture loss throughout the 30-day storage period (Figure 4.5). During the first 12 days, bulbs stored at 30°C experienced rapid weight loss at 14.72% in darkness, 16.98% under 9,000 lux, and 20.20% under 18,000 lux illumination. By day 30, weight loss in these treatments reached 25-27%. In contrast, bulbs stored at 15°C showed slower moisture loss, 20.18% in darkness, 21.67% under 9,000 lux, and 25.43% under 18,000 lux illumination.

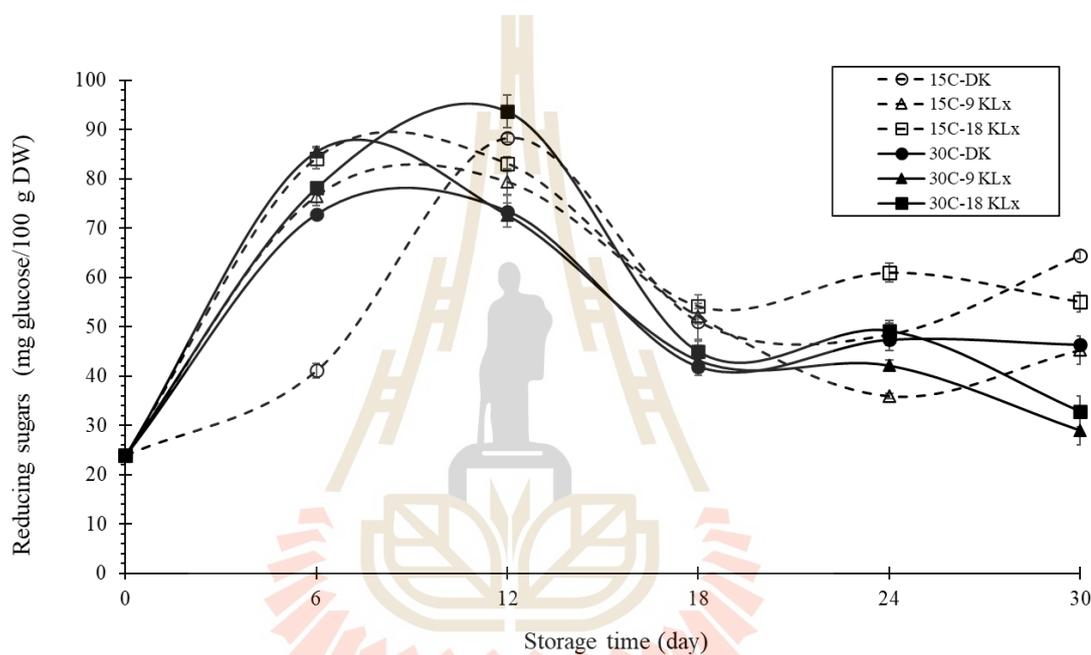
The data reveal that temperature had a more pronounced effect during the early storage phase, with 30°C treatments showing 1.5-1.8 times higher weight loss rates than 15°C treatments. Light exposure also contributed to increased weight loss, particularly at lower temperatures, suggesting that photostimulation may accelerate metabolic processes linked to water loss.

Complex interactions between environmental and physiological factors of shallot bulbs may be the factors that influence the different quality responses of shallot bulbs. At 30°C, the combination of high metabolic activity and increased vapor pressure deficit creates optimal conditions for rapid moisture loss (Islam et al., 2019). The relatively smaller effect of light at high temperatures suggests that thermal stress predominates over light-induced metabolic responses, while at moderate temperatures (15°C), light-induced physiological changes become more apparent (Galsurker et al., 2018).

These findings align with previous studies indicating that elevated temperatures are the primary factor accelerating weight loss through several related mechanisms, such as increasing vapor pressure deficit, compromising cuticle integrity, and enhancing respiration. Water evaporation rates increase proportionally with temperature and storage duration (Sharma and Lee, 2016). Higher temperatures induce cell wall structural deterioration and compromise the integrity of the cuticle layer (Lara, Heredia, and Domínguez, 2019), thereby diminishing the barrier function of outer scale layers and resulting in reduced moisture retention in shallots. Additionally, higher temperatures enhance respiratory metabolism, resulting in carbohydrate and water depletion (Singh, Dhanapal, and Yadav, 2020). Light exposure had a lesser impact than temperature, but still exerted a minor influence, particularly at 15 °C. Its effects may stimulate light-stimulated biochemical activities (Goa et al., 2020) and light-induced cuticle modifications in the surface tissues (Riglet, Gatti, and Moyround, 2021) that further exacerbate water loss. Therefore, cool (15°C) dark storage conditions offer the optimal strategy for moisture retention and commercial viability.

#### 4.2.2 Effect of Storage Conditions on Reducing Sugars

Reducing sugar are essential for flavor and nutritional quality. Their concentration reflects enzymatic activity, carbohydrate metabolism, and stress responses during storage. Figure 4.6 illustrates significant reducing sugar changes under different temperatures and light intensities during the 30-day storage period, revealing complex metabolic dynamics in stored shallots.



**Figure 4.6** Changes in reducing sugar content of shallots during 30-day storage under different temperature and light conditions.

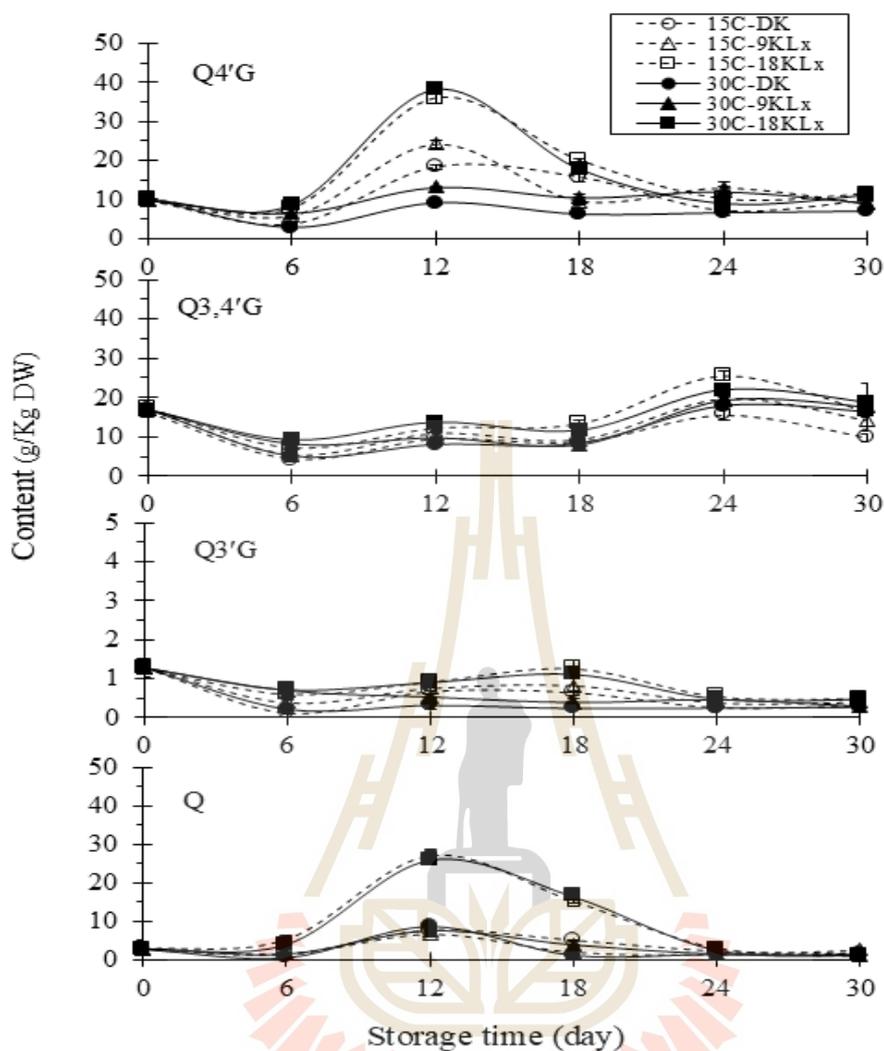
Initial reducing sugar content (23.92 mg/100 g DW) increased significantly during the first 12 days, peaking at 30°C-18,000 lux (93.71 mg/100 g DW) and at 15°C-9KLx (84.19 mg/100 g DW). During the accumulation phase (day 0–12), temperature had a stronger influence on sugar concentration than light intensity. The 30°C treatments achieved higher peak levels (93.71 and 73.42 mg/100 g DW with and without light) compared to 15°C treatments (86.10 and 82.83 mg/100 g DW). During

the decline phase (days 12-30), sugar levels dropped sharply under high temperature and light at 30°C-18KLx (32.89 mg/100 g DW). In contrast, bulbs stored at 15°C in darkness retained higher sugar levels (64.51 mg/100 g DW).

The biphasic pattern, initial accumulation followed by degradation, likely reflects initial fructan hydrolysis and enzyme activation (Raines, Henson, and Havey, 2009; Chope, Cools, Hammond, Thompson and Terry, 2012; Petropoulos, Ntatsi, and Ferreira, 2016;) followed by increased respiration, secondary metabolite synthesis (Shivakumar and Chandrashekar, 2014), and tissue deterioration under stress conditions (Galsurker, Doron-Faigenboim, Teper-Bamnolker, Daus, Lers, and Eshel, 2018). The dramatic fluctuations at 30°C with light exposure (decreasing from 93.71 to 32.89 mg/100 g DW) indicate significant metabolic instability, consistent with previous studies showing negative impacts of light on storage quality (Martínez-Sánchez, Tudela, Luna, Allende, and Gil, 2011). Therefore, storing shallots at 15°C in darkness preserves reducing sugar stability (64.51 mg/100 g DW), which is critical for maintaining sweetness and overall product quality throughout the supply chain. Thus, storage at 15°C in darkness preserved reducing sugar content, supporting the sweetness and nutritional integrity of the stored shallot bulbs.

#### **4.2.3 Effect of Storage Conditions on Quercetin Compound Profiles**

Quercetin and its glycosides are key flavonoids with antioxidant properties. Their stability and transformation during storage are influenced by enzymatic activity, temperature, and light exposure. The quercetin compound profiles demonstrated 4 major distinct patterns during storage, with each compound responding differently to temperature and light conditions, as shown in Figure 4.7.



**Figure 4.7** Changes in quercetin compounds in shallot bulbs during 30-day storage under different temperatures and light conditions.

Q4'-glucoside content exhibited a marked increase from day 0 to day 12, with the highest peak observed at 30°C under 18,000 lux (38.41 g/kg DW). After day 12, all treatments exhibited declining trends, with final values ranging from 6.89 to 11.43 g/kg DW by day 30.

Q3,4'-diglucoside content demonstrated the most stability throughout storage, with levels ranging from 4.23 to 25.63 g/kg DW. The 15°C under 18,000 lux showed

the highest accumulation at day 24 (25.63 g/kg DW), while dark storage conditions generally maintained lower and more consistent levels.

Q3-glucoside content showed a gradual decline from initial values across all treatments, dropping from initial values of 1.27-1.28 g/kg DW to final values of 0.27-0.46 g/kg DW by day 30. The highest intermediate concentration was observed at 15°C under 18,000 lux treatment on day 18 (0.49 g/kg DW). Temperature apparently had minimal effect on this compound's degradation compared to the influence of light conditions.

The free quercetin aglycone displayed the most dramatic changes, a rapid increase from near-zero initial level to peak concentration by day 12. At 15°C, under 18,000 lux, the highest peak (27.00 g/kg DW) was achieved, followed by a steep decline to a minimal level by day 30.

Quercetin transformations during storage are primarily controlled by the balance between glucosidase and glucosyltransferase enzyme activities (Sharma and Lee, 2016). At elevated temperatures (30°C), enhanced glucosidase activity converted glycosylated forms to free quercetin, explaining the rapid accumulation observed until day 12. This temperature-dependent enzyme activation aligns with previous studies demonstrating increased flavonoid metabolism at higher storage temperatures (Sharma and Lee, 2016). However, the subsequent decline after day 12 reflects a shift from enzymatic conversion to oxidative degradation processes. Free quercetin undergoes autooxidation and peroxidase-mediated breakdown during cellular senescence (Takahama, 2004), resulting in the observed compound instability in later storage phases. This biphasic pattern—initial accumulation followed by degradation—illustrates the complex interplay between beneficial enzyme activation and detrimental oxidative processes.

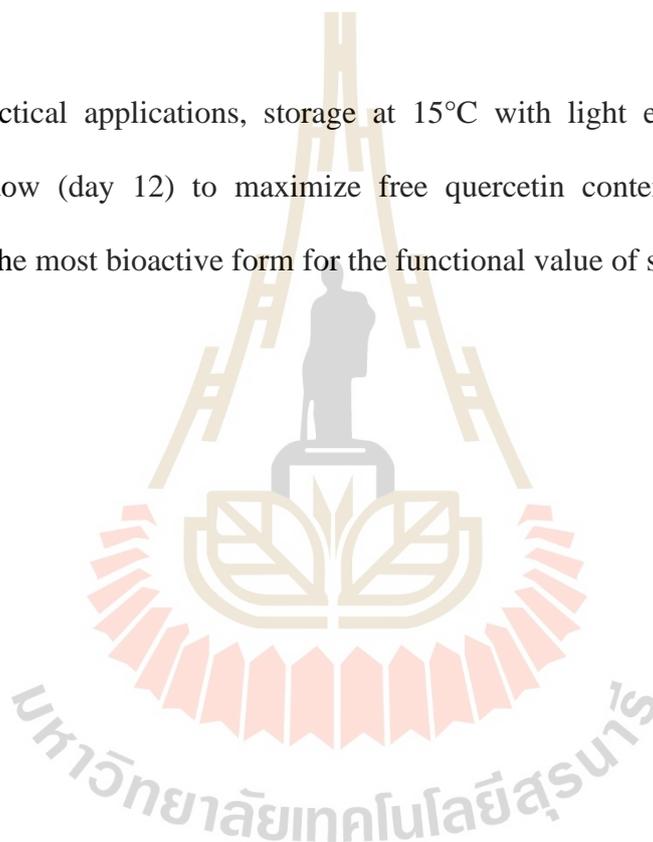
In contrast to temperature effects, light exposure primarily functions as a stress signal that activates protective quercetin synthesis pathways (Yoo, Lee, and Patil, 2013). This response increases Q4'-glucoside and Q3,4'-diglucoside levels as cellular defense mechanisms against photo-oxidative damage. Although this protective response enhances initial compound levels, prolonged light exposure ultimately contributes to metabolic instability, as evidenced by the dramatic fluctuations observed under combined high temperature and light conditions.

The subsequent glucosides decline after day 12 likely reflects the continuous oxidation and degradation of quercetin compounds through non-enzymatic browning reactions. The oxidation mechanism of quercetin has been confirmed in various systems to occur through autoxidation and non-enzymatic processes (Zhou and Sadik, 2008). In previous research, quercetin concentrations were elevated under light conditions compared to dark treatments, particularly showing increases in Q4'-glucoside and Q3,4'-diglucoside. This pattern reflects light-induced stress reactions that activate quercetin synthesis pathways, providing cellular protection from ultraviolet damage (Yoo et al., 2013). The results demonstrate distinct stability patterns among quercetin compounds, likely due to differences in their chemical structures and reactivities. These differential stability patterns are consistent with previous studies, which show that flavonoid glycosides exhibit greater stability than their corresponding aglycones during digestion processes (Xie et al., 2022).

Q3-glucoside content showed a rapid decline in the early storage period under most conditions but demonstrated relative stability in the later phases. Storage conditions significantly affected these transformation patterns throughout the storage period. The superior stability of Q3,4'-diglucoside may be attributed to its dual glucose substitution pattern. These two glucose molecules provide enhanced protection of

quercetin from oxidation and degradation compared to Q3-glucoside, which contains only one glucose molecule (Xie et al. 2022). This study demonstrates that controlling temperature and light during storage affects the changes in quercetin compounds in shallot bulbs. These findings are consistent with previous studies, which have shown that when onion bulbs are exposed to ambient temperatures after storage, internal changes occur, followed by metabolic and chemical alterations (Petropoulos et al., 2016).

For practical applications, storage at 15°C with light exposure provides the optimal window (day 12) to maximize free quercetin content (27.00 g/kg DW), representing the most bioactive form for the functional value of shallots.



# CHAPTER V

## CONCLUSION

### 5.1 Conclusions

This study provides a comprehensive understanding of the physiological and biochemical characteristics of shallot bulbs in relation to bulb size, tissue maturity, and postharvest storage conditions (temperature and light intensity). The findings reveal that these factors significantly influence dry matter distribution, reducing sugar content, pungency, and quercetin compound profiles, both at harvest and during storage.

#### 5.1.1 Fresh Shallot Bulbs

At harvest, large bulbs exhibited a gradient of dry matter and reducing sugars from outer to inner tissues, whereas small and extra-small bulbs concentrated these compounds in the outer layers. Pyruvic acid, indicative of pungency, was highest in the outer tissues of larger bulbs, suggesting that flavour intensity correlates with tissue maturity and bulb size. Quercetin glycosides, particularly Q3,4'-diglucoside and Q4'-glucoside, were most abundant in small bulbs and outer tissues. Enzyme analyses indicated that flavanol synthase (FLS) was inactive, and glucosyltransferase activity was minimal. In contrast, glucosidases—especially Q4 'G-glucosidase—were highly active in the outer tissues, supporting the conclusion that deglucosidation, rather than de novo synthesis, is the primary pathway regulating flavonol transformations in shallots.

### 5.1.2 Weight Loss

During postharvest storage, the weight loss of shallot bulbs was significantly influenced by temperature and light intensity. Shallots subjected to elevated temperatures (45°C during curing and 30°C during storage) exhibited significantly higher weight loss compared to those stored at lower temperatures (30°C during curing and 15°C during storage). Light intensity further exacerbated moisture loss, especially under 18,000 lux. The lowest weight loss was consistently observed under 1°C in darkness, including that cool and shaded conditions are optimal for moisture retention.

### 5.1.3 Reducing Sugar Content

For reducing sugars, the content increased during the initial phase of curing and storage, peaking at day 6 and day 12, respectively. The highest accumulation was observed under high temperature and light exposure (45°C and 18,000 lux during curing and 30°C and 18,000 lux during storage). However, prolonged exposure led to a decline in sugar content, suggesting that temperature and light accelerate both hydrolysis and subsequent degradation. Reducing sugar changes followed a biphasic pattern, with an initial accumulation phase (0–12 days) driven by fructan hydrolysis and enzyme activation, followed by a decline phase (12–30 days) due to respiration and tissue deterioration. Shallot storage at 15°C in darkness preserves sugar stability most effectively.

### 5.1.4 Quercetin Compound Profiles

Quercetin glycosides and free quercetin responded distinctly to postharvest conditions. High temperature and light exposure promoted enzymatic conversion of glycosides to free quercetin, with peak levels observed at day 6 of

curing and day 12 of storage. However, extended exposure resulted in degradation of both glycosides and aglycones. Quercetin compounds displayed a similar biphasic trend. The compounds then declined due to oxidative degradation and non-enzymatic browning processes. The most stable quercetin profiles were maintained under 15 °C with moderate light exposure, indicating that controlled conditions are essential for preserving antioxidant compounds.

Overall, the study demonstrated that shallot quality is dynamically shaped by both intrinsic factors (bulb size, tissue maturity, enzyme activity) and extrinsic conditions (temperature, light intensity). Temperature emerged as the dominant factor influencing postharvest physiology, with light acting as a secondary stressor that modulates enzymatic and oxidative pathways. Curing at 45°C for 6 days with controlled light exposure enhances biochemical attributes, while storage at 15°C in darkness minimizes physiological deterioration. These conditions are recommended for maintaining commercial and nutritional quality during postharvest handling.

## **5.2 Recommendations**

Based on the results obtained, the following recommendations are proposed for practical application and future research:

### **5.2.1 Curing Protocol**

Shallot bulbs should be cured at 45°C for 6 days under controlled light intensity (9,000–18,000 lux) to promote sugar accumulation and quercetin synthesis without excessive moisture loss.

### **5.2.2 Storage Conditions**

Post-curing storage should be conducted at 15°C in darkness to minimize weight loss and preserve reducing sugars and quercetin compounds. Light exposure during storage should be avoided to prevent biochemical degradation.

### **5.2.3 Harvest Timing for Functional Compounds**

For maximum retention of free quercetin and reducing sugars, shallots should be processed or marketed at day 6 of curing or day 12 of storage, corresponding to peak compound concentrations.

### **5.2.4 Future Research**

Further investigation into the enzymatic pathways involved in fructan hydrolysis and quercetin glycoside conversion is recommended to elucidate the underlying biochemical mechanisms.

Exploration of advanced storage technologies, such as modified atmosphere packaging or natural coatings, may provide additional strategies for extending shelf life and preserving quality.

Comparative studies across different shallot cultivars are recommended to determine genotype-specific responses to curing and storage treatments.

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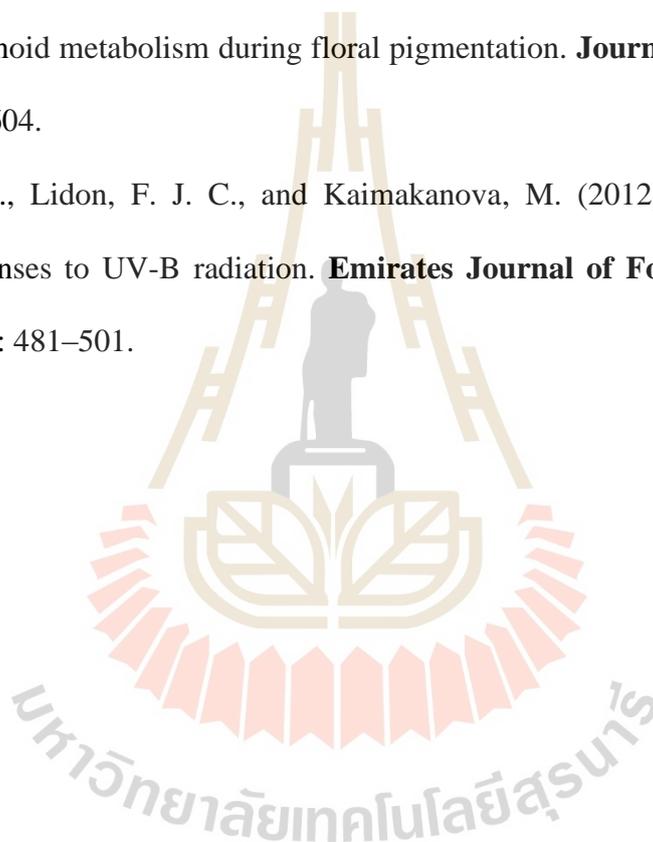
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Phattharaporn Yuthachit was born in Nakhonpanom, Thailand. In 1990, she received a Bachelor of Science degree in Biology from Prince of Songkla University, Songkla, Thailand. She worked at Tim Food Co., Ltd., in the quality control unit for frozen fruit and vegetables for one year. She then worked at Sun Yang Food Co., Ltd., in quality control, research and development of fruit and vegetable canning for four years. In 1998, she received a Master's degree in Food Technology at Prince of Songkla University, Thailand. She worked at Nakhon Ratchasima Rajabhat University as a lecturer in the Food Science and Technology Program, Faculty of Science and Technology for eight years. In 2007-2010, she received the Strategic Scholarships for Frontier Research Networks from Commission on Higher Education, Ministry of Education Fund, to study for a Doctor of Philosophy degree in Food Technology at Suranaree University of Technology. During her graduate study, she received a scholarship from Nakhon Ratchasima Rajabhat University to pursue her research works at the Department of Food Science, Purdue University, USA.