

CHANGES OF FLAVOR COMPONENTS OF ONION (*ALLIUM CEPA* L.) IN A SALINE ENVIRONMENT

by

PAI-TSANG CHANG

(Under the Direction of William M. Randle)

ABSTRACT

Salinity may affect the intensity of onion (*Allium cepa* L.) because onion is classified as a salt-sensitive crop. To better understand these flavor changes, two experiments were conducted to evaluate changes of flavor components in “Granex 33” onion in a salinity environment. The first experiment reported that S-alk(en)yl cysteine sulfoxides (ACSOs) change in response to salinity levels. Methyl cysteine sulfoxide (MCSO) increased as a percentage of total ACSOs and the ratio of 1-propenyl cysteine sulfoxide (1-PRENC SO) to total ACSOs decreased as NaCl increased.

To speculate on the result of first experiment, we thought that sequential addition of NaCl would affect onion flavor intensity. A second experiment was conducted to investigate whether onion flavor quality changes were associated with sequential addition of NaCl. Results indicated that during the active bulbing stage, onion started to accumulate ACSOs. It is thought that active bulbing would be a key metabolic step for biosynthesis of onion flavor.

INDEX WORDS: Onions, NaCl, S-alk(en)yl cysteine sulfoxides, Alliinase, Lachrymatory factor, γ -L-Glutamyl-S-(1-propenyl)-L-cysteine sulfoxide, S-2 Carboxypropyl glutathione, (+) S-Methyl cysteine sulfoxide, *trans*-(+)-S-(1-Propenyl)-L-cysteine sulfoxide, (+)-S-Propyl-L-cysteine sulfoxide, HPLC

CHANGES OF FLAVOR COMPONENTS OF ONION (*ALLIUM CEPA* L.) IN A
SALINE ENVIRONMENT

by

PAI-TSANG CHANG

B.S., Chung-Hsing University, Taiwan, 1996

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2003

© 2003

Pai-Tsang Chang

All Rights Reserved

CHANGES OF FLAVOR COMPONENTS OF ONION (*ALLIUM CEPA* L.) IN A
SALINE ENVIRONMENT

by

PAI-TSANG CHANG

Major Professor: William M. Randle

Committee: Bruce Haines
Marc W. van Iersel

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2003

ACKNOWLEDGEMENTS

As a foreign student studying in the United States, my intention at the beginning of my degree program was to communicate more with all instructors and to take advantage of their knowledge. Their advice, suggestions and encouragement help me to achieve my degree. I would like to thank you my major professor, Dr. William Randle, for his patient, effort and contributions, and my committee members, Dr. Bruce Haines and Dr. Marc van Iersel. You are all excellent mentors and not only be my teachers but also important to me as friends. It is a good experience to work my career with you.

I also would like to thank you those individuals who helped a lot and gave me advice. Dr. Harry Mills gave me his time and knowledge of plant nutrition, Dr. Mark Rieger provided his time and salinity equipment. Mila Pearce provided me with everything I needed for working at the greenhouse. My colleague, Tim Coolong, helped me out everything I do not understand. Without the support of my family, I would not successfully make the first step. To all my friends and horticulture graduate students in here or in Taiwan who helped, listened and were just there when I needed, I say thank you.

Thank you all,

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
CHAPTER	
1 INTRODUCTION.....	1
2 LITERATURE REVIEW	3
The History of Alliums.....	3
The Botany of The Onion.....	5
Sulfur and Sulfate Assimilation	7
Onion Flavor Chemistry.....	12
ACSO Biosynthesis	15
Environmental Factors and Flavor Intensity.....	18
Literature Cited.....	19
3 ONION FLAVOR DEVELOPMENT: THE INFLUENCE OF NACL GRADIENT IN NUTRIENT SOLUTION.....	28
Abstract	29
Introduction	30
Materials and Methods	32
Results and Discussion.....	37

	Literature Cited.....	44
4	GROWTH, DEVELOPMENT, AND FLAVOR OF ALLIUM CEPA L. (ONION): EFFECTS OF SEQUENTIAL ADDITIONS OF NACL STRESS.....	53
	Abstract	54
	Introduction	54
	Materials and Methods	56
	Results and Discussion.....	60
	Literature Cited.....	64
5	CONCLUSIONS	78

LIST OF TABLES

	Page
Table 1: The effect of NaCl concentration on Na ⁺ and Cl ⁻ content and the average leaf and bulb FW of ‘Granex 33’ onion (<i>Allium cepa</i> L.) bulbs.....	50
Table 2: The effect of NaCl concentration on total pyruvate (TPY), soluble solids content (SSC), total bulb sulfur (S), total bulb sulfate (SO ₄ ⁻²), and organic-S (O-S) of ‘Granex 33’ onion (<i>Allium cepa</i> L.) bulbs.....	51
Table 3: The effect of NaCl concentration on bulb flavor precursors S-alk(en)l cysteine sulfoxides (ACSOs), methyl cysteine sulfoxide (MCSO), 1-propenyl cysteine sulfoxide (1-PRENC SO), propyl cysteine sulfoxide (PCSO), and the intermediates 2-carboxypropyl glutathione (2-CARB) and γ-glutamyl propenyl cysteine sulfoxide (γGPRENC SO) of ‘Granex 33’ onion(<i>Allium cepa</i> L.) bulbs ...	52

LIST OF FIGURES

	Page
Figure 1: Different Pathways of sulfur assimilation.(A-C) Sulfide or thiosulfide is produced from APS through three pathways.(D) Cysteine synthesis pathway.....	9
Figure 2: Pathway for the synthesis of glutathione (γ -glutamylcysteinylglycine) in plant. The enzymes involved are (1) γ -glutamylcysteine synthetase and (2) glutathione synthetase	11
Figure 3: The synthesis of volatile sulfur compounds in onion.(1) S-alk(en)yl cysteine sulfoxide, (2) S-alk(en)yl sulfenic acid, (3) thiopropanal S-oxide, (4) thiosulfinate, (5) monosulfide, (6) disulfide, (7) thiosulfonate, (8) dimethyl thiophene, (9) trisulfide, (10) pyruvate, (11) propanal. R = methyl, propyl, or propenyl	14
Figure 4: The synthesis of γ -glutamyl peptides and flavor precursors (ACSOs) in onion	17
Figure 5: Changes in bulb and leaf FW from mature ‘Granex 33’ bulbs when NaCl concentration was applied sequentially at 14 d intervals beginning 74 d before harvest. Bulb (●) and leaf (○) FW were decreased linearly (Bulb FW = $318.4761 - 52.9571 \text{ day}$, $r^2 = 0.91$), (Leaf FW = $165.4226 - 27.6607 \text{ day}$, $r^2 = 0.92$) respectively.....	68

Figure 6: Changes in soluble solids content (SSC)(●) and total pyruvate (TPY)(Δ) from mature ‘Granex 33’ bulbs when NaCl concentration was applied sequentially at 14 d intervals beginning 74 d before harvest. Soluble solids content responded quadratically ($SSC = 8.2107 + 0.09810d - 0.1696 d^2$, $R^2 = 0.55$), while total pyruvate was response cubic ($TPY = 5.5142 + 0.1129 d - 0.005 d^2 + 0.00005 d^3$, $R^2 = 0.55$).....70

Figure 7: Changes in bulb sulfur (S) (●), bulb sulfate (SO_4^{-2}) (▲), and organic-S (O-S) (□) from mature ‘Granex 33’ bulbs when NaCl concentration was applied sequentially at 14 d intervals beginning 74 d before harvest. The response of bulb sulfur was quadratic ($Bulb S = 5.1708 - 0.0383 d + 0.0002 d^2$, $R^2 = 0.76$), while bulb sulfate was not significant changed, organic-S was linear ($O-S = 3.8325 - 0.0244 d$, $R^2 = 0.56$).....72

Figure 8: Changes in alk(en)yl cysteine sulfoxides (ACSOs) (●), methyl cysteine sulfoxide (MCSO)(■), 1-propenyl cysteine sulfoxide (1-PRENCISO) (▲), and propyl cysteine sulfoxide (PCSO) (◆), from mature ‘Granex 33’ bulbs when NaCl was applied at 14 d intervals beginning 74 d before harvest. The response of ACSOs was quadratic ($ACSOs = 3.0687 - 0.0454 d + 0.0005561 d^2$, $R^2 = 0.58$), MCSO was quadratic ($MCSO = 2.0255 - 0.0277 d + 0.0003414 d^2$, $R^2 = 0.41$), 1-PRENCISO was also quadratic ($1-PRENCISO = 0.8942 - 0.0163 d + 0.0001939 d^2$, $R^2 = 0.61$), PCSO was unaffected.....74

Figure 9: Changes in 2carboxypropyl glutathione (2-CARB) (●), and γ-glutamyl propenyl cysteine sulfoxide (γGPRENCISO)(■) from mature ‘Granex 33’ bulbs when NaCl was applied at 14 d intervals beginning 74 d before harvest. The

response of 2-CARB was quadratically ($2\text{-CARB} = 0.6004 + 0.0054 d - 0.0001073 d^2$, $R^2 = 0.52$), and $\gamma\text{GPRENCSO}$ changed quadratically ($\gamma\text{GPRENCSO} = 1.6574 + 0.0281 d - 0.0004676 d^2$, $R^2 = 0.34$).....76

CHAPTER 1

INTRODUCTION

Onions (*Allium cepa* L.) ranked number two of horticultural crops in economic value because of their particular flavor characteristics. Flavor intensities have a wide range from very pungent to mild, even to sweet. Onion flavor is caused by cellular damage when the enzyme alliinase (EC 4.4.1.4) in the vacuole is released to hydrolyze the flavor precursors, S-alkyl(en)yl cysteine sulfoxides (ACSOs), in the cytoplasm (Lancaster and Boland, 1990). Three flavor precursors are found in onions; (+)-S-methyl-L-cysteine sulfoxide (MCSO), (+)-S-propyl-L-cysteine sulfoxide (PCSO), and *trans*-(+)-S-1-propenyl-L-cysteine sulfoxide (1-PRENCISO) (Lancaster and Boland, 1990). In general, 1-PRENCISO has the highest concentration in onions and it is also responsible for tearing and pungency; MCSO has a lower concentration; and PCSO has the lowest concentration (Randle et al., 1995). When ACSOs are hydrolyzed by alliinase, onions produce unstable volatile sulfenic acids, pyruvate, and ammonia. The volatile compounds form the lachrymatory factor (LF) and thiosulfinates which are responsible for the different flavor strength (Block, 1992; Randle et al., 1994).

Onion absorbs sulfate (SO_4^{2-}) from the medium, which can be reduced to sulfide. Sulfide is then assimilated to cysteine. Cysteine can further form the S-containing amino acid, methionine, and glutathione in onions. Through glutathione, sulfur can be incorporated into different peptide pathways that lead to the synthesis of the three onion flavor precursors (Block, 1992).

In onions, the ACSOs and their peptide intermediates are influenced by environmental factors. A primary factor that affects onion flavor intensity is S fertility (Freeman and Mossadeghi, 1970; Randle, 1992; Randle et al., 1994). Generally, 1-PRENCISO is the main flavor precursor found to dominate onion flavor strength. However, under low SO_4^{2-} concentrations, MCSO accumulates in higher amounts, which then predominates onion flavor (Randle et al., 1995).

Onion is classified as a salt sensitive crop which can produce at up to 1.2 dS/m salinity in soil (Palif, 1960). Even though onion is salt-sensitive, there is no evidence to illustrate a relationship among Na and Cl stress and S metabolism. Glutathione was found to respond to other plant physiological stress e.g. heat, cold and drought stress (Smith et al., 1990).

To understand the relationship between salt stress and flavor changes, compounds in the flavor biosynthetic pathway were measured under different NaCl concentrations in our first study. In a second experiment, NaCl was applied at different growth stages to study flavor quality changes at different growth stage. Sulfate fertility applied at different onion growth stages was shown to affect onion flavor intensity and quality (Randle et al., 2002).

CHAPTER 2

LITERATURE REVIEW

The History of Alliums

It is believed that *Alliums* have been cultivated for at least 5000 years or more in the Middle and Far East. However, there are no records to prove exactly when and where onions originated because various regions were found to have onions. Today, *Allium* is widely spread in the northern hemisphere; most are grown in warm areas, but some are found in arctic regions. Some researchers suggested onions originated from central Asia in the regions of Iran and West Pakistan (Hanelt, 1990).

While the place and time of the onion's origin is still unsure, there are many documents, from very early times, which describe the importance of onions as food and their use in art, medicine and mummification.

The earliest record of onion was found in Egypt 3500 years ago. Onions had significance in holy worship in Egypt. The Egyptians used onions for funeral decorations and in carvings on pyramid walls and in tombs (2700B.C.). They saw eternal life in the anatomy of the onion because of its circle-within-a-circle structure. Onions were also found in mummies, because the strong scent of the onion would prompt the dead to breathe or be handy in the afterlife (per.com., National Onion Association).

In India, the onion was mentioned as early as the 6th century B.C. At that time the onion was used as a diuretic, and was believed to promote a healthy heart, eyes and joints (Hanlet, 1990).

Carbonized onion was found when Pompeii was excavated (date to 79 A.D.). It showed that onions had been grown during the Roman Empire. It is thought that the Romans ate and carried onions on their journeys to England and Germany. Hence onions were very likely introduced to Europe at that time (Hanlet, 1990).

Onions became widespread in Europe by the Middle Ages. As onions expanded into other areas of the world, they were still more than just food. Physicians prescribed onions to alleviate headaches, snakebites, and even hair loss.

Onions, valued as both medicine and food, traveled with the Pilgrims who settled the New World. At the same time, they found that Native American Indians used wild onions for cooking, seasoning, or as vegetables.

The onion we use today became popular in the sixteenth century. A variety of onion phenotypes were found including red, yellow, white, oblate, globe, spindle, sweet, and pungent onions (Fenwick and Hanley, 1985).

In 1925, Henry Jones started the beginning of modern onion breeding. Crossing different breeding lines could produce new and better varieties or hybrids, which lets us plant onions under specific or various conditions today.

Today, onions still have an outstanding place in our diet. Furthermore in 1986 Block stated that garlic was used as an antibiotic in Russia and Japan. The National Cancer Institute has reported that onions contain antioxidants that help block cancer and appear to lower cholesterol (Carper, 1991).

The Botany of The Onion

Historically the common onion (*Allium cepa* L.) was classified as a member of the *Liliaceae*. However, the taxonomic position of *Allium* is still a controversy. An acceptable hierarchy of the common onion is: Class *Monocotyledones*, Superorder *Liliiflorae*, Order *Asparagales*, Family *Alliaceae*, Tribe *Allieae*, and Genus *Allium*. The onion is a biennial crop grown for bulb production and then for seed production in second season (Hanelt, 1990).

The leaves of the onion are hollow and emerge in two ranks at 180 degrees to each other. Onion leaves consist of two parts. One is leaf base or sheath, and the other is a blade. The leaf sheath is a hollow tube which encircles the apical meristem and encloses the shoot apex. The leaf blade is also a hollow tube and is closed at the tip (DeMason, 1990). Succeeding leaves increase in size which leads to the formation of the bulb. The leaf sheathes and young leaf blades are formed in concentric rings called the pseudostem, which is distinguished from the true stem at the onion base (Hanelt, 1990; Brewster, 1994).

The primary root of the onion emerges from the seed, but it is short lived. The subsequent root system comes out from the stem base. Basically, the roots of the onion are shallow, sparsely branched, and grow no more than 50 cm into the soil (Weaver and Brunner, 1927).

The onion is a biennial crop and usually goes through a separate flowering season of growth to produce seed. The inflorescence occurs when the inflorescence axis is elongated from the shoot apical meristem. When elongation occurs, the true stem is extended through the leaf sheathes. The extended floral axis encloses the floral apex

called the spathe (DeMason, 1990). When the floral axis elongates between 1 to 2 meters, the spathe splits. At that time, the shoot apical meristem ceases leaf production and the spathe forms an inflorescence (Hanelt, 1990).

The bulb, or the edible part of the onion, consists of the vegetative stem axis and the leaf bases. The onion bulb is formed when leaf bases swell. While bulbing, the leaf bases thicken. During advanced bulbing, young developing leaves stop forming new blades and start to translocate photosynthate to the leaf bases, which results in enlarging the bulb scales (Rubatzky and Yamaguchi, 1997). When the bulb is mature, the outer 2 or 3 sheathes lose moisture and become scaly (Hanelt, 1990; Brewster, 1994).

Onion bulbing is photoperiod-sensitive. There are three kinds of onions, based on the daylength required for bulb formation. Short-day varieties start forming an enlarged bulb when days are 10 to 13 hours long; long-day varieties do not form a bulb until days are 14 to 16 hours long, and intermediate-day varieties require 13 to 14 hours of light per day to bulb. For all types, bulb enlargement is arrested during hot, freezing, or dry weather. Near the equator, where days are 11-13 hours long throughout the year, long-day onions will never form a bulb, and in Canada where days are 14-20 hours long during the growing season short-day onions produce only small bulbs (Brewster, 1990).

Light intensity and quality are also important for onion bulbing. Bulb scales could be initiated earlier with increasing light intensity (Brewster, 1990). Furthermore, when the onion is exposed to different ratios of red light (600 nm) and far red light (730 nm), the lower red : far red ratio will enhance more bulbing (Mondai et al., 1986).

Sulfur and Sulfate Assimilation

The onion and other *Alliums* are valued because of their particular flavor. When organic sulfur compounds were isolated from garlic, flavor chemistry in *Alliums* attracted many investigations (Block, 1992). The particular flavor of the onion and other *Allium* is due to a large number of organosulfur compounds existing in onions. Therefore, understanding sulfur metabolism is quite important to comprehend onion flavor chemistry.

The most important source of sulfur for higher plants to utilize is sulfate (SO_4^{-2}). Generally, plant roots absorb sulfate from a medium against an electrochemical gradient by a membrane localized proton driven SO_4^{-2} transporter. This mechanism is established by the plasma membrane protein adenosine tri-phosphatase (ATPase) (Leustek et al., 2000). The ability to take up sulfate is regulated not only by internal sulfate concentrations (Anderson, 1990), but also by cysteine concentrations (Datko and Mudd, 1984).

Once sulfate is absorbed by the roots, sulfate is transported through the xylem via the transpiration stream. Through this pathway, sulfate is delivered to older, mature leaves and stored in the vacuole as a sulfur source. Sulfur is loaded and transported via phloem to sinks such as young developing leaves or shoot apex as glutathione (Bergmann and Rennenberg, 1993). SO_4^{-2} is able to transport across the plasma membrane into cells because there are several sulfate permeable channels which act as $\text{H}^+ / \text{SO}_4^{-2}$ co-transporters to move SO_4^{-2} across the cell membrane, some of them having low affinities and others having high affinities for SO_4^{-2} (Takahashi et al., 1997; Takahashi et al., 2000).

When sulfate crosses into the cells, it has to be transported into chloroplasts for reduction to sulfide (S^{2-}) (Saito, 2000). The first step of sulfur assimilation in higher plants is the reduction of sulfate to the cysteine. Sulfate is activated by ATP in the plastids which leads to form adenosine phosphosulfate (APS) and pyrophosphate (PP_i).

From APS, there are three pathways of sulfate assimilation (Figure 1) (Taiz and Zeiger, 1998). The APS can react with ATP to form phosphoadenine phosphosulfate (PAPS). PAPS is then reduced to sulfite (SO_3^{2-}) and then to sulfide (S^{2-}) (Figure 1 A). Another way is to convert APS to thiosulfonate ($R-SO_3^{2-}$) and then to thiosulfide ($R-S^-$) (Figure 1 B). The other pathway is also from APS, which can be directly reduced to sulfite and then to sulfide (Figure 1 C).

The new thio groups react with acetylserine to form cysteine and acetate (Figure 1 D). Cysteine then can act as a precursor for synthesis of other organic compounds containing reduced sulfur, such as methionine, proteins, and glutathione in the plant (Marschner, 1999).

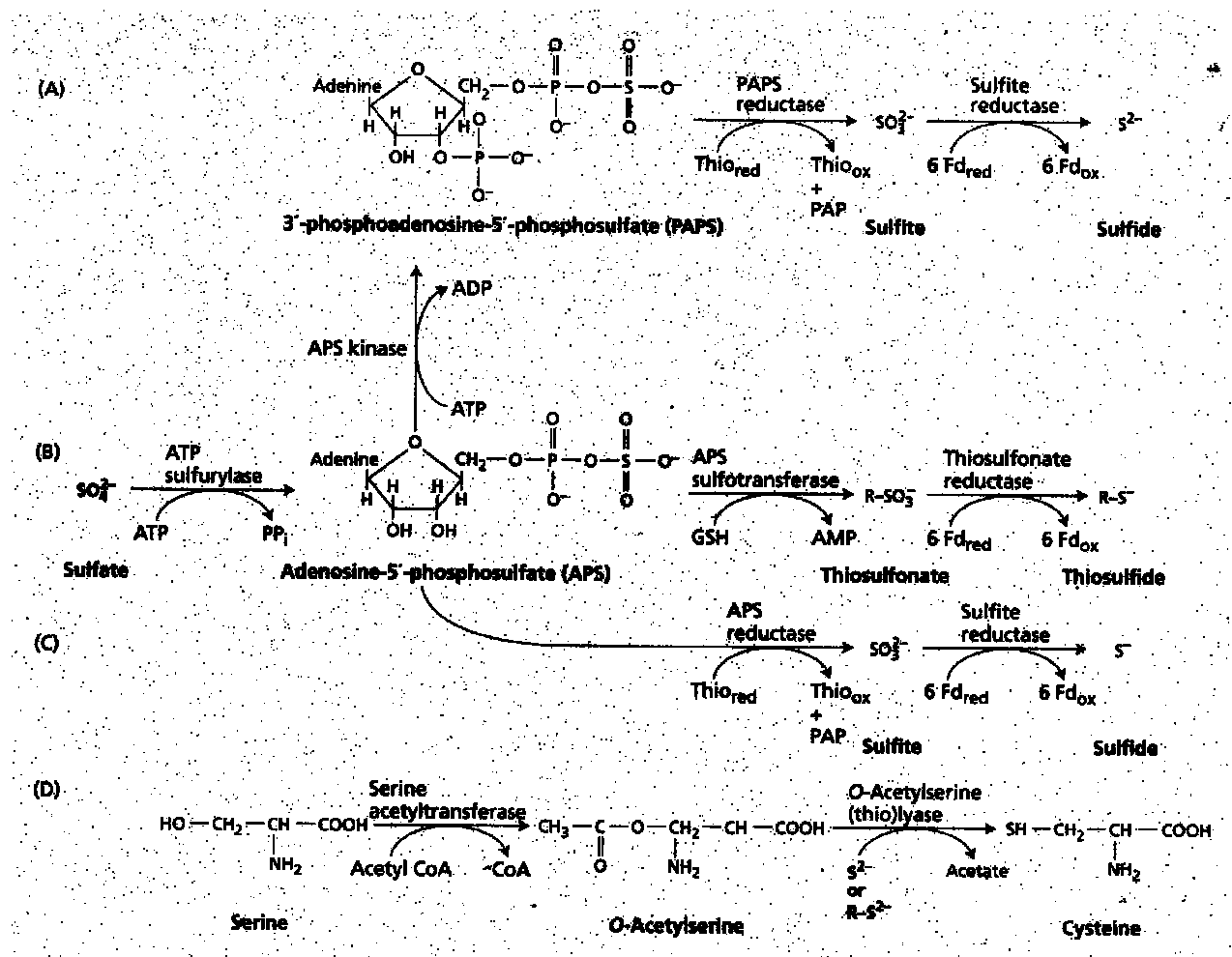


Figure. 1. Different pathways of sulfur assimilation. (A-C) Sulfide or thiosulfide is produced from APS through three pathways. (D) Cysteine synthesis pathway (Taiz and Zeiger, 1998).

According to Bergmann and Rennenberg (1993), the reductive assimilation of sulfate into cysteine occurs in green leaves because the reaction is light stimulated. In green leaves, the principal soluble organic sulfur compound is glutathione, a tripeptide, which can be transported to sites of protein synthesis. Glutathione is also a major intermediate in the production of the organosulfur flavor compounds in *Allium*. The synthesis of glutathione is a two-step process that occurs in both the cytosol and the chloroplast (Leustek et al., 2000) (Figure 2). In the first reaction, cysteine and glutamate are incorporated with an ATP dependent reaction which is catalyzed by γ -glutamylcysteine synthetase to produce the dipeptide glutamylcysteine. Later, glycine is coupled to glutamylcysteine with another ATP dependent reaction forming glutamylcysteinylglycine, or glutathione. This reaction is catalyzed by glutathione synthetase (Rennenberg, 1982). In plants, glutathione is used not only as an antioxidant in response to oxidative stress (Smith et al., 1990) but is important in storing reduced sulfur and in maintaining cysteine concentration (Schütz et al., 1991; Schmidt and Jäger, 1992).

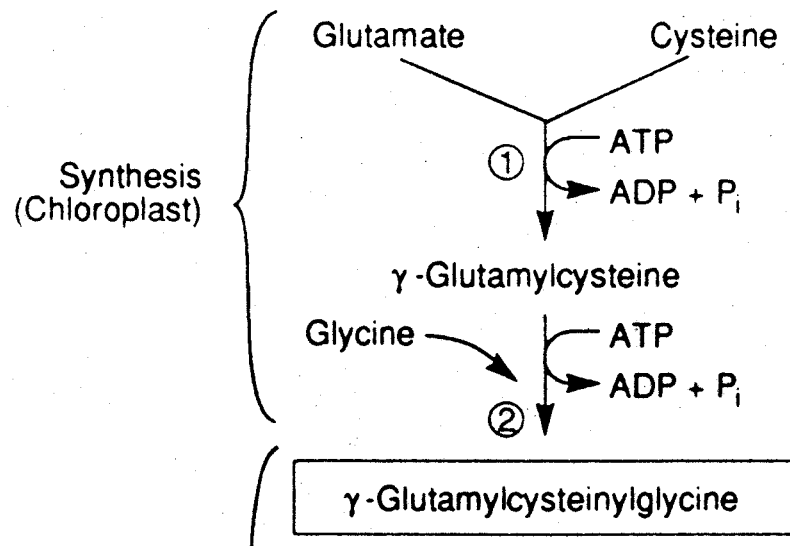


Figure. 2. Pathway for the synthesis of glutathione (γ-glutamylcysteinylglycine) in plants. The enzymes involved are (1) γ-glutamylcysteine synthetase and (2) glutathione synthetase (Anderson, 1990).

Onion Flavor Chemistry

Onions (*Allium cepa* L.) have an important status among vegetables because of their particular flavors and their ability to enhance the flavor of other foods. Previous studies have demonstrated that many sulfur compounds contribute to flavor synthesis and the development of flavor precursors. It is agreed that onion flavor arises from the enzymatic decomposition of S-alk(en)yl cysteine sulfoxides when onion tissue is disrupted. The products of the decomposition process, such as lachrymatory factor and thiosulfinates, present onion's unique flavor (Randle, 1997).

Generally, undamaged onions are odorless but, when onion tissues are cut or disrupted, the enzyme alliinase (EC 4.4.1.4) is released to hydrolyze the S-alk(en)yl cysteine sulfoxides to produce pyruvate, ammonia, and volatile sulfur compounds. The volatile sulfur compounds play the most important role in the flavor chemistry (Lancaster and Boland, 1990). In 1992, Block pointed out that alliin (S-alk(en)yl cysteine sulfoxides) are the major flavor precursors to produce the odor flavor of the *Allium* species when cells are damaged. Schnug (1993) concurred that alliin is the most important sulfur-containing compounds of the secondary metabolism of the *Allium*.

Flavor Precursors

Today, four ACSOs have been found naturally in *Alliums*. According to Lancaster and Boland (1990) and Breu (1996), S-methyl cysteine sulfoxide (MCSO) and S-propyl cysteine sulfoxide (PCSO) were the first two flavor precursors identified in onions by Virtanen and Matikkala in 1959. Subsequently, S-1 propenyl cysteine sulfoxide (1-PRENCOSO) was isolated from onions by Virtanen and Spare in 1961. One flavor precursor, S-2 propenyl cysteine sulfoxide (2-PRENCOSO), which exists in garlic

and other *Alliums* but not in onions, was isolated by Stoll and Seebeck in 1947 (Lancaster and Boland, 1990). In general, 1-PRENCISO, found in highest concentration in onions, is responsible for tearing and pungency. MCSO generally is found in the second most abundant concentration in onions, and causes a cabbage and fresh onion taste. PCSO, found in the lowest concentration, has a raw onion and chive sensation (Randle, 1997).

Alliinase and Flavor Formation

The alliinase (EC 4.4.1.4.), or S-alk(en)yl cysteine sulfoxides lyase, which occurs in the *Alliums* is the enzyme responsible for the hydrolysis of S-alk(en)yl cysteine sulfoxides. Lachrymatory factor and sulfenic acids are the first products of the enzymatic reaction (Block, 1992). These products of the reaction are unstable. After nonenzymatic rearrangements, the volatile substrates are produced, giving off the characteristic aroma of alliums. Thiosulfinates are formed from sulfenic acids or thiopropanal S-oxide which subsequently degrade to monosulfides, disulfides, and thiosulfonates (Lancaster and Boland, 1990) (Figure 3).

Pyruvate is used as an indicator of onion pungency because it is produced by hydrolysis of all ACSOs. In 1961, Schwimmer and Weston were the first two to use enzymatic pyruvate (EPY) to measure onion pungency. Randle and Bussard (1993) streamlined the method. Using EPY to predict onion pungency is quick; however, it can be used just for determining gross flavor intensity in onions.

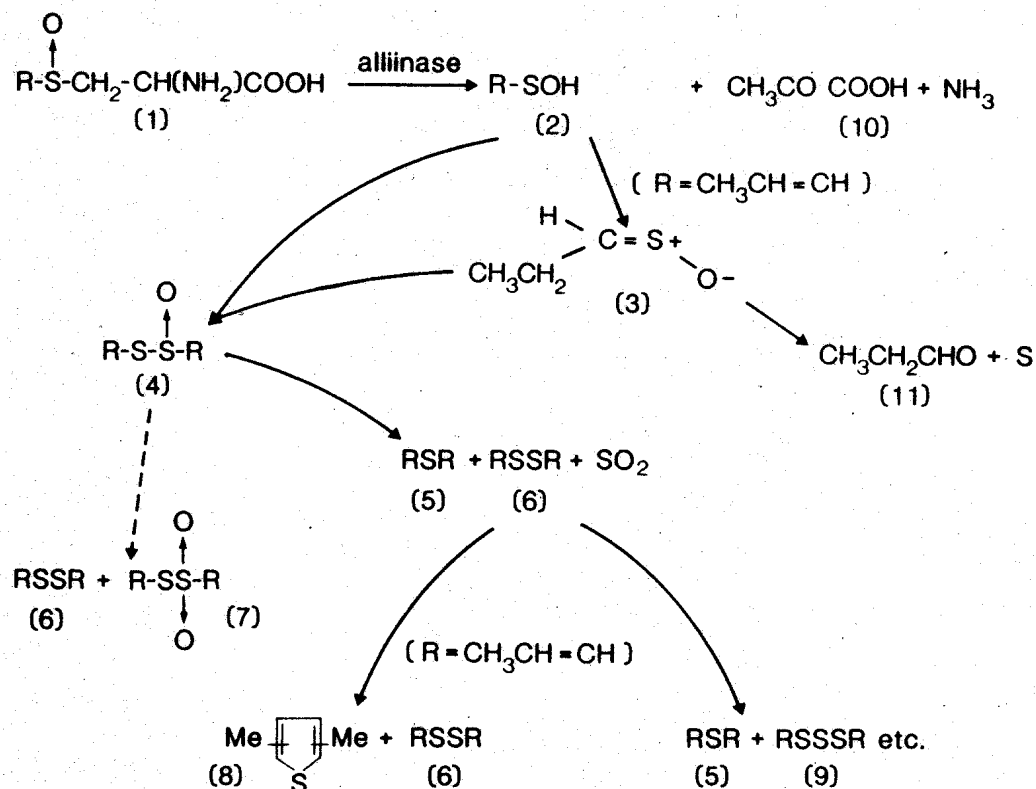


Figure 3. The synthesis of volatile sulfur compounds in onion. (1) S-alk(en)yl cysteine sulfoxide, (2) S-alk(en)yl sulfenic acid, (3) thiopropanal S-oxide, (4) thiosulfinate, (5) monosulfide, (6) disulfide, (7) thiosulfonate, (8) dimethyl thiophene, (9) trisulfide, (10) pyruvate, (11) propanal. R = methyl, propyl, or propenyl (Lancaster and Boland, 1990).

γ -glutamyl Peptides

In the biosynthetic pathways of flavor precursors in onion, a number of intermediates exist. In onions, 24 γ -glutamyl peptides have been found, 18 of which contain sulfur (Lancaster and Boland, 1990). They were thought to function as nitrogen and sulfur reservoirs and transport amino acids in cells (Randle and Lancaster, 2002). These sulfur-containing γ -glutamyl peptides, which are not metabolized by the action of alliinase, do not contribute flavor and pungency directly (Shaw et al., 1989). Matikkala and Virtanen in 1965 indicated that γ -glutamyl peptides are hydrolyzed by γ -glutamyl transpeptidase during the biosynthesis of flavor precursors (Lancaster and Boland, 1990). In 1989, Lancaster and Shaw showed that S^{35} was incorporated into γ -glutamyl peptides before alk(en)yl cysteine sulfoxides.

ACSO Biosynthesis

In 1989, Lancaster and Shaw pointed out that labeled S^{35} was first incorporated into γ -glutamyl peptides before the synthesis of S-alk(en)yl cysteine sulfoxides. Later, Parry and Lii in 1991 added methacrylic acid to glutathione which produced the γ -glutamyl-S-2-carboxypropyl glutathione. Sequential hydrolysis results in γ -glutamyl-S-2-carboxy propenyl cysteine, and followed by decarboxylation gives γ -glutamyl-S-1-carboxy propenyl cysteine. Then oxidation to γ -glutamyl-S-1-propenyl cysteine sulfoxides and cleavage by γ -glutamyl transpeptidase leads to 1-PRENCISO (Block, 1992; Edwards et al, 1994).

1-PRENCISO is thought to be the most abundant flavor precursor in onions, which dominates onion flavor. A similar reaction was applied to synthesis of MCSO. The

MCSO pathway is found in all *Allium* species (Lancaster and Boland, 1990).

Methylation of glutathione leads to S-methyl glutathione, which is cleaved to produce γ -glutamyl-S-methyl cysteine. Subsequent oxidation leads to γ -glutamyl-S-methyl cysteine sulfoxides. Finally, cleavage of γ -glutamyl-S-methyl cysteine sulfoxides results in MCSO (Lancaster and Boland, 1990; Block, 1992). Lancaster and Shaw (1989) indicated a possible way of synthesizing PCSO via saturation of the double bond from γ -glutamyl-S-propenyl cysteine (Randle and Lancaster, 2002) (Figure 4).

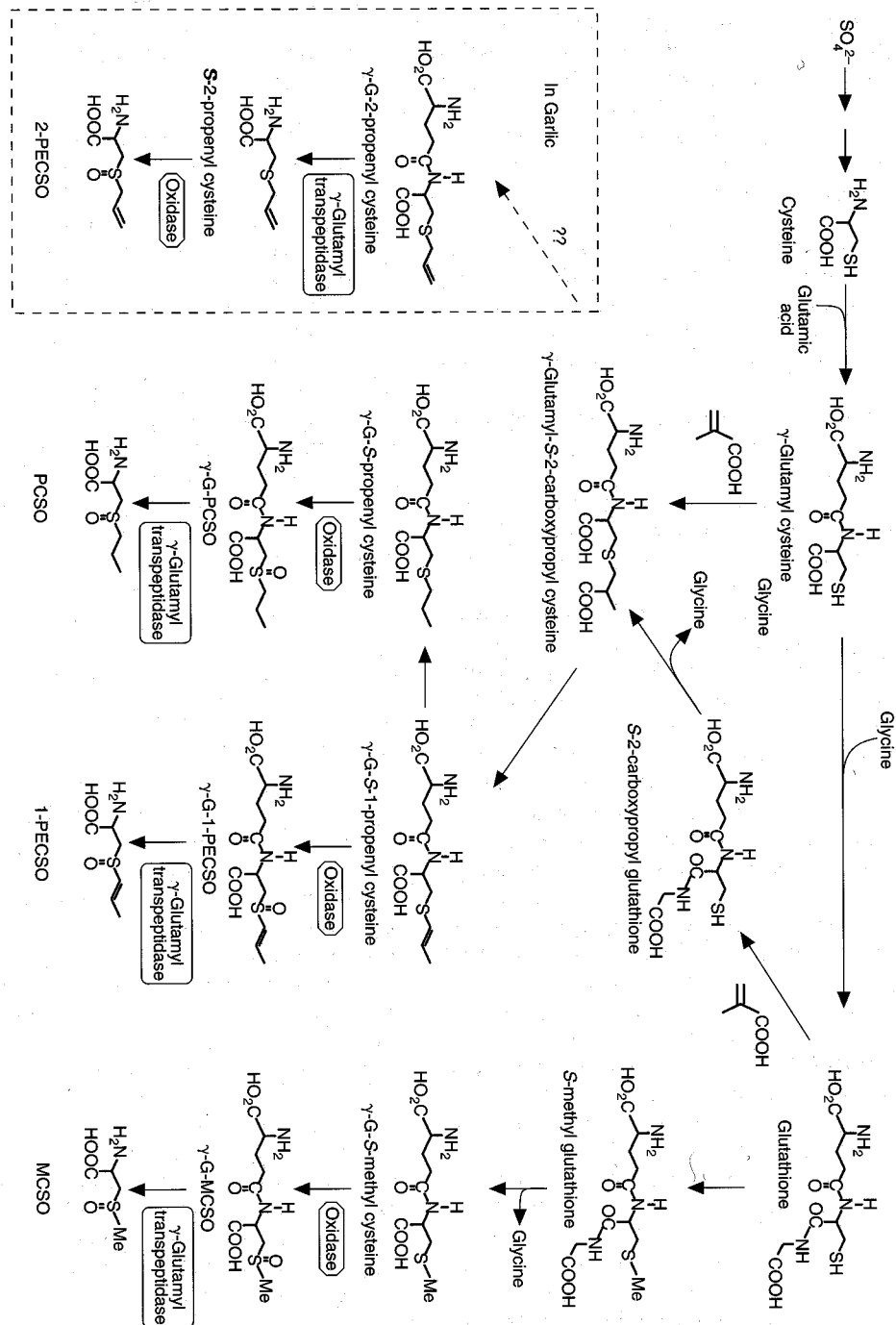


Figure 4. The synthesis of γ -glutamyl peptides and flavor precursors (ACSOs) in onion (Randle and Lancaster, 2002).

Environmental Factors and Flavor Intensity

Different onion varieties have different qualities and flavor intensity. Besides genetic differentiation, the growing environment plays an important role in onion flavor. Lancaster (1988) indicated that the same cultivar, grown in different locations, had different flavors. Furthermore, environmental factors such as sulfur fertility, nitrogen concentration, irrigation, and temperature affect flavor intensity.

Sulfur, described above, is an important element of onion flavor. Previous studies have reported that increased S fertility resulted in increasing onion pungency (Freeman and Mossadeghi, 1970, Randle and Bussard 1993). However, when sulfur is applied above sufficient levels, onion pungency is not changed (Hamilton et al., 1998). In 1995, Randle et al., indicated that different S fertility rates influence the ratio of individual ACSOs, and total ACSO accumulation in onions. Furthermore, selenium thought to compete with S in plants has been found to influence onion pungency. Kopsell and Randle (1999) reported that increasing sodium selenate would decrease onion pungency.

Nitrogen concentration was also reported to influence onion flavor. Randle (2000) reported that high nitrogen concentration in hydroponic solution results in high levels of MCSO. In addition, Randle et al, (1995) showed that low level of S fertility causes MCSO to become the dominant flavor precursor in onions. Therefore, low S and high N availability can cause an increase in the partitioning of organically bound S into the MCSO pathway.

Other growth conditions reported to affect onion flavor are water supply and temperature (Freeman and Mossadeghi, 1973; Platenius and Knott 1941; Randle et al., 1993). Increased temperature increases volatile sulfur content which leads to onion

pungency enhancement. A similar result was found when growing onions with restricted water availability.

Salinity levels were reported to influence onion growth (Wannamaker and Pike, 1987) and bulb quality (Malik et al., 1982). There were no significant differences in onion germination until salt levels up to at 30 dS/m, but, onion growth was significant reduced at 1.4 dS/m. Furthermore, increased salinity levels decreases onion sulfur content and sugar content which suggests that salinity also affects bulbs quality.

Literature Cited

Anderson, J.W. 1990. Sulfur metabolism in plants. p. 327-381. In: B. J. Miflin and P. J. Lea (eds.). Vol. 16: The Biochemistry of Plants, A Comprehensive Treatise. Academic Press. New York, NY.

Bergmann, L., and H. Rennenberg. 1993. Glutathione Metabolism in Plants, p. 102-123. In: Sulfur nutrition and assimilation in higher plants, Regulatory, agricultural and environmental aspects. L. J. DeKok, I. Stulen, H. Rennenberg, C. Brunold, and W. E. Rauser (eds.). SPB Acad. Publ. The Hague, Netherland.

Block, E. 1986. The chemistry of garlic and onions. Sci. Amer. 252:114-119.

Block, E. 1992. The organosulfur chemistry of the genus *Allium*. Implications for the organic chemistry of sulfur. Angew. Chemistry. International. Edition. Englishl., 31:1135-1178.

Breu, W. 1996. *Allium cepa* L. (Onion) Part 1: Chemistry and analysis. *Phytomedicine* 3:293-306.

Brewster, J.L. 1990. Physiology of Crop Growth and Building, p. 53-81. In: H.D. Rabowitch and J. L. Brewster (eds.). Vol. 1: Onions and Allied Crops. CRC Press. Boca Raton, FL.

Brewster, J.L. 1994. Onions and other vegetable Alliums, p. 19-35.

Carper, J. 1991. Onions-Their Place in Your Diet, *Onion World*, p. 24.

Datko, A.H., and S.H. Mudd. 1984. Responses of sulfur-containing compounds in *Lemna paucicostata* Hegelm 6746 to changes in availability of sulfur sources. *Plant Physiol.* 75:474-479.

Demason, D.A. 1990. Morphology and Anatomy of Allium, p. 27-52. In: H.D. Rabowitch and J. L. Brewster (eds.). Vol. 3: Onions and Allied Crops CRC Press. Boca Raton, FL.

Edwards, S.J., G. Britton, and H.A. Collin. 1994. The biosynthetic pathways of the S-alk(en)yl-L-cysteine sulfoxides (flavor precursors) in species of *Allium*. *Plant Cell. Tissue and Organ Culture.* 38:181-188.

Fenwick, G.R., and A.B. Hanley. 1985. The genus *Allium*-part 1. CRC Critical Rev. In: Food Sci. and Nutri. 22:1-73.

Freemman, G.G., and N. Mossadeghi. 1970. Effect of sulphate nutrition on flavor components of onion (*Allium cepa*). J. Sci. Food Agric. 21:610-615.

Freemman, G.G., and N. Mossadeghi. 1973. Studies on the relationship between water regime and flavor strength in water cress (*Rorippa nasturtium-aquaticum* [L.] Hayek), cabbage (*Brassica oleracea* var. capitata) and onion (*Allium cepa*). J. Hort. Sci. 48:465-378.

Freeman, G.G., and R. J. Whenham. 1975. A survey of volatile components of some *Allium* species in terms of S-alk(en)yl-L-cysteine sulfoxides present as flavor precursors. J. Sci. Fd. Agric. 26:1869-1886.

Hamilton, B.K., K.S. Yoo, and L.M. Pike. 1998. Changes in pungency of onions by soil type, sulphur nutrition and bulb maturity. Sci. Hort. 74:249-256.

Hanelt, P. 1990. Taxonomy, Evolution and History, p. 1-26. In: H. D. Rabowitch and J. L. Brewster (eds.). Vol. 1: Onions and Allied Crops. CRC Press. Boca Raton, FL.

- Kospell, D.A., and W.M. Randle. 1999. Selenium affects the S-alk(en)yl cysteine sulfoxides among short-day onion cultivars. *J. Amer. Soc. Hort. Sci.* 124:307-311.
- Lancaster, J.E., and M.J. Boland. 1990. Flavor biochemistry, p.33-72. In: H.D. Rabowitch and J. L. Brewster (eds.). Vol. 3: Onions and Allied Crops. CRC Press. Boca Raton, FL.
- Lancaster, J.E., and M.L. Shaw. 1989. γ -glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulfoxides (flavor precursors) in *Allium*. *Phytochemistry*. 28:455-460.
- Lancaster, J.E., P.F. Reay, J.D. Mann, W.D. Bennett, and J.R. Sedcole. 1988. Quality in New Zealand-growing onion bulbs—a survey of chemical and physical characteristics. *N. Z. J. Exp. Agric.* 16:279-285.
- Leustek, T., M.N. Martin, J.A. Bick, and J.P. Davies. 2000. Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* 51:141-165.
- Marschner, H. 1995. Mineral Nutrition of Higher Plants. Second ed. Academic Press. London, UK.

Malik, Y.S., K. Singh, and M.L. Pandia. Effect of salinity on quality of bulb and chemical composition of leaves in onion (*Allium cepa* L.). 1982. Haryana J. Hort. Sci. 11:226-230.

Mondai, M.F., J.L. Brewster, G.E.L. Norris, and H.A. Butler. 1986. Bulb development in onion (*Allium cepa* L.) II. The influence of red:far red spectral ratio and of photon flux density, Ann. Bot. 58:197-206.

Palif, G. 1960. Seed germination of crop in saline media. Novenytermneles. 9:359-368.

Platenius, H., and J. E. Knott. 1941. Factors affecting onion pungency. J. Agric. Res. 62:371-379.

Randle, W.M. 1992. Onion germplasm interacts with sulfur fertility for plant sulfur utilization and bulb pungency. Euphytica 59:151-156.

Randle, W.M. 1997. Onion Flavor Chemistry and Factors Influencing Flavor Intensity, p. 41-52. In: Spices Flavor Chemistry and Antioxidant Properties. S. J. Rish and C. T. Ho (eds.). American Chem. Soc. Washington, DC.

Randle, W.M. 2000. Increasing nitrogen concentration in hydroponic solution affects onion flavor and bulb quality. J. Amer. Soc. Hort. Sci. 125:254-259.

Randle, W.M., and M.L. Bussard. 1993. Streamlining onion pungency analysis. HortScience. 28:60.

Randle, W.M., and M.L. Bussard. 1993. Pungency and sugars of short-day onions as affected by sulfur nutrition. J. Amer. Soc. Hort. Sci. 118:766-770.

Randle, W.M., and J.E. Lancaster. 2002. Sulphur compounds in alliums in relation to flavor quality, p. 329-356. In: H. D. Rabinowitch and L. Currah (eds.). Allium crop science: Recent advances. CAB International, Wallingford, UK.

Randle, W.M., E. Block, M. Littlejohn, D. Putman, and M.L. Bussard. 1994. Onion (*Allium cepa* L.) thiosulfinates respond to increasing sulfur fertility. J. Agric. Food Chem. 42:2085-2088.

Randle, W.M., J.E. Lancaster, M.L. Shaw, K.H. Sutton, R.L. Hay, and M.L. Bussard. 1995. Quantifying onion flavor compounds responding to sulfur fertility: sulfur increases levels of alk(en)yl cysteine sulfoxides and biosynthetic intermediates. J. Amer. Soc. Hort. Sci. 120:1075-1081.

Rennenberg, H. 1982. Glutathione metabolism and possible biological roles in higher plants. Phytochemistry. 21:2771-2781.

Rubatzky, V.E., and M. Yamaguchi. 1997. *World Vegetables, Principles, Production, and Nutritive Values*: Second ed. Chapman and Hall. New York, NY.

Satio, K. 2000. Regulation of sulfate transport and synthesis of sulfur-containing amino acids. *Curr. Opin. Plant Biol.* 3:188-195.

Schütz, B., L. J. Dekok, and H. Rennenberg. 1991. Thiol accumulation and cysteine desulphydrase activity in H₂S-fumigated leaves and leaf homogenates of cucurbit plant. *Plant Cell Physiol.* 32:733-736.

Schmidt, A., and K. Jäger. 1992. Open question about sulfur metabolism in plants. *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* 43:325-349.

Schnug, E. 1993. Physiological Functions and Environmental Relevance of Sulfur-containing Secondary Metabolism, p.179-190. In: *Sulfur Nutrition and Assimilation in Higher Plants*. L. J. DeKok, I. Stulen, H. Rennenberg, C. Brunold, and W. E. Rauser (eds.). SPB Acad. Publ. The Hague, Netherland.

Schwimmer, S., and W.J. Weston. 1961. Enzymatic development of pyruvic acid in onion as a measure of pungency. *J. Agric. Food Chem.* 9:301-304.

Smith, I.K., A. Polle, and H. Rennenberg. 1990. Adaptation and Acclimation Mechanisms, p. 291-215. In: stress response in plants. R. Alscher and I. Cuning (eds.). Wiley-Liss. New York.

Shaw, M.L., J.E. Lancaster, and G.A. Lane. 1989. Quantitative analysis of the major γ glutamyl peptides in onion bulbs (*Allium cepa*). J. Sci. Food Agric. 48:459-467.

Takahashi, H., M. Yamazaki, N. Sasakura, A. Watanabe, T. Leustek, J.D.A. Engler, G. Engler, M. VanMonagu, and K. Satio. 1997. Regulation of sulfur assimilation in higher plants: A sulfate transporter induced in sulfate-starved roots plays a central role in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. 94:11102-11107.

Takahashi, H., A. Watanabe-Takahashi, F.W. Smith, M. Blake-Kalff, M.J. Hawkersford, and K. Satio. 2000. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. Plant J. 23:171-182.

Taiz, L., and E. Zeiger. 1998. Plant Physiology. Second ed. Sinauer Associate, Inc. Sunderland, Massachusetts.

Wannamaker, M.J., and L.M. Pike. 1987. Onion responses to various salinity levels. J. Amer. Soc. Hort. Sci. 112:49-52.

Weaver, J.E., and W.E. Brunner. 1927. Root development of vegetable crops.
McGrawhill, New York.

Yoo, K.S., and L.M. Pike. 1998. Determination of flavor precursor compound S-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Scientia Horticulturae*. 75:1-10.

**ONION FLAVOR DEVELOPMENT: THE INFLUENCE OF NACL
GRADIENT IN NUTRIENT SOLUTION¹**

¹ Chang, P.T. and W.M. Randle. To be submitted to the Journal of the American Society for Horticultural Science.

Abstract

Onion flavor intensity has been a topic studied in response to various growing conditions. Little known about its response to salt stress, although onion is classified as a salt-sensitive crop. The purpose of this investigation was to study the effects of increasing NaCl levels on onion growth, mineral nutrient relations, and flavor development. 'Granex 33' onions were grown at six different concentrations of sodium chloride ranging from 0 (control) to 125 mM. Increasing NaCl concentrations not only affected onion fresh weight but altered onion flavor quality in mature plants. Leaf and bulb FW responded linearly to NaCl concentrations. The minerals Na^+ and Cl^- also increased linearly with increasing NaCl concentration. Total bulb S and sulfate responded quadratically to NaCl used in this study. Though bulb soluble solids content was not influenced by NaCl concentrations, pungency was affected by NaCl concentrations. Total alk(en)yl cysteine sulfoxides, methyl cysteine sulfoxide, 1-propenyl cysteine sulfoxide, and propyl cysteine sulfoxide showed quadratic responses to NaCl levels. MCSO accumulated in the highest concentrations of all precursors measured under salt stress. While NaCl affected onion flavor, severe reductions in growth would prevent onion production under those conditions. Therefore, this experiment may be an implication to evaluate flavor variation when onions are grown in salinized areas.

Introduction

Soil salinity is a major agricultural problem which directly affects crop productivity and quality. Using FAO world salinity maps, the total area of saline soils is estimated at 397 million ha with 45 million ha under irrigation (Oldeman et al., 1991). Through continued irrigation with saline water, productive land can become salinized (Epstein et al., 1980).

Onion (*Allium cepa* L.), valued for its characteristic flavors, is classified as a salt sensitive crop which has a 1.2 dS/m electrical conductivity (EC) threshold (Palif, 1960; Mass and Hoffman, 1977). Previous studies showed that onion flavor is influenced by environmental factors such as sulfate availability (Freeman and Mossadeghi, 1970), sulfur fertility (Randle, 1992; Randle et al., 1995), nitrogen concentrations (Randle, 2000), temperature (Platenius and Knott, 1941), and water supply (Freeman and Mossadeghi, 1973). Changes in onion flavor intensity are not known when onions are grown under saline conditions.

Higher plants are thought to have evolved chemical defenses as protective mechanisms when exposed to environmental stress (Rennenberg, 1982; Bergmann and Rennenberg, 1993). Rennenberg (1984) indicated that glutathione could serve as a temporary storage for reduced S which could be used to maintain a cellular cysteine levels (Schmidt and Jäger, 1992). Noctor et al., (1998) confirmed that glutathione not only functioned as a storage and transported form of reduced S, but also as a signal for the regulation of sulfur assimilation. Salt stress induced an increase in glutathione biosynthesis in *Brassica napus* to ameliorate salt-induced oxidative stress (Ruiz and

Blumwald, 2002). Glutathione and its derivatives γ -glutamyl peptides, are also biosynthetic intermediates of S-alk(en)yl cysteine sulfoxides (ACSOs) flavor precursors in *Allium* (Lancaster and Shaw, 1989).

The flavor pathway of onion begins with sulfate (SO_4^{-2}) uptake followed by its reduction to sulfide, and assimilation into cysteine (Leustek et al., 2000). Cysteine is then available for incorporation into glutathione. Onion flavor is primarily determined from the enzymatic decomposition of the S-alk(en)yl cysteine sulfoxides (ACSOs) by alliinase (EC 4.4.1.4) when onion tissue is broken or damaged. There are three principal flavor precursors found in onion, and each imparts different flavor characteristics to an onion (Block, 1992). Generally, 1-propenyl cysteine sulfoxide (1-PRENCISO) is the main precursor found in onion, and responsible for the mouth burn and lachrymatory sensations. Methyl cysteine sulfoxide (MCSO), is found in lesser abundance but under certain condition, can accumulate in large amounts (Randle et al., 1995; Kopsell and Randle, 1999; Randle, 2000). MCSO imparts fresh onion and cabbage-like flavors. Propyl cysteine sulfoxide (PCSO) is found in the lowest concentration in onion and produces onion and chive-like notes when decomposed (Lancaster and Boland, 1990).

The primary products from the hydrolysis of ACSOs are volatile and unstable thiosulfinates, pyruvate, and ammonia. Pyruvate, because it is stable, has been used as a measure of overall flavor intensity (Wall and Corgan, 1992).

Because onions are grown on saline soils world-wide and S metabolism has been implicated in salt-stress amelioration, we investigated how onion flavor would be influenced by increasing levels of NaCl availability.

Materials and Methods

PLANT CULTURE. On 10 Jan. 2002, seeds of 'Granex 33' onion (Asgrow Seeds, Kalamazoo, Mich.) were planted in growing cubes (Grodan; Hedenhusene, Denmark) covered with vermiculite, and watered as needed. After cotyledons emerged, seedlings were fertilized with 400 mL of Peters 20N-20P-20K nutrient solution (Scotts-Sierra Co., Marysville, OH) at a rate of $200 \text{ mg} \cdot \text{L}^{-1}$ once a week. After five true leaves had emerged, the seedlings were transplanted to 30 L tubs (Rubbermaid, Inc., Wooster, OH) in a greenhouse under natural photoperiods ($\approx 34^\circ \text{ N}$ latitude) with day and night temperature set points of 20 and 12°C , respectively. Onion seedlings were grown hydroponically with half-strength modified of Hoagland solution (Hoagland and Arnon, 1950) for two weeks before salt (NaCl) treatments were applied.

Each tub was filled with 28 liters of deionized water and all plants received a nutrient solution of $0.47 \text{ g} \cdot \text{L}^{-1} \text{ Ca} (\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $0.30 \text{ g} \cdot \text{L}^{-1} \text{ KNO}_3$, $0.057 \text{ g} \cdot \text{L}^{-1} \text{ NH}_4\text{H}_2\text{PO}_4$, $0.246 \text{ g} \cdot \text{L}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.08 \text{ mg} \cdot \text{L}^{-1} \text{ CuSO}_4 \cdot 5\text{H}_2\text{O}$, $0.02 \text{ mg} \cdot \text{L}^{-1} \text{ H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$, $0.22 \text{ mg} \cdot \text{L}^{-1} \text{ ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $1.81 \text{ mg} \cdot \text{L}^{-1} \text{ MnCl}_2 \cdot 4\text{H}_2\text{O}$, $2.86 \text{ mg} \cdot \text{L}^{-1} \text{ H}_3\text{BO}_3$ and $10.0 \text{ mg} \cdot \text{L}^{-1} \text{ Fe}$ chelate. The experiment was set as a completely randomized design with six NaCl treatments (0, 25, 50, 75, 100, and 125 mM) arranged in individual tubs with ten plants per treatment, and four replications. Tubs were refilled with deionized water daily. The nutrient solutions were renewed every two weeks to initial treatment levels. Water potential was determined with the initial treatment using a thermocouple psychrometer (model SC-10, Decagon Devices Inc., Pullman, WA.) equipped with a nanovoltmeter

(model NT-3, Decagon Devices Inc.). Water potential was calculated against a sodium chloride standard curve.

Plants were harvested at maturation stage when 80% of onions had soft pseudostems. Bulb and leaf FW were measured at harvest. Bulbs were dried at ambient greenhouse temperature for one week before analyses.

PLANT ANALYSIS. The eight most uniform bulbs of each treatment were used for analysis. Each bulb was cut longitudinally into three 5 mm thickness wedges and the tissue from the eight bulbs combined. One wedge group was used to measure total pyruvic acids (TPY) and soluble solids content (SSC), a second wedge group was used for ACSOs and γ -glutamyl peptides (γ GPs) analysis, and a third wedge group was used to calculate bulb sodium (Na^+), chloride (Cl^-), total sulfur (S) and sulfate (SO_4^{-2}).

SOLUBLE SOLIDS CONTENT AND TOTAL PYRUVIC ACID. Eight bulb wedges were squeezed in pneumatic press (Univ. Georgia design). A few drops of the fresh juice were placed on a refractometer (Kernco, Tokyo, Japan) to measure SSC. Generally, pyruvic acid, a decomposition product of S-alk(en)yl cysteine sulfoxides hydrolysis, is used to indicate gross flavor intensity (Schwimmer and Weston, 1961). Pungency of the onion was determined by the method of Randle and Bussard (1993). Total pyruvic acid was measured with 1 mL of juice diluted 40 fold, reacted with 1 mL 2,4-dinitrophenyl hydrazine, and put into a 37 °C water bath for 10 min. Five mL of 0.6 N sodium hydroxide was then added, and the solution read on a Spectronic 21D spectrophotometer (Milton Roy, Rochester, N.Y.) at 420 nm. Pyruvic acid content was quantified against a sodium pyruvate standard curve.

SULFUR AND ION ANALYSIS. The bulb wedges were dried in an oven (Linberg Blue, Asheville, N.C.) at 65 °C for 3 days. Dried tissue was ground through a 0.5 mm screen using a Cyclotec Mill (model 1093, Tector, Hoganas, Sweden). Approximately 0.25 g dried of tissue was mixed with 0.1 g of vanadium pentoxide accelerant (Leco corp.). Total bulb sulfur (S) was determined by a Leco 232 Sulfur determinator (Leco corp., St. Joseph, Minn.).

Bulb SO_4^{2-} concentration was measured by anion analysis through high performance liquid chromatography (HPLC). 0.25 g of ground tissue was dissolved with 50 mL of HPLC grade water in 125 mL Erlenmeyer flasks. The suspension solutions were shaken for 30 min at 150 rpm. The solution was filtered through 0.22 μm nylon syringe filters (Fisher Scientific, Pittsburg, Pa.) into 1 mL plastic vials (National Scientific Company, Lawrenceville, Ga). Samples were run on a Waters 2690 Separations Module with a Waters 432 Conductivity Detector (Waters Corp., Milford, Mass.). Forty μL of the extract solution were injected into an IC-PAK Anion HR column linked to an IC-PAK Anion Guard Pak (Waters corp.). Column temperature was set at 30 °C and an isocratic sodium borate-gluconate eluent was used at a flow rate of 1 $\text{mL} \cdot \text{min}^{-1}$. Peaks were quantified and integrated on Millennium Chromatography Software (Version 3.05, Waters corp.). Quantification was performed using 10 ppm sodium sulfate as an external standard.

Sodium was measured by cation analysis through HPLC. 0.1 g of oven-dried, ground tissue was ashed at 500 °C for 4 hr. 10 mL of HPLC grade water was added to the ash and shaken at 75 rpm for 1 hr. The extracts were filtered through a 0.22 μm nylon syringe filter (Fisher Scientific, Pittsburg, PA.) into 1 mL plastic vials (National Scientific

Company, Lawrenceville, Ga). Samples were run on a Waters 2690 Separations Module with a Waters 432 Conductivity Detector (Waters Corp.). Forty μL of extract solution were injected into an IC-PAK Cation HR column, linked to an IC-PAK Cation Guard Pak (Waters corp.). Column temperature was set at 30 °C, and 0.1 mmolar EDTA mixed 189 μL nitric acid eluent was used at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. Peaks were quantified and integrated on Millennium Chromatography Software (Version 3.05, Waters corp.).

Chloride amount was measured according to Rieger and Litvin (1998). Chloride was measured with a solid-state electrode (Fisher Scientific, Pittsburgh, PA), using a silver-silver chloride, double junction reference electrode (Fisher Scientific, Pittsburgh, PA), and a corning 350 ion analyzer (Corning Inc., NY). 0.5 g of dried-ground tissue was dissolved in 20 mL of 0.5 N HNO_3 for 10-15 min. Concentrations were measured 1 min after the electrode was inserted.

ACSOS AND PEPTIDE INTERMEDIATES. The content of ACSOs and γGPs from onion bulbs was measured according to Randle (2000). Fresh wedges were weighed and were extracted twice in 12:3 methanol/water (5 $\text{mL}\cdot\text{g}^{-1}$ FW) and then once in 12:3 ethanol/water solution (5 $\text{mL}\cdot\text{g}^{-1}$ FW). One mL of γ -glutamyl glutamic acid (γG ; 0.2 $\text{mg}\cdot\text{g}^{-1}$ FW.), and ($\pm$)-S-butyl-cysteine sulfoxide (BCSO; 1.0 $\text{mg}\cdot\text{g}^{-1}$ FW.) were used as internal standards and added into 15 mL of the combined extracts (1.0 g FW equivalent).

The solutions were dried by forced air and redissolved in 1 mL of deionized water. 0.5 mL of rehydrated solution was placed on a 10 x 40 ion exchanged column (Bio-Rad, Hercules, CA.) with 3 mL Dowex 1 x 8 resin (200 to 400 mesh; Bio-Rad). Fractionation of the samples was done using 0.1, 0.2, 2.0, and 5.0 M acetic acid. The 0.1 and 2.0 M fractions contained the ACSO and γGP compounds, respectively, and were dried by

forced air. The fractions were rehydrated with 1 mL deionized water, and 100 μ L solution was placed in a 1.5 mL microcentrifuge vial and vacuumed dry using a Labconco Centrivap Concentrator (Kansas City, Mo.). 250 μ L of a ethanol: triethylamine (TEA): deionized water (1:1:1) solution was added and then dried again. Samples were then derivatized by adding 100 μ L 7:1:1:1 ethanol: TEA: phenylisothiocyanate (PITC): deionized water. Vials were immediately flushed with nitrogen, capped and stored at room temperature for 19 min. The derivatizing samples were dried under vacuum. Dried samples were redissolved in 1 mL of 7:2 deionized water: acetonitrile and transferred to 1.5 mL glass vials before HPLC analysis.

Samples were analyzed by a Waters 2690 separator module (Waters corp.) with a 996 photodiode array detector (Waters corp.). A forty μ L sample was injected into a 5 μ m, 250 x 4.6 mm column (Spheri-5 RP-18; Applied biosystems, Foster City, CA.) fitted with a 15 x 3.2 mm, 7 μ m guard column (RP-18 Newgard; Applied biosystems) for separation. Column temperature was maintained at 30 °C. Eluents were A) aqueous acetonitrile (60%), B) 0.14 M sodium acetate with 0.05% TEA buffered to pH 6.35 using glacial acetic acid. All eluents were filtered through 0.45 μ m nylon filters (Millipore, Molsheim, France). Flow rate was 1 mL $\cdot$ min⁻¹. The eluent gradient was 15 % A for 1.1 min, 15-45 % A for next 21.1 min, 45-100 % A over 1 min, and 100 % A for 14 min. The gradient was returned to the initial 15 % A and 85 % B over 1 min. and the column was conditioned and equilibrated for 12.9 min before the next sample was injected.

STATISTICAL ANALYSIS. Data were subjected to analysis of variance (ANOVA) and linear and polynomial regression procedures using SAS statistical software (Version 8.2, SAS, Cary, NC.).

Results and Discussion

PLANT PHYSICAL RESPONSE. Generally plants react to salinity by a reduction in growth. Onion plant growth was visibly affected by increasing NaCl concentrations in the nutrient solutions within one week. Onion growth was more stunted at higher NaCl concentration. Onion roots were smaller. The size of older leaves was much reduced, followed by wilting and necrosis at 125mM NaCl concentration. At 100 mM NaCl concentration, tips were chlorotic and curly. At 75 and 50 mM NaCl concentration, older leaves showed light yellow coloration but root length was longer at 50 mM than at 75 mM. There was no salt toxicity shown at 25 mM NaCl compared to control. Due to the long-term nature of the experiment, onions grown at the highest NaCl did not survive. Therefore, no data of 125 mM NaCl treatment were available.

BULB AND LEAF FRESH WEIGHT. Increasing NaCl concentrations reduced bulb ($P \leq 0.001$) and leaf FW ($P \leq 0.001$), respectively (Table 1). Bulb FW decreased linearly (Bulb FW = $356.124 - 3.129 \text{ NaCl}$, $r^2 = 0.93$) from 350 g per bulb to 44.8 g per bulb as NaCl levels increased. Leaf FW also decreased linearly with increased NaCl concentrations (Leaf FW = $171.505 - 1.514 \text{ NaCl}$, $r^2 = 0.91$) and ranged from 156 g per bulb to 21.6 g per bulb. High levels of NaCl have been shown to restrict onion growth, which decreases bulb yield (Malik et al., 1978). Little change was noted between plants grown in the control and 25mM NaCl treatments. Low concentrations of Na^+ and Cl^- are essential for onion to regulate stomatal movement.

SODIUM AND CHLORIDE CONTENT. Onion is classified as salt-sensitive with a low salt tolerance threshold ($\text{EC} = 1.2 \text{ dS/m}$)(Mass and Hoffman, 1977) which is

equivalent to $\sim 12 \text{ mM NaCl} \cdot \text{L}^{-1}$ (Marschner, 1995). As salinity increased in the solutions, sodium and chloride increased in bulbs. Bulb sodium ($P \leq 0.001$) and chloride ($P \leq 0.001$) content were significantly increased with increasing NaCl concentrations, respectively (Table 1). The relationships were linear (Bulb Na = $-0.0371 + 0.0262 \text{ NaCl}$, $r^2 = 0.75$; and Bulb Cl = $-1.6735 + 0.0987 \text{ NaCl}$, $r^2 = 0.80$). While NaCl increased in the external medium, the onions possibly accumulated those ions to balance internal osmotic potential. Chloride is more of an indicator than sodium because it is readily taken up by plants, and it is stored in plants. The chloride content of onion was greater than sodium, which was in agreement with reports of many salt-sensitive species, such as maize, cress, sunflower, pepper, and bean, have higher chloride than sodium in their leaves (Alam, 1999). In most plants, K^+ is thought to be responsible for changes of turgor pressure in the guard cells during stomatal movement. To adjust K^+ in vacuoles, counteranions, such as Cl^- or malate⁻² are used (Marschner, 1995). However, in onion, starch is lacking in the guard cells, and only Cl^- accumulates to compensate K^+ charges for stomatal regulation (Schnabl and Ziegler, 1977). Na^+ stress suppresses plant growth through a variety of mechanisms. High Na^+ concentrations in the medium cause greater negative osmotic potential, which inhibited water uptake by citrus (Lea-Cox and Syvertsen, 1993). In addition, high Na^+ concentration disturbed metabolism in soybean (Nonami et al., 1995). Furthermore, high Na^+ content interrupted the absorption of other cations in tomato (Al-Karaki, 2000). Generally, the Na^+ content in plants is $\sim 1 \text{ mg/g}$ on a dry weight basis. In our study, sodium accumulation was significantly higher as more NaCl was supplied. Therefore, the symptoms of Na^+ toxicity were easily observed.

SOLUBLE SOLIDS CONTENT AND TOTAL PYRUVATE. According to ANOVA, bulb SSC was not affected by increasing NaCl levels ($P = 0.07$)(Table 2). Bulb pungency, as measured by TPY, was affected by NaCl concentrations ($P \leq 0.001$) (Table 2). The response of TPY to NaCl levels was quadratic ($TPY = 6.3152 - 0.0532 \text{ NaCl} + 0.0006976 \text{ NaCl}^2$, $R^2 = 0.71$). Flavor intensity decreased from $6.25 \mu\text{mol}\cdot\text{g}^{-1}$ FW to $5.37 \mu\text{mol}\cdot\text{g}^{-1}$ FW and increased to $8.19 \mu\text{mol}\cdot\text{g}^{-1}$ FW. Flavor intensity differences are perceivable at approximately $1 \mu\text{mole}$ increments (Wall and Corgan, 1992; personal observation).

TOTAL BULB SULFUR AND SULFATE. Sulfur is the major element to affect onion flavor intensity (Randle and Lancaster, 2002). Previous studies have found that different levels of S fertility can influence the quantity of precursors. Therefore, sulfur accumulation in onions will result in changing onion flavor intensity (Randle, 1992). Plants are able to take up sulfur as SO_4^{-2} , which can be reduced to sulfide and then incorporated into cysteine. Through cysteine, organic sulfur compounds are metabolized into methionine, glutathione, and other compounds (Leustek et al., 2000). Lancaster and Boland (1990) indicated that various S-compounds are intermediates of the flavor biosynthetic pathway. Hence, factors which affect S uptake or influence the S metabolic activities are important to onion flavor development. According to ANOVA, total bulb S responded significantly to increasing NaCl concentrations ($P = 0.03$). A significant quadratic decrease and subsequent increase was found for total bulb S with increasing NaCl levels (Bulb S = $5.0847 - 0.0311 \text{ NaCl} + 0.00018857 \text{ NaCl}^2$, $R^2 = 0.46$) (Table 2). Bulb S ranged from $5.04 \text{ mg}\cdot\text{g}^{-1}$ DW with no NaCl to $3.69 \text{ mg}\cdot\text{g}^{-1}$ DW at 75 mM NaCl. Another fate of SO_4^{-2} is that it can be stored in the vacuole for later incorporated into

organic-S compounds. In this study, bulb SO_4^{-2} was significantly influenced by increasing NaCl levels ($P = 0.05$). A quadratic increase was found for bulb SO_4^{-2} with increasing NaCl levels (Bulb $\text{SO}_4^{-2} = 0.6516 + 0.009 \text{ NaCl} + 0.00000571 \text{ NaCl}^2$, $R^2 = 0.30$) (Table 2). The amount of bulb SO_4^{-2} increased from $0.68 \text{ mg}\cdot\text{g}^{-1}$ DW with no NaCl to $1.76 \text{ mg}\cdot\text{g}^{-1}$ DW at 100 mM NaCl concentration. Randle et al., (1999) indicated that organic-S could be estimated by subtracting bulb SO_4^{-2} from bulb S. The amount of organic-S was significantly affected by NaCl treatments as well ($P = 0.01$) and the response was similar to the bulb S, (Organic-S = $4.4277 - 0.0406 \text{ NaCl} + 0.0001834 \text{ NaCl}^2$, $R^2 = 0.55$) (Table 2). The amount of organic-S ranged from $4.36 \text{ mg}\cdot\text{g}^{-1}$ DW to $2.16 \text{ mg}\cdot\text{g}^{-1}$ DW. Therefore, as NaCl increased, lower amounts of S accumulated in the bulb and a greater percentage of S remained as SO_4^{-2} .

FLAVOR PRECURSORS AND INTERMEDIATES. To understand the influence of NaCl on onion flavor development and quality, total and individual flavor precursors and their related peptide intermediates were measured. Different flavor precursors result in various flavor sensations. Thus, changing the concentrations or ratios of individual flavor precursors can affect flavor quality of the onion (Randle et al., 1994).

Total flavor precursors were significantly affected by NaCl concentrations ($P = 0.001$). Total ACSO concentrations ranged from $2.45 \text{ mg}\cdot\text{g}^{-1}$ FW to $3.32 \text{ mg}\cdot\text{g}^{-1}$ FW. The response of total ACSO to NaCl levels was a quadratic decrease then increase (ACSOs = $2.5204 - 0.0117 \text{ NaCl} + 0.0001898 \text{ NaCl}^2$, $R^2 = 0.62$) (Table 3). This result was similar to the TPY curve and could be expected because pyruvic acid should be produced proportionally to the amounts of ACSOs (Schwimmer and Weston, 1961).

MCSO was influenced significantly by NaCl concentrations and was found in highest concentration among the individual flavor precursors ($P \leq 0.001$). The concentration of MCSO ranged from $1.49 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ to $2.15 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ and the response was quadratic to increased NaCl levels ($\text{MCSO} = 1.5204 - 0.0038 \text{ NaCl} + 0.00009446 \text{ NaCl}^2$, $R^2 = 0.59$) (Table 3). MCSO was found to accumulate at high levels when onions were grown with low S fertility (Randle et al., 1995), with high sodium selenate (Kopsell and Randle, 1999), and with high N fertility (Randle, 2000). The thiosulfinates that result from decomposition of MCSO cause a fresh onion and cabbage-like taste (Randle et al, 1994). Therefore, higher NaCl concentration should enhance above flavors.

NaCl also affected concentrations of 1-PRENCISO ($P = 0.01$). The amounts of 1-PRENCISO ranged from $0.69 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ to $0.90 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$. 1-PRENCISO is a quadratic trend with increasing NaCl concentrations ($1\text{-PRENCISO} = 0.8558 - 0.0046 \text{ NaCl} + 0.00005214 \text{ NaCl}^2$, $R^2 = 0.34$) (Table 3). The decomposition of 1-PRENCISO produces the lachrymatory factor which results in mouth burning and tearing sensations (Randle et al., 1994).

PCSO was found in the lowest concentrations among the individual ACSOs and was affected significantly by NaCl concentrations ($P = 0.05$). The concentrations of PCSO ranged from $0.10 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ to $0.27 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$, and the response was quadratic ($\text{PCSO} = 0.1776 - 0.004 \text{ NaCl} + 0.00004826 \text{ NaCl}^2$, $R^2 = 0.40$) (Table 3). The decomposition of PCSO produces thiosulfinates that impart fresh onion and chive-flavor sensations (Randle et al., 1994).

NaCl dissolved in nutrient solutions caused more negative external water potential which resulted in an increase of total ACSOs in onions. Similar results were reported when onions were grown at lower water potential conditions, which resulted in increasing total pyruvate (Freeman and Mossadeghi, 1973). In our study, MCSO was found in highest average concentrations among three ACSOs in response to increasing NaCl levels. MCSO averaged about 60 % of the total ACSOs, 1-PRENCISO was about 33 %, and PCSO was maintained about 5 % of the ACSOs under saline conditions. From 25 mM to 75 mM NaCl levels, MCSO changed little, but bulb FW was significantly affected. Therefore, NaCl had a more negative effect on onion growth than it did on flavor at these concentrations. However, at 100 mM NaCl both MCSO and bulb FW changed dramatically, indicating the severity of the NaCl condition.

Two measurable intermediates in the ACSO synthesis were also affected by NaCl concentration (Table 3). The concentration of 2-carboxypropyl glutathione (2-CARB) was significantly influenced by increasing NaCl levels ($P = 0.002$). The concentration of 2-CARB ranged from $0.48 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ to $0.29 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$, and the response was quadratic ($2\text{-CARB} = 0.4754 - 0.0005924 \text{ NaCl} - 0.00001329 \text{ NaCl}^2$, $R^2 = 0.62$) (Table 3). A decrease in 2-CARB would support the result that more S was metabolized through the MCSO pathway, a result reported by Randle (2000). 2-CARB is not thought to be a part of the MCSO biosynthetic pathway. Gamma-glutamyl propenyl cysteine sulfoxide (γ GPRECSO) was also significantly affected by increasing NaCl concentrations ($P = 0.05$). The amounts of γ GPRECSO ranged from $0.63 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$ to $1.17 \text{ mg} \cdot \text{g}^{-1} \text{ FW}$, with a quadratic response to increasing NaCl concentrations ($\gamma\text{GPRECSO} = 1.1154 + 0.00338 \text{ NaCl} - 0.00007869 \text{ NaCl}^2$, $R^2 = 0.37$) (Table 3). γ GPRECSO concentrations were higher than 2-CARB when

compared at the same NaCl levels. Similar results were found when onions were grown with increased S and N solution, respectively (Randle et al., 1995; Randle, 2000). At severe NaCl stress, 2-CARB and γ GPRECSO were at relatively low concentrations relative to ACSO concentration which suggested S was rapidly metabolized through the biosynthetic pathway. Theoretically, the response of γ GPRECSO and 1-PRENCISO to increasing NaCl concentrations would be similar because γ GPRECSO is the penultimate peptide in the synthesis of 1-PRENCISO (Lancaster and Boland, 1990). However, in this study the quadratic response of γ GPRECSO and 1-PRENCISO had moderated negative correlation ($r = -0.61$). In addition, γ GPRECSO is thought to be a sink for S in the flavor biosynthetic pathway (Randle and Lancaster, 2002); therefore, a similar trend of bulb S and γ GPRECSO would be expected. However, this association was also poor ($r = 0.5$).

In this experiment, we investigated how adding NaCl in nutrient solutions influenced onion flavor development and intensity. As millions of irrigation lands are subjected to salinization, it is necessary to understand the interaction between onion flavor synthesis and NaCl accumulation. NaCl did affect onion physiology as reflected in bulb FW, sodium and chloride accumulation, total ACSO accumulation, the concentration of individual flavor precursors and their peptide intermediates when concentrations were above 25mM. Higher NaCl concentration resulted in lower concentration of γ GP intermediates, and this led to synthesize more MCSO than 1-PRENCISO. Flavor intensity and quality changes would result from shifts in precursors concentration and composition. The mechanism of changes of S metabolism under various conditions is still unsure and needs further investigation. Although onion could survive at concentrations up to 100

mM NaCl, the reduction in growth would prevent onion production in extensively saline areas.

Literature Cited

Alam, S.M. 1999. Nutrient uptake by plants under stress conditions, p. 285-313. In: M. Pessarakli. Handbook of Plant and Crop Stress. Marcel Dekker, Inc. New York, NY.

Al-Karaki, G.N. 2000. Growth, water use efficiency, and sodium and potassium acquisition by tomato cultivars grown under salt stress. Journal of Plant Nutrition. 23:1-8.

Block, E. 1992. The organosulfur chemistry of the genus *Allium*—Implications for the organic chemistry of sulfur. Angew. Chemistry International. Edition. English. 31:1135-1178.

Bergmann, L. and H. Rennenberg. 1993. Glutathione metabolism in plants, p.109-123. In: L. J. De Kok, I. Stulen, H. Rennenberg, C. Brunold and W. Rauser (eds.). Sulfur Nutrition and Assimilation in Higher Plants. SPB Acad. Publ. The Hague, The Neth.

Epstein, E., J.D. Norlyn, D.W. Rush, W. Kigsbury, C.B. Kelly, G.A. Cunningham, and A.F. Wrona. 1980. Saline culture of crops: A genetic approach. Science 210:399-404.

Freeman, G.G. and N. Mossadeghi. 1970. Effect of sulphate nutrition of flavor components of onion (*Allium cepa* L.). J. Sci. Food Agric. 21:610-615.

Freeman, G.G. and N. Mossadeghi. 1973. Studies on relationship between water regime and flavor strength in watercress (*Rorippa nasturtium-aquaticum* (L) Hayek), cabbage (*Brassica oleracea capitata*) and onion (*Allium cepa*). J. Hort. Sci. 48:365-378.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. 347:4-32.

Kopsell, D.A. and W.M. Randle. 1999. Selenium affects the S-alk(en)yl cysteine sulfoxides among short-day onion cultivars. J. Amer. Sci. Hort. Sci. 124:307-311.

Lancaster, J.E. and M.J. Boland. 1990. Flavor biochemistry, p. 33-72. In: H.D. Rabinowitch and J.L. Brewster (eds.) Vol. 3: Onions and Allied Crops. CRC Press, Boca Raton, FL.

Lancaster, J.E. and M.L. Shaw. 1989. γ -glutamyl peptides in the biosynthesis of S-alk(en)yl cysteine sulfoxides (flavor precursors) in *Allium*. Phytochemistry. 28:455-460.

Lancaster, J.E., M.L. Shaw, and W. M. Randle. 1998. Differential hydrolysis of alk(en)yl cysteine sulfoxides by alliinase in onion macerates: Flavor implications. J. Sci. Food Agric. 78:367-372.

Leustek, T., M.N. Martin, J-A. Bick, and J.P. Davies. 2000. Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51:141-165.

Lea-Cox, J.D. and J.P. Syvertsen. 1993. Salinity reduces water use and Nitrate-N-use of citrus. *Annals of Botany.* 72:47-54.

Maas, E.V. and G.J. Hoffman. 1977. Crop salt tolerance-Current assessment. *J. Irrig. Drainage. Am. Soc. Civ. Eng.* 103:115-134.

Malik, Y.S., K. Singh, and M.L. Pandita. 1982. Effect of salinity on quality of bulb and chemical composition of leaves in onion (*Allium cepa* L.) varieties. *Haryana J. Hort. Sci.* 11:226-230.

Marchner, H. 1995. Mineral Nutrition of Higher Plants. Second ed. Academic Press. London, UK.

Noctor, G., ACM. Arisi, L. Jouanin, K.J. Kunert, H. Rennenberg, and C.H. Foyer. 1998. Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. *J. Exp. Bot.* 49:623-647.

Nonami, H., K. Tanimoto, A. Tabuchi, T. Fukuyama, and Y. Hashimoto. 1995. Salt stress under hydroponic conditions causes changes in cell wall extension during growth. *Acta Horticulturae*. 396:91-98.

Oldeman, L.R., R.T.A. Hakkeling, and W.G. Sombroek. 1991. World map on the status of human induced soil degradation. Soil Reference and Information Center. Wageningen, pp. 35.

Palif, G. 1960. Seed germination of crop plants in saline media. *Novenytemnles* 9:359-368.

Platenius, H. and J.E. Knott. 1941. Factors affecting onion pungency. *J. Agric. Res.* 62:371-379.

Randle, W.M. 1992. Onion germplasm interacts with sulfur fertility for plant sulfur utilization and bulb pungency. *Euphytica*. 59:151-156.

Randle, W.M. 2000. Increasing nitrogen concentration in hydroponic solution affects onion flavor and bulb quality. *J. Amer. Hort. Sci.* 125:254-259.

Randle, W.M. and M.L. Bussard. 1993. Streamlining onion pungency analyses. *Hort. Sci.* 28:60.

Randle, W.M., E. Block, M.H. Littlejohn, D. Putman, and M.L. Bussard. 1994. Onion (*Allium cepa* L.) thiosulfinates respond to increasing sulfur fertility. J. Agric. Food Chem. 42:2085-2088.

Randle, W.M., and J.E. Lancaster. 2002. Sulphur compounds in alliums in relation to flavor quality, p. 329-356. In: H. D. Rabinowitch and L. Currah (eds.). Allium crop science: Recent advances. CAB International, Wallingford, UK.

Randle, W.M., D.E. Kopsell, D.A. Kopsell, and R.L. Snyder. 1999. Total sulfur and sulfate accumulation in onion is affected by sulfur fertility. Journal of Plant Nutrition. 22:45-51.

Randle, W.M., J.E. Lancaster, M.L. Shaw, K.H. Sutton, R.L. Hay, and M.L. Bussard. 1995. Quantifying onion flavor compounds responding to sulfur fertility—Sulfur increases levels of alk(en)yl cysteine sulfoxides and biosynthetic intermediates. J. Am. Soc. Hort. Sci. 120:1075-1081.

Rennenberg, H. 1982. Glutathione metabolism and possible biological roles in higher plants. Phytochemistry. 21:2771-2781.

Rennenberg, H. 1984. The fate of excess sulfur in higher plants. Annu. Rev. Plant Physiology. 35:121-153.

Rieger, M. and P. Litvin. 1998. Ion selective electrodes for measurement of sodium and chloride in salinity experiment. *Journal of Plant Nutrition*. 21 (2):205-215.

Ruiz, J.M. and E. Blumwald. 2002. Salinity-induced glutathione synthesis in *Brassica napus*. *Planta*. 214:965-969.

Schmidt, A. and K. Jäger. 1992. Open questions about sulfur metabolism in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:325-349.

Schwimmer, S. and W.J. Weston. 1961. Enzymatic development of pyruvic acid in onion as a measure of pungency. *J. Agri. Food Chem.* 9:301-304.

Wall, M.M. and J.N. Corgan. 1992. Relationship between pyruvate analysis and flavor perception for onion pungency determination. *HortScience*. 27:1029-1030.

Table 1 Effects of increasing NaCl concentration on mean leaf and bulb FW and on Na⁺ and Cl⁻ content of ‘Granex 33’ onion (*Allium cepa* L.) bulbs.

NaCl	Leaf FW	Bulb FW	Bulb (Na)	Bulb (Cl)
(mM)	(g·plant ⁻¹)	(g·plant ⁻¹)	(mg·g ⁻¹ DW)	(mg·g ⁻¹ DW)
0	156.67	342.62	ND	0.23
25	152.12	306.00	0.72	1.66
50	104.87	191.87	1.12	2.53
75	43.55	94.02	1.92	4.44
100	21.62	44.87	2.64	9.20
Contrast ^z	L	L	L	L

^zSingle degree-of-freedom contrast linear (L), non detectable (ND)

P ≤ 0.001.

Table 2 Effects of increasing NaCl concentration on mean total pyruvate (TPY), soluble solids content (SSC), total bulb sulfur (S), total bulb sulfate (SO₄⁻²), and organic-S (O-S) of ‘Granex 33’ onion (*Allium cepa* L.) bulbs.

NaCl	TPY	SSC	Bulb (S)	Bulb (SO ₄ ⁻²)	Organic-S (OS)
(mM)	(μmol·g ⁻¹ FW)	(%)	(mg·g ⁻¹ DW)	(mg·g ⁻¹ DW)	(mg·g ⁻¹ DW)
0	6.3	8.8	5.04	0.68	4.36
25	5.4	8.0	4.52	0.74	3.79
50	5.9	8.8	4.03	1.51	2.52
75	5.6	9.3	3.69	1.06	2.63
100	8.2	8.5	3.92	1.76	2.16
Contrast ^z	Q**	NS	Q*	Q*	Q*

^zSingle degree-of-freedom contrast, linear (L) quadratic (Q) or not significant (NS).

** * P ≤ 0.001, 0.05 respectively.

Table 3 Effects of increasing NaCl concentration on mean flavor precursors (alk(en)yl cysteine sulfoxides, methyl cysteine sulfoxide, 1-propenyl cysteine sulfoxide, and propyl cysteine sulfoxide), and the intermediates (2-carboxypropyl glutathione and γ -Glutamyl propenyl cysteine sulfoxide) of ‘Granex 33’ onion (*Allium cepa* L.) bulbs.

NaCl	ACSO	MCSO	1-PRENC SO	PCSO	2-CARB	γ GPRESSO
(mM)	(mg·g ⁻¹ FW)	(mg·g ⁻¹ FW)	(mg·g ⁻¹ FW)	(mg·g ⁻¹ FW)	(mg·g ⁻¹ FW)	(mg·g ⁻¹ FW)
0	2.51	1.49	0.85	0.17	0.48	1.17
25	2.45	1.51	0.81	0.13	0.44	1.02
50	2.46	1.67	0.69	0.10	0.43	1.14
75	2.59	1.60	0.87	0.12	0.35	0.99
100	3.32	2.15	0.90	0.27	0.29	0.63
Contrast ^z	Q***	Q***	Q**	Q*	Q**	Q*

^zSingle degree of freedom contrast, quadratic (Q) or not significant (NS).

*** ** * P ≤ 0.001, 0.01, 0.05 respectively.

**GROWTH, DEVELOPMENT, AND FLAVOR OF ALLIUM CEPA L.
(ONION): EFFECTS OF SEQUENTIAL ADDITIONS OF NACL STRESS¹**

¹ Chang, P.T. and W.M. Randle. To be submitted to the Journal of the American Society for Horticultural Science.

Abstract

Onion (*Allium cepa* L.) is classified as a salt-sensitive crop. Application of NaCl at an early stage decreases onion growth, but increases bulb pungency in the late developmental stage. The objective of this research was to study how sequentially adding NaCl during onion growth and development would affect flavor intensity and quality of 'Granex 33' onion. NaCl concentration (100 mM) was applied in nutrient solutions to onions starting 74 days before harvest at bi-week intervals in a greenhouse experiment. Bulb and leaf FW were measured at harvest. Bulbs were analyzed for soluble solids content (SSC), total pyruvate (TPY), bulb S and SO_4^{2-} accumulation, and flavor precursors and biosynthetic intermediates. As NaCl addition was delayed during the experiment, bulb and leaf FW increased linearly. While bulb S was affected significantly by sequentially addition of NaCl, bulb SO_4^{2-} was unaffected during the experiment, indicating that NaCl would inhibit accumulation of SO_4^{2-} as S pool. TPY was significantly affected as NaCl was added. When the total concentration of flavor precursors significantly changed in response to adding NaCl during the experiment, the concentration of methyl cysteine sulfoxide (MCSO) and 1-propenyl cysteine sulfoxide (1-PRENCISO) were quadratically changed. When NaCl was applied, changes in flavor biosynthetic pathway appeared to be associated with the growth stage.

Introduction

Onion (*Allium cepa* L.) is prized because of its unique flavor and its ability to season other foods. Onion flavor is dominated by sulfur compounds, which are known as S-alk(en)yl cysteine sulfoxides (ACSOs) (Block, 1992). Onion flavor is produced when

onion tissue is damaged or destroyed. Alliinase (EC 4.4.1.4) is then released to hydrolyze the ACSOs to form the lachrymatory factor and thiosulfinates which are responsible for tearing and onion flavors (Randle, 1997). The three flavor precursors found in onions which give rise to different characteristic flavors are methyl cysteine sulfoxide (MCSO), 1-propenyl cysteine sulfoxide (1-PRENCISO), and propyl cysteine sulfoxide (PCSO) (Block et al., 1992).

In the past decades, onion flavor and its development have attracted much attention. In addition to cultivar differences, growing environment and some mineral elements were identified as factors affecting onion flavor intensity and quality. These include sulfur fertility (Freeman and Mossadeghi, 1970), sulfate availability (Randle et al., 1993; Randle et al., 1995; and Randle et al., 1999), nitrogen concentration (Randle, 2000), selenium level (Kopsell and Randle, 1999), temperatures (Platenius and Knott, 1941), and water supply (Freeman and Mossadeghi, 1973). Historically, 1-PRENCISO was reported to accumulate in the highest concentration and to dominate onion flavor. However, under different environmental conditions such as lower sulfur, high temperature, or high nitrogen, MCSO became the dominant precursor. The above reports provide evidence that environmental factors have a dramatic impact on onion flavor and can alter onion flavor.

Though onion is classified as a salt sensitive crop, it can still survive at the electrical conductivity threshold of $EC = 1.2$ dS/m salt concentration (Maas and Hoffman, 1977). In previous work, onions were exposed to increasing levels of NaCl (up to 100 mM in a nutrient solution) and flavor was affected. MCSO became the dominant precursor as NaCl concentration increased (Chang, master thesis 2003). Onion is greatly

affected when exposed to salt in the early stage of growth and development (Wannamaker and Pike, 1987). Conversely as the plant grows and matures, it becomes more tolerant to salt exposure. The object of the experiment, therefore, was to investigate how sequentially adding NaCl during onion growth and development would affect final flavor quality and intensity at harvest.

Materials and Methods

PLANT CULTURE. Seeds of ‘Granex 33’ (Asgrow Seeds, Kalamazoo, Mich.) were planted on 10 Jan. 2002 in Grodan cubes (DK-2640, Hedenhusene, Denmark) and watered as needed. The seedlings were grown at day/night set point temperatures of 20/12 °C for 6 weeks under natural photoperiods and $\approx 34^\circ$ N latitude in a greenhouse. Seedlings were fertilized with 400 ml of Peters 20N-20P-20K soluble fertilizer (Scotts-Sierra Co., Marysville, OH) at a concentration of 200 mg·L⁻¹ weekly. Seedlings were then transplanted to 30 L plastic tubs (Rubbermaid, Inc., Wooster, OH) on 24 Feb. 2002. Ten uniform plants were placed in each tub lid. The tub was filled with 28 liters of a half-strength Hoagland’s solution (Hoagland and Arnon, 1950). Each solution contained 0.47 g·L⁻¹ Ca (NO₃)₂·4H₂O, 0.30 g·L⁻¹ KNO₃, 0.057 g·L⁻¹ NH₄H₂PO₄, 0.25 g·L⁻¹ MgSO₄·7H₂O, 0.08 mg·L⁻¹ CuSO₄·5H₂O, 0.02 mg·L⁻¹ H₂MoO₄·H₂O, 0.22 mg·L⁻¹ ZnSO₄·7H₂O, 1.81 mg·L⁻¹ MnCl₂·4H₂O, 2.86 mg·L⁻¹ H₃BO₃ and 10 mg·L⁻¹ Fe chelate (EDTA). The experimental design was a completely randomized design using four replications and six treatments dates when 100 mM NaCl was added. Compressed air was forced through airstones placed on the container bottom for solution aeration. Wire mesh was set on each lid to support the foliage.

Nutrient solutions were changed every two weeks and reestablished with new solutions. Deionized water was added to maintain 28 liters daily. On 22 Mar. 2002 (74 days before harvest), the first NaCl treatment was applied to one tub in each replication and maintained at that level until the experiment ended. Every two weeks thereafter NaCl was applied to one new tub in each replication. Five sequential NaCl adding treatments were applied at 74, 60, 46, 32, and 18 days, before harvest, respectively. As a control, no NaCl was applied to four tubs. The developmental stages associated with the sequential treatment dates were pre-bulbing at ~ 74 days, early bulbing at ~ 50 days, active bulbing at ~ 30 days and bulb maturation at ~ 7 days before harvest. At harvest, the roots and foliage were removed from the bulbs. The bulbs were cured at ambient greenhouse temperatures for one week before analyses.

The eight most uniform bulbs from each replication were used for analysis. Each bulb was cut longitudinally to three wedges, 5 mm thick. The combined tissue of each eight-bulb treatment replication was used for all analyses. One wedge group was used to measure total pyruvic acid (TPY) and soluble solids content (SSC), a second wedge group was used for the ACSOs and γ -glutamyl peptides (γ GPs) determination, and a third wedge group was used to calculate bulb total sulfur (S) and sulfate (SO_4^{2-}) content.

SOLUBLE SOLIDS CONTENT AND PYRUVIC ACID. Wedges from each eight-bulb group were juiced in a pneumatic press (Univ. Georgia design). Several drops of the fresh juice were placed on a hand-held refractometer (Kernco, Tokyo, Japan) to measure soluble solids content (SSC). Gross flavor intensity was measured using TPY (Randle and Bussard, 1993) by taking 1 ml of juice diluted 40-fold, reacting it with 2, 4-dinitrophenyl hydrazine, and incubating the solution at 37 °C in a water bath for 10 min.

Five ml of 0.6 N sodium hydroxide was then added, and then absorbance was measured on a Spectronic 21D spectrophotometer (Milton Roy, Rochester, N.Y.) at 420 nm.

Pyruvic acid content was quantified against a sodium pyruvate standard curve.

SULFUR AND SULFATE ANALYSIS. The bulb wedges were dried in an oven (Linberg Blue, Asheville, N.C.) at 65 °C for 3 days. Dried tissue was then ground through a 0.5 mm screen with a Cyclotec Mill (model 1093, Tector, Hoganas, Sweden).

Approximately 0.25 g of tissues was mixed with 0.1 g of vanadium pentoxide accelerant (Leco corp.). Total bulb S was determined by a Leco 232 Sulfur determinator (Leco corp., St. Joseph, Minn.).

Sulfate concentrations were measured using anion analysis through high performance liquid chromatography (HPLC). 0.25 g of ground tissue was dissolved with 50 ml of HPLC grade water in 125 ml Erlenmeyer flasks. Suspensions were shaken for 30 min at 150 rpm and then filtered through a 0.22 µm nylon syringe filter (Fisher Scientific, Pittsburg, Pa.) into 1 ml plastic vials. Samples were run on a Waters 2690 Separations Module using a Waters 432 Conductivity Detector (Waters Corp., Milford, Mass.). Forty µl of extract solution were injected into an IC-PAK Anion HR column, linked to an IC-PAK Anion Guard Pak (Waters corp., Milford, Mass.). Column temperature was set at 30 °C, and an isocratic sodium borate-gluconate eluent was used at a flow rate of 1 ml·min⁻¹. The solvent contained 16 g sodium gluconate, 18 g boric acid, 25 g sodium tetraborate and 250 ml glycerin in 1 L of water. Peaks were quantified and integrated on Millennium Chromatography Software (Version 3.05, Waters corp.). Quantification was performed using a 10 ppm concentration of the sodium sulfate as an external standard.

ACSOS AND PRECURSORS INTERMEDIATES. The total precursors and their intermediates were extracted twice in 12:3 methanol/water (5 ml·g⁻¹ fresh weight) and then once in a 12:3 ethanol/water solution (5 ml·g⁻¹ fresh weight). One ml of γ -glutamyl glutamic acid (γ G; 0.2 mg·g⁻¹ fresh wt.), and ($\pm$)-S-butyl-cysteine sulfoxide (BCSO; 1 mg·g⁻¹ fresh wt.) were used as internal standards and added to 15 ml of the combined extracts.

The combined solutions were dried by forced air and redissolved in 1 ml of deionized-distilled water. A 0.5 ml of rehydrated solution was placed into a 10 x 40 ion exchange column (Bio-Rad, Hercules, CA.) with 3 ml Dowex 1 x 8 resin (200 to 400 mesh; Bio-Rad), and 0.1, 0.2, 2.0, and 5.0 M acetic acid was used to separate the different fractions. The 0.1 M and 2.0 M fractions containing the ACSO and the γ GPs were collected and dried using forced air. Samples were derivatized according to Randle et al. (1995). 100 μ l of each solution was pipetted into 1.5 ml microcentrifuge vials and vacuum dried using a Labconco Centrivap Concentrator (Kansas City, Mo.). A 250 μ l of ethanol: triethylamine (TEA): deionized water (1:1:1) was added and dried again. Samples were then derivatized by adding 100 μ l of 7:1:1:1 ethanol: TEA: phenylisothiocyanate (PITC): deionized water. Vials were flushed with nitrogen immediately, capped and stored at room temperature for 19 min. The derivatized samples were then dried by vacuum. Dried samples were redissolved in 1 ml of 7:2 deionized water: acetonitrile and transferred to 1.5 ml glass vials before HPLC analysis.

A Waters 2690 separator module (Waters corp.) with a 996 photodiode array detector (Waters corp.) was used for analysis. A 5 μ m, 250 x 4.6 mm column (Spheri-5 RP-18; Applied biosystems, Foster City, CA.) fitted with a 15 x 3.2 mm, 7 μ m guard

column (RP-18 Newgard; Applied biosystems) was used for separation. Column temperature was maintained at 30 °C. The flavor compounds were detected at 254 nm.

Eluents were A) aqueous acetonitrile (60%), and B) 0.14 M sodium acetate with 0.05% TEA buffered to pH 6.35 using glacial acetic acid. All eluents were filtered through 0.45 µm nylon filters (Millipore, Molsheim, France). Forty µl of sample were injected into the column. A flow rate was used at 1 ml ·min⁻¹. The eluent gradient was 15 % A for 1.1 min, 15-45 % A for the next 21.1 min, 45-100 % A over 1 min, and 100 % A for 14 min. The gradient was returned to the initial 15 % A and 85 % B over 1 min. and the column was conditioned and equilibrated for 12.9 min before the next sample was injected.

STATISTIC ANALYSIS. Data were subjected to analysis of variance and linear and polynomial regression using SAS statistical software (Version 8.2, SAS, Cary, NC.).

Results and Discussion

BULB AND LEAF FRESH WEIGHT. Bulb and leaf FW responded significantly to the sequential addition of NaCl during onion growth and development ($P \leq 0.001$). Bulb FW increased linearly as the application of NaCl was delayed during plant growth (Bulb FW = $318.4761 - 52.9571 \text{ day}$, $r^2 = 0.91$, Fig. 5). A similar result was found for leaf FW. Leaf FW increased linearly as NaCl application was delayed (Leaf FW = $165.4226 - 27.6607 \text{ day}$, $r^2 = 0.92$, Fig. 5). Onions have been classified as a salt-sensitive crop and our results show that the duration of salt exposure is negatively correlated with growth.

SOLUBLE SOLIDS CONTENT AND TOTAL PYRUVIC ACID. Bulb SSC responded significantly to the sequential addition of NaCl during onion growth and development ($P \leq 0.001$). The overall response of SSC was quadratic ($\text{SSC} = 8.2107 + 0.9810 \text{ day} - 0.1696 \text{ day}^2$, $R^2 = 0.55$, Fig. 6). Soluble solids content increased to 46 days before harvest, and then decreased. Adding NaCl during the bulbing process caused the onions to have higher SSC than the control plants. Interestingly, the maximum SSC was found when NaCl was added prior to or during active bulbing. Randle and Bussard (1993) indicated the higher SSC in the mild-type onions was caused by the higher amounts of sucrose, glucose and fructose. Therefore, onion bulbs would be expected to be sweeter under NaCl stress compared to the control plants.

Gross flavor intensity as measured by TPY was statistically affected by sequential addition of NaCl ($P = 0.003$). The higher TPY content, the more intense the onion flavor is (Wall and Corgan, 1992). The response of TPY to sequentially adding NaCl during onion growth and development was cubic ($\text{TPY} = 5.5142 + 0.1129 \text{ day} - 0.005 \text{ day}^2 + 0.00005 \text{ day}^3$, $R^2 = 0.55$, Fig. 6). TPY decreased during early bulbing and then increased during active bulbing compared to the control plants.

TOTAL BULB SULFUR AND SULFATE. Lancaster and Boland (1990) indicated that S-compounds are intermediates of the flavor biosynthetic pathway. Randle (1992) reported that the amount of S in onions would change onion flavor intensity. Bulb S responded significantly to the sequential addition of NaCl during onion growth ($P \leq 0.001$). Bulb S increased quadratically as NaCl application was delayed during plant growth ($\text{Bulb S} = 5.1708 - 0.0383 \text{ day} + 0.0002 \text{ day}^2$, $R^2 = 0.76$, Fig. 7). Similarly, it was shown that bulb S decreased in response to increasing NaCl concentration (Chang,

master thesis 2003). As NaCl concentration increased from 0 mM to 100 mM, bulb S was decreased quadratically.

Bulb SO_4^{-2} accumulation was statistically unaffected by sequential addition of NaCl ($P = 0.81$). In addition, no meaningful trend was found for bulb SO_4^{-2} accumulation. In a previous study, bulb SO_4^{-2} response was quadratic to increasing NaCl concentrations (Chang, master thesis 2003). However, bulb SO_4^{-2} was not affected at high constant salt stress during plant growth which implied that either onions were able to efficiently metabolize SO_4^{-2} to organic S, or onions were inhibited to accumulate SO_4^{-2} as S pooled at high salt stress.

A previous study reported that organic-S could be estimated by subtracting bulb SO_4^{-2} from bulb S (Randle et al., 1999). NaCl concentration was found to influence organic-S accumulation significantly (Chang, master thesis 2003). In this study, organic-S was found to increase significantly with earlier addition of NaCl during plant growth ($P = 0.002$). A linear trend was established for organic-S accumulation when the addition of NaCl was delayed ($\text{Organic-S} = 3.8325 - 0.0244 \text{ day}$, $R^2 = 0.56$, Fig. 7). More organic-S accumulated during the later stage, which implied that more organic-S is synthesized during active bulbing.

FLAVOR PRECURSORS AND INTERMEDIATES. Total flavor precursors responded significantly to the sequential addition of NaCl during the growth and development of the onions ($P \leq 0.001$). The response of the precursors was quadratic ($\text{ACSOs} = 3.0687 - 0.0454 \text{ day} + 0.0005561 \text{ day}^2$, $R^2 = 0.58$, Fig. 8). In general, NaCl decreased ACSO content when compared to the control. The lowest ACSOs content occurred when NaCl was added following early-bulbing.

MCSO also responded significantly to the sequential addition of NaCl during onion growth and development ($P \leq 0.001$) and the trend was quadratic ($\text{MCSO} = 2.0255 - 0.0277 \text{ day} + 0.0003414 \text{ day}^2$, $R^2 = 0.41$, Fig. 8). MCSO accumulated in the highest concentration of the three onion flavor precursors. High MCSO was also found when onions were grown in lower SO_4^{-2} (Randle et al., 1995), high nitrogen (Randle, 2000), and high NaCl (Chang, master thesis 2003). In this study, MCSO and ACSOs had a similar trend ($r = 0.97$). As MCSO increased, more methyl thiosulfinates are produced, which gives onion a cabbage-like and fresh onion flavor (Randle, 1997).

1-PRENCISO responded significantly to the sequential addition of NaCl during onion growth and development ($P \leq 0.001$) and the response was quadratic ($1\text{-PRENCISO} = 0.8942 - 0.0163 \text{ day} + 0.0001939 \text{ day}^2$, $R^2 = 0.61$, Fig. 8). Enzymatic decomposition of 1-PRENCISO produces the lachrymatory factor which is responsible for tearing and mouth burning when the onion is cut and consumed (Randle, 1997).

Although PCSO changes were significant in response to sequential addition of NaCl ($P = 0.002$), the amounts of PCSO were the lowest (Fig. 8). However, no reliable trend was found between PCSO and sequential NaCl treatment. When PCSO is enzymatically hydrolyzed, a raw, fresh-onion flavor is produced (Randle, 1997).

2-Carboxypropyl glutathione (2-CARB), one of the two measurable intermediates in ACSO synthesis responded significantly to the sequential addition of NaCl ($P = 0.008$). 2-CARB responded quadratically to the sequential addition of NaCl ($2\text{-CARB} = 0.6004 + 0.0054 \text{ day} - 0.0001073 \text{ day}^2$, $R^2 = 0.52$, Fig. 9). An increase in 2-CARB was accompanied by an increase in 1-PRENCISO. This relationship should be expected because 2-CARB is an intermediate of 1-PRENCISO synthesis. γ -Glutamyl propenyl

cysteine sulfoxide (γ GPRESSO) is the other measurable intermediate. γ GPRESSO responded significantly to the sequential addition of NaCl ($P = 0.04$) and the response was quadratic (γ GPRESSO = $1.6574 + 0.0281 \text{ day} - 0.0004647 \text{ day}^2$, $R^2 = 0.34$, Fig. 9). Lancaster and Boland (1990) indicated that γ GPRESSO is the penultimate peptide in the synthesis of 1-PRESSO. In this study, the addition of NaCl after pre-bulbing causes much more γ GPRESSO to accumulate than 1-PRESSO. While γ GPRESSO does not directly contribute to onion flavor, Kopsell et al., (2002) reported that it is apparently converted to 1-PRESSO during maceration and subsequently hydrolyzed to form the LF.

The significant changes in bulb FW, bulb S and SO_4^{2-} , TPY, flavor precursors, and γ GPs with sequential addition of NaCl suggest that onion exposure to NaCl should be avoided, especially for long periods. Exposure to NaCl could stimulate flavor precursors metabolism within 5 weeks before harvest. However, adding NaCl too early decreases bulb FW and TPY. Changes in the flavor biosynthetic pathways as a result of NaCl exposure, therefore, depend on developmental stage.

Literature Cited

Block, E. 1992. The organosulfur chemistry of the genus *Allium*—Implications for the organic chemistry of sulfur. *Angew. Chemistry International. Edition. English.* 31:1135-1178.

Freeman, G.G., and N. Mossadeghi. 1970. Effect of sulphate nutrition of flavor components of onion (*Allium cepa* L.). J. Sci. Food Agric. 21:610-615.

Freeman, G.G., and N. Mossadeghi. 1973. Studies on relationship between water regime and flavor strength in watercress (*Rorippa nasturtium-aquaticum* (L) Hayek), cabbage (*Brassica oleracea capitata*) and onion (*Allium cepa*). J. Hort. Sci. 48:365-378.

Hoagland, D.R., and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. 347:4-32.

Kopsell, D.A., and W.M. Randle. 1999. Selenium affects the S-alk(en)yl cysteine sulfoxides among short-day onion cultivars. J. Amer. Soc. Hort. Sci. 124:307-311.

Lancaster, J.E., and M.J. Boland. 1990. Flavor biochemistry, p. 33-72. In: H.D. Rabinowitch and J. L. Brewster (eds.). Vol. 3: Onions and Allied Crops CRC Press, Boca Raton, FL.

Leustek, T., M.N. Martin, J-A. Bick, and J.P. Davies. 2000. Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:141-165.

Maas, E.V., and G.J. Hoffman. 1977. Crop salt tolerance-Current assessment. J. Irrig. Drainage. Am. Soc. Civ. Eng. 103:115-134.

Platenius, H., and J. E. Knott. 1941. Factors affecting onion pungency. *J. Agric. Res.* 62:371-379.

Randle, W.M. 1992. Onion germplasm interacts with sulfur fertility for plant sulfur utilization and bulb pungency. *Euphytica*. 59:151-156.

Randle, W.M. 1997. Onion flavor chemistry and factors influencing flavor intensity, p. 41-52. In: S. J. Risch and C. Ho (eds.). *Species: Flavor chemistry and antioxidant properties*. ACS Symposium Series 660, Washington, D.C.

Randle, W.M. 2000. Increasing nitrogen concentration in hydroponic solution affects onion flavor and bulb quality. *J. Amer. Hort. Sci.* 125:254-259.

Randle, W.M., and M.L. Bussard. 1993. Streamlining onion pungency analyses. *HortScience*. 28:60.

Randle, W.M., D.E. Kopsell, and D.A. Kopsell. 2002. Sequentially reducing sulfate fertility during onion growth and development affects bulb flavor at harvest. *HortScience*. 37:118-121.

Randle, W.M., D.E. Kopsell, D.A. Kopsell, and R. L. Snyder. 1999. Total sulfur and sulfate accumulation in onion is affected by sulfur fertility. *Journal of Plant Nutrition*. 22:45-51.

Randle, W.M., J.E. Lancaster, M. L. Shaw, K. H. Sutton, R. L. Hay, and M. L. Bussard. 1995. Quantifying onion flavor compounds responding to sulfur fertility—Sulfur increases levels of alk(en)yl cysteine sulfoxides and biosynthetic intermediates. *J. Amer. Soc. Hort. Sci.* 120:1075-1081.

Wannamaker, M.J., and L.M. Pike. 1987. Onion responses to various salinity levels. *J. Amer. Soc. Hort. Sci.* 112:49-52.

Fig. 5. Changes in bulb and leaf FW from mature ‘Granex 33’ bulbs when NaCl concentration was applied sequentially at 14 d intervals beginning 74 d before harvest. Bulb (●) and leaf (○) FW were decreased linearly (Bulb FW = $318.4761 - 52.9571 \text{ day}$, $r^2 = 0.91$), (Leaf FW = $165.4226 - 27.6607 \text{ day}$, $r^2 = 0.92$), respectively.

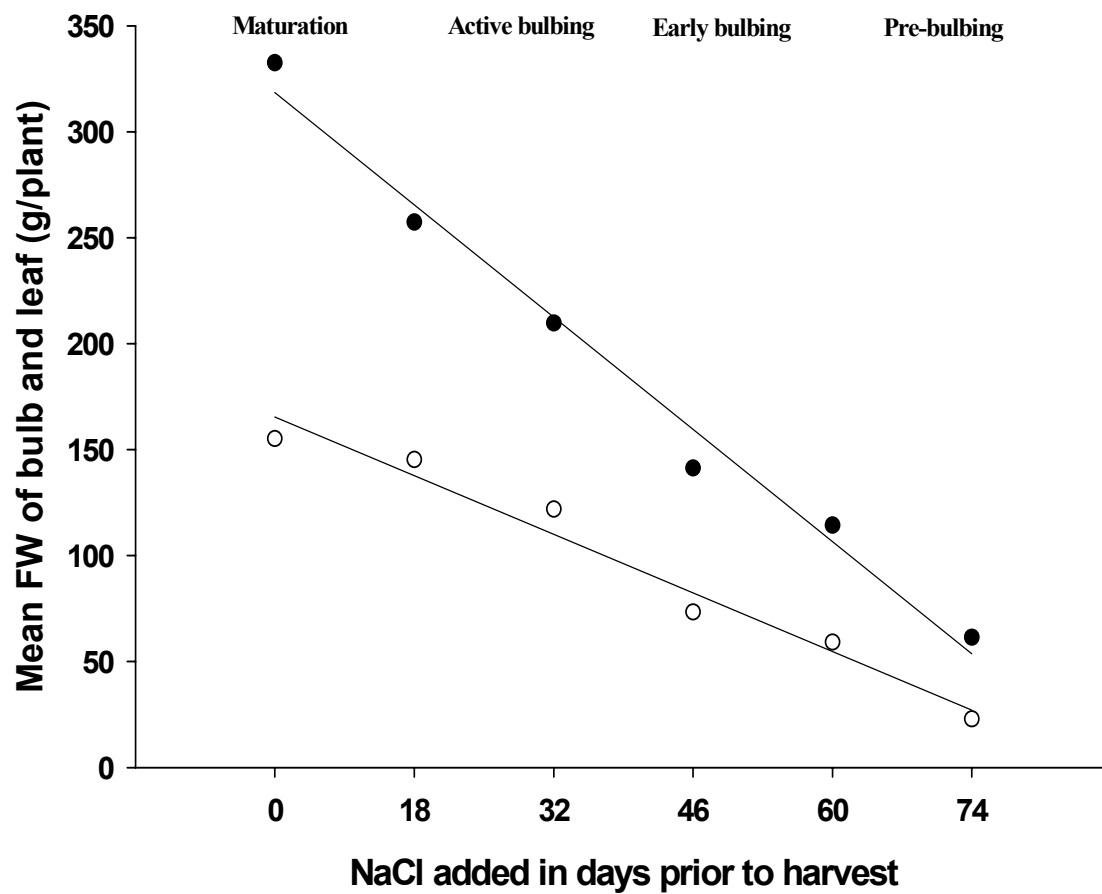


Fig. 6. Changes in soluble solids content (SSC) (●) and total pyruvate (TPY) (Δ) from mature ‘Granex 33’ bulbs when NaCl concentration was applied sequentially at 14 d intervals beginning 74 d before harvest. Soluble solids content responded quadratically ($SSC = 8.2107 + 0.9810 \text{ day} - 0.1696 \text{ day}^2$, $R^2 = 0.55$), while total pyruvate was response cubic ($TPY = 5.5142 + 0.1129 \text{ day} - 0.005 \text{ day}^2 + 0.00005 \text{ day}^3$, $R^2 = 0.55$).

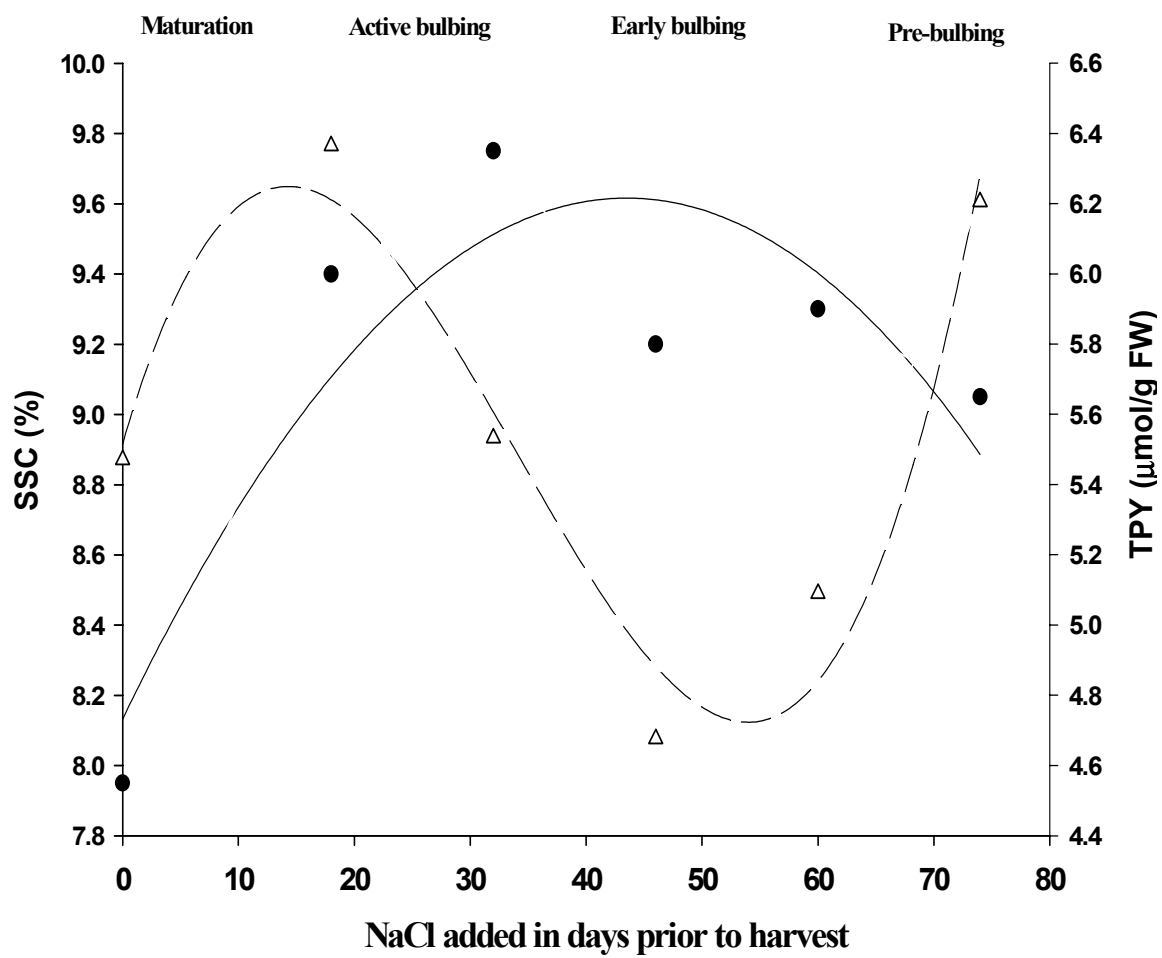


Fig. 7. Changes in bulb sulfur (S) (●), bulb sulfate (SO_4^{-2}) (▲), and organic-S (O-S) (□) from mature ‘Granex 33’ bulbs when NaCl concentration was applied sequentially at 14 d intervals beginning 74 d before harvest. The response of bulb sulfur was quadratic ($\text{Bulb S} = 5.1708 - 0.0383 \text{ day} + 0.0002 \text{ day}^2$, $R^2 = 0.76$), while bulb sulfate was not significant changed, organic-S was linear ($\text{O-S} = 3.8325 - 0.0244 \text{ day}$, $R^2 = 0.56$).

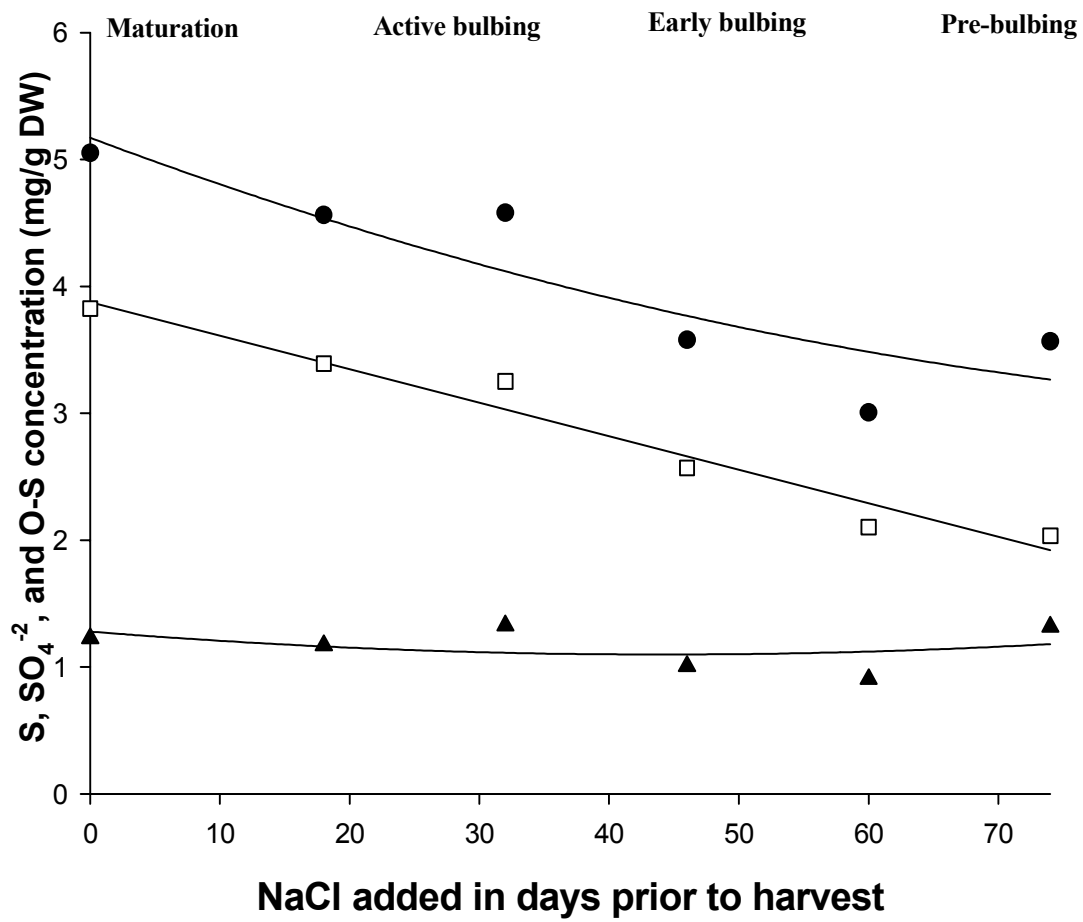


Fig. 8. Changes in alk(en)yl cysteine sulfoxides (ACSOs) (●), methyl cysteine sulfoxide (MCSO)(■), 1-propenyl cysteine sulfoxide (1-PRENCISO) (▲), and propyl cysteine sulfoxide (PCSO) (◆), from mature ‘Granex 33’ bulbs when NaCl was applied at 14 d intervals beginning 74 d before harvest. The response of ACSOs was quadratic ($ACSOs = 3.0687 - 0.0454 \text{ day} + 0.0005561 \text{ day}^2$, $R^2 = 0.58$), MCSO was quadratic ($MCSO = 2.0255 - 0.0277 \text{ day} + 0.0003414 \text{ day}^2$, $R^2 = 0.41$), 1-PRENCISO was also quadratic ($1-PRENCISO = 0.8942 - 0.0163 \text{ day} + 0.0001939 \text{ day}^2$, $R^2 = 0.61$), PCSO was unaffected.

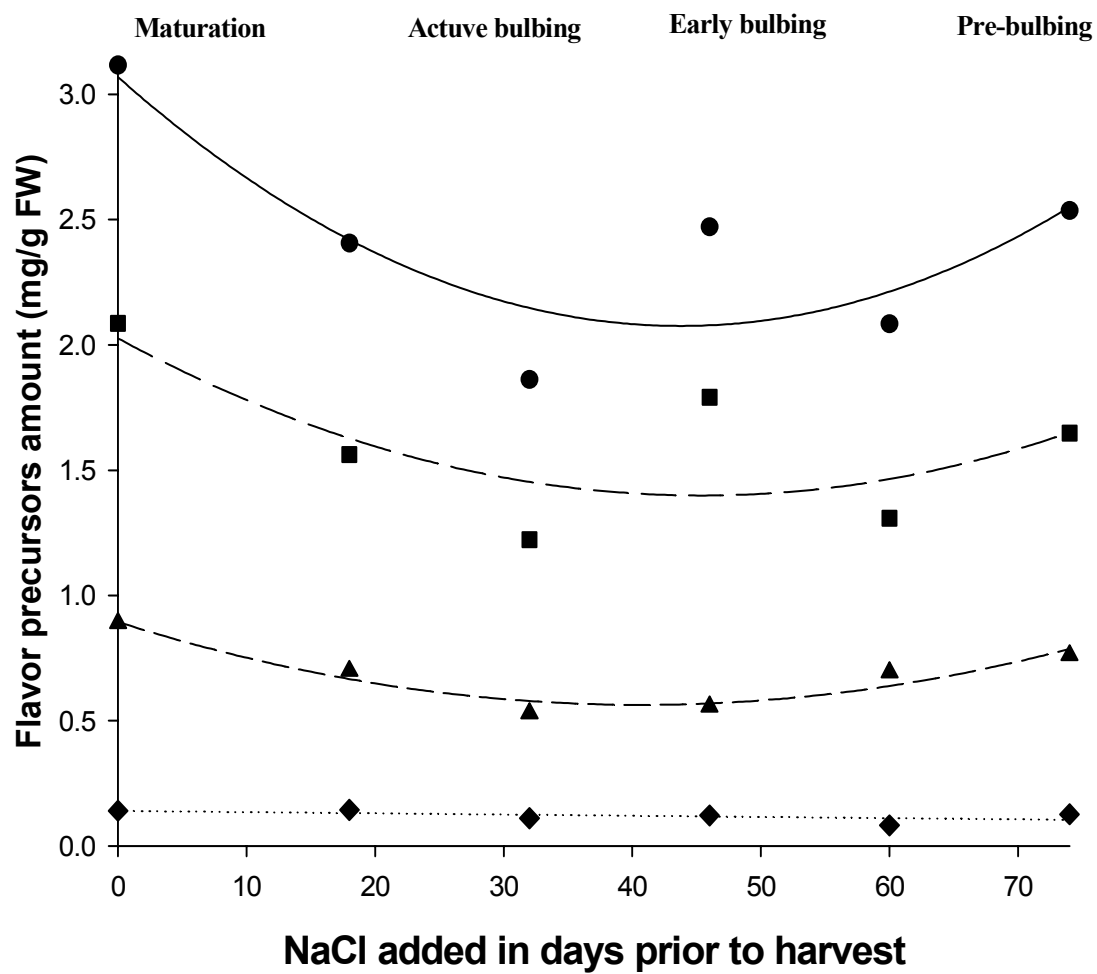
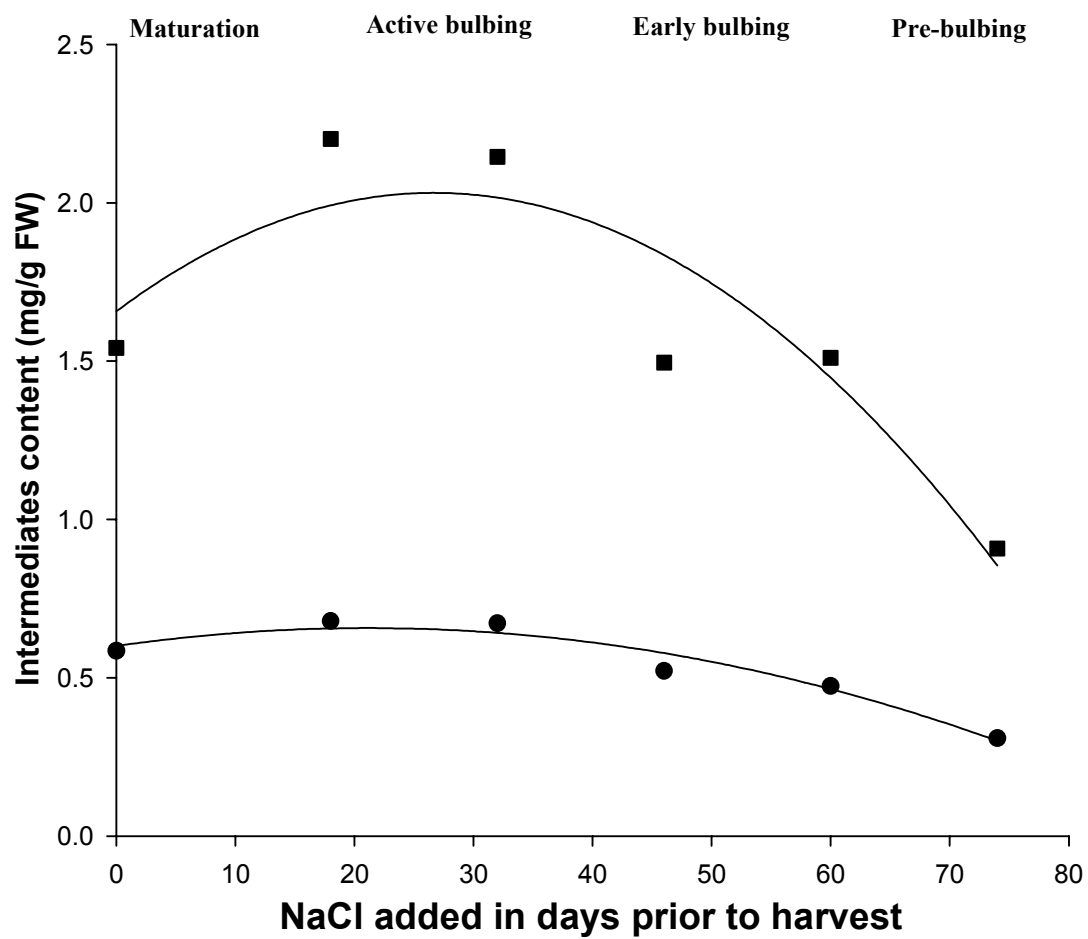


Fig. 9. Changes in 2 carboxypropylglutathione (2-CARB)(●) and γ -glutamyl propenyl cysteine sulfoxide (γ GPRENCSO)(■) from mature ‘Granex 33’ bulbs when NaCl was applied at 14 d intervals beginning 74 d before harvest. The response of 2-CARB was quadratically ($2\text{-CARB} = 0.6004 + 0.0054 \text{ day} - 0.0001073 \text{ day}^2$, $R^2 = 0.52$) and γ GPRENCSO changed quadratically ($\gamma\text{GPRENCSO} = 1.6574 + 0.0281\text{day} - 0.0004647 \text{ day}^2$, $R^2 = 0.34$)



CHAPTER 5

CONCLUSIONS

Onion is classified as a salt-sensitive crop. Our first study pointed out that NaCl concentrations affected onion flavor quality and intensity which enhances onion flavor but has more cabbage-like taste. This experiment showed that different NaCl concentrations caused different rates of metabolism in the onion flavor pathway and that the ACSOs and their biosynthetic intermediates were changed. ACSO concentrations increased significantly in 'Granex 33' when NaCl level was up to 100 mM. Under salt treatments three individual flavor precursors showed different proportion of total ACSOs. Adding NaCl in hydroponic culture would result in changes of osmotic potential, which may have affected water usage of onions. Previous reports indicated that water deficits resulted in changes in onion flavor intensity. Adding NaCl to external solution causes more negative water potential, which results in a similar conclusion. Since onion flavor change at high NaCl concentration, growers should not consider the soil salinity and onion flavor. However, onion production is decreased when onions are grown in saline environment. Therefore, it is not advised to grow onion in saline environment or avoid using high saline water for irrigation

The data revealed that ACSO composition changed when NaCl was applied. 1-PRENCISO amounts increased as NaCl increased; however, the ratio of 1-PRENCISO to total ACSOs was decreased as NaCl increased. In contrast, MCSO not only increased in concentration but also increased as a percentage of total ACSOs. By exposing the plants

to different NaCl concentration treatments, our data showed that ACSOs and MCSO accumulate at highest amounts at 100 mM NaCl concentration.

To speculate on the results of the first experiment, we thought that active bulbing would be a key metabolic step for biosynthesis of onion flavor. During active bulbing stage, onions started to accumulate ACSOs. It was previously shown that sulfur-containing peptides, such as glutathione, were increased when plants are grown in saline environment. However, we did not measure glutathione and do not know how glutathione was partitioned among the flavor compounds and antioxidation activity. Besides, why MCSO accumulates at high rates when compared to 1-PRENCISO in a saline environment is unclear. As a result of onion production, long-term exposure of saline environment is not recommended.

Since I have studied onion flavor for only two years, it is not quite clear to me how to explain these results and how they fit into overall flavor development. Onion flavor development looks like simple chemistry, but it involves many factors. Because irrigation lands are getting salinized every year, my experiments and data hopefully can be informative for future onion growing in the saline environment and help predicting onion flavor intensity from salt stress.