EFFECTS OF BRASSIN-LIKE SUBSTANCE AND ROASTING ON QUALITY OF COFFEE FROM EARLY GERMINATED COFFEE BEAN

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DOCTOR OF PHILOSOPHY
IN HORTICULTURE

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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN HORTICULTURE

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หัวข้อดุษฎีนิพนธ์	ผลของสารคล้ายบราสซินและการคั่วต่อคุณภาพของกาแฟจากเมล็ดในระยะเริ่มงอก	
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บทคัดย่อ

ในการศึกษาผลของสารคล้ายบราสซินและการคั่วต่อคุณภาพของกาแฟจากเมล็ดในระยะเริ่มงอก ได้แบ่งการทดลองออกเป็นสามส่วน ดังนี้ การทดลองที่ 1 การศึกษาผลของสารคล้ายบราสซินต่อการงอกของกาแฟอราบิก้า (*Coffea arabica* L.) โดยวางแผนการทดลองแบบสุ่มสมบูรณ์ ประกอบด้วย 4 กรรมวิธี คือ นำเมล็ดกาแฟมาแช่ในสารคล้ายบราสซินที่ความเข้มข้นแตกต่างกัน 4 ระดับ (0 (แช่น้ำกลั่น), 0.5, 1.0 และ 2.0 มิลลิกรัมต่อลิตร) เป็นเวลา 24 ชั่วโมง ก่อนนำไปเพาะที่อุณหภูมิ 30 องศาเซลเซียส ในตู้ควบคุมสภาพแวดล้อม จากการทดลองพบว่าเมล็ดกาแฟที่แช่ในสารคล้ายบราสซิน ความเข้มข้น 1.0 และ 2.0 มิลลิกรัมต่อลิตร ทำให้เมล็ดมีการดูดซึมน้ำในปริมาณสูงกว่ากรรมวิธีอื่น ในช่วง 3 และ 6 ถึง 15 วัน พบว่าหลังจากแช่สาร เปอร์เซ็นต์การงอกของเมล็ดสูงที่สุดในกรรมวิธีที่แช่เมล็ดในสารคล้ายบราสซิน 1.0 และ 2.0 มิลลิกรัมต่อลิตร ค่าเวลาเฉลี่ยในการงอกมีค่าน้อยที่สุดในกรรมวิธีที่แช่เมล็ดในสารคล้ายบราสซิน 1.0 และ 2.0 มิลลิกรัมต่อลิตร ส่วนค่าดัชนีความแข็งแรงของต้นกล้า I มีค่ามากที่สุด ในเมล็ดที่แช่ในสารคล้ายบราสซิน 2.0 มิลลิกรัมต่อลิตร และค่าดัชนีความแข็งแรงของต้นกล้า II มีค่ามากที่สุดในเมล็ดที่แช่ในสารคล้ายบราสซิน 1.0 และ 2.0 มิลลิกรัมต่อลิตร

การทดลองที่ 2 การศึกษาผลของสารคล้ายบราสซินและการงอกต่อการเปลี่ยนแปลงองค์ประกอบทางเคมีของกาแฟในระยะเริ่มของของกาแฟอราบิก้า มีวิธีการเตรียมเมล็ดเช่นเดียวกับการทดลองที่ 1 ในการทดลองนี้มีการวางแผนการทดลองแบบสุ่มสมบูรณ์ในแฟคทอเรียล ปัจจัยที่ 1 ประกอบด้วย การแช่เมล็ดกาแฟ ในสารคล้ายบราสซิน ที่ระดับความเข้มข้นแตกต่างกัน 4 ระดับ คือ 0

(แช่น้ำกลั่น), 0.5, 1.0 และ 2.0 มิลลิกรัมต่อลิตร เป็นเวลา 24 ชั่วโมงก่อนนำไปเพาะในตู้ควบคุมสภาพแวดล้อม ที่อุณหภูมิ 30 องศาเซลเซียส และปัจจัยที่ 2 จำนวนวันหลังเพาะเมล็ด (ก่อนเพาะ, จำนวนวันหลังจากเพาะ 2, 4, 6 และ 8 วัน) จากผลการทดลองพบว่าความเข้มข้นของสารคล้ายบราสซินและจำนวนวันหลังเพาะเมล็ดมีผลต่อการเปลี่ยนแปลงองค์ประกอบทางเคมีภายในเมล็ดกาแฟ ดังนี้ พบปริมาณโปรตีนมากที่สุดในเมล็ดกาแฟที่แช่ในสารคล้ายบราสซิน ความเข้มข้น 1.0 และ 2.0 มิลลิกรัมต่อลิตร ในวันที่ 4 หลังการเพาะเมล็ด ปริมาณน้ำตาลรวมในเมล็ดที่แช่ในสารคล้ายบราสซินความเข้มข้น 1.0 และ 2.0 มิลลิกรัมต่อลิตรในวันที่ 4 หลังจากเพาะมีค่ามากที่สุด และน้อยที่สุดในเมล็ดที่ไม่ผ่านการเพาะ พบปริมาณไขมันในเมล็ดมากที่สุดในเมล็ดกาแฟที่แช่ในสารคล้ายบราสซินความเข้มข้น 1.0 และ 2.0 มิลลิกรัมต่อลิตร ในวันที่ 4 หลังการเพาะเมล็ด และปริมาณไขมันในเมล็ดลดลงอย่างต่อเนื่องจนถึงวันที่ 8 หลังการเพาะ สารคล้ายบราสซินมีผลทำให้ปริมาณคาเฟอีนลดลงในระหว่างการเพาะ ปริมาณกรดคลอโรจีนิกมีค่ามากที่สุดในเมล็ดที่แช่ในสารคล้ายบราสซินความเข้มข้น 2.0 มิลลิกรัมต่อลิตรในวันที่ 4 หลังการเพาะเมล็ด และมีค่าลดลงอย่างต่อเนื่องไปจนถึงวันที่ 8 หลังการเพาะ ปริมาณสารประกอบฟีนอลรวมมีค่าลดลงในเมล็ดที่แช่ในสารคล้ายบราสซิน และพบว่า กิจกรรมการต้านอนุมูลอิสระด้วยวิธี DPPH (2′, 2′-diphenyl-1-pycrylhydrazyl) ของเมล็ดกาแฟเพิ่มขึ้นหลังจากที่ทำการเพาะต่อเนื่องไปจนถึงวันที่ 8 หลังการเพาะเมล็ด

การทดลองที่ 3 การศึกษาผลของการคั่วต่อการเปลี่ยนแปลงองค์ประกอบเคมีและคุณภาพของเมล็ดกาแฟในระยะเริ่มงอก โดยแช่เมล็ดกาแฟ นำมาเพาะ จากนั้นนำเมล็ดที่เพาะได้มาคั่วตามกรรมวิธีโดยเตรียมเมล็ด และเพาะเช่นเดียวกับการทดลองที่ 1 และวิเคราะห์องค์ประกอบทางเคมีเช่นเดียวกับการทดลองที่ 2 วางแผนการทดลองแบบสุ่มสมบูรณ์ในแฟคทอเรียล ประกอบด้วย 2 ปัจจัย คือ ปัจจัยที่ 1 ชนิดของเมล็ดกาแฟงอกที่นำมาคั่ว (เมล็ดที่ไม่ผ่านการเพาะ, เมล็ดแช่ในน้ำกลั่นก่อนนำมาเพาะเมื่อ 4 วันหลังเพาะ, เมล็ดที่แช่ในสารคล้ายบราสซินความเข้มข้น 1.0 มิลลิกรัมต่อลิตรก่อนนำมาเพาะเมื่อ 4 วันหลังเพาะ และเมล็ดที่แช่ในสารคล้ายบราสซินความเข้มข้น 1.0 มิลลิกรัมต่อลิตรก่อนนำมาเพาะ เมื่อ 6 วันหลังเพาะ) และปัจจัยที่ 2 ระดับการคั่วเมล็ดกาแฟ คั่วเมล็ดกาแฟที่อุณหภูมิ 240 องศาเซลเซียส (ระดับอ่อน 6 นาที, ระดับกลาง 7 นาที และระดับเข้ม 9 นาที) ภายหลังกระบวนการคั่ว พบการสูญเสียน้ำหนักเมล็ดกาแฟมากที่สุดในระดับการคั่วเข้ม และน้อยที่สุดในระดับการคั่วอ่อน จากการวัดสีเมล็ดกาแฟภายหลังการคั่ว ไม่พบความแตกต่างกันทางสถิติระหว่างชนิดของเมล็ดกาแฟ และพบว่าค่าความสว่าง (L*) ของเมล็ด มีค่ามากที่สุดในการคั่วระดับอ่อน และมีค่าน้อยที่สุดในการคั่วระดับเข้ม นำเมล็ดกาแฟที่คั่วจากแต่ละกรรมวิธีมาวิเคราะห์ปริมาณองค์ประกอบเคมีภายในตามวิธีการในการทดลองที่ 2 จากผลการวิเคราะห์ พบว่าปริมาณโปรตีนมีมากที่สุดในเมล็ดที่แช่ใน

สารคล้ายบราสซิน 1.0 มิลลิกรัมต่อลิตร เพาะแล้ว 4 วันในระดับคั่วอ่อน และมีค่าน้อยที่สุดในเมล็ดกาแฟที่ไม่ผ่านการเพาะ เมื่อนำมาคั่วในระดับเข้ม พบปริมาณน้ำตาลรวมมากที่สุดในเมล็ดกาแฟที่แช่ในสารคล้ายบราสซิน 1.0 มิลลิกรัมต่อลิตร เมื่อ 6 วัน หลังเพาะในระดับคั่วอ่อน ปริมาณไขมันในเมล็ดคั่วเข้มมากกว่าการคั่วกลางและคั่วอ่อน และเมล็ดที่แช่ในสารคล้ายบราสซิน 1.0 มิลลิกรัมต่อลิตร เมื่อ 4 วันหลังเพาะ มีปริมาณไขมันมากกว่าเมล็ดกาแฟในกรรมวิธีอื่น ปริมาณคาเฟอีนในเมล็ดมีการลดลงในระหว่างการคั่ว และเมล็ดที่ไม่ผ่านการเพาะมีปริมาณคาเฟอีนมากกว่าเมล็ดที่ผ่านการเพาะ ปริมาณกรดคลอโรจินิกมีค่าลดลงตามระดับการคั่วที่เพิ่มขึ้น จึงมีปริมาณมากที่สุดในเมล็ดกาแฟที่ไม่ผ่านการเพาะ และเมล็ดที่แช่ในสารคล้ายบราสซิน 0 มิลลิกรัมต่อลิตร (แช่ในน้ำกลั่น) เพาะเป็นเวลา 6 วัน ปริมาณสารประกอบฟีนอลรวมมีค่าลดลงตามระดับการคั่วที่เพิ่มมากขึ้น พบปริมาณสารประกอบฟีนอลรวมมากที่สุดในเมล็ดที่แช่ในกลั่น แล้วเพาะเป็นเวลา 4 วันในระดับคั่วอ่อนและน้อยที่สุดในเมล็ดที่แช่ในสารคล้ายบราสซิน 1.0 มิลลิกรัมต่อลิตรแล้วเพาะเป็นเวลา 6 วันในระดับคั่วเข้ม นอกจากนี้ ระดับการคั่วที่เพิ่มขึ้นยังมีผลให้ค่ากิจกรรมการต้านอนุมูลอิสระ DPPH ลดลง ดังนั้นในเมล็ดที่แช่ในสารคล้ายบราสซิน 1.0 มิลลิกรัมต่อลิตรแล้วเพาะเป็นเวลา 4 วันในระดับคั่วอ่อน จึงมีค่ากิจกรรมการต้านอนุมูลอิสระ DPPH สูงที่สุดสำหรับ ค่า pH มีค่ามากขึ้นเมื่อคั่วเมล็ดกาแฟในระดับสูงขึ้น โดยเมล็ดกาแฟในกรรมวิธี ที่แช่ในสารคล้ายบราสซิน 1.0 มิลลิกรัมต่อลิตร เมื่อ 4 และ 6 วัน ในระดับการคั่วเข้ม มีค่า pH มากที่สุด และในการทดสอบคุณภาพจากการชิม พบคะแนนรวมการชิมมากที่สุดในเมล็ดที่แช่ในสารคล้ายบราสซิน 1.0 มิลลิกรัมต่อลิตรที่เพาะเป็นเวลา 4 วัน ในระดับคั่วกลาง

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ABSTRACT

The study emphasized on the effects of brassin-like substance (BS) and roasting on quality of coffee from early germinated coffee bean. This thesis was carried out in 3 experiments. Experiment 1: the effects of BS on seed germination of arabica coffee (*Coffea arabica* L.). The experimental design was completely randomized design (CRD) with 4 treatments and 4 replications. The seeds were soaked with BS at the concentrations of 0 (distilled water), 0.5, 1.0 and 2.0 mg/L for 24 hours before germination test at 30ºC in a growth chamber. The results showed that the coffee seeds soaked in BS 1.0 and 2.0 mg/L had the highest imbibition curve at 3, 6 to 15 days after soaking. The highest percentage of seed germination and germination index were obtained from 1.0 and 2.0 mg/L BS treatments. The mean germination time were significantly shortened by BS 1.0 and 2.0 mg/L applications. The highest seedling vigor index I was obtained in the seeds treated by BS 2.0 mg/L. Also, the maximum of seedling vigor index II were observed in 1.0 and 2.0 mg/L of BS treatments.

Experiment 2 was the effects of brassin-like substance and germination on changes of chemical composition of arabica coffee. Germination of coffee seeds was prepared in the same procedure as experiment 1. The experiment was used 4×5 factorial in completely randomized design (CRD) with three replications. Factor 1 was BS concentration: 0 (distilled water), 0.5, 1.0 and 2.0 mg/L, and factor 2 was germination

time: before soaking, 2, 4, 6 and 8 days. The results showed that BS concentration and germination time significantly affected on the level of biochemical compositions and antioxidant activity. Maximum protein content in seed was found in 1.0 and 2.0 mg/L BS treatments at 4th day after germination. Total sugars content in 1.0 and 2.0 mg/L BS treatments reached the highest point at day 8 after germination, while the lowest total sugars was found in seed before soaking. The results indicated that soaking the seed at BS 1.0 and 2.0 mg/L could increase the greater fat content of arabica coffee than germinated seed from control treatment and 0.5 mg/L of BS treatment. BS applications significantly decreased the content of caffeine in coffee seed during germination. Caffeine content was significantly degraded by days of germination. BS concentration and germination time also affected chlorogenic acid content in seed. The maximum chlorogenic acid content in seed was achieved from 2.0 mg/L of BS treatment at day 4 after germination. The minimum content was achieved from 2.0 mg/L of BS treatment at day 8 after germination. Total phenol content in seeds was significantly decreased by treatment of BS. Coffee seeds soaked with 1.0 and 2.0 mg/L of BS exhibited higher antioxidant activities than treatments of distilled water and 1.0 mg/L of BS. During the germination process, DPPH radical scavenging activity in the coffee seed increased gradually upon germination time.

Experiment 3 was the effect of roasting on changes of chemical composition of and qualities of early germinated arabica coffee. The aim of this experiment was to study the effect of different germinated coffee and degrees of roasting (light, medium and dark) on chemical composition of arabica coffee beans. Seeds were germinated by the conditions used in experiment 1. The experimental design was a factorial based on complete randomized design with three replications. Factor 1 was germinated bean type: non-germinated bean, germinated bean soaked in 0 mg/L of BS (distilled water) at 4 days after germination (DAG), germinated bean soaked in 1.0 mg/L of BS at 4 DAG, and germinated bean soaked in 1.0 mg/L of BS at 6 DAG. Factor 2 was roasting level: light roast (6 min), medium roast (7 min) and dark roast (9 min). Early germinated coffee beans were roasted at 240ºC. After roasting, the results showed that the maximum weight loss was recorded for dark roast and minimum was recorded for light roast. The color analysis of roasted coffee was not significantly different between coffee

bean types. The lightness values (L*) decreased with an increase of roasting level. Maximum L* value was found in light roasted bean, while the minimum value was found in dark roasted one. The chemical compositions from these roasted beans were analyzed likewise Experiment 2. Protein content was significantly affected by the interaction between roasting level and type of germinated coffee bean. Maximum protein contain in roasted bean was recorded for BS 1.0 mg/L at 4^{th} DAG in light-roasted condition, and minimum protein content was observed in dark-roasted non-germinated bean. The highest total sugars content in roasted bean was recorded for BS 1.0 mg/L BS at 6^{th} DAG with light-roasted coffee. The fat content from dark roasted bean had the highest value. Non-germinated bean, BS 0 and1.0 mg/L at 4^{th} DAG beans demonstrated significantly higher fat contents than the bean from BS 1.0 mg/L 6^{th} DAG. Caffeine content of all bean types tended to decrease after roasting. The caffeine contents in non-germinated and BS 0 mg/L beans had higher contents than other treatments. Chlorogenic acid content in non-germinated and BS 0 mg/L at 4^{th} DAG were greater than the average chlorogenic acid contents in BS 1.0 mg/L at 4^{th} DAG and BS 1.0 mg/L at 6^{th} DAG. The chlorogenic acid concentration decreased upon roasting levels. The results also showed that chlorogenic acid content was higher in light roasting level than the medium and dark roasting conditions. The total phenol contents of roasted coffee decreased with roasting level. The highest total phenol content was showed in non-germinated bean with light roasted, while the lowest amount was observed in BS 1.0 mg/L at 6^{th} DAG with dark roasted bean. Increasing roasting degrees led to a decrease in DPPH radical scavenging activity. The highest DPPH radical scavenging activity was observed in germinated coffee from BS 1.0 mg/L at 4^{th} DAG treatment upon light roasted level. The pH value of roasted coffee significantly increased upon degree of roasting. The highest pH value was showed in 1.0 mg/L at 4^{th} DAG and BS 1.0 mg/L at 6^{th} DAG with dark roasted level. The BS 1.0 mg/L at 4^{th} DAG bean with moderately roasted gave the highest aroma score and overall score of cupping test.

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LIST OF ABBREVIATIONS

µg/mL	Microgram per Milliliter
µm	Micrometer
µM Trolox/g DW	Micromolar Trolox Equivalent per Gram Dry Weight
ABA	Abscisic Acid
BRs	Brassinosteroids
BS	Brassin-like Substance
CRD	Completely Randomized Design
CV	Coefficient of Variation
DAG	Day After Germination
DMRT	Duncan's Multiple Range Test
DPPH	2´, 2´-diphenyl-1-pycrylhydrazyl
g	Gram
GA_3	Gibberellic Acid
HPLC	High Performance Liquid Chromatography
M	Molarity
mM	Millimolar
mg GAE/g DW	Milligram Gallic Acid Equivalent per Gram Dry Weight
mg/L DW	Milligram per Liter on Dry Weight basis
mg/mL	Milligram per Milliliter
mL	Milliliter
mm	Millimeter
N	Normality
nm	Nanometer
NS	Not Significant Different
RH	Relative Humidity
rpm	Rounds per Minute

LIST OF ABBREVIATIONS (continued)

USD	United States Dollar
UV/Vis	Ultraviolet-Visible
w/v	Weight per Volume

LIST OF SYMBOLS

Ø	Diameter
ºC	Degrees Celsius

ข้อความแห่งการริเริ่ม

1) วิทยานิพนธ์นี้เป็นงานวิจัยแรกที่ได้นำสารคล้ายบราสซินมาใช้ในการส่งเสริมการงอกของเมล็ดกาแฟอราบิก้า

2) วิทยานิพนธ์นี้เป็นงานวิจัยแรกที่ได้นำสารคล้ายบราสซินมาใช้ในการศึกษาการเปลี่ยนแปลงองค์ประกอบทางเคมีภายในเมล็ดกาแฟอราบิก้าในระหว่างกระบวนการงอก

3) วิทยานิพนธ์นี้เป็นงานวิจัยแรกที่มีการนำเอาเมล็ดกาแฟอราบิก้ามาผ่านกระบวนการงอกเพื่อให้เกิดการเปลี่ยนแปลงองค์ประกอบทางเคมีภายในเมล็ด ส่งผลต่อคุณภาพของเมล็ดกาแฟต่อไป

4) งานวิจัยนี้ได้ยืนยันว่าการใช้สารคล้ายบราสซินและกระบวนการงอก มีผลต่อคุณภาพของเมล็ดกาแฟทั้งก่อนและหลังกระบวนการคั่วกาแฟ

STATEMENTS OF ORIGINALITY

1) This thesis is the first study that introduces the effects of brassin-like substance on germination and seedling growth of arabica coffee.

2) This thesis is the first study that introduces effects of brassin-like substance changes of chemical compositions of arabica coffee during germination.

3) This thesis is the first study that introduces effects of germination on changes of chemical compositions of arabica coffee.

4) This research confirms that treating arabica coffee seed with brassin-like substance before germination has significantly effects on changes of chemical composition of coffee before and after roasting.

CHAPTER 1

Introduction

Coffee is one of the most important beverage crop as it serves an enormous demand in the world market. Thailand is the third largest coffee producer in Asia after Vietnam and Indonesia. In 2015, Thai Office of Agricultural Economics reported that coffee export value worth up to 3.1 million USD. (Office of Agricultural Economics (Thailand), 2015). In Thailand, arabica coffee (*Coffea arabica* L.) is typically grown in the mountainous areas of Northern region where the land elevation is located approximately at 800 to 1,200 meters above mean sea level.

Coffee has been considered as the most commercialized product for decades. It is widely consumed throughout the world. This popularity increases through coffee variety selections, breeding and appropriate cultivation that significantly improve cup quality. A presentation of coffee's image through commercial and academic publications of health benefits from coffee consumption also enhances coffee notability. Nowadays, coffee is considered a functional food due to its high content of several potential bioactive compounds that exhibit antioxidant activity and other beneficial biological effects (Farah, 2012). In addition to biological aspect, the characteristic flavor and richness of coffee aroma also greatly make it a unique beverage that is mostly influenced by over a thousand volatile compounds once roasted (Yeretzian *et al.*, 2003).

Coffee roasting is an important process that literally turns the beans into beverage (Eggers *et al.*, 2002). During roasting at temperature of 200 ºC to 300ºC, several chemical reactions take place and result in a pleasant flavor and aroma of the roasted coffee. The beans change their color along with an increase of bean volume by 50% to 80%, while simultaneously lose 13% to 20% from its mass at the expense of moisture. Seed compositions change dramatically during roasting as a consequence of

notable reactions: pyrolysis, caramelization, and maillard reactions (Antonio *et al*., 2011).

The quality of coffee used for beverage is typically related to the chemical compositions of roasted beans, which is greatly affected by green beans quality and roasting condition. The key criteria commonly used to evaluate coffee beans quality include bean size, color, shape, roasting condition, processing procedure and cup quality (Banks *et al*., 1999). In which, cup-testing is an evaluation of coffee aroma and taste characteristics. Evaluation usually includes the following basic coffee characteristics such as aroma, taste, body and acidity (Angkasith and Warrit, 1999).

Germinated seed has become a healthy food since last decades (Frias *et al*., 2007). The practice involved in germination has been used to improve seeds nutritional quality (Wang and Fields, 1978). Seed germination provides important effects to biochemical composition, nutritional value and sensory characteristics of germinated seeds. Germination is a biological process that metabolic enzymes, such as amylase and proteinases are activated. Some storage materials, namely, proteins, fats and carbohydrates can be vastly released to synthesis other organic compounds as well as utilization of the new cells. Therefore, the seed nutrition can be enhanced, altered to other compounds, and improved in digestibility, thus, germination is literally a crucial biological procedure that improve nutritional quality of the seeds (Gulewicz *et al*., 2008).

In commercial aspect, coffee bean price can be increased over 100 times (from 10 USD to 1,100 USD per Kg) by using coffee berries that have been eaten and partially digested by animals, for example, the civet coffee (also known as kopi Luwak in Indonesia) and the elephant's poop coffee (in Thailand). Scanning electron microscopy (SEM) revealed that civet coffee bean possessed surface micro-pitting caused by the action of gastric juices and digestive enzymes during digestion. Gastric juices and moisture were entering into the beans and modifying the micro-structural properties of these beans, for example, protein, fat and caffeine content. This process might be similar to germination process (Marcone, 2004). However, in hygienic aspect, number of consumers somehow concern on consumption of coffee bean that passed from feces of animals.

Despite of considerable effort at vegetative and micro-vegetative propagation of coffee plants, they still are primarily propagated by seedlings produced directly from seeds. Undesired traits of coffee seeds are their slow and asynchronous germination, which are difficult to obtain seedlings of desirable quality. Little work has been done to understand coffee seed germination and its regulation. In recent years, several plant growth-substances have been used in attempts to promote coffee seed germination and seedling growth, however, exogenous gibberellic acid (GA_3) inhibits coffee seed germination (Da Silva *et al*., 2008). Valio (1976) also found that endogenous abscisic acid-like substances and exogenous abscisic acid (ABA) cause the inhibition of germination by preventing embryonic growth. Thus, gibberellins and ABA obviously provide no enhance effect on seed germination.

Brassinosteroids (BRs) are the class of steroid hormones that control various growth and developmental processes in plants, including cell division and expansion, photomorphogenesis, xylem differentiation, floral development and seed germination (Clouse and Sasse, 1998; Bajguz, 2007). BRs are found to increase secondary metabolites synthesis such as phenolics, tannins, flavonoids and anthocyanins (Xi *et al*., 2013; Choudhary *et al*., 2011; Swamy and Rao, 2011) They also help to increase quality of the yield as well as improve content of antioxidants in seeds (Bajguz and Hayat, 2009). Moreover, brassinolide and other natural BRs such as 28-homocastasterone present a broad antiviral spectrum against RNA and DNA viruses. Consequently, BRs have the medical prospect as potential cure for cancer, fungal, bacterial, and viral infections (Renu *et al*., 2012). However, very little work has reported on the effect of brassin-like substance or BS (the synthetic brassinosteroids which have similar chemical structure like brassinosteroids) on seed germination and changes of chemical compositions in arabica coffee seed during germination. Therefore, the purpose of this study is to evaluate the effect of different brassin-like substance concentrations on seed germination, and to determine the influence of BS application on germination process and seed chemical composition changes occurred during germination and roasting of arabica coffee.

Propose of study

1. To study effects of brassin-like substance on germination and chemical compositions of coffee seed and bean.
2. To study quality of early germinated coffee beans.

CHAPTER 2

Literature Review

2.1 Arabica coffee

Coffee trees belong to the genus *Coffea*, family Rubiaceae, and order Rubiales. Arabica coffee plant is a woody perennial evergreen with small shrub characteristic. There are two economically important species of *Coffea* namely *Coffea arabica* L. (arabica coffee) and *Coffea canephora* Pierre A. ex Froehner (robusta coffee). Coffee is one of the most valuable cash crops in Thailand, there are two main types of coffee cultivated in Thailand: arabica in the Northern region and robusta in the South. Arabica coffee is mainly grown in the highlands at approximately 800-1,200 m above mean sea level. The Catimor cultivar is recommended as it has a rust resistant ability. Growing arabica coffee provides cash income for hill tribe farmers and reduces the old-fashioned slash-and-burn shifting agriculture. In Thailand, both shaded and full sun coffees are grown. With the policy of natural resource conservation and the limitation of land area, the hill-tribe farmers are strongly recommended to grow coffee to sustain natural resources on the highlands (Angkasith, 2002).

2.1.1 Coffee fruit

Coffee fruits are categorized as a “drupe” with a pulpy mesocarp and lignified endocarp (De Castro and Marraccini, 2006). Two coffee seeds (beans) are located within the drupes, being the most inner part of the coffee berry. They are surrounded by a silver-colored skin, followed by a hard cover, the pericarp, which consists of the 3 parts called endocarp (parchment), mesocarp (pulp) and exocarp (skin). Mesocarp is also called mucilage where the sticky and sugar-like substances are accumulated (Figure 2.1).

The exocarp is usually dark when coffee cherry reaches its fully ripe. The color of the berries thus changes from green to red during 1-2 months of ripening period (Holzapfel, 2014). Coffee fruit has to be removed its pulp in order to obtain the green coffee beans, which are dried and roasted thereafter.

2.1.1.1 Pericarp

The pericarp is composed of exocarp (peel), mesocarp and endocarp (Avallone, 1999).

2.1.1.1.1 Exocarp (skin)

The exocarp, also referred to as the peel, skin, or epicarp, is the outermost layer of the coffee fruit. It is formed by a single layer of compact parenchyma cells (cells with thin primary walls that contain chloroplasts and are capable of absorbing water). The color of the exocarp at the beginning of fruit development is green due to the presence of chloroplasts which then disappear as the fruit matures (De Castro and Marracini, 2006). The change in color is due to the disappearance of chlorophyll pigments and anthocyanin accumulation during the last stages of fruit maturation (Marin-Lopez *et al*., 2003).

2.1.1.1.2 Mesocarp (Mucilage)

The mesocarp is commonly referred to a "true pulp". It is rich in reducing sugars and water. With 0.5-2.0 mm thickness, it can be divided into external and internal parts. The part is formed by parenchyma cells with compact and dense cell wall during green fruits, and become thinner during maturation, probably due to pectin modifications (Ouguerram, 1999). In the wet processing method, this mucilage layer is removed through a controlled fermentation. In the dry method, the layer along with the exocarp and endocarp are typically left intact during drying.

2.1.1.1.3 Endocarp (Parchment)

The endocarp is generally called parchment layer or "pergaminho". It is the innermost layer of the pericarp and is the hull that envelops the coffee bean. It is formed by three to seven layers of sclerenchyma cells (fibrous cells that serve as the principal support cells in plants). The endocarp is a hard and lignified tissue with

thickness of 150 µm (Mendes, 1942). The cells of the endocarp harden during coffee fruit maturation due to a self-defense mechanism of coffee seed against digesting enzymes from the gut of frugivorous animals (Urbaneja *et al*., 1996).

2.1.1.2 Silver skin

The silver skin, also called the perisperm or spermoderm, is the outermost layer that wraps the seed. It is formed from nucellus or central portion of the ovule. Generally some remnants of the silver skin remain on the bean pre-roast, and come off during coffee roasting as a husk. Silver skin may be polished off from the bean, however, it is generally accepted that this practice can partially diminish coffee flavor. (Rogers *et al*., 1999; Marraccini *et al*., 2001)

2.1.2 Coffee seed

The coffee seed or coffee bean is an elliptical or plano-convex egg shape that has a longitudinal furrow on the flat surface covered by the silver skin with a thin pellicle remnant from the perisperm (about 70 µm in thickness) referred to the integument or spermoderm (Dedecca, 1957; Estanislau, 2002). The endosperm is a group of living tissues formed by polygonal and rectangular cells in different parts of the seed with the endosperm cap being formed by smaller and thinner cell walls compared to the rest of the endosperm where the radicle ultimately protrudes (Dedecca, 1957; Dentan, 1985; Da Silva *et al*., 2004). In terms of chemical composition, the endosperm contains several nutritive components, i.e. proteins, lipids and minerals (Dentan, 1985). Nonetheless, the main storage reserves possess high levels of polysaccharides, cellulose and hemicellulose are commonly deposited in the cell walls (Wolfrom *et al*., 1961; Wolfrom and Patin, 1964; Da Silva *et al*., 2004).

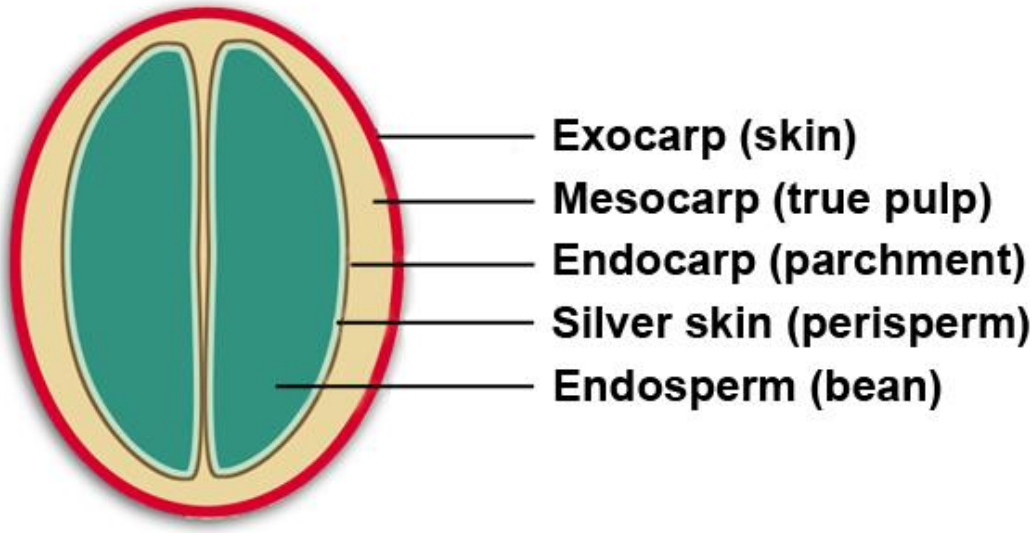

Figure 2.1 Coffee fruit structure (Modified from Wintgens, 2009)

2.1.2.1 Endosperm

Endosperm is a major reserve tissue of the seed. The biochemical compounds within endosperm are the utmost importance since they are the precursors of coffee flavor and aroma. The chemical compounds found in the endosperm can be classified as soluble and insoluble in water. The water-soluble compounds are caffeine, trigonelline, nicotinic acid (niacin), at least 18 chlorogenic acids, mono-, di-, and oligosaccharides, some proteins, minerals and carboxylic acids. Insoluble components typically include cellulose, polysaccharides, lignin, hemicellulose and lipids (Borem *et al*., 2008).

2.1.2.2 Embryo

The embryo of coffee is very small which is about 3 to 4 mm long. It is composed of an axis and two adherent cordiform cotyledons which are localized close to the convex surface of the seed (Dedecca, 1957; Huxley, 1964; Rena *et al*., 1986). The embryo itself has a very few storage reserves, it therefore, depends upon endosperm reserving nutrients until the seedling become autotrophic (Giorgini and Campos, 1992).

2.2 Coffee propagation

Reproduction of coffee can be performed in a sexual or asexual alternative. Although coffee can be vegetatively propagated either by grafting, stem-cutting, or in vitro propagation, which provides high rate of autogamy, the most appropriate and easiest method of arabica coffee reproduction is seed propagation. Thus, arabica plantations have been established from seeds for many years (Wintgens and Zamarripa, 2004).

2.2.1 Germination of seed

2.2.1.1 Definition of germination

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development (Sangronis *et al.*, 2006). During this period, reserved materials are gradually degraded and commonly used for respiration and synthesis of new cells prior to developing embryo (Vidal-Valverde *et al*., 2002). The process begins with an uptake of water (imbibitions) by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually appeared as a radical (Bewley and Black, 1994).

2.2.1.2 Germination of coffee seed

Generally, coffee seeds germinate slowly in the field (Rena *et al*., 1986). In a warm temperature, arabica coffee seedlings emerge from the soil within 50 to 60 days after sowing (Maestri and Vieira, 1961). The seedlings however take up to 90 days for emerging from the soil under low temperature (Went, 1957).

Radicle protrusion in arabica coffee seeds under optimal conditions (30ºC, in the dark lighting condition) starts around day 5 or 6 of imbibition and 50% of the seed population displays radicle protrusion by day 10. At day 15 of imbibition, most of the seeds have completed their germination processes (Valio, 1980; Da Silva *et al*., 2004).

Staging coffee seedling growth based on morphological changes (Da Rosa *et al*., 2010)

Seed imbibition 1 – imbibed seed

A fully imbibed seed with no visible protuberance usually occurs up to day 3 of imbibition. The initial step necessary for germination is water uptake and, under optimal supply conditions, this process has a triphasic pattern (Camargo, 1998). In living seeds without an endocarp, phase I is typically completed after 6 days and phase III begins after approximately 9 days following to the onset of imbibition. During stage *Seed Imbibition 1*, changes in structure and size occur as the egg-shaped and plano-convex seed increases in all dimensions. Thus the seed becomes a rather rounder shape (Da Rosa *et al*., 2010).

Seed imbibition 2 – visible protuberance

A visible protuberance can be noticed on the endosperm cap at five days which is a result of the embryo growing inside the endosperm. Although the region from which the radicle will protrude is noticeable, the radicle does not penetrate through the outer layer of the endosperm at this stage. Theoretically, endosperm cells expand as a result of water uptake during the first three days of germination (Da Silva, 2002). And at the third day, the cells are literally turgid, indicating that phase II of the germination process is initiated. The next event is seedling growth which begins when germination *sensu stricto* is completed and includes the mobilization of storage reserves necessary for transforming the seedling into an independent autotrophic organism that is capable of photosynthesis (Da Rosa *et al*., 2010).

Phase III imbibition (Germinated seed)

The radicle penetrates through the outer endosperm layer completing *sensu stricto* germination at approximately 7 days. Before this process is completed, several metabolic processes take place in preparation for radicle emergence, such as protein hydration, sub-cellular structural changes, respiration, macromolecular syntheses and cell elongation (Bewley and Black, 1994). Da Silva (2002) reported that about half of coffee seed population germinated at 10 days after imbibition. While during imbibition, the fresh weight of intact seeds increased to a plateau at day 3, and unchanged until day 15, when phase III imbibition was achieved. As a result, the author suggested the phases of coffee seed germination were not concurrent with the phases of

water uptake as found in most seeds. Coffee seeds probably failed to have water uptake phases in conjunction with germination phases in his study because of abnormal growth since these seedlings did not develop into normal seedlings. After the lag phase, only germinating seeds and normal seedlings enter into phase III imbibition as apparently signaled by radicle elongation and the increase in water uptake. The phase III is initially related to changes in radicle cells requiring an increase of cell turgidity for further extension (Bewley and Black, 1994).

Seedling 1 – hypocotyl root axis

From the germinated seed stage, embryo is considered a seedling that greatly depends on storage reserves inside the seed. Subsequent physiological and morphological activities are considered post-germination events. After germination, and at approximately 9 days of imbibition, a hypocotyl grows and possesses a distinct pink color from the white radicle. The radicle appears as an arrow shape which is a result of the enlarged junction between the hypocotyl and root caused by the growing root primordium. Root primordia are not noticeable, but lateral roots later appear in this region. Thus, the enlarged junction between the hypocotyl and root contains an organized series of cells in their earliest stages of differentiation (Da Rosa *et al*., 2010).

Seedling 2 – junction root primordia

This stage is characterized by the presence of root primordia at the junction between the hypocotyl and primary root that increase in size at approximately day 12. The radicle, an embryonic root at the lower end of the hypocotyl, continues to grow and develops into the primary root (Sutton and Tinus, 1983).

Seedling 3 – junction lateral roots

At *Seedling* 3, about 15 days of germination, the first two or three lateral roots apparently arise at the junction between hypocotyl and primary root together with a more root primordia and root hairs form around the surface of primary root. The primary root and the root cap are well developed, and a zone of cell division and elongation can be differentiated with no root hairs, but root hairs can be detected in the zone of maturation. Root hairs develop from epidermal cells on the root surface in the

zone of maturation and they are absent in other regions of the roots, such as root apex (Raven and Johnson, 2002).

Seedling 4 – lateral roots

Starting at approximately day 20 to day 30, lateral roots are initiated from the primary root. The increases in size and number and root hairs are detected in every root in the zone of maturation, but they are not appeared on the root apices. Lateral roots that initiated at the junction between the hypocotyl and primary root begin to form the secondary roots. A lateral root is defined as a root that arisen by cell division in the pericycle of the parent roots, and typically depends on the rank of the root developing lateral roots (Sutton and Tinus, 1983). They are physiologically designated as secondary roots (laterals on a primary root) and tertiary roots (laterals on a secondary root) (Esau, 1977; Sutton and Tinus, 1983).

Seedling 5 – cotyledonary leaves

Beginning at approximately 30 days, seedling continues to grow and form a more substantial root system with a hypocotyl increasing in height. The only significant morphological change occurring during this period is the consumption of the endosperm by the cotyledonary leaves. At approximately 45 days, the seedling finally has the opened cotyledons, and the remaining endosperm tissue appears in a thin layer covering the cotyledonary leaves, indicating that the endosperm has been substantially consumed (Da Rosa *et al*., 2010).

2.3 Plant growth regulators for coffee germination

The germination of arabica coffee seed is also influenced by exogenous and endogenous hormones and their levels. It has been found that gibberellic acid (GA_3) and abscisic acid (ABA) inhibit coffee seed germination, while kinetin stimulates the process when these regulators are exogenously applied during the imbibition of the intact seeds (Valio, 1976). In arabica coffee seed, Da Silva *et al*. (2004) found that endogenous ABA-like substances and exogenous ABA caused inhibition of germination by preventing embryonic growth. Contrary to many reports on the stimulatory effect of

GA_3 during seed germination and tissue elongation, GA_3 inhibited radicle protrusion (Takaki and Dietrich, 1979).

2.4 Brassinosteroids

Brassinosteroids (BRs) play an important role in hormone signaling in plants and in the physiological responses of plants to environmental stimuli in processes such as seed germination, growth, cell division and differentiation, root and stem elongation (Clouse and Sasse, 1998). BRs positively regulate seed germination by controlling the inhibitory effects of ABA on seed germination and seedling elongation (Zheng *et al*., 2009). Serna *et al*. (2012) reported that applying brassinosteroid analogues; DI-31 and DI-100, effectively increased total yield, total antioxidant activity and total phenols of field grown endives. Total antioxidant activity and total phenols increased significantly in endive treated with BR analogues. BRs also increased the soluble proteins of radish, as well as nucleic acids (DNA and RNA), carbohydrates in terms of reducing sugars, non-reducing sugars and starch. (Vardhini *et al*., 2012). The content of antioxidants (tocopherols, trocotrienols, ascorbic acid, β-carotene) as well as soluble proteins, total fats and soluble sugars were notably increased in pea and lupine seeds after application of 24-epibrassinolide (Biesaga-Koscielniak *et al*., 2014).

2.5 Brassinosteroids promotes seeds germination

Endogenous brassinosteroids have been shown to break dormancy and increase germination of several seed genera (Bewley and Black, 1982, 1985), including pea (Yokota *et al*., 1996), Arabidopsis (*Arabidopsis thaliana)* (Schmidt *et al*., 1997) and red catchfly (Lychnis viscaria) (Friebe *et al*., 1999). Brassinosteroids and gibberellins are coherently promote seed germination of arabidopsis (*Arabidopsis thaliana*), tobacco (*Nicotiana tabacum*), and of parasitic angiosperms (*Orobranche* and *Striga* species). Meanwhile, the regulators counteract with absicisic acid (ABA) in the inhibition of seed germination (Leubner-Metzger, 2003). Furthermore, brassinosteroids, gibberellic acid and ethylene are able to increase the ability of embryos to grow out of the seed by enhanced rupturing of endosperm and antagonistically interacting with ABA (Finch-Savage and Leubner-Metzger, 2006). The BRs alone also increase seed germination by enhancing the growth of embryonic cells (Leubner-Metzger, 2001).

Seed treated by brassinosteroids applications have been found to improve the seedling growth in many plant species. Pretreatment of BRs to barley seeds effectively accelerates seedling growth (Gregory, 1981). Pre-sowing seed with brassinolide stimulates germination and seedling emergence of aged rice seeds (Yamaguchi *et al*., 1987). In tobacco (*Nicotiana tabacum*), treatment the seeds with brassinosteroids can enhance the accumulation of xyloglucan endotransglycosylases (XET) enzyme activity especially in the embryo. Brassinosteroids are also known to promote endosperm rupture of tobacco (Leubner-Metzger, 2001). Therefore, it is possible that XET enzyme in the endosperm and in the embryo mediates the promotion of seed germination by brassinosteroids via cell wall loosening. Srivastava *et al*. (2011) reported that in the case of moong bean (*Vigna radiata*) seeds, application of brassinosteroids provided significant increases in seed germination, germination rate index, speed of germination, coefficient of velocity of germination, germination index, relative seed germination, root and shoot length, relative root elongation, fresh and dry weight, seedling growth, and vigor index.

2.6 Brassin-like substance

Brassin-like substance is a synthetic brassinosteroid which has similar chemical structure to brassinosteroids. Pankasemsuk (2007) reported that application of brassin-like substance or BS developed by Division of Horticulture, Faculty of Agriculture, Chiang Mai University at the concentration between 0.01 to 0.50 mg/L on longan, papaya and mango could increase fruit size and quality. Moreover, Praphutphitthaya and Pankasemsuk (2012) reported that the coffee fruit treated with BS 1.5 mg/L gave the largest fruit and bean sizes.

2.7 Germinated seed

Seed germination is an important phenomenon that greatly influences on the chemical compositions, nutritive value and acceptability characteristics of the products. Germination is a biological process, in which metabolic enzymes, such as proteinases are activated (Sangronis *et al.*, 2006). During this period, reserve materials are

degraded, commonly used for respiration and synthesis of new cells prior to developing embryo (Vidal-Valverde *et al.*, 2002).

Recently, several attempts involved in germination method have been believed to increase seed quality including dietary fibers, essential amino acids, proteins, carbohydrates, secondary metabolites, vitamins and other essential phytochemicals. Germination significantly alters nutrient compositions of the seed, causing marked increase in calorific value, crude protein, soluble carbohydrate, cellular and organic cellular contents, cellulose, lignin, non-nutritive matter, total oxalate and phytic acid contents of the seed (Gulewicz *et al.*, 2008).

The observed reduction in the polyphenols particularly after germination is a result of formation of hydrophobic association of tannins with seed proteins and enzymes. The reason for loss of tannins during germination process is binding of polyphenols with other organic substances such as carbohydrate or protein. Apart from that, during the period of soaking prior to germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols. Germination process causes a reduction percent in total phenolic compounds ranged from 32.8% after 24 hours of germination to 60.8% after 120 hours of germination in kidney bean (*Phaseolus vulgaris*). Moreover, a longer period of germination literally causes significant greater losses in total phenolic compounds in kidney bean. In the case of soybean (*Glycine max*), the reduction percentage in total phenol content is 27.0% after 24 hours of germination and reaches 45.0% after 96 hours of germination (Naveena and Bhaskarachary, 2013).

The mung bean displays the great losses of phenolic compounds which reach 66.8% after 48 hours of germination (Rasha *et al*., 2011). A decrease in polyphenol contents was observed by Giami *et al*., (2001) in germinated cowpea (*Vigna unguiculata*) (41.5 to 51.7%), and for Indian pulses by Khandelwal *et al*., (2010). The reduction of total phenolic compounds during germination may be attributed to the presence of polyphenol-oxidase and enzymatic hydrolysis (Rao and Deosthale, 1982).

For Australian sweet lupine (*Lupinus angustifolius* L.), germination significantly increases crude fiber contents by 450% (dry weight), total protein contents by 38% (dry

weight), however, the fat content is decreased by 70% (dry weight) at day 9 of seed germination (Rumiyati *et al*, 2012).

2.8 Roasting of coffee

The desired aroma and flavor of coffee are developed by roasting process, thus this is considered the most important process in coffee processing. In this process, the beans undergo notable series of reactions leading to the desired changes in physical properties and chemical compositions (Pittia *et al*., 2001; Illy and Viani, 2005). Nevertheless, this process is highly complicated since the amount of heat transferred to the bean is greatly crucial. In coffee roasting, the green beans are heated to high temperature, ranging between 200ºC to 240ºC for different times, depending on the desired characteristics of the final products (Pittia *et al*., 2001). During the roasting process, coffee beans are subjected to a steady weight loss (Hernandez *et al*., 2007; Jokanovic *et al*., 2012) due to water and volatile materials losses, while significant increase is typically noted in coffee volume (Dutra *et al*., 2001). Consequently, the coffee bean density decreases and the typical porous structure of roasted coffee bean is formed (Pittia *et al*., 2001). In addition, coffee beans undergo other major changes in terms of color, form, pH, flavor and aroma (Noor Aliah *et al*, 2015).

Coffee roasting is one of the most important stages in the process that turns the beans into a beverage (Eggers *et al*., 2002). In an initial phase of roasting, free water evaporates. When the seed temperature reaches 130ºC, sucrose caramelizes, and the seed begins to brown and swell. Chemical changes in this phase are relatively small compared to those changes occurred at the end roasting. At temperature greater than 160ºC, several series of exothermic and endothermic reactions take place, the seed becomes light brown, seed volume increases considerably, and aroma formation begins. The chemical reactions responsible for aroma and flavor of roasted coffee are triggered at approximately 190ºC. During the Maillard and Strecker reactions, carbohydrates (reducing sugar), proteins, other classes of compounds, low and high molecular weight compounds such as melanoidins are simultaneously degraded and produced. During this process the light brown seed gradually turns to almost a black one (Clarke, 2003). The quality of coffee used for beverage is related to the chemical compositions of the roasted beans, which is affected by the compositions of the green beans and roasting

conditions. The criteria commonly used to evaluate the quality of coffee beans include bean size, color, shape, roasting, processing procedure and cup quality (Wondimu, 1998).

2.9 Coffee chemical composition

2.9.1 Non-volatile compounds

Non-volatile fraction of green coffee is composed primarily of water, carbohydrates and fiber, proteins and free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline and caffeine. The aforementioned compounds are most likely to be bioactive, and they may also be the important contributors to coffee flavor after roasting (Farah, 2012).

2.9.1.1 Water

The water content of green seeds of arabica coffee generally varies from approximately 8.5% to 12%. These moisture levels are considered undesirable for both aroma/flavor quality and health effects since they increase water activity, and therefore the probability of microbial growth. On the other hand, too low moisture level usually produces cracks in the seeds, and decreases their viability to germinate (Farah, 2012).

After roasting, moisture content of roasted coffee is considerably lower than the green one (1.5%-5.0%). However, moisture content varies depending on roasting degree (Trugo and Macrae, 1984a; Farah, 2004).

2.9.1.2 Protein

In the cup quality, amino acids are very important because they react with sugar moieties through "Maillard" reactions (Maillard, 1912) to produce aromatic compounds. They serve as precursors for the formation of volatile compounds such as furans, pyridines, pyrazines, pyrrols, aldehydes and melanoidins. The melanoidins are responsible for coffee's color and also act as antioxidant compounds. There are several reports describing the importance of proteins in beverage quality (Amorim and Josepson, 1975; Melo and Amorim, 1975; Montavon *et al*., 2003). In mature beans of *Coffea arabica* var. Caturra, protein content represents 10% of dry weight with 45%

of storage proteins (Rogers *et al*., 1999). Glutamic acid and asparagine are the most abundant, representing nearly 40% of free amino acids in coffee beans (Arnold and Ludwig, 1996). Both are also the major forms of nitrogen transport in xylem sap of coffee (Mazzafera and Goncalves, 1999). Some differences exist in the composition of free amino acids in green coffee beans among different species, for example with glutamic acid content being higher in arabica (Arnold *et al*., 1994).

During roasting, a portion of coffee protein is degraded, and free amino acids and peptides are consumed by Strecker reactions. Some of the amino acids react with reducing sugars (via Maillard reaction) to form low-molecular-weight compounds and melanoidins that incorporate into their structures and other components, such as chlorogenic acids, galactomannans and arabinogalactan-proteins (Bekedam *et al*., 2008). Melanoidin polymers are responsible for the brown color of roasted coffee (Nicoli *et al*, 1997a). Furthermore, several studies suggest that melanoidins are partially responsible for antioxidant activity, antibacterial and metal-chelating properties of coffee beverages (Nicoli *et al*., 1997b, Daglia *et al*., 2000).

2.9.1.3 Total sugar

Sucrose accounts for up to 9% of arabica coffee dry weight. Small amounts of simple carbohydrates such as fructose, glucose, mannose, arabinose and rhamnose, together with some oligosaccharides such as raffinose and stachyose have been also identified in green coffee (Flament *et al*., 1968; Kolling-Speer and Speer, 2005). Sugar is a precursor for the typical Maillard reaction which are important to color and aroma establishments. Sugar molecules are then able to react with amino acids to produce aliphatic acids, hydroxymethylfurfural, other furan derivatives, pyrazine and carbonyl compounds, which are the essential contributors to coffee flavor, either as volatile aromatic compounds, or as non-volatile taste compounds (Grosch, 2001; Homma, 2001). They also contribute to the acidity of the brew after coffee roasting. Higher sucrose content is one of the reasons for bringing superior aroma and signature flavor of arabica coffee. High-molecular-weight polysaccharides give body to the brew. Of the main polysaccharides in coffee, galactomannan and arabinogalactan are water soluble but not for cellulose (Nunes and Coimbra, 2001; De Maria *et al*., 1994).

During roasting, sucrose is consumed by caramelization and Maillard reactions (after inversion). Soluble fiber is partially degraded and incorporated into melanoidins. The brew acidity may increase as levels of aliphatic acids (formic, acetic, glycolic and lactic acids) rise through degradations of sucrose, polysaccharides and other compounds, especially during a short-high temperature roasting (Clarke, 1985; Ginz *et al.*, 2000).

2.9.1.4 Lipid

Lipids are the major components of coffee. The lipid fraction of coffee is composed mainly of triacylglycerols (approximately 75%), free fatty acids (1%), sterols (2.2% unesterified and 3.2% esterified with fatty acids) and tocopherols (0.05%), which are typically found in edible vegetable oils. This fraction also contains diterpenes of the kaurene family in proportions of up to 20% of the total lipid fraction (Trugo and Macrae, 1984a; Folstar, 1985; Kolling-Speer and Speer, 2005). Total lipid content in arabica seed is about 14 g/100 g DW (Stephanucci, 1979). Fatty acids in coffee are found primarily in combined forms; most are esterified with glycerol in the triacylglycerol fraction, 20% esterified with diterpenes, and a small proportion in sterol esters. Most fatty acids in coffee are unsaturated. Linoleic acid, oleic acid and linolenic acid are found in the amounts of 43.0%-54.0%, 7.0%-14.0%, and 1.0%-2.6% of the triacylglycerol fraction, respectively. The aforementioned acids also found in the amounts of 46.0%, 11.0%, and 1.0% of the free fatty-acid fractions, respectively (Folstar, 1985; Lercker *et al*., 1996; Nikolova-Damyanova *et al*., 1998; Kolling-Speer and Speer, 2005). Fatty acids are not only important for health, but also help keeping coffee fresh and avoid the staleness caused by hydrolysis and oxidation of triacylglycerols (Toci *et al.*, (2008). The major categories of sterols in coffee are 4-desmethylsterols (accounting for 93% of total sterols), 4-methylsterols (2%) and 4,4-dimethyl-sterols (5%). Sitosterol belongs to the first category, and accounts for up to 54% of the sterol fraction. While, stigmasterol and campesterol individually account for about 20% of the sterol fraction (Flament *et al*., 1968; Speer and Kolling-Speer, 2006). The average amount of tocopherols in coffee has been reported as 11.9 mg/100 g in green coffee (Ogawa *et al*,. 1989). Although most lipids are located in the endosperm of green coffee seeds, the coffee wax is located in the outer layer. This fraction accounts

for 0.2%-0.3% of the coffee seed's weight. The main components of coffee wax are carboxylic acid-5-hydroxytryptamides, which are amides of serotonin and fatty acids (Speer and Kolling-Speer, 2006).

Lipids are important components to coffee beverage flavor and aroma as it greatly proposed to an increase of cup quality. Coffee beans grown at higher altitudes have higher "fat matter" content (Decazy *et al*., 2003; Vaast *et al*., 2006). Lipids are expelled with roasting to the bean surface, forming a layer which may trap volatile aromas, impairing the immediate loss of these compounds (Clifford, 1985a; Arnaud, 1988). The most important commercial use for this oil is the aromatization of soluble coffee (Clarke, 1985). Moreover, tocopherols content also decreases during roasting. (Speer and Kolling-Speer, 2006).

2.9.1.5 Caffeine

Caffeine is an alkaloid of the methylxanthine family. Its chemical formula is $C_8H_{10}N_4O_2$, and its systematic name is 1,3,7-trimethyl-xanthine. A purine alkaloid is a secondary metabolite of coffee plant. Biosynthesis of this compound starts from xanthosinemonophosphate (Arnaud, 1987). In the metabolic pathway, subsequent methylation steps occur with different N-methyl transferases, methionine being a methyl donor. The purine catabolism of caffeine comprises its degradation via successive demethylation, resulting in carbon dioxide and ammonia.

Caffeine stimulates central nervous system as an adenosine-receptor antagonist. Although caffeine is widely consumed and studied psychoactive substance in history, its effects on health are controversial. While caffeine intake has been associated with high blood cholesterol, coronary diseases and cancer, other studies suggest that its consumption may lower the incidence of suicide and hepatic cirrhosis (Arnaud, 1987).

In coffee bean processing, caffeine is slightly altered during roasting, small losses may occur due to sublimation. However, an increase in caffeine content may be observed due to the degradation of other compounds (Farah, 2012).

2.9.1.6 Total phenol

Phenolic compounds are secondary metabolites, and generally involve in plant adaptation to environmental stress conditions. Chlorogenic acids and related compounds are the main components of the phenolic fractions in green coffee beans, reaching up to about 14% of coffee dry matter. Phenolic compounds are presented predominantly as a family of esters formed between certain hydroxycinnamic acids and quinic acid, collectively known as chlorogenic acids (Clifford, 1985b). Other phenolic compounds, such as tannins, lignans and anthocyanins are also observed in coffee seeds, although in minor amounts. The most common type chlorogenic acids in coffee is caffeic acid (3,4-dihydroxy-cinnamic acid), followed by ferulic acid (3-metoxy, 4-hydroxy-cinnamic acid) and *p*-coumaric acid (4-hydroxy-cinnamic acid) (Clifford *et al*., 2003).

Total chlorogenic acid content of green coffee beans may vary according to species, cultivars, degree of maturation, and less importantly, agricultural practices, climate and soil conditions (Clifford, 1985b, Guerrero *et al*., 2001; Camacho-Cristobal *et al*., 2002, Farah *et al*., 2005). Chlorogenic acid (CGA) is a key factor determining coffee quality and plays an important role in the formation of coffee flavor (Carelli *et al*., 1974; Clifford and Wright, 1976; Trugo and Macrae, 1984a; Variyar *et al*., 2003, Farah *et al*., 2006a). Moreover, this compound also provides several health-promoting properties, mainly expressed in its antioxidant activity. In addition, the compound also exhibits hypoglycemic, antiviral, hepatoprotective and antispasmodic activities (Basnet *et al*., 1996; Trute *et al*., 1997; Grace *et al*., 1998; Trugo, 2001; Natella *et al*., 2002; Pereira *et al*., 2003, Moreira *et al*., 2005).

In the coffee processing, chlorogenic acid takes part in the generation of color, flavor and aroma of coffee during roasting (Trugo and Macrae, 1984a; Moreira *et al*., 2000; Montavon *et al*., 2003). Due to their thermal instability, chlorogenic acid may be completely degraded into phenol derivatives when submitted to intense roasting conditions. During roasting, chlorogenic acid is partially isomerized and transformed into quinolactones due to dehydration and formation of an intramolecular bond, while the rest part is somewhat hydrolyzed and degraded into low molecular weight compounds (Trugo, 1984; Trugo and Macrae, 1984a; Leloup *et al*., 1995; Clifford,

2000; Farah *et al*., 2005). Chlorogenic acid also participates in the formation of polymeric material like melanoidins (Menezes, 1994; Steinhart and Luger, 1997). Drastic roasting conditions may lead to a vast losses of chlorogenic acid, up to 95% (Trugo, 1984; Clifford, 1997, 1999, 2000). Total chlorogenic acid content in commercial roasted coffee typically ranges from about 0.5 to 7.0%, depending on the type of processing, roasting degree, blend and analytical conditions (Farah *et al*., 2001; Clifford, 2000).

2.9.2 Volatile compounds

The most abundant classes of volatile compounds in green bean are alcohols, esters, hydrocarbons, aldehydes, ketones, pyrazines, furans and sulfur compounds. The complex aroma of coffee is formed by pyrolysis, Strecker degradation and Maillard reaction in roasting process. The variety and concentrations of volatile compounds in roasted coffee depend on the compositions of non-volatile compounds in the raw seeds, and on roasting conditions (Farah, 2012).

2.10 Antioxidant capacity of coffee

Coffee is a very popular beverage that enriches in non-enzymatic and bioactive constituents with high antioxidant capacity. Beneficial health effects of coffee are usually attributed to its high level of free radical scavenging activity (an ability of oxidation process inhibition). Antioxidant activity of coffee is highly related to chlorogenic, ferulic, caffeic and p-coumaric acids contents (Nicoli *et al*, 1997a). In roasted coffee, melanoidins (brown pigments) are synthesized and considered as strong antioxidants (Steinhart *et al*., 2001). Some literatures reported that caffeine and trigonelline are considered as natural antioxidants found in typical coffee seeds. Moreover, phenylalanine, another compound appeared when roasted, also exhibits high level antioxidant activity (Farah and Donangelo, 2006).

CHAPTER 3

Effects of Brassin-like Substance on Seedling Growth of Arabica Coffee

3.1 Materials and methods

3.1.1 Plant material

Fresh ripened fruit of Arabica coffee (*Coffea arabica* L. cv. Catimor) were harvested from an experimental field at Khun Chang Kien Station site A, Highland Research and Training Center, Faculty of Agriculture, Chiang Mai University (at the altitude 1,200 m above mean sea level). The fruit were manually de-pulped and left to ferment for 48 hours. The mucilage were removed thereafter and rinsed off by running water. Then, the seeds were dried until moisture content reached 12%. Finally, an endocarp was removed by hand prior to conducting germination procedure.

3.1.2 Germination

For germination and seedling growth, all coffee seeds were disinfected by dipping in 1.0% sodium hypochloride (NaOCl) solution for 2 minutes, and subsequently rinsed off by distilled water. Afterward, the seeds were divided into four soaking treatments, namely, soaking in distilled water (control), soaking in 0.5, 1.0 and 2.0 mg/L brassin-like substance (BS) solutions for 24 hours. Germination test was conducted by using a completely randomized design with four replications. Each replication consisted of 100 seeds. In germination procedure, the seeds were placed between papers in an aluminum tray (width×length: 18×29 cm). Each germination unit was moistened using distilled water at the quantity of 2.5 times of dry paper weight. All germination trays were subsequently placed in a germination chamber (30.0ºC ± 1.0ºC) under dark lighting condition for 15 days (Valio, 1976). Seeds were considered germinated according to the rules for seed snalysis described by Brasil (2009).

Figure 3.1 Arabica coffee seeds (endocarp removed)

Figure 3.2 Soaking the arabica coffee seed before germination

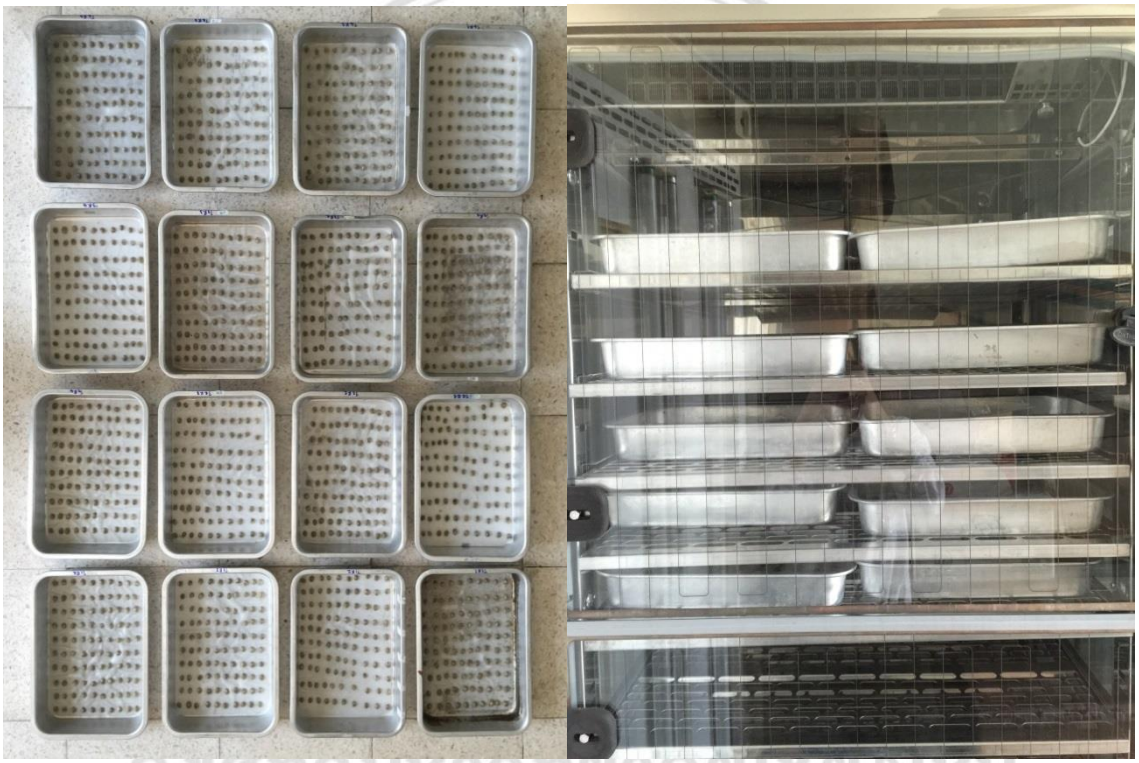

Figure 3.3 Coffee seeds placed in a growth chamber for germination (30ºC, RH 85%)

3.1.3 Determination of imbibition curves

Seeds without endocarps (parchments) were conducted to the determination of moisture content before germination test. Every 24 hours, seeds were removed from a growth chamber and weighed on an analytical balance (capable of weighing 0.0001 g) for detection of seed weight. Weight gain of seed was an estimate of the amount of water absorbed (Bewley and Black, 1982). First count and final count were made on the first day to day 15 after soaking.

3.1.4 Germination percentage

Physiological germination was assessed at day 15 of the germination test. Seeds with at least 2.0 mm root protrusion were counted. The results were expressed in percentage unit. In this study, seeds considered germinated according to the rules for seed analysis as explained by Brasil (2009).

$$\text{Germination percentage} = \left(\frac{n}{N}\right)\times 100$$

Where;

n = the number of germinated seeds

N = the number of total seeds (ISTA, 1996)

3.1.5 Speed of germination

$$\text{Speed of germination} = \sum D\times\left(\frac{n}{N}\right)$$

Where;

n = the number of germinated seeds at each day

D = the number of days after the start of the experiment (ISTA, 1996)

3.1.6 Mean germination time

The mean germination time was calculated using the method of Yoursheng and Sziklai (1985) as follows;

$$\text{Mean germination time} = \sum_{i=1}^{n}\left(\frac{n_i d_i}{n}\right)$$

Where;

n_i = number of seed germinated on day

d_i = day during germination period

n = total number of seeds germinated during experimental period

3.1.7 Seedling vigor index

Nursery experiment was conducted to study the effects of brassin-like substance concentrations on seedling vigor index of arabica coffee seed. After soaking the seeds with different concentrations of brassin-like substance solution for 24 hours, seeds from each treatment were sown in the black perforated polyethylene bags (width × length : 3″ × 7″) filled with growing media. The medium composed of soil (100 kg), manure (20 kg), super phosphate (200 g), dolomite (200 g) and 3% Furadan (25 g) (Angkasith and Warit, 1999). One seedling was sown directly per bag, and watered daily. Coffee seedling production was tested in a nursery under ambient environmental conditions. Seedling length was measured using a digital vernier caliper (capable of measuring 0.01 mm).

One hundred seedlings were randomly taken from each replication after 45 days of sowing. The following measurements of seedling vigor characters were made as follows:

1) Length of seedling (cm)
2) Fresh weight of seedling (g)

Seedlings were weighed immediately in order to obtain fresh weight information, and oven-dried for 24 hour at 105ºC. Then, dry weight was recorded. Seedling vigor was calculated using formula described by Abdul-Baki and Anderson (1973) as follows;

Vigor index I = Germination% × Seedling length

Vigor index II = Germination% × Seedling dry weight

3.1.8 Statistical analysis

Means and one-way ANOVA were calculated using software the PASW® Statistics 18. Duncan's multiple range test (DMRT) was used to interpret significant difference among the means ($p < 0.05$).

3.2 Results and discussion

3.2.1 Determination of imbibition curves

Water uptake is an essential requirement for initiation of seed germination. Rapid initial absorption of a dry seed is a consequence of water potential difference between the seed and the substrate, which is caused by high matrix potential of the seed. This water movement always occurs spontaneously along a decrease of water potential gradient (Villela *et al*., 1991). This phenomenon is the same in either viable or non-viable seeds (Bewley and Black, 1994).

Uptake of water by coffee seed is triphasic; "Phase I" is characterized by a relatively fast water uptake. At high concentration of BS (1.0 and 2.0 mg/L), imbibition of seed occurred rapidly at day 2 to day 4 after soaking. Seed fresh weight increased up to 43% when it reached nearly to "Phase II"; plateau phase (metabolic processes) after approximately 5 days of imbibition (Table 3.1).

During "Phase II", seeds remained on average; with the slight increases their fresh weight until 10 days after soaking. At the end of "Phase II", seed fresh weight increased again, as a consequence of radicle protrusion of the first (fastest) seed. Figure

3.4 showed significant increases in water uptake during the imbibitions observed in BS 1.0 and 2.0 mg/L treatments.

The beginning of "Phase III" for the population average was around day 12. In which, it was about 10 to 15 days after soaking. According to the imbibition curves, BS application at 1.0 and 2.0 mg/L stimulated the rate of water uptake; weight gain of the seeds. The highest rate of water uptake was relatively higher than the control and 0.5 mg/L of BS treatment. A further increase in water uptake (Phase III) probably appears only when germination is completed, as the embryo axis elongates and breaks through its covering structures (Manz *et al*., 2005). Water uptake is the essential requirement for the initiation and completion of seed germination. Sufficient water and oxygen, and appropriate temperature are necessary for a mature or non-dormant seed to complete germination. The completion of germination is demonstrated as radicle protrusion. The emergence of the radicle depends on embryo expansion, which is a process driven by water uptake and cell wall loosening, thus results in radicle penetration through all seed-covering layers (Bewley, 1997). Results in this experiment agreed with Valio (1980) and Da Silva *et al*. (2004), whom also reported that radicle protrusion in arabica coffee seeds under optimal conditions (30ºC, in the dark) started around day 5 or 6 of imbibition, and 50% of the seed population displays radicle protrusion by day 10. At day 15 of imbibition, most of the seeds had completed their germination.

Table 3.1 Effect of brassin-like substance on water imbibition of arabica coffee seed

Treatment	Fresh weight of coffee seed (mg)							
	Days after germination							
	Before soaking	**1**	**2**	**3**	**4**	**5**	**6**	**7**
BS 0 mg/L	248.34	289.33±0.75	301.97±1.56^{B}	317.94±1.86^{B}	333.48±0.89^{D}	349.90±0.84^{B}	351.16±0.96^{B}	351.27±0.97^{B}
BS 0.5 mg/L	248.34	288.30±0.66	303.00±0.65^{B}	320.03±1.53^{B}	342.55±0.97^{C}	347.60±0.90^{B}	352.28±1.17^{B}	352.32±1.11^{B}
BS 1.0 mg/L	248.34	290.03±0.72	311.25±0.72^{A}	330.29±0.79^{A}	355.73±0.69^{A}	357.00±1.11^{A}	357.11±1.04^{A}	357.13±1.04^{A}
BS 2.0 mg/L	248.34	290.59±0.66	311.34±0.76^{A}	330.21±0.85^{A}	350.13±1.49^{B}	354.73±1.04^{B}	356.03±0.96^{A}	356.16±0.95^{A}
F-test		NS	*	*	*	*	*	*
C.V. (%)		3.41	4.57	5.82	4.31	4.19	4.15	4.07

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.

The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 3.1 Effect of brassin-like substance on water imbibition of arabica coffee seed (continued)

Treatment	Fresh weight of coffee seed (mg)							
	Days after germination							
	8	**9**	**10**	**11**	**12**	**13**	**14**	**15**
BS 0 mg/L	351.36±1.28^{B}	351.57±0.87^{B}	351.60±1.02^{B}	351.73±0.73^{C}	351.98±1.05^{B}	353.82±0.93^{D}	360.90±0.94^{B}	363.00±0.98^{B}
BS 0.5 mg/L	352.32±1.25^{B}	352.39±1.12^{B}	352.45±1.11^{B}	354.44±0.97^{B}	353.89±1.13^{B}	357.99±0.83^{C}	361.59±0.93^{B}	365.03±0.91^{B}
BS 1.0 mg/L	357.28±1.20^{A}	357.33±0.94^{A}	357.48±1.13^{A}	358.09±0.94^{AB}	360.68±1.00^{A}	363.33±0.85^{A}	366.10±0.77^{A}	370.33±0.99^{A}
BS 2.0 mg/L	356.25±0.96^{A}	356.28±0.85^{A}	356.54±0.89^{A}	356.61±1.00^{A}	358.15±0.96^{A}	360.55±0.84^{B}	364.70±0.94^{A}	368.37±0.87^{A}
F-test	*	*	*	*	*	*	*	*
C.V. (%)	4.71	3.79	4.16	3.65	4.11	3.40	3.49	3.62

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.

The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

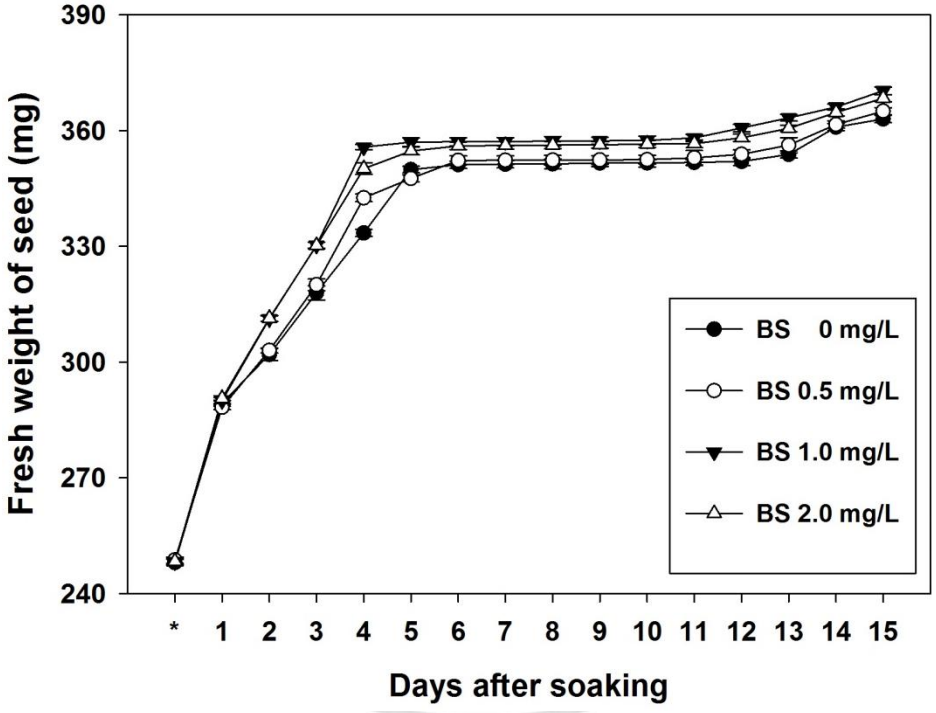

Figure 3.4 Effect of brassin-like substance on imbibition of seed triphasic pattern of water uptake of arabica coffee

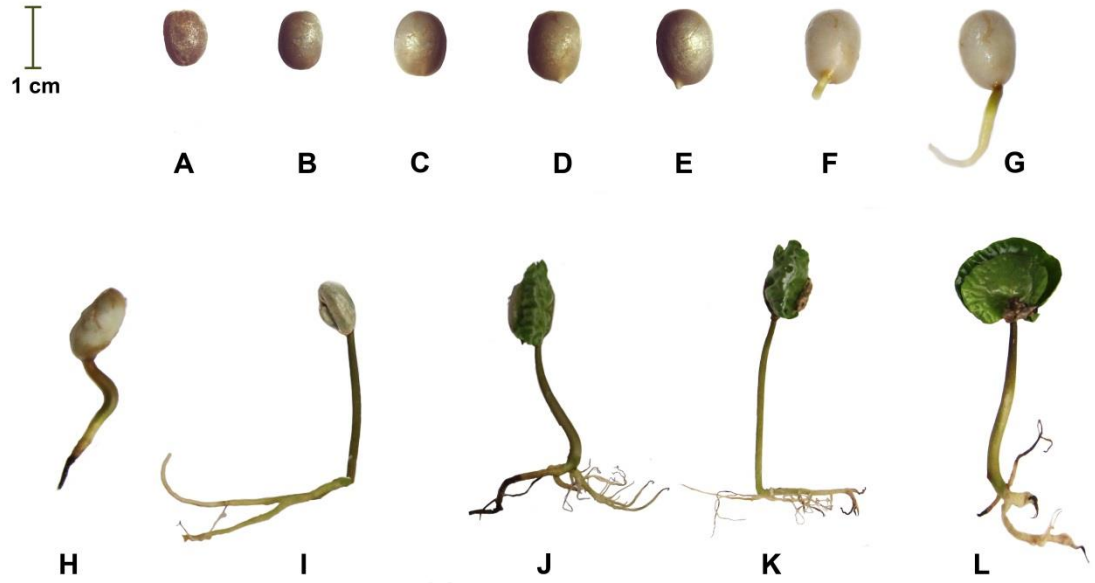

Figure 3.5 Coffee seed germination: (A) before soaking, (B) 2 days, (C) 4 days, (D) 6 days, (E) 8 days, (F) 10 days, (G) 12 days, (H) 15 days, (I) 25 days, (J) 30 days, (K) 40 days and (L) 45 days

3.2.2 Germination percentage

Table 3.2 and Figure 3.6 showed germination results of coffee seeds treated by different BS concentrations. Statistically, the highest percentage of germination was achieved in seeds treated by 2.0 and 1.0 mg/L of BS which were 94.00 and 93.50%, respectively.

Several literatures show that plant brassinosteroids play a role in promoting germination. It has been recognized that seed dormancy and germination are regulated by the plant hormones called abscisic acid (ABA) and gibberellin (GA). These two hormones act antagonistically to each other. ABA typically induces seed dormancy in maturing embryos and inhibits seed germination. While, GA breaks seed dormancy and promotes germination. Brassinosteroid is needed to overcome inhibition of germination by ABA (Steber and McCourt, 2001). Ethylene and BR promote seed germination and also counteract ABA effects (Birgit *et al*., 2005). Brassinosteroid application has been reported to enhance germination of several angiosperms (Takeuchi *et al*., 1991, 1995), cereals (Gregory, 1981; Yamaguchi *et al*., 1987), Arabidopsis (Steber and McCourt, 2001) and tobacco (Leubner-Metzger, 2001). Pretreatment with brassinolide also stimulates germination and seedling emergence of aged rice seeds (Yamaguchi *et al*., 1987). Brassinolide application also accelerates subsequent seedling growth of barley (*Hordeum vulgare* L.) (Gregory, 1981). The seed treatment with brassinolide has been proofed to improve germination percentage in several plant species. Similarly, brassinosteroids promoted seed germination in case of rape (*Brassica napus*) (Chang and Cai, 1988), rice (*Oryza sativa*) (Dong *et al*., 1989), wheat (*Triticum aestivum* L. cv. HD-2204) (Hayat *et al*., 2003) and tobacco (*Nicotiana tabacum* L. cv. Havana 425) (Leubner-Metzger, 2001).

Table 3.2 Effect of brassin-like substance on percent germination of arabica coffee (Physiological germination was assessed at 15 days)

Treatment	Germination (%)
BS 0 mg/L	91.25 ± 0.48 ^{B}
BS 0.5 mg/L	91.25 ± 0.48 ^{B}
BS 1.0 mg/L	93.50 ± 0.29 ^{A}
BS 2.0 mg/L	94.00 ± 0.41 ^{A}
F-test	*
C.V. (%)	0.90

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.

The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

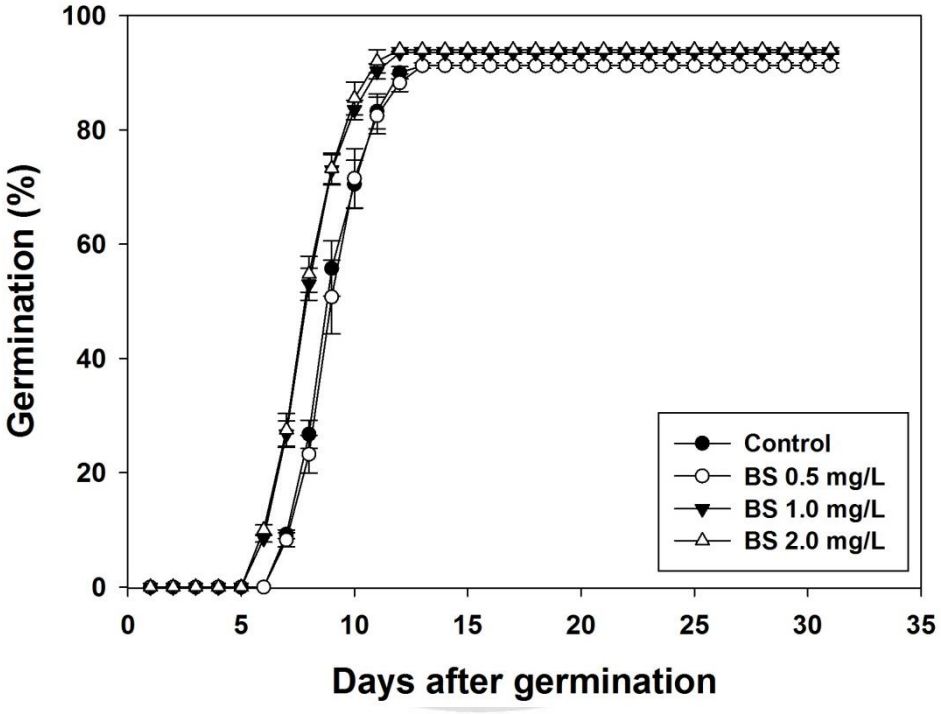

Figure 3.6 Effect of brassin-like substance on germination of arabica coffee

3.2.3 Mean germination time

The mean germination time represents the mean time a seed requires to initiate and end germination. Concentration of BS had a significant effect ($p < 0.05$) on mean germination time (Figure 3.7). Seeds soaked in distilled water and 0.5 mg/L of BS solution had significantly longer mean germination time than those of the seeds soaked in 1.0 and 2.0 mg/L of BS solutions (Table 3.3). Similar results literally reported that the germination rate was improved, and mean time germination of ailanthus (*Ailanthus altissima*) seeds was shortened after the seeds were soaked with brassinolide (Kai-rong, *et al*., 2005).

Table 3.3 Effect of brassin-like substance on mean germination time of arabica coffee seed

Treatment	Mean germination time (day)
BS 0 mg/L	8.32 ± 0.18^{A}
BS 0.5 mg/L	8.45 ± 0.22^{A}
BS 1.0 mg/L	7.42 ± 0.10^{B}
BS 2.0 mg/L	7.35 ± 0.10^{B}
F-test	*
C.V. (%)	3.96

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.

The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

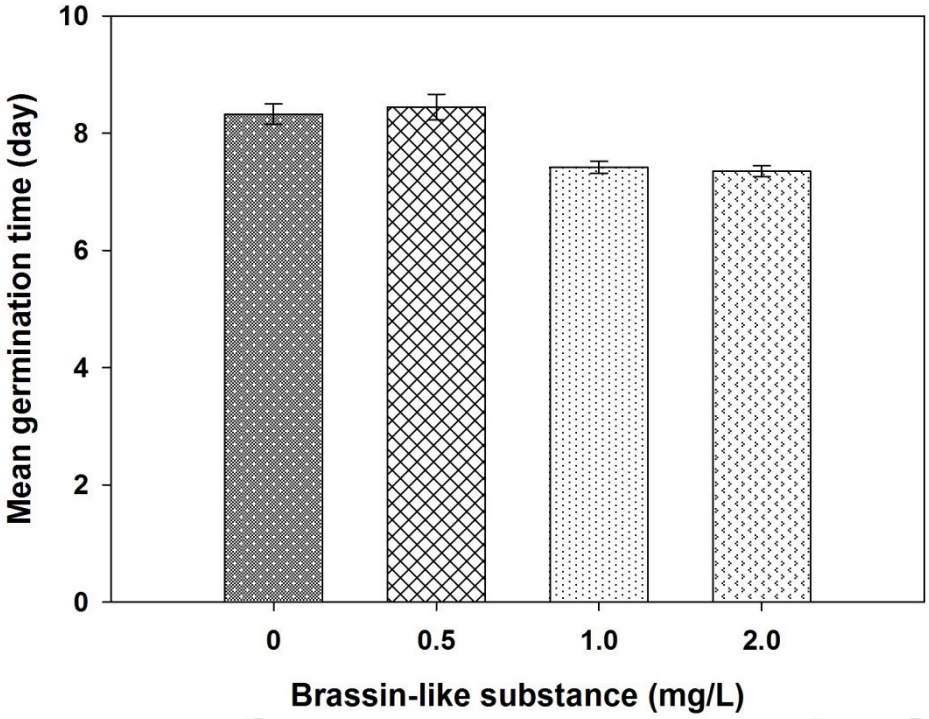

Figure 3.7 Effect of brassin-like substance on mean germination time of arabica coffee seed

3.2.4 Speed of germination index

Germination speed has been used to evaluate germination enhancement in primed seeds and as an indicator of seed vigor (Hacisalihoglu *et al*., 1999). However, our experimental results showed no significant difference in speed germination index among brassin-like substance concentrations (Table 3.4).

Table 3.4 Effect of brassin-like substance on speed of germination index of arabica coffee seed

Treatment	Speed of germination index
BS 0 mg/L	11.30 ± 0.19
BS 0.5 mg/L	11.13 ± 0.29
BS 1.0 mg/L	11.86 ± 0.33
BS 2.0 mg/L	11.61 ± 0.15
F-test	NS
C.V. (%)	4.38

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.

The results were expressed as mean value ± standard error.

(NS): not significantly different

3.2.5 Seedling vigor index I and II

BS treatments significantly affected on both of seedling vigor indexes I and II. The seedling vigor index I increased upon brassin-like substance concentration. The highest and the lowest of seedling vigor index I were obtained in the seeds applied by BS concentration of 2.0 mg/L (914.72) and distilled water (813.28), respectively (Table 3.5, Figure 3.8). Likewise previous results, the maximum of seedling vigor index II were observed in seeds treated by 1.0 and 2.0 mg/L of BS solutions (59.97 and 60.16, respectively), while a minimum value was obtained from seeds in control treatment (46.93). Both of brassinosteroid and gibberellins are known to regulate shoot elongation and photomorphogenesis of seedlings, but antagonize to the growth inhibiting actions of abscisic acid (ABA) (Altmann, 1999; Neff *et al*., 2000; Bishop and Koncz, 2002). Brassinolide application causes pronounced elongation of hypocotyls, epicotyls, and peduncles of dicots, as well as coleoptiles and mesocotyls of monocots (Mandava, 1988; Sasse, 1990; Clouse, 1996).

In this study, seedling vigor index was found to be synchronized with the emergence performance of seedlings. Accordingly, seedlings from BS concentrations of 1.0 and 2.0 mg/L showed better performance in terms of germination percentage and mean time germination. The seedlings were also found to be more vigorous than ones treated by BS concentration of 0.5 mg/L and distilled water. In another word, seedlings that emerged earlier were more vigorous than the late germinated ones.

Table 3.5 Effect of brassin-like substance on seedling vigor index I and II of arabica coffee seed

Treatment	Seedling vigor index I	Seedling vigor index II
BS 0 mg/L	813.28 ± 5.10 ^{D}	46.93 ± 0.56 ^{C}
BS 0.5 mg/L	846.59 ± 6.69 ^{C}	53.23 ± 0.65 ^{B}
BS 1.0 mg/L	878.74 ± 6.42 ^{B}	59.97 ± 0.66 ^{A}
BS 2.0 mg/L	914.72 ± 6.77 ^{A}	60.16 ± 0.67 ^{A}
F-test	*	*
C.V. (%)	10.29	16.39

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.

The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

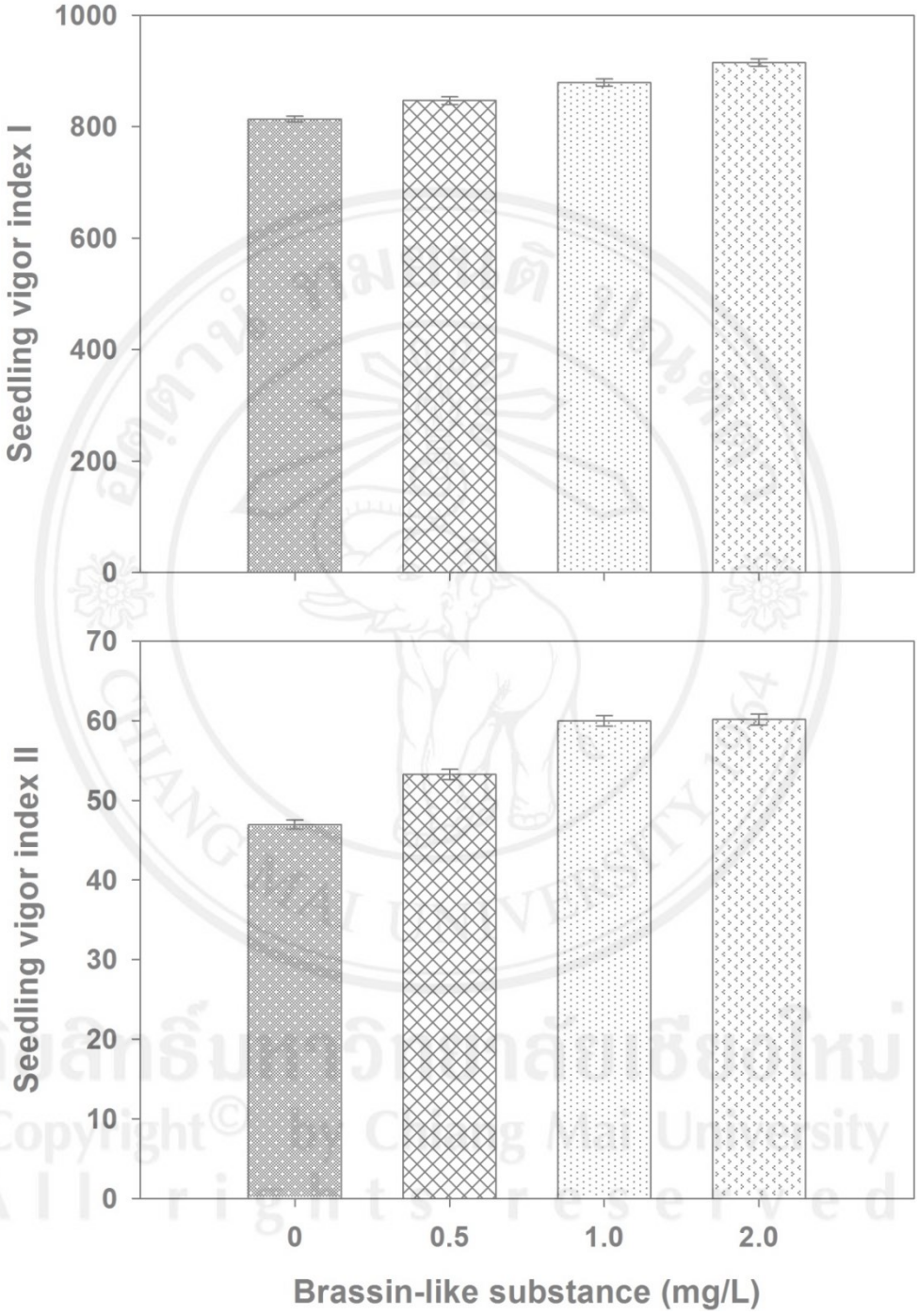

Figure 3.8 Effect of brassin-like substance on seedling vigor index I and II of arabica coffee seed

3.3 Conclusion

Soaking coffee seeds with 1.0 mg/L brassin-like substance for 24 hours was the most effective and efficient for improving germination percentage, mean germination time and seedling vigor index.

CHAPTER 4

Effects of Brassin-like Substance and Germination on Changes of Chemical Composition of Arabica Coffee

4.1 Materials and Methods

All seeds were soaked in brassin-like substance (BS) solution for 24 hours prior to conducting germination procedure as described in a previous experiment. One hundred coffee seeds were randomly selected from three replications of BS soaking treatments (0, 0.5, 1.0 and 2.0 mg/L). In order to study the changes of biochemical compositions during germination, the coffee seeds were collected at day 0, 2, 4, 6 and 8.

The experiment was designed as 4×5 factorial in completely randomized design (CRD) with three replications, in which 100 seeds per replicate were used. The following determinations were expressed as milligram per gram of dry weight basis (mg/g DW). Total phenolics content was expressed in milligram gallic acid equivalent per gram dry weight (mg GAE/g DW) and DPPH radical scavenging activity were expressed as micromolar trolox equivalent per gram dry weight (μM Trolox/g DW).

4.1.1 Protein content was analyzed by the method of Lowry *et al*. (1951). (Appendix A)

4.1.2 Total sugars content was analyzed according to James (1995). (Appendix B)

4.1.3 Fat content was analyzed according to AOAC, (2000) (Appendix C)

4.1.4 Caffeine content was determined by high performance liquid chromatography (HPLC) (Wang *et al*., 2000). (Appendix D)

4.1.5 Chlorogenic acid content was determined by high performance liquid chromatography (HPLC) (Ky *et al*., 1997) (Appendix E)

4.1.6 Total phenols content was analyzed according to Folin and Ciocalteu (1927) (Appendix F)

4.1.7 Oxidation activity (DPPH radical scavenging activity) was analyzed according to (Brand-Willium *et al*., 1995) (Appendix G)

4.1.8 Statistical analysis

Means of each data combinations were analyzed by using statistical analysis software; the PASW® Statistics 18. Duncan's multiple range test (DMRT) was used to interpret significant difference among the means ($p < 0.05$).

4.2 Results and discussion

4.2.1 Protein content

Figure 4.1 showed the effect of brassin-like substance pretreatments on protein content of arabica coffee seeds during germination.

Initial protein content of coffee seeds were 74.10 mg/g DW. The protein content of seeds from all BS treatments tended to increase and reach their peaks at day 4 of germination (Table 4.1 and 4.2). Nonetheless, the protein content gradually declined during day 6 to day 8 of germination. This diminution was probably caused by reserve proteins degradation. Degraded proteins were commonly used for respiration and synthesis of new cells prior to developing embryo (Vidal-Valverde, 2002).

Brassin-like substance concentration had significant effect on protein content in seed during germination. Coffee seeds which were soaked with BS concentrations of 1.0 and 2.0 mg/L for 24 hours before germination greatly produced higher protein content (101.71 and 100.23 mg/g DW, respectively) than the coffee seeds that soaked with distilled water (control) and BS concentration of 1.0 mg/L (82.08 and 92.96 mg/g DW, respectively).

Kalinich *et al*. (1986) suggested that the increase in protein content under brassinosteroid influence probably was a result of the enhanced activity of RNA and DNA polymerases that engaged in a physiological response to the BRs hormone. Furthermore, their report also showed the increases in protein content after BRs application to pea and lupine plants.

In this study, there was a significant interaction between brassin-like substance concentrations and germination times on protein content in coffee seeds. At day 4 of germination, the seeds in 1.0 and 2.0 mg/L BS treatments gave the highest protein content which were 135.74 and 134.27 mg/g DW, respectively (Table 4.2, Figure 4.1).

Generally, during germination process, storage protein breakdown usually occurs as the plant uses them as sources of nitrogen and carbon for bio-molecule synthesis. According to Wang *et al*. (2007), plants accumulate and store proteins in protein storage vacuoles during seed development and maturation. Upon seed germination, these storage proteins are gradually degraded and mobilized in order to provide nutrient supplements for seedling growth. The increases in protein content after germination are also found in other plants such as germinated rice, germinated brown rice (*Oryza sativa*) (Trachoo *et al*. 2006), sweet lupin (*Lupinus angustifolius*) (Rumiyati *et al*., 2012), soybean (*Glycine max*) (Mostafa *et al*., 1987) and mung bean (*Vigna radiata*) (Sangronis and Machado, 2007).

Table 4.1 Effect of brassin-like substance concentrations and germination on protein content in arabica coffee seeds

Treatment		Protein content (mg/g DW)
BS concentration	BS 0 mg/L	82.08 ± 4.80 ^{C}
	BS 0.5 mg/L	92.96 ± 6.95 ^{B}
	BS 1.0 mg/L	101.71 ± 9.05 ^{A}
	BS 2.0 mg/L	100.23 ± 9.06 ^{A}
Days after germination	Before soaking	74.10 ± 0.16 ^{e}
	Day 2	99.44 ± 6.32 ^{b}
	Day 4	122.42 ± 7.79 ^{a}
	Day 6	91.79 ± 4.90 ^{c}
	Day 8	83.48 ± 4.10 ^{d}
BS concentration		*
Days after germination		*
BS concentration×*Days after germination*		*
C.V. (%)		9.82

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

Table 4.2 Interaction of effect of brassin-like substance concentrations and germination on protein content in arabica coffee seeds

Treatment		Protein content
BS concentration	**Days after germination**	**(mg/g DW)**
BS 0 mg/L	Before soaking	73.91 ± 3.03 ^{H}
	Day 2	81.20 ± 4.16 ^{HI}
	Day 4	102.96 ± 6.12 ^{CDE}
	Day 6	77.17 ± 1.86 ^{I}
	Day 8	75.19 ± 6.98 ^{I}
BS 0.5 mg/L	Before soaking	74.14 ± 4.54 ^{I}
	Day 2	100.74 ± 3.76 ^{CDEF}
	Day 4	116.71 ± 2.24 ^{B}
	Day 6	95.25 ± 1.49 ^{EFG}
	Day 8	77.99 ± 4.87 ^{HI}
BS 1.0 mg/L	Before soaking	74.55 ± 4.18 ^{I}
	Day 2	108.44 ± 1.65 ^{BC}
	Day 4	135.74 ± 4.94 ^{A}
	Day 6	97.53 ± 3.67 ^{DEFG}
	Day 8	92.34 ± 2.44 ^{FG}
BS 2.0 mg/L	Before soaking	73.85 ± 2.93 ^{I}
	Day 2	107.39 ± 3.15 ^{BCD}
	Day 4	134.27 ± 2.89 ^{A}
	Day 6	97.24 ± 2.36 ^{DEFG}
	Day 8	88.43 ± 2.71 ^{GH}
F-test		*
C.V. (%)		9.91

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

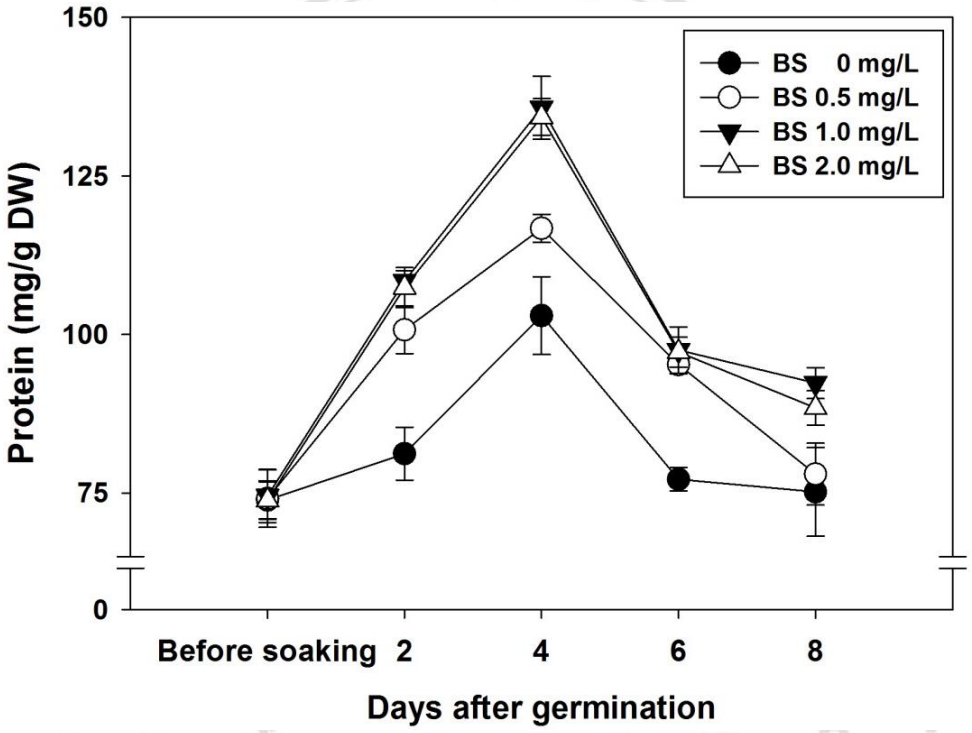

Figure 4.1 Effect of brassin-like substance concentrations and germination on protein content in arabica coffee seeds

4.2.2 Total sugars content

The changes in total sugars content in seed during germination are presented in Table 4.3. During germination, brassin-like substance concentration and germination time significantly affected on the total sugars in germinated coffee seeds. The seeds which were soaked with BS 1.0 mg/L had significantly higher total sugars (210.90 mg/g DW) than seeds treated by other BS concentrations.

The results of the BS application showed that total sugars in arabica coffee seeds had the increased tendency during day 2 to day 8 after germination. Final amount of total sugars (day 8) in germinated coffee seeds was much higher than the amount of non-germinated seeds (before soaking), which were 239.79 and 116.37 mg/g DW, respectively (Table 4.3, Figure 4.2).

There were significant interactions between brassin-like substance soaking concentrations and germination times on total sugars content (Table 4.4). The highest amount of total sugars content during germinating (8 days) was obtained when treated the seeds by 1.0 and 2.0 mg/L of BS (255.41 and 253.40 mg/g DW, respectively). The lowest total sugars was found in seeds before soaking (116.37 mg/g DW). Similar results were reported by Satyanarayana *et al.* (2011). The authors studied the changes of soluble sugars in Gum karaya (*Sterculia urens* Roxb.) seeds during germination and found that there was an increase in total soluble sugars upon day 6, and the sugars decreased thereafter until day 15 of seed germination. This might be due to the requirement of energy in a growing plant at initial stages of seed germination.

Table 4.3 Effect of brassin-like substance concentrations and germination on total sugars content in arabica coffee seeds

Treatment		Total sugars content (mg/g DW)
BS concentration	BS 0 mg/L	176.62 ± 18.81 ^{D}
	BS 0.5 mg/L	183.94 ± 18.92 ^{C}
	BS 1.0 mg/L	210.90 ± 24.32 ^{A}
	BS 2.0 mg/L	193.72 ± 22.39 ^{B}
Days after germination	Before soaking	116.37 ± 0.10 ^{e}
	Day 2	187.81 ± 13.97 ^{d}
	Day 4	196.67 ± 10.85 ^{c}
	Day 6	215.83 ± 5.41 ^{b}
	Day 8	239.79 ± 8.45 ^{a}
BS concentration		*
Days after germination		*
BS concentration×*Days after germination*		*
C.V. (%)		2.14

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.
The results were expressed as mean value ± standard error.
(*) : significantly different between treatments

Table 4.4 Interaction of effect of brassin-like substance concentrations and germination on total sugars content in arabica coffee seeds

Treatment		Total sugars content
BS concentration	**Days after germination**	**(mg/g DW)**
BS 0 mg/L	Before soaking	116.52 ± 0.90 ^{J}
	Day 2	163.04 ± 1.40 ^{I}
	Day 4	171.56 ± 0.99 ^{H}
	Day 6	207.19 ± 0.94 ^{E}
	Day 8	224.80 ± 3.37 ^{C}
BS 0.5 mg/L	Before soaking	116.30 ± 0.79 ^{J}
	Day 2	174.59 ± 0.60 ^{H}
	Day 4	193.24 ± 1.16 ^{F}
	Day 6	210.00 ± 1.69 ^{E}
	Day 8	225.57 ± 0.79 ^{C}
BS 1.0 mg/L	Before soaking	116.11 ± 0.91 ^{J}
	Day 2	227.19 ± 4.04 ^{BC}
	Day 4	224.41 ± 2.23 ^{C}
	Day 6	231.38 ± 2.04 ^{B}
	Day 8	255.41 ± 1.99 ^{A}
BS 2.0 mg/L	Before soaking	116.55 ± 0.91 ^{J}
	Day 2	186.44 ± 0.79 ^{G}
	Day 4	197.49 ± 1.06 ^{F}
	Day 6	214.74 ± 0.60 ^{D}
	Day 8	253.40 ± 0.92 ^{A}
F-test		*
C.V. (%)		2.14

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

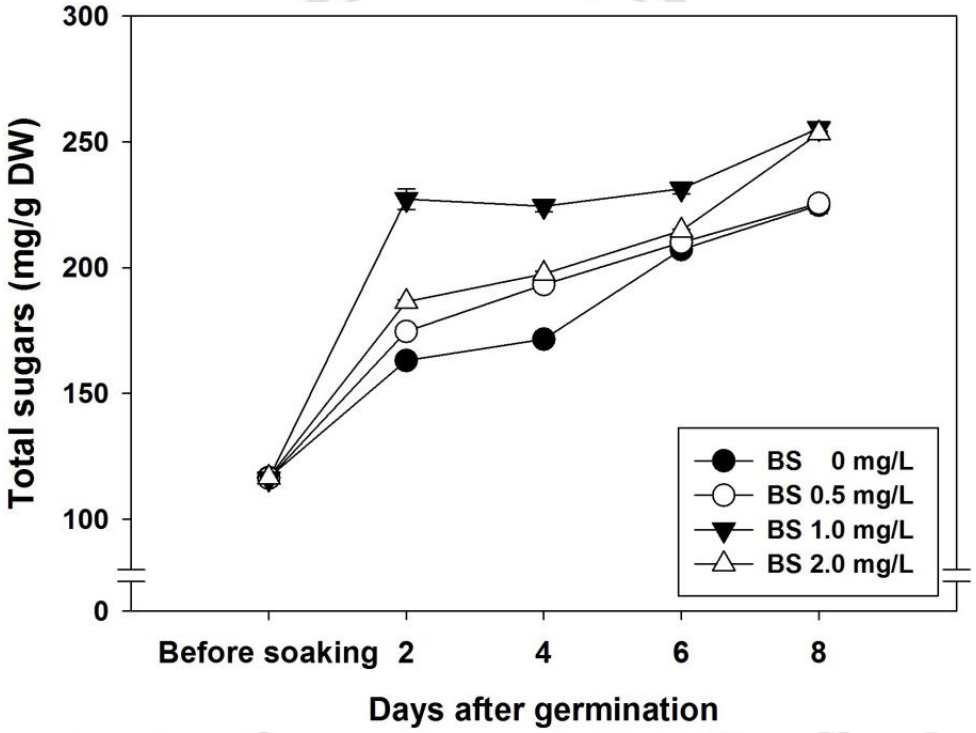

Figure 4.2 Effect of brassin-like substance concentrations and germination on total sugars content in arabica coffee seeds

4.2.3 Fat content

The effect of brassin-like substance and germination time on fat content in germinated coffee seeds are shown in Table 4.5. Soaking the seeds with BS solution at concentrations of 1.0 and 2.0 mg/L provided higher fat content in arabica coffee rather than using BS solution at concentration of 0.5 mg/L and distilled water.

Fat content gradually increased upon the first four days of germination (from 97.77 mg/g DW to 105.33 mg/g DW) (Table 4.5). However, the content declined until day 8 of germination as illustrated in Figure. 4.3. Similarly results were observed in the seeds of gum karaya *(Sterculia urens L.)* (Satyanarayana *et al.*, 2011). The lipid content was high at initial stages of germination (0-6 days), but it gradually declined by day 15 of germination. During this phase, the fat declining is usually observed in germinated seeds of several plants, such as mung bean (*Vigna radiata*) and pea (*Pisum sativum*) (El-Adawy *et al*., 2003), germinated lentil (*Lens culinaris*) and chick pea (*Cicer arietinum*) (Ghavidel and Prakash, 2007) and germinated sesame (*Sesamum indicum*) (Hahm *et al*., 2008). Bau *et al*. (1997) reported that the fat content in the seed decreased with an increase in the time of germination. This is because fat was used as the major source of carbon for seed growth. The fatty acids are oxidized to carbon dioxide and water to generate energy for seed germination (Hahm *et al*., 2008).

There was no significant interaction between the concentration of brassin-like substance and germination time on fat content in germinated arabica coffee seeds (Table 4.6).

Table 4.5 Effect of brassin-like substance concentrations and germination on fat content in arabica coffee seeds

Treatment		Fat content (mg/g DW)
BS concentration	BS 0 mg/L	88.37 ± 6.56 ^{B}
	BS 0.5 mg/L	89.02 ± 6.77 ^{B}
	BS 1.0 mg/L	96.98 ± 2.65 ^{A}
	BS 2.0 mg/L	95.01 ± 4.38 ^{A}
Days after germination	Before soaking	97.77 ± 0.11 ^{b}
	Day 2	97.17 ± 1.49 ^{b}
	Day 4	105.33 ± 0.72 ^{a}
	Day 6	83.73 ± 3.74 ^{c}
	Day 8	77.71 ± 5.33 ^{c}
BS concentration		*
Days after germination		*
BS concentration×*Days after germination*		NS
C.V. (%)		8.67

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 4.6 Interaction of effect of brassin-like substance concentrations and germination on fat content in arabica coffee seeds

Treatment		Fat content
BS concentration	**Days after germination**	**(mg/g DW)**
BS 0 mg/L	Before soaking	97.71 ± 0.25
	Day 2	93.50 ± 6.26
	Day 4	104.26 ± 4.82
	Day 6	77.30 ± 2.91
	Day 8	69.07 ± 2.42
BS 0.5 mg/L	Before soaking	98.02 ± 0.88
	Day 2	96.04 ± 0.37
	Day 4	104.72 ± 5.24
	Day 6	77.44 ± 0.59
	Day 8	68.86 ± 6.19
BS 1.0 mg/L	Before soaking	97.52 ± 0.88
	Day 2	100.10 ± 2.76
	Day 4	104.90 ± 9.81
	Day 6	91.77 ± 8.45
	Day 8	90.62 ± 6.96
BS 2.0 mg/L	Before soaking	97.84 ± 1.81
	Day 2	99.02 ± 2.71
	Day 4	107.45 ± 4.32
	Day 6	88.42 ± 2.97
	Day 8	82.29 ± 4.60
F-test		NS
C.V. (%)		8.62

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(NS): not significantly different

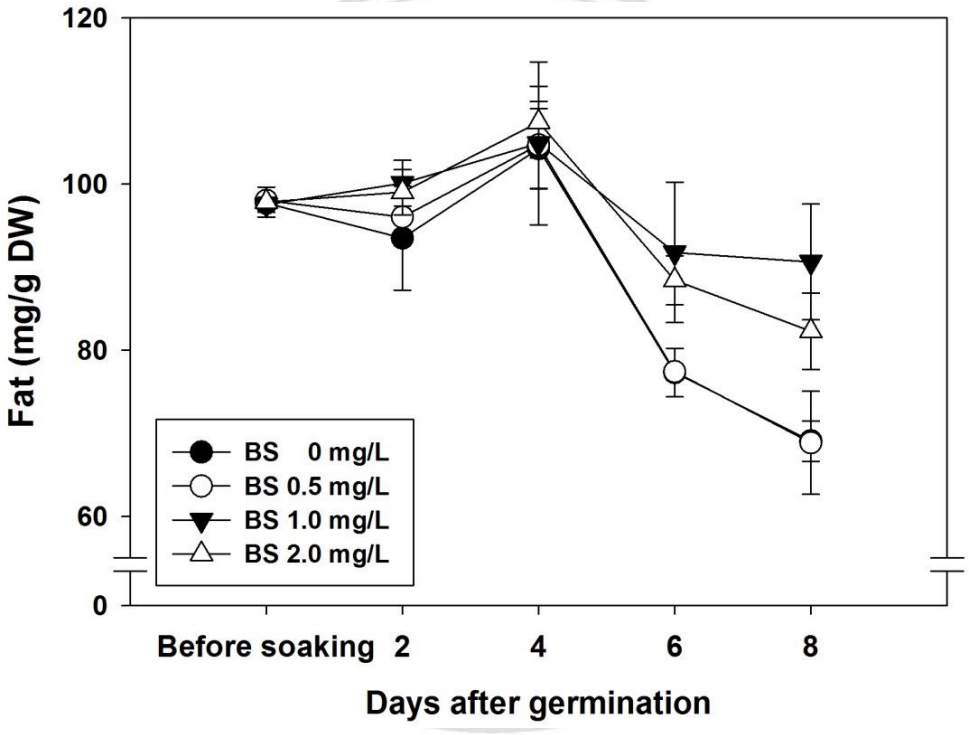

Figure 4.3 Effect of brassin-like substance concentrations and germination on fat content in arabica coffee seeds

4.2.4 Caffeine content

Changes of caffeine content of coffee seeds pretreated by different BS concentrations were monitored during early germination. The results were presented in Table 4.7. Caffeine content in seeds obtained from all treatments showed the obvious diminutions upon germination time (11.80 mg/g DW at initial date to 6.12 mg/g DW at day 8 of germination) (Figure 4.4).

BS concentrations significantly decreased the content of caffeine in coffee seeds during germination. Coffee seeds in control treatment and 0.5 mg/L of BS application showed higher caffeine content than the seeds soaked in BS concentrations of 1.0 and 2.0 mg/L. Nonetheless, there were no significant interactions among brassin-like substance concentration and germination time on the caffeine content in arabica coffee seeds (Table 4.8).

On the contrary, Baumann and Gabriel (1984) reported that during germination of coffee seeds, the caffeine content in young seedling considerably increased. Baumann (2006) reported that during the first 2 to 3 weeks of germination, primary root and hypocotyls emerged from the seed, and the cotyledons had already started to invade and resorb the endosperm. However, caffeine content in endosperm was neither degraded nor additionally formed during the invasion of developing cotyledons (Petermann and Baumann, 1983).

Table 4.7 Effect of brassin-like substance concentrations and germination on caffeine content in arabica coffee seeds

Treatment		Caffeine content (mg/g DW)
BS concentration	BS 0 mg/L	8.48 ± 1.07 ^{A}
	BS 0.5 mg/L	8.42 ± 0.98 ^{A}
	BS 1.0 mg/L	7.99 ± 1.03 ^{B}
	BS 2.0 mg/L	7.91 ± 1.12 ^{B}
Days after germination	Before soaking	11.88 ± 0.06 ^{a}
	Day 2	9.03 ± 0.30 ^{b}
	Day 4	7.45 ± 0.17 ^{c}
	Day 6	6.52 ± 0.14 ^{d}
	Day 8	6.12 ± 0.20 ^{d}
BS concentration		*
Days after germination		*
BS concentration×*Days after germination*		NS
C.V. (%)		9.77

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 4.8 Interaction of effect of brassin-like substance concentrations and germination on caffeine content in arabica coffee seeds

Treatment		Caffeine content
BS concentration	**Days after germination**	**(mg/g DW)**
BS 0.0 mg/L	Before soaking	11.99 ± 0.37
	Day 2	9.84 ± 0.48
	Day 4	7.61 ± 0.39
	Day 6	6.43 ± 0.44
	Day 8	6.53 ± 0.27
BS 0.5 mg/L	Before soaking	11.87 ± 0.49
	Day 2	9.10 ± 0.18
	Day 4	7.86 ± 0.13
	Day 6	6.94 ± 0.03
	Day 8	6.33 ± 0.13
BS 1.0 mg/L	Before soaking	12.04 ± 0.23
	Day 2	8.60 ± 0.21
	Day 4	7.17 ± 0.22
	Day 6	6.43 ± 0.44
	Day 8	6.02 ± 0.05
BS 2.0 mg/L	Before soaking	11.94 ± 0.52
	Day 2	8.57 ± 0.41
	Day 4	7.14 ± 0.41
	Day 6	6.30 ± 0.26
	Day 8	5.61 ± 0.21
F-test		NS
C.V. (%)		9.77

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(NS): not significantly different

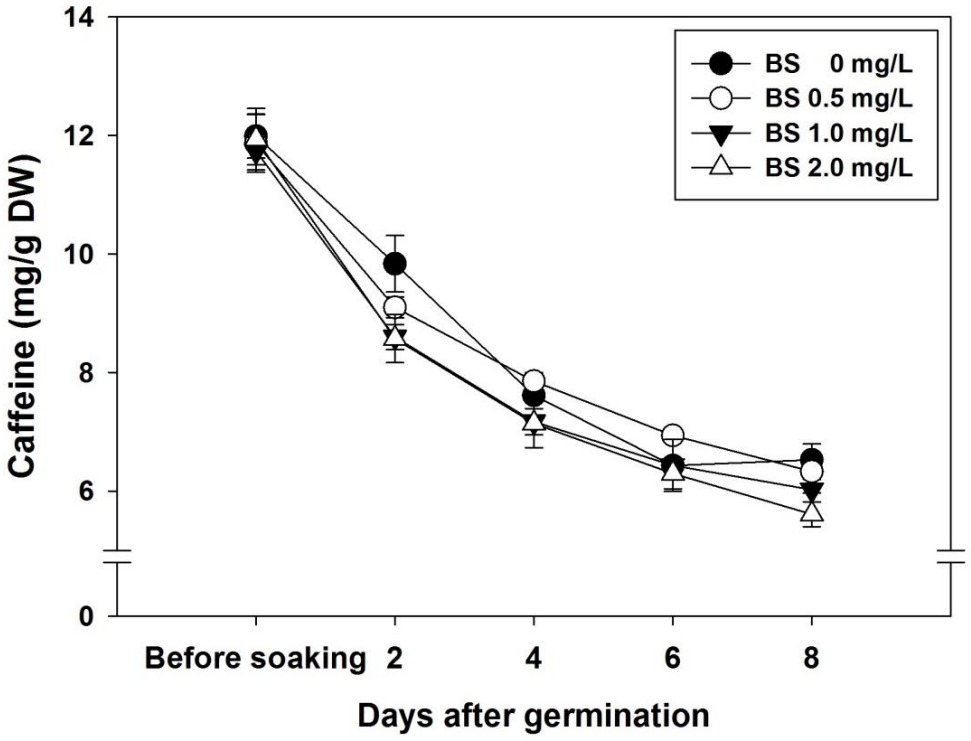

Figure 4.4 Effect of brassin-like substance concentrations and germination on caffeine content in arabica coffee seeds

4.2.5 Chlorogenic acid content

Chlorogenic acids are one of the important groups of phenolic metabolites produced by certain plant species. They are considered as signature components of coffee (*Coffea* spp.) (Lallemand *et al.*, 2012).

The results showed that chlorogenic acids content in arabica coffee seeds significantly increased during the first 4 days of germination (from 45.07 to 52.54 mg/g DW). The contents, however, rapidly declined upon the rest of early germination (Table 4.9) (Figure 4.5).

Brassin-like substance concentrations significantly affected on chlorogenic acid. Chlorogenic acid content in germinated coffee seeds influenced by different concentration of brassin-like substance, and the results were presented in Table 4.9. Chlorogenic acids in germinated coffee seeds observed in control and 0.5 mg/L of BS soaking treatments (43.23 and 42.95 mg/g DW, respectively) were greater than those of content from coffee seeds treated by BS at concentrations of 1.0 and 2.0 mg/L (41.56 and 41.80 mg/g DW, respectively).

Significant interactions among brassin-like substance concentration and germination time on chlorogenic acid content in germinated coffee seeds were observed. The maximum chlorogenic acid content in seed was achieved from 2.0 mg/L of BS treatment at day 4 of germination (54.86 mg/g DW). While, the minimum chlorogenic acid content in seeds were obtained from 2.0 and 1.0 mg/L of BS treatments at day 8 of germination, which were 30.51 and 31.21 mg/g DW, respectively (Table 4.10).

Aerts and Baumann (1994) revealed that during the first 10 weeks of arabica coffee seed germination, the chlorogenic acid content dropped to one-third of the original level after seed germination due to the cotyledons invaded the endosperm and expanded. The loss of chlorogenic acid was not recovered in the other organs of the seedling. The drop in chlorogenic acid content coincided with the increase amount of cell wall-bound phenolic polymers in the developing cotyledons, which could be extracted after thioglycolic acid derivatization. Likewise Aerts and Baumann (1994), Canella and Bernardi (1983) studied phenolic tendency during sunflower seeds gemination and reported that the chlorogenic acid falled rapidly during germination.

Table 4.9 Effect of brassin-like substance concentrations and germination on chlorogenic acid content in arabica coffee seeds

Treatment		Chlorogenic acid content (mg/g DW)
BS concentration	BS 0 mg/L	43.23 ± 2.81 ^{A}
	BS 0.5 mg/L	42.95 ± 3.21 ^{A}
	BS 1.0 mg/L	41.56 ± 3.75 ^{B}
	BS 2.0 mg/L	41.80 ± 4.28 ^{B}
Days after germination	Before soaking	45.07 ± 0.02 ^{b}
	Day 2	45.17 ± 0.49 ^{b}
	Day 4	52.54 ± 0.81 ^{a}
	Day 6	36.33 ± 0.96 ^{c}
	Day 8	32.83 ± 1.16 ^{d}
BS concentrations		*
Days after germination		*
BS concentration×Days after germination		*
C.V. (%)		4.65

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

Table 4.10 Interaction of effect of brassin-like substance concentrations and germination on chlorogenic acid content in arabica coffee seeds

Treatment		Chlorogenic acid content
BS concentration	**Days after germination**	**(mg/g DW)**
BS 0 mg/L	Before soaking	45.11 ± 0.15 ^{C}
	Day 2	46.11 ± 1.00 ^{C}
	Day 4	51.06 ± 0.65 ^{B}
	Day 6	38.51 ± 0.78 ^{D}
	Day 8	35.34 ± 0.64 ^{EF}
BS 0.5 mg/L	Before soaking	45.08 ± 0.03 ^{C}
	Day 2	45.89 ± 1.09 ^{C}
	Day 4	52.20 ± 1.08 ^{B}
	Day 6	37.36 ± 0.54 $^{D E}$
	Day 8	34.24 ± 0.68 ^{F}
BS 1.0 mg/L	Before soaking	45.07 ± 0.15 ^{C}
	Day 2	44.55 ± 1.42 ^{C}
	Day 4	52.03 ± 0.78 ^{B}
	Day 6	34.95 ± 0.55 ^{F}
	Day 8	31.22 ± 0.77 ^{G}
BS 2.0 mg/L	Before soaking	45.01 ± 0.11 ^{C}
	Day 2	44.11 ± 0.98 ^{C}
	Day 4	54.86 ± 0.84 ^{A}
	Day 6	34.50 ± 0.55 ^{F}
	Day 8	30.51 ± 1.37 ^{G}
F-test		*
C.V. (%)		4.64

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

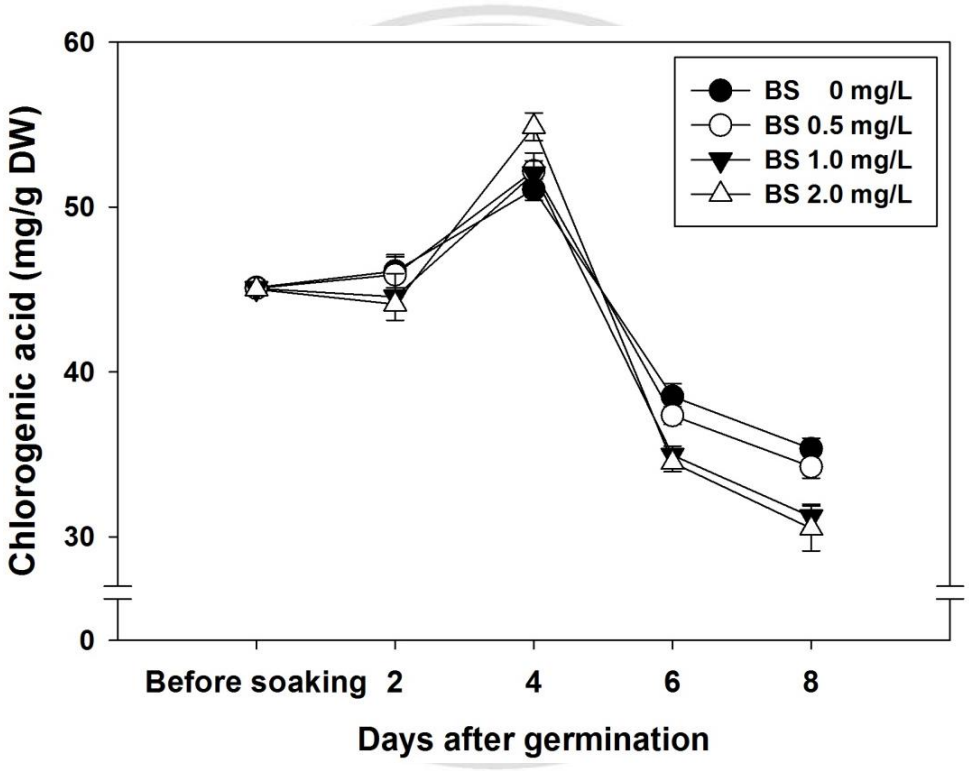

Figure 4.5 Effect of brassin-like substance concentrations and germination on chlorogenic acid content in arabica coffee seeds

4.2.6 Total phenols content

Total phenols content in seeds treated by brassin-like substance sharply decreased upon the whole period of early germination (Table 4.11). The contents were found to be significantly lower than the value from seeds in control treatment.

Figure 4.6 illustrated the obvious decreases in total phenols content among all treatments from day 2 to day 8 of germination. Saxena *et al*. (2003) reported that during germination of legume seeds, an enzyme called polyphenol oxidase might be activated, and resulted in degradation and consequent losses of polyphenols. The decrease of free total phenolics content during germination might be able to explain according to Khandelwal *et al*. (2010) in terms of leaching of water-soluble phenolic compounds into the water, small phenolic molecules polymerized into insoluble forms, binding with other organic substances such as carbohydrate or protein. Thus, the decline of bound phenolic content was mainly due to the reduction of the phenolic acid.

This experiment also found the interaction between brassin-like substance and germination time upon the changes of total phenols content. Total phenols content of all germinated seeds of this study were much lower than their initial values (Table 4.12).

Germination of lotus (*Nelumbo nucifera*) seeds caused the total phenolics of the seeds to decrease significantly at 95% confidence level. The observed reduction in polyphenols particularly after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. Tannins might be binding with other polyphenols and/or other organic substances such as carbohydrates and proteins, resulting in degradation and consequent losses of polyphenols. In kidney bean (*Phaseolus vulgaris*), germination process led to a reduction of total phenolic compounds, from 2.8% after 24 hours of germination to 60.8% after 120 hours of germination. Moreover, a longer period of germination greatly caused a further loss of total phenolic compounds (Rasha *et al*., 2011). Mung bean (*Vigna radiata*) also displayed high losses of phenolic compounds which reached 66.8% after 48 hours of germination (Rasha *et al*., 2011). The rapid decrease in polyphenol contents were also observed by Giami *et al*. (2001) for germinated cowpea (*Vigna unguiculata*) (41.5 to 51.7%), and for Indian pulses by Khandelwal *et al*. (2010). The reduction of phenolic

compounds during germination significantly associated with the presence of polyphenol-oxidase and enzymatic hydrolysis (Rao and Deosthale, 1982).

Table 4.11 Effect of brassin-like substance concentrations and germination on total phenols content in arabica coffee seeds

Treatment		Total phenols content (mg GAE/g DW)
BS concentration	BS 0 mg/L	55.52 ± 6.51 ^{A}
	BS 0.5 mg/L	49.49 ± 6.45 ^{B}
	BS 1.0 mg/L	48.65 ± 6.68 ^{B}
	BS 2.0 mg/L	48.93 ± 6.65 ^{B}
Days after germination	Before soaking	72.74 ± 0.03 ^{a}
	Day 2	55.84 ± 4.03 ^{b}
	Day 4	48.24 ± 1.68 ^{c}
	Day 6	39.86 ± 1.81 ^{d}
	Day 8	36.58 ± 0.76 ^{d}
BS concentration		*
Days after germination		*
BS concentration×*Days after germination*		*
C.V. (%)		2.45

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.
The results were expressed as mean value ± standard error.
(*) : significantly different between treatments

Table 4.12 Interaction of effect of brassin-like substance concentrations and germination on total phenols content in arabica coffee seeds

Treatment		Total phenols content
BS concentration	**Days after germination**	**(mg GAE/g DW)**
BS 0 mg/L	Before soaking	72.66 ± 0.16 ^{A}
	Day 2	67.90 ± 0.08 ^{B}
	Day 4	53.28 ± 0.19 ^{C}
	Day 6	45.28 ± 0.43 ^{E}
	Day 8	38.51 ± 1.14 ^{F}
BS 0.5 mg/L	Before soaking	72.77 ± 0.20 ^{A}
	Day 2	52.30 ± 2.47 ^{CD}
	Day 4	46.88 ± 0.64 ^{E}
	Day 6	38.48 ± 0.30 ^{F}
	Day 8	37.03 ± 0.50 ^{FG}
BS 1.0 mg/L	Before soaking	72.79 ± 0.12 ^{A}
	Day 2	51.14 ± 0.34 ^{D}
	Day 4	46.37 ± 0.42 ^{E}
	Day 6	37.81 ± 0.34 ^{F}
	Day 8	35.19 ± 0.80 ^{G}
BS 2.0 mg/L	Before soaking	72.74 ± 0.06 ^{A}
	Day 2	52.03 ± 0.14 ^{CD}
	Day 4	46.43 ± 0.53 ^{E}
	Day 6	37.88 ± 0.40 ^{F}
	Day 8	35.57 ± 0.59 ^{G}
F-test		*
C.V. (%)		2.45

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

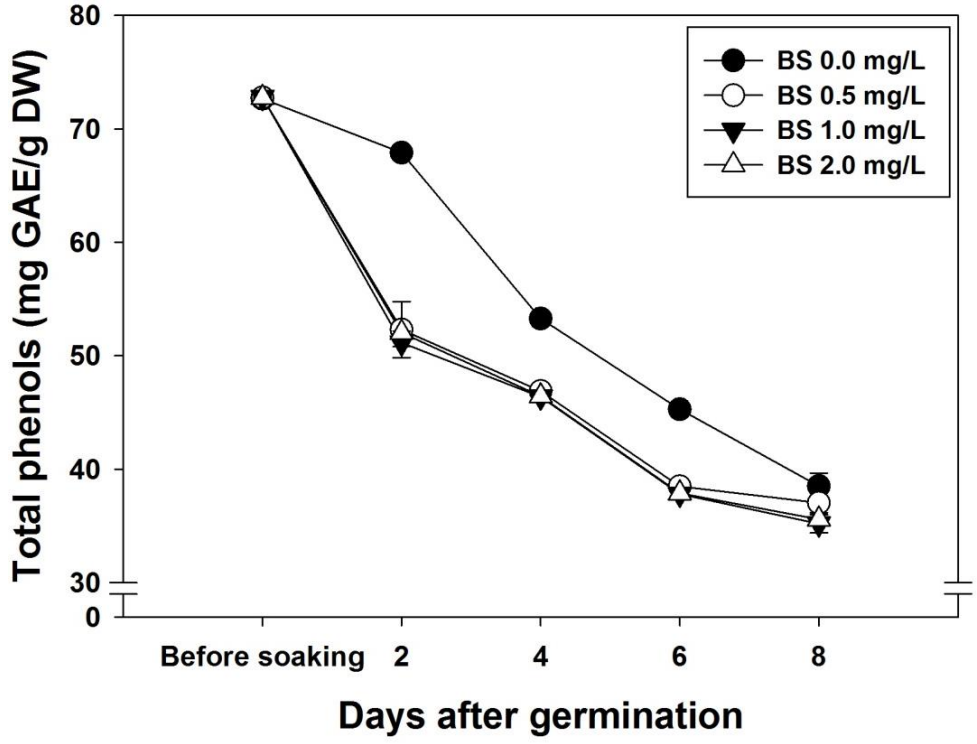

Figure 4.6 Effect of brassin-like substance concentrations and germination on total phenols content in arabica coffee seeds

4.2.7 Oxidation activity (DPPH radical scavenging activity)

Coffee seeds soaked with 1.0 and 2.0 mg/L of brassin-like substance exhibited higher antioxidant activities (DPPH values of 781.94 and 786.29 μM Trolox/g DW, respectively) than the seeds soaked with distilled water and 1.0 mg/L of brassin-like substance (DPPH values of 728.33 and 728.06 μM Trolox/g DW, respectively) (Table 4.13).

Initial value of DPPH radical scavenging activity in seeds was about 704.25 μM trolox/g DW. During germination process, the DPPH radical scavenging activity in the coffee samples increased gradually and reached the amount of 789.97 μM Trolox/g DW at day 8 of germination (Figure 4.7). However, there was no significant difference among combinations of two studied factors (Table 4.14).

Nowadays, it is widely known that antioxidant activity of beverage is related to high phenolic content. Phenolic compounds are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidative damages (Amic *et al*., 2003). Coffee seeds are abundant in substances with antioxidant properties, including plant phenolics especially for chlorogenic acid form. Early germinated arabica coffee is a potential source of bioactive components which exhibit high level of antioxidant activities.

Table 4.13 Effect of brassin-like substance concentrations and germination on DPPH radical scavenging activity in arabica coffee seeds

Treatment		DPPH radical scavenging activity (μM Trolox/g DW)
BS concentration	BS 0 mg/L	728.33 ± 8.84 ^{B}
	BS 0.5 mg/L	728.06 ± 10.01 ^{B}
	BS 1.0 mg/L	781.94 ± 20.63 ^{A}
	BS 2.0 mg/L	786.29 ± 21.71 ^{A}
Days after germination	Before soaking	704.25 ± 0.70 ^{d}
	Day 2	749.49 ± 21.92 ^{c}
	Day 4	759.86 ± 19.25 ^{bc}
	Day 6	777.21 ± 18.51 ^{ab}
	Day 8	789.97 ± 21.32 ^{a}
BS concentration		*
Days after germination		*
BS concentration×Days after germination		NS
C.V. (%)		3.59

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 4.14 Interaction of effect of brassin-like substance concentrations and germination on DPPH radical scavenging activity in arabica coffee seeds

Treatment		DPPH radical scavenging activity
BS concentration	**Days after germination**	**(µM Trolox/g DW)**
BS 0 mg/L	Before soaking	705.78 ± 0.68
	Day 2	711.22 ± 35.19
	Day 4	731.63 ± 8.90
	Day 6	743.20 ± 11.86
	Day 8	751.36 ± 26.62
BS 0.5 mg/L	Before soaking	703.06 ± 1.18
	Day 2	711.90 ± 7.67
	Day 4	722.11 ± 15.33
	Day 6	747.28 ± 30.60
	Day 8	754.76 ± 14.06
BS 1.0 mg/L	Before soaking	705.10 ± 1.18
	Day 2	784.69 ± 11.24
	Day 4	788.10 ± 16.00
	Day 6	806.46 ± 7.10
	Day 8	826.19 ± 4.91
BS 2.0 mg/L	Before soaking	703.06 ± 2.36
	Day 2	790.14 ± 21.38
	Day 4	797.62 ± 9.60
	Day 6	811.90 ± 9.81
	Day 8	827.55 ± 14.14
F-test		NS
C.V. (%)		3.58

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(NS): not significantly different

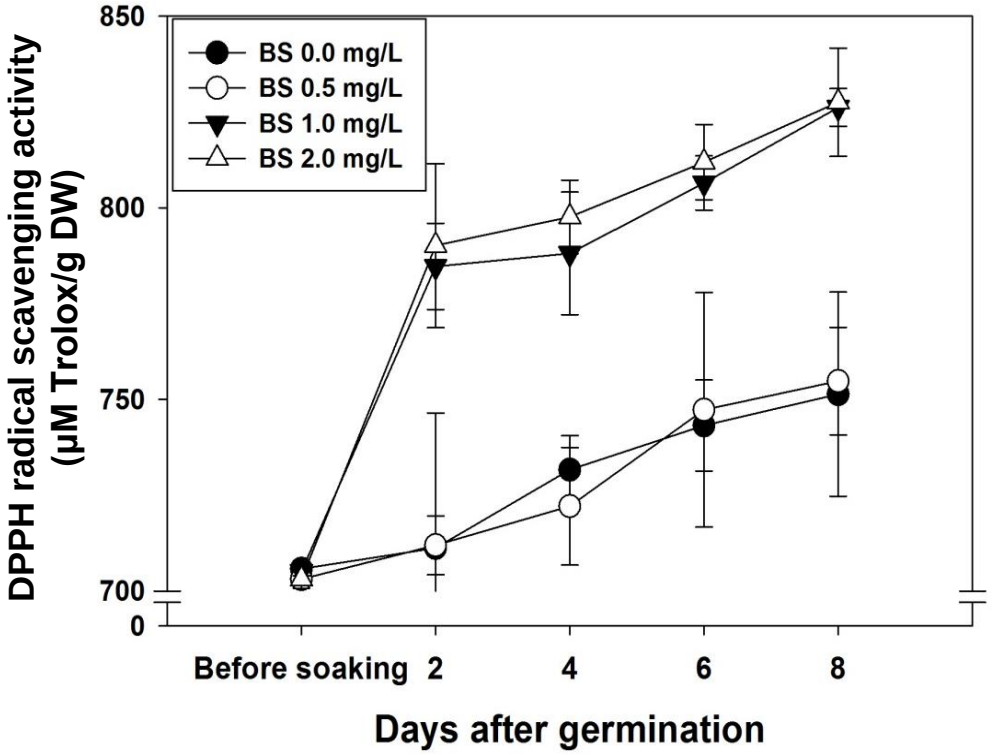

Figure 4.7 Effect of brassin-like substance concentrations and germination on DPPH radical scavenging activity in arabica coffee seeds

4.3 Conclusion

Germination time is one of the most important factors affecting the level of biochemical compositions and antioxidant activity. BS applications significantly increased protein content, total sugars, fat content and cholorogenic acid in arabica coffee seed during germination. Caffeine and total phenols content in seed were significantly degraded by days of germination.

CHAPTER 5

Effects of Roasting on Chemical Compositions of Early Germinated Arabica Coffee

5.1 Materials and Methods

5.1.1 Raw materials

Early germinated arabica coffee beans treated with appropriate brassin-like substance (BS) concentration from previous experiment were used to study the effects of roasting levels on beans chemical compositions. Figure 5.1 showed the coffee bean type used in this experiment; non-germinated seeds, BS 0 mg/L (distilled water) at 4th DAG (Day after germination), BS 1.0 mg/L at 4th DAG and BS 1.0 mg/L at 6th DAG.

Figure 5.1 Appearance of early germinated arabica coffee beans used in roasting experiment; (A) Non-germinated, (B) Distilled water 4th DAG, (C) BS 1.0 mg/L 4th DAG and (D) BS 1.0 mg/L 6th DAG

5.1.2 Roasting

The aforementioned types of early germinated coffee beans were collected and roasted at 240ºC. Three degrees of roasting were made up by varying the length roasting time: light roast (6 min), medium roast (7 min) and dark roast (9 min) (Farah *et al*., 2006b). All germinating seeds were used to determined weight loss, color analysis, biochemical analysis and cupping test. The experiment was conducted as 4×3 factorial in completely randomized design (CRD) with three replications. In which, the first factor was coffee bean type, and the second factor was roasting level.

5.1.3 Weight loss of roasted coffee bean

The roast weight loss was determined by weighing the green beans and roasted beans. Numbers were used to calculate roast weight loss according to the following equation;

$$\text{Roast weight loss (\%)} = \frac{(\text{Weight of green coffee} - \text{Weight of roasted coffee}) \times 100}{\text{Weight of green coffee}}$$

5.1.4 Color analysis of roasted coffee beans

After roasting, the roasted coffee were ground and placed into a color analytical dish. Color analysis of the ground coffee was carried out using a tristimulus colorimeter equipped with a Chromameter measuring head (CR400, Minolta, Osaka, Japan). The instrument was standardized against a white tile prior to running color measurement. Color was expressed in L*, a* and b* according to Comission Internationale de Eclairage (CIE) scale parameters. Ten measurements were made on each sample.

Chemical compositions of roasted coffee beans were analyzed according the methods described in Experiment 2.

5.1.5 Protein content was analyzed by the method of Lowry *et al*. (1951). (Appendix A)

5.1.6 Total Sugars content was analyzed according to James (1995). (Appendix B)

5.1.7 Fat content was analyzed according to AOAC (2000). (Appendix C)

5.1.8 Caffeine content was determined by a high performance liquid chromatography (HPLC) (Wang *et al*., 2000). (Appendix D)

5.1.9 Chlorogenic acid content was determined by a high performance liquid chromatography (HPLC) (Ky *et al*., 1997). (Appendix E)

5.1.10 Total phenols content was analyzed according to methods of Folin and Ciocalteu (1927). (Appendix F)

5.1.11 Oxidation activity (DPPH radical scavenging activity) was analyzed according to Brand-William *et al*. (1995). (Appendix G)

5.1.12 pH measurement

The pH value of coffee sample was determined by the method described by Ramalakshmi and Rahhavan (2000). One gram of ground coffee was dissolved in 10 mL of distilled water, and mixed thoroughly in a hot plate. The sample solution was cooled to room temperature and subsequently analyzed its pH using pH meter.

5.1.13 Cupping test

Cup-testing is the process for evaluating coffee aroma and taste characteristics. In this experiment, the test evaluated a variety of basic coffee characteristics such as aroma, taste, body and acidity in order to provide the overall score of acceptability (Angkasith and Warrit, 1999) (Appendix H).

5.1.14 Statistical Analysis

Means of each data combination were statistically analyzed by analysis software, the PASW® Statistics 18. Duncan's multiple range test (DMRT) was used to interpret significant difference among the means ($p < 0.05$).

5.2 Results and discussion

5.2.1 Weight loss of roasted coffee bean

The changes in weight and total weight loss (measured from 100 beans) are presented in Table 5.1 and Figure 5.2. The results revealed significant differences in percentage of weight loss due to roasting levels. The maximum weight loss was recorded for dark roast (16.31%), while the minimum value was recorded for light roast (8.51%) (Table 5.2). During the heating (roasting process), the processed coffee beans typically lose significant mass due to the water and volatile materials losses (Franca *et al*., 2005b).

Table 5.1 Effect of roasting on weight loss of coffee from early germinated arabica coffee bean

Treatment		Weight loss (%)
Coffee bean type	Non-germinated	12.42 ± 2.31
	BS 0 mg/L 4th DAG	12.28 ± 2.13
	BS 1.0 mg/L 4th DAG	12.49 ± 2.52
	BS 1.0 mg/L 6th DAG	12.61 ± 2.05
Roasting level	Light	8.51 ± 0.21 ^{c}
	Medium	12.53 ± 0.13 ^{b}
	Dark	16.31 ± 0.16 ^{a}
Coffee bean type		NS
Roasting level		*
Coffee bean type×*Roasting level*		NS
C.V. (%)		11.56

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 5.2 Interaction of effect of roasting on weight loss of coffee from early germinated arabica coffee bean

Treatment		Weight loss (%)
Coffee bean type	**Roasting level**	
Non-germinated	Light	8.34 ± 0.32
	Medium	12.60 ± 0.47
	Dark	16.32 ± 0.58
BS 0 mg/L 4th DAG	Light	8.67 ± 0.40
	Medium	12.16 ± 0.42
	Dark	16.03 ± 0.51
BS 1.0 mg/L 4th DAG	Light	8.03 ± 0.48
	Medium	12.68 ± 0.56
	Dark	16.75 ± 0.51
BS 1.0 mg/L 6th DAG	Light	9.02 ± 0.32
	Medium	12.68 ± 0.41
	Dark	16.13 ± 0.41
F-test		NS
C.V. (%)		11.56

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.
(NS): not significantly different

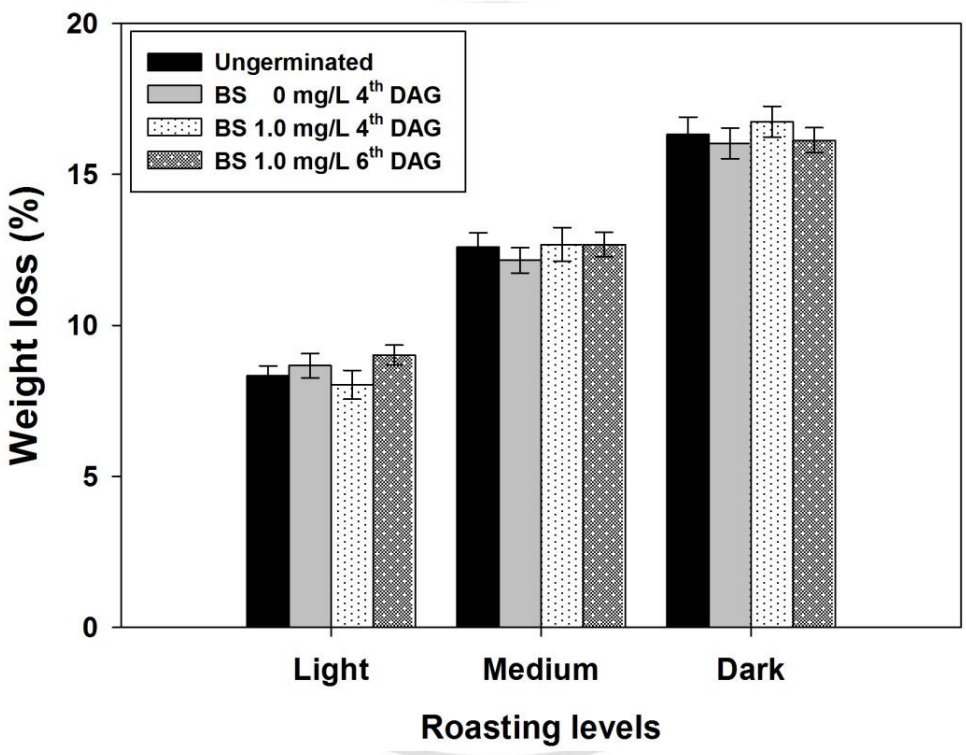

Figure 5.2 Effect of roasting on weight loss of coffee from early germinated arabica coffee bean

5.2.2 Color analysis of roasted coffee

Changes in color of early germinated arabica coffee beans during roasting are presented in Table 5.3 and Table 5.4. Generally, the L* represents lightness (contribution of black or white varying between 0 and 100), a* represents the contribution of green or red (positive or negative), and b* represents the contribution of yellow or blue (positive or negative).

The color analysis in terms of L* a* and b* values were not significantly different between coffee bean types (Table 5.4).

The color of coffee under different levels of roasting showed a statistically significant difference. The lightness values (L*) decreased upon the increase of roasting from light to dark roasted (45.05 to 28.12, respectively) (Figure 5.3). The a* values also decreased significantly along roasting level (6.88 to 2.16, respectively). Likewise, L* and a*, the b* values gradually declined along roasting level (9.72 to 1.76, respectively), meaning the disappearance of yellow color of coffee beans.

During roasting, temperature is one of the important causes of color degradation in dehydrated products (Lozano and Ibarz, 1997). The color changes in coffee beans occur upon the non-enzymatic browning and pyrolysis reactions (Hernandes *et al*, 2007). It is in agreement with results reported by Wanyo *et al*. (2011), who reported that degradation of certain bioactive compounds in the coffee beans might be related to the decreasing of bioactivity.

The interaction effect between coffee bean type and roasting level were analyzed. However, there was no interaction between coffee bean type and roasting level treatments on color parameter: L*, a* and b* of roasted bean (Table 5.5).

Table 5.3 Roasted coffee color

Coffee bean type	Roasting levels		
	Light	***Medium***	***Dark***
Non-germinated			
BS 0 mg/L 4th DAG			
BS 1.0 mg/L 4th DAG			
BS 1.0 mg/L 6th DAG			

Table 5.4 Effect of roasting on color parameter of coffee from early germinated arabica coffee bean

Treatment		Color parameter		
		L*	**a***	**b***
Coffee bean type	Non-germinated	37.22±5.14	4.34±1.40	5.18±2.42
	BS 0 mg/L 4th DAG	38.15±4.95	4.24±1.39	5.17±2.46
	BS 1.0 mg/L 4th DAG	38.02±5.16	4.28±1.35	5.22±2.43
	BS 1.0 mg/L 6th DAG	38.03±4.98	4.29±1.38	4.91±2.21
Roasting level	Light	45.05±0.41[a]	6.88±0.04[a]	9.72±0.18[a]
	Medium	40.39±0.17[b]	3.83±0.02[b]	3.88±0.07[b]
	Dark	28.12±0.35[c]	2.16±0.02[c]	1.76±0.04[c]
Coffee bean type		NS	NS	NS
Roasting level		*	*	*
Coffee bean type×*Roasting level*		NS	NS	NS
C.V. (%)		5.69	19.90	21.04

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 5.5 Interaction of effect of roasting on color parameter of coffee from early germinated arabica coffee bean

Treatment		Color parameter		
Coffee bean type	**Roasting level**	**L***	**a***	**b***
Non-germinated	Light	43.87 ± 0.60	6.98 ± 0.32	9.82 ± 0.61
	Medium	40.67 ± 0.55	3.84 ± 0.09	4.08 ± 0.20
	Dark	27.11 ± 0.60	2.20 ± 0.12	1.65 ± 0.17
BS 0 mg/L 4th DAG	Light	45.20 ± 0.52	6.85 ± 0.54	9.94 ± 0.55
	Medium	40.65 ± 0.56	3.76 ± 0.17	3.85 ± 0.30
	Dark	28.59 ± 0.99	2.11 ± 0.14	1.72 ± 0.21
BS 1.0 mg/L 4th DAG	Light	45.64 ± 0.74	6.82 ± 0.35	9.95 ± 0.32
	Medium	40.24 ± 0.54	3.85 ± 0.16	3.86 ± 0.19
	Dark	28.18 ± 0.73	2.18 ± 0.13	1.85 ± 0.19
BS 1.0 mg/L 6th DAG	Light	45.51 ± 0.74	6.87 ± 0.44	9.19 ± 0.48
	Medium	39.99 ± 0.58	3.86 ± 0.15	3.72 ± 0.29
	Dark	28.61 ± 0.84	2.14 ± 0.17	1.81 ± 0.20
F-test		NS	NS	NS
C.V. (%)		5.69	19.90	21.04

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.
(NS): not significantly different

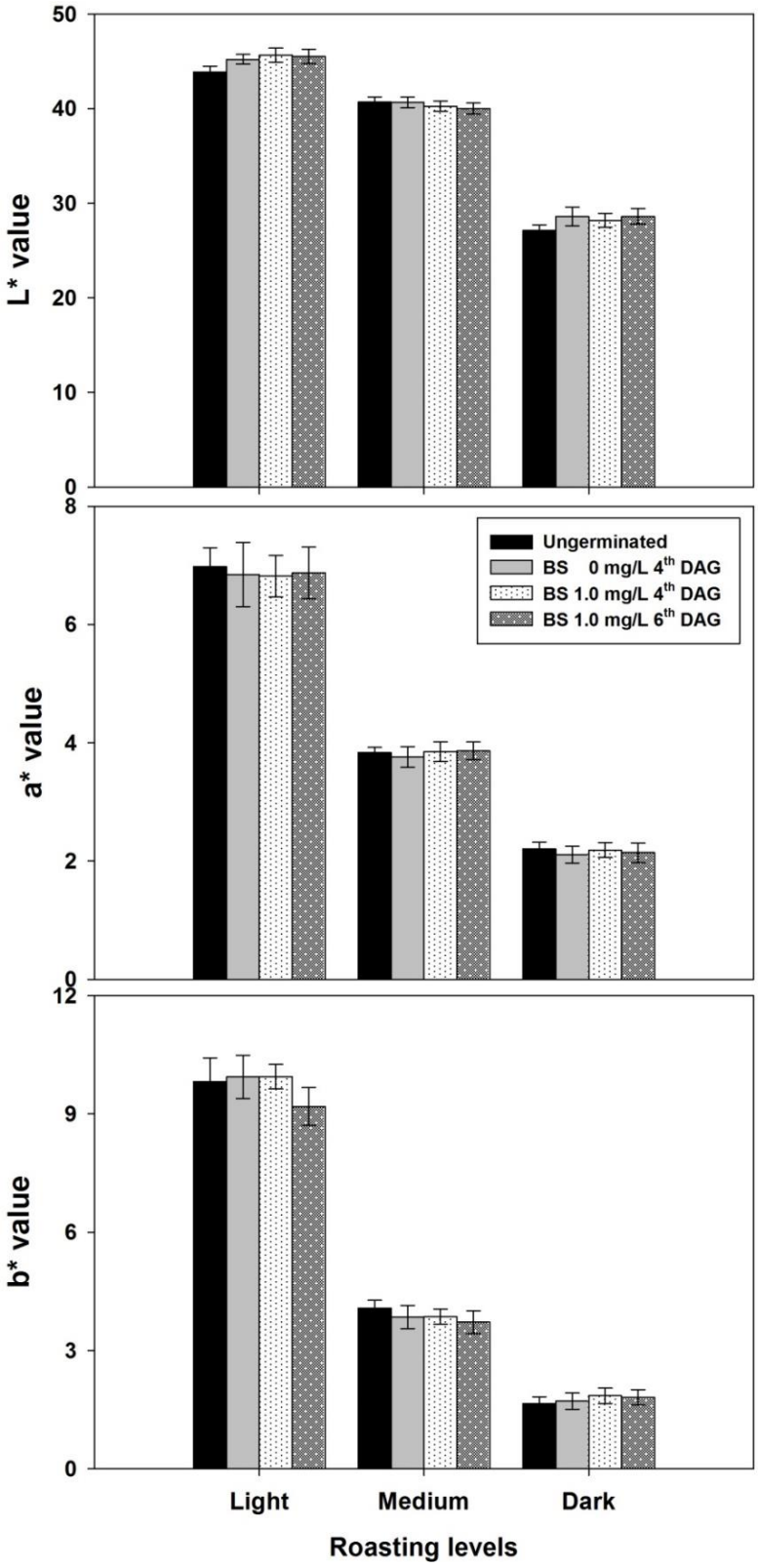

Figure 5.3 Effect of roasting on color parameter of coffee from early germinated coffee bean

5.2.3 Protein content

The effect of germination was found to be significant on protein content. The maximum protein content was recorded for BS 1.0 mg/L at 4th DAG of coffee bean (55.27 mg/g DW), followed by germinated coffee from BS 0 mg/L at 4th DAG and 1.0 mg/L at 6th DAG (49.41 and 49.01 mg/g DW, respectively). The minimum protein content was recorded for non-germinated bean, which was 44.99 mg/g DW (Table 5.6).

The results revealed significant differences in protein content due to roasting levels. The maximum protein content was recorded for light roasted beans (74.94 mg/g DW), while a minimum content was recorded for the dark roasted ones (33.50 mg/g DW) (Table 5.6).

Protein content was significantly affected by the interaction between roasting level and type of germinated coffee bean (Table 5.7, Figure 5.4). Maximum protein contained in roasted bean was recorded for soaking in 1.0 mg/L BS at 4th DAG (87.56 mg/g DW) when the coffee was roasted lightly. The minimum protein content was observed in dark roasted non-germinated bean (28.46 mg/g DW). Therefore, roasting greatly affected on protein degradation in arabica coffee beans. Typically, the green coffee bean contains all ingredients necessary for later development of coffee flavor. Moreover, it is now widely accepted that free amino acids and peptides are required for the generation of coffee aroma (Montavon *et al*., 2003).

Table 5.6 Effect of roasting on protein content of coffee from early germinated arabica coffee bean

Treatment		Protein content (mg/g DW)
Coffee bean type	Non-germinated	44.44 ± 12.14 ^{C}
	BS 0 mg/L 4th DAG	49.41 ± 11.22 ^{B}
	BS 1.0 mg/L 4th DAG	55.27 ± 16.41 ^{A}
	BS 1.0 mg/L 6th DAG	49.01 ± 11.67 ^{B}
Roasting level	Light	74.94 ± 4.30 ^{a}
	Medium	40.16 ± 1.60 ^{b}
	Dark	33.50 ± 1.73 ^{c}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		*
C.V. (%)		6.48

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

Table 5.7 Interaction of effect of roasting on protein content of coffee from early germinated coffee bean

Treatment		Protein content
Coffee bean type	**Roasting level**	**(mg/g DW)**
Non-germinated	Light	68.25 ± 1.29 ^{C}
	Medium	36.60 ± 1.15 ^{FG}
	Dark	28.46 ± 0.78 ^{H}
BS 0 mg/L 4th DAG	Light	71.67 ± 0.83 ^{BC}
	Medium	40.68 ± 1.11 ^{DE}
	Dark	35.88 ± 2.87 ^{FG}
BS 1.0 mg/L 4th DAG	Light	87.56 ± 0.91 ^{A}
	Medium	44.25 ± 0.54 ^{D}
	Dark	34.00 ± 0.72 ^{G}
BS 1.0 mg/L 6th DAG	Light	72.26 ± 1.29 ^{B}
	Medium	39.10 ± 0.81 ^{EF}
	Dark	35.68 ± 1.70 ^{FG}
F-test		*
C.V. (%)		6.48

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

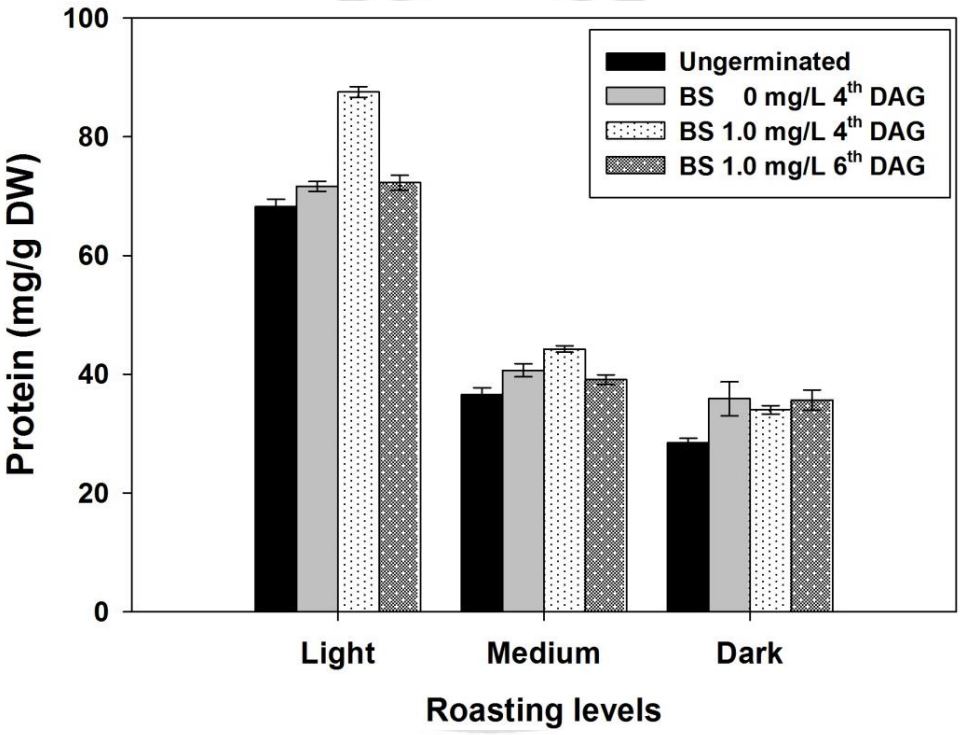

Figure 5.4 Effect of brassin-like substance and roasting on protein content of coffee from early germinated coffee bean

5.2.4 Total sugars content

Table 5.8 showed that total sugars of coffee bean decreased upon roasting level (duration of 6, 7 and 9 minutes). The effect of germination was found to be significant on total sugars content. The highest total sugars was recorded for coffee bean soaked with BS 1.0 mg/L at 6^{th} DAG (22.55 mg/g DW), followed by germinated coffee from BS 1.0 and BS 0 mg/L at 4^{th} DAG (19.02 and 10.65 mg/g DW, respectively). Lastly, non-germinated coffee bean had the lowest total sugars content which was 9.60 mg/g DW.

The results also indicated that roasting level negatively affected on the total sugars content in coffee bean. The dark roasted bean had the lowest mean value of total sugars content (13.58 mg/g DW), while the highest total sugars was obtained from bean with light roasting level (17.22 mg/g DW).

According to Table 5.9, interaction between roasting level and germinated bean type was indicated. The three roasting levels noticeably resulted in different mean total sugars content for each germinated bean. The highest amount of total sugars content was obtained from the bean treated by 1.0 mg/L BS at 6^{th} DAG when roasted lightly (Figure 5.5).

Bradbury (2001) categorized the reactions that sucrose probably decreased during roasting into three main types: fragmentation, dehydration and Millard reaction. These reactions somewhat produced different volatile and non-volatile compounds. Many of them were known for their contributions to the organoleptic properties of coffee beverages. Generally, sugars available in coffee beans are precursors of acids during roasting. Thermal degradation of sugars results in the formation of volatile compounds which can act as precursors to important odorants of the sweetish, caramel and earthy groups. The Maillard reaction occurs between free amino acids or protein and reducing sugars that are important contributors to the aroma, taste and brown pigment of coffee (Bradbury, 2001).

Table 5.8 Effect of roasting on total sugars content of coffee from early germinated arabica coffee bean

Treatment		Total sugars content (mg/g DW)
Coffee bean type	Non-germinated	9.60 ± 0.57 ^{D}
	BS 0 mg/L 4th DAG	10.65 ± 0.90 ^{C}
	BS 1.0 mg/L 4th DAG	19.02 ± 1.30 ^{B}
	BS 1.0 mg/L 6th DAG	22.55 ± 1.45 ^{A}
Roasting level	Light	17.22 ± 3.45 ^{a}
	Medium	15.57 ± 3.27 ^{b}
	Dark	13.58 ± 2.79 ^{c}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		*
C.V. (%)		7.04

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

Table 5.9 Interaction of effect of roasting on total sugars content of coffee from early germinated arabica coffee bean

Treatment		Total sugars content
Coffee bean type	**Roasting level**	**(mg/g DW)**
Non-germinated	Light	10.58 ± 0.32 ^{G}
	Medium	9.60 ± 0.15 ^{GH}
	Dark	8.61 ± 0.17 ^{H}
BS 0 mg/L 4th DAG	Light	12.26 ± 0.18 ^{F}
	Medium	10.54 ± 0.14 ^{G}
	Dark	9.16 ± 0.43 ^{H}
BS 1.0 mg/L 4th DAG	Light	21.15 ± 0.34 ^{C}
	Medium	19.25 ± 0.24 ^{D}
	Dark	16.66 ± 0.72 ^{E}
BS 1.0 mg/L 6th DAG	Light	24.89 ± 0.92 ^{A}
	Medium	22.88 ± 0.37 ^{B}
	Dark	19.89 ± 0.54 ^{D}
F-test		*
C.V. (%)		7.04

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

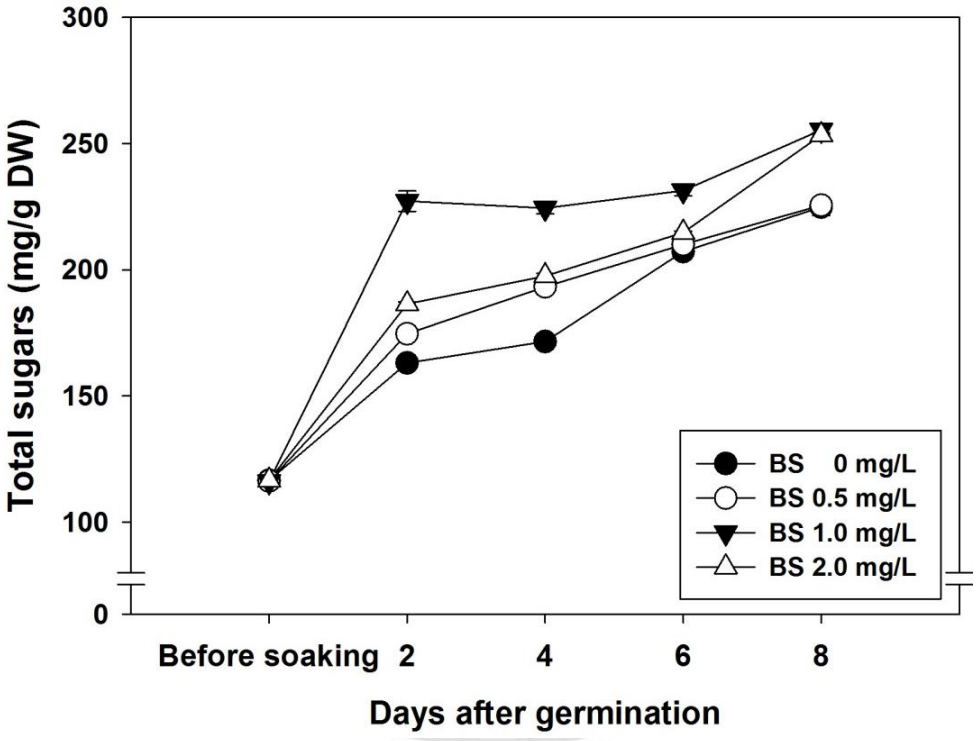

Figure 5.5 Effect of roasting on total sugars content of coffee from early germinated arabica coffee bean

5.2.5 Fat content

The fat content of dark roasted bean had the highest amount (140.34 mg/g DW), while the content in light roasted bean had the lowest value (78.53 mg/g DW) (Table 5.10). All of coffee bean types, namely, non-germinated, BS 0 and 1.0 mg/L at 4th DAG, and BS 1.0 mg/L at 6th DAG showed significant differences in the fat content after roasting. Non-germinated beans, BS 0 and 1.0 mg/L at 4th DAG beans demonstrated significantly higher fat content compared to the bean treated by BS 1.0 mg/L at 6th DAG. However, there was no significant interaction between roasting level and germinated bean for the fat content (Table 5.11, Figure 5.6).

The increase in total fat content in the darker coffee samples is actually related and may be attributed to the losses of other organic compounds, such as carbohydrates, proteins, trigonelline and chlorogenic acids (Trugo, 2003; Toci *et al*., 2006). The total lipid content observed in the samples roasted at light-medium and dark-medium degrees were 102 mg/g DW and 140 mg/g DW, respectively. These values agreed with those contents from Trugo (2003), who reported that roasted arabica coffee bean contained about 110 to 200 mg/g DW of fat content. Furthermore, Toci *et al*., (2006) also reported similar range (110 to 160 mg/g DW) of fat content in light-medium and dark-medium roasted coffee beans.

Table 5.10 Effect of roasting on fat content of coffee from early germinated arabica coffee bean

Treatment		Fat content (mg/g DW)
Coffee bean type	Non-germinated	121.03 ± 18.85 ^{A}
	BS 0 mg/L 4th DAG	120.73 ± 19.54 ^{A}
	BS 1.0 mg/L 4th DAG	116.25 ± 18.37 ^{A}
	BS 1.0 mg/L 6th DAG	103.58 ± 19.54 ^{B}
Roasting level	Light	78.53 ± 3.46 ^{c}
	Medium	127.31 ± 7.49 ^{b}
	Dark	140.34 ± 1.54 ^{a}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		NS
C.V. (%)		9.84

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 5.11 Interaction of effect of roasting on fat content of coffee from early germinated arabica coffee bean

Treatment		Fat content
Coffee bean type	**Roasting level**	**(mg/g DW)**
Non-germinated	Light	83.41 ± 2.58
	Medium	137.94 ± 3.52
	Dark	141.76 ± 7.83
BS 0 mg/L 4th DAG	Light	81.67 ± 1.95
	Medium	138.89 ± 1.66
	Dark	141.61 ± 8.27
BS 1.0 mg/L 4th DAG	Light	80.78 ± 6.40
	Medium	125.70 ± 11.01
	Dark	142.27 ± 6.83
BS 1.0 mg/L 6th DAG	Light	68.28 ± 3.55
	Medium	106.71 ± 6.71
	Dark	135.73 ± 9.72
F-test		NS
C.V. (%)		9.84

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.
(NS): not significantly different

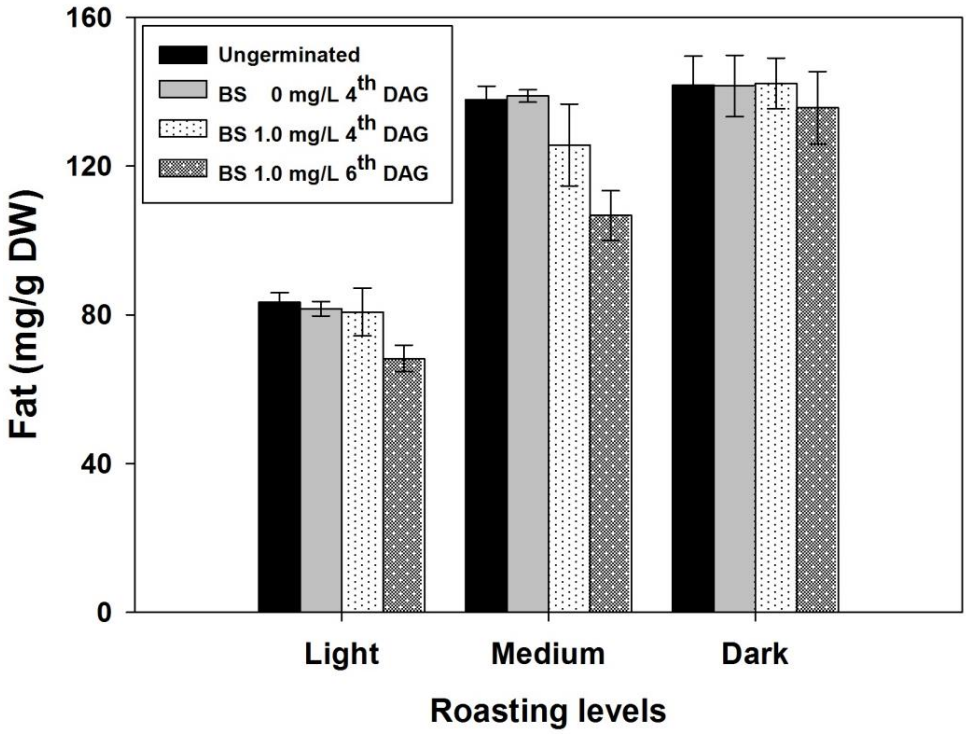

Figure 5.6 Effect of roasting on fat content of coffee from early germinated arabica coffee bean

5.2.6 Caffeine content

The caffeine content of all bean types tended to decrease after roasting. Non-germinated bean and the bean treated by BS 0 mg/L had greater caffeine content than the beans in other treatments (Table 5.12).

There was no any interaction among coffee bean type and roasting level (Table 5.13) (Figure 5.7). Wanyika *et al*. (2010) found that dark roasted coffee had less caffeine than lighter roasts, because the roasting process reduces the bean's caffeine content. Arabica coffee had the highest caffeine content in light roasted coffees (0.66-2.55%), while the lowest caffeine content was determined in green coffee beans (0.66%). Light roasted coffee contained about 2.55% of caffeine, which was the highest overall value in all coffee.

Table 5.12 Effect of roasting on caffeine content of coffee from early germinated arabica coffee bean

Treatment		Caffeine content (mg/g DW)
Coffee bean type	Non-germinated	9.54 ± 0.19 ^{A}
	BS 0 mg/L 4th DAG	9.23 ± 0.43 ^{A}
	BS 1.0 mg/L 4th DAG	7.11 ± 0.32 ^{B}
	BS 1.0 mg/L 6th DAG	5.86 ± 0.03 ^{C}
Roasting level	Light	8.29 ± 0.92 ^{a}
	Medium	8.03 ± 0.95 ^{a}
	Dark	7.49 ± 0.77 ^{b}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		NS
C.V. (%)		6.93

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 5.13 Interaction of effect of roasting on caffeine content of coffee from early germinated arabica coffee bean

Treatment		Caffeine content
Coffee bean type	**Roasting level**	**(mg/g DW)**
Non-germinated	Light	9.79 ± 0.25
	Medium	9.65 ± 0.15
	Dark	9.17 ± 0.10
BS 0 mg/L 4th DAG	Light	9.71 ± 0.23
	Medium	9.61 ± 0.15
	Dark	8.37 ± 0.23
BS 1.0 mg/L 4th DAG	Light	7.72 ± 0.22
	Medium	7.00 ± 0.08
	Dark	6.62 ± 0.19
BS 1.0 mg/L 6th DAG	Light	5.92 ± 0.37
	Medium	5.86 ± 0.20
	Dark	5.81 ± 0.34
F-test		NS
C.V. (%)		6.93

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.
(NS): not significantly different

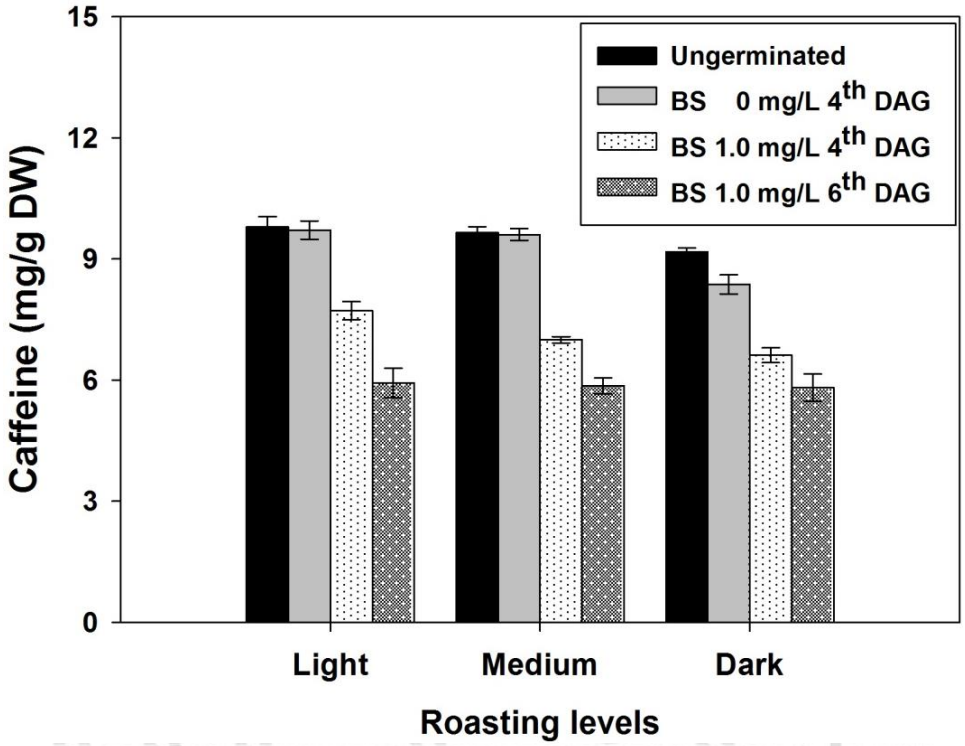

Figure 5.7 Effect of roasting on caffeine content of coffee from early germinated arabica coffee bean

5.2.7 Chlorogenic acid

The results obtained from HPLC analysis for the concentration of chlorogenic acid in mg/g DW of the different coffee bean samples are shown in Table 5.7.

The content of chlorogenic acid in roasted beans of non-germinated and BS 0 mg/L at 4th DAG, BS 1.0 mg/L at 4th DAG and BS 1.0 mg/L at 6th DAG at different roast level are presented in Table 5.14. The chlorogenic acid content in non-germinated roasted bean and BS 0 mg/L at 4th DAG were 21.14 and 21.01 mg/g DW, respectively. The amounts were higher than the average chlorogenic acid content in BS 1.0 mg/L at 4th DAG and BS 1.0 mg/L at 6th DAG (19.63 and 18.40 mg/g DW, respectively).

The chlorogenic acid concentration decreased with increased roasting. The result showed that the chlorogenic content was higher in light roasted (23.36 mg/g DW) than in the medium (19.92 mg/g DW) and dark roasted (16.85 mg/g DW) coffee beans. Nonetheless, chlorogenic acid was not significantly affected by the interaction between roasting level and type of coffee bean (Table 5.15).

During roasting process, a mixture of chemical compounds in green coffee seed is occurred or formed. Green coffee bean enriched in several phenolic compounds. Chlorogenic acid is one of the major phenolic compounds found in arabica coffee. They belong to ester group which are formed between quinic acid and cinnamic acids such as caffeic, ferulic and p-coumaric acid (Illy and Viani, 1988). During roasting, coffee beans are typically heated at 200-240ºC. And roasting levels are indicated upon roasting time; roasting light (6 minutes), medium (7 minutes) and dark roast (9 minutes). Chlorogenic acids are degraded proportional with the roasting degree; the loss is higher in dark roasted coffee. Degradation of chlorogenic acids leads to quinic acid and other phenol derivatives found in coffee's aroma (Guyot *et al*., 1985). Chlorogenic acids break down during roasting (Leloup *et al*., 1995; Farah *et al*., 2005; Moon *et al*., 2009). According to Trugo and Macrae (1984a) who reported that chlorogenic acids break down greatly depends on roasting temperature and time. Losses of about 60 percent have been observed in medium roasts, and up to 100 percent breakdown in dark roasts. Other studies reported that a light roast led to about 45%-54% chlorogenic acid breakdown, however, depending on the country of origin and genetics (Moon *et al*., 2009). Perrone *et al*. (2012) also showed that coffee roasted for only six minutes

retained 47% of its chlorogenic acids, and they could still be found, primarily non-degraded (44%) in the brewed coffee.

During the roasting process, as part of the dissolution of these molecules, caffeic and quinic acid, components of chlorogenic acids split off. Thus, the actual effect of chlorogenic acids on the taste of coffee might be in particular from the products of their breakdown. Quinic and caffeic acids are often formed by product of different chlorogenic acids, which have been shown to further degrade into phenol and catechols, among almost 30 other chemical compounds (Farah *et al*., 2005; Moon and Shibamoto, 2010).

Table 5.14 Effect of roasting on chlorogenic acid content of coffee from early germinated arabica coffee bean

Treatment		Chlorogenic acid content (mg/g DW)
Coffee bean type	Non-germinated	21.14 ± 1.76 ^{A}
	BS 0 mg/L 4th DAG	21.01 ± 1.80 ^{A}
	BS 1.0 mg/L 4th DAG	19.63 ± 2.12 ^{B}
	BS 1.0 mg/L 6th DAG	18.40 ± 1.90 ^{C}
Roasting level	Light	23.36 ± 0.69 ^{a}
	Medium	19.92 ± 0.54 ^{b}
	Dark	16.85 ± 0.79 ^{c}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		NS
C.V. (%)		4.73

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

(NS): not significantly different

Table 5.15 Interaction of effect of roasting on chlorogenic acid content of coffee from early germinated arabica coffee bean

Treatment		Chlorogenic acid content
Coffee bean type	**Roasting level**	**(mg/g DW)**
Non-germinated	Light	24.31 ± 0.21
	Medium	20.87 ± 0.58
	Dark	18.24 ± 0.54
BS 0 mg/L 4th DAG	Light	24.21 ± 0.19
	Medium	20.85 ± 0.58
	Dark	17.96 ± 0.42
BS 1.0 mg/L 4th DAG	Light	23.59 ± 0.19
	Medium	18.94 ± 0.27
	Dark	16.35 ± 0.37
BS 1.0 mg/L 6th DAG	Light	21.33 ± 0.26
	Medium	19.04 ± 0.35
	Dark	14.84 ± 0.36
F-test		NS
C.V. (%)		4.73

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.
(NS): not significantly different

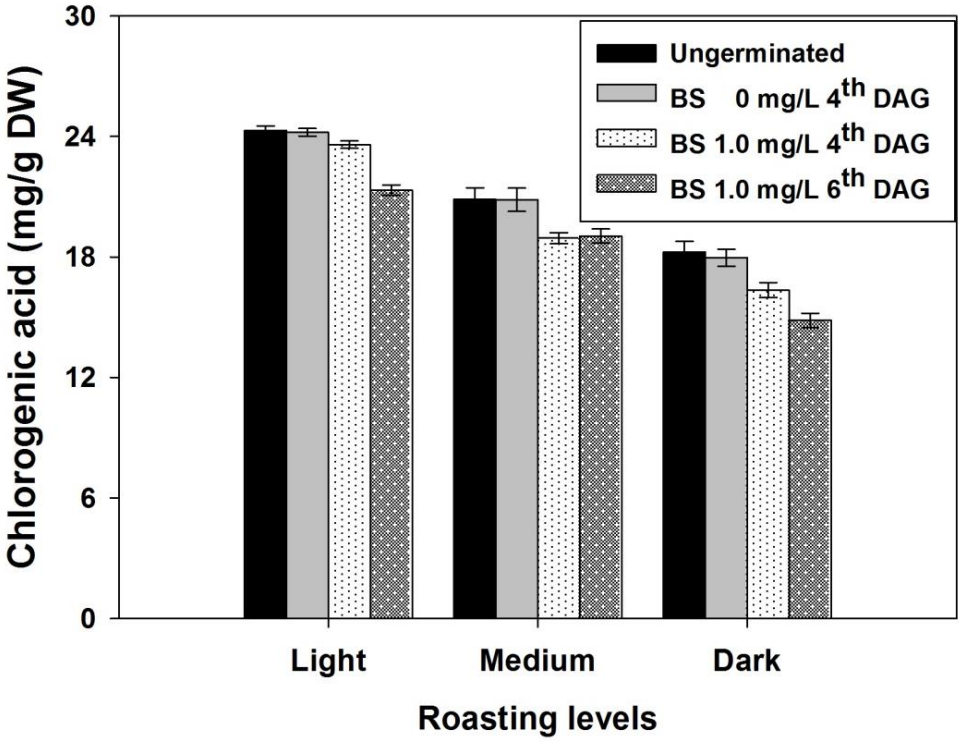

Figure 5.8 Effect of roasting on chlorogenic acid content of coffee from early germinated arabica coffee bean

5.2.8 Total phenols content

After roasting, significantly changes in total phenols content in roasted bean occurred in all coffee treatments. The non-germinated coffee had higher total phenols content than the bean treated by BS 0, 1.0 mg/L at 4th DAG and BS 1.0 mg/L at 6th DAG, respectively. Total phenols content of roasted coffee decreased with upon roasting level (Table 5.16).

As for factors interaction, the germinated bean and roasting levels significantly influenced on total phenols content. The highest total phenols content was observed in non-germinated bean with light roasted level, while the lowest total phenols content was found in BS 1.0 mg/L at 6th DAG with dark roasted level (Table 5.17, Figure 5.9).

Green coffee beans are rich in several phenolic compounds exemplified by chlorogenic acid, caffeic acid, ferulic acid and p-coumaric acid. Coffee is the major source of chlorogenic acid in human diet. On the basis of 10 g of coffee per cup of brew, a cup approximately contains 15-325 mg of chlorogenic acid (Richelle *et al*. 2001; De Castillo *et al*. 2002). Total phenolic content of coffee brews decreased with the increase of roasting degree due to the degradation of phenolic compounds (Shan *et al*, 2015).

Table 5.16 Effect of roasting on total phenols content of coffee from early germinated arabica coffee bean

Treatment		Total phenols content (mg GAE/g DW)
Coffee bean type	Non-germinated	29.52 ± 3.36 ^{A}
	BS 0 mg/L 4th DAG	27.56 ± 3.25 ^{B}
	BS 1.0 mg/L 4th DAG	26.28 ± 2.54 ^{C}
	BS 1.0 mg/L 6th DAG	20.48 ± 0.91 ^{D}
Roasting level	Light	29.98 ± 3.02 ^{a}
	Medium	26.49 ± 1.81 ^{b}
	Dark	21.40 ± 1.04 ^{c}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		*
C.V. (%)		5.94

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

Table 5.17 Interaction of effect of roasting on total phenols content of coffee from early germinated arabica coffee bean

Treatment		Total phenols content
Coffee bean type	**Roasting level**	**(mg GAE/g DW)**
Non-germinated	Light	35.33 ± 0.96 ^{A}
	Medium	29.52 ± 0.89 ^{CD}
	Dark	23.70 ± 1.12 ^{F}
BS 0 mg/L 4th DAG	Light	33.05 ± 0.51 ^{B}
	Medium	27.82 ± 0.40 ^{DE}
	Dark	21.80 ± 0.05 ^{G}
BS 1.0 mg/L 4th DAG	Light	30.04 ± 0.47 ^{C}
	Medium	27.37 ± 0.39 ^{E}
	Dark	21.44 ± 0.05 ^{G}
BS 1.0 mg/L 6th DAG	Light	21.51 ± 0.58 ^{G}
	Medium	21.26 ± 0.69 ^{G}
	Dark	18.67 ± 0.43 ^{H}
F-test		*
C.V. (%)		5.94

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

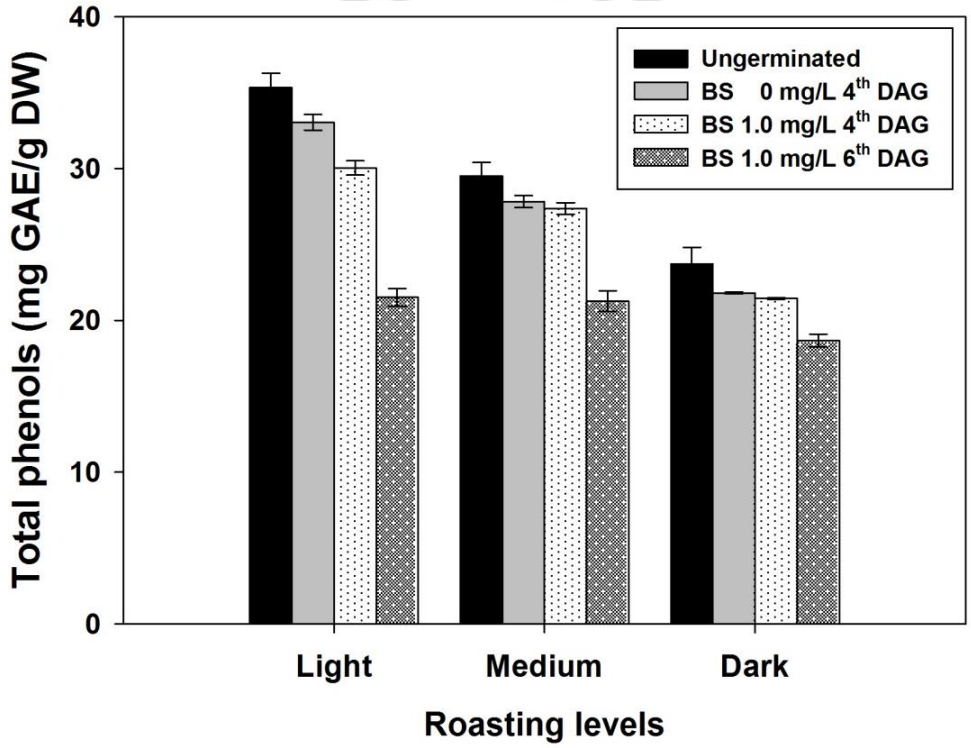

Figure 5.9 Effect of roasting on total phenols content of coffee from early germinated arabica coffee bean

5.2.9 Oxidation activity (DPPH radical scavenging activity)

The increase of roasting degrees led to a decrease in DPPH radical scavenging activity. Maximum radical-scavenging activity was obtained from the light-roasted coffee bean (642.82 μM Trolox/g DW), followed by medium-roasted coffee (553.16 μM Trolox/g DW) and dark-roasted coffee (512.29 μM Trolox/g DW) (Table 5.18).

The highest activity (625.17 μM Trolox/g DW) was found in germinated roasted coffee from BS 1.0 mg/L at 4th DAG treatment (Table 5.18).

The interactions between germinated coffee and roasting level were observed. The highest DPPH radical scavenging activity (734.35 μM Trolox/g DW) was obtained from light roasted germinated coffee of BS 1.0 mg/L at 4th DAG treatment (Table 5.19, Figure 5.10).

The light roasted coffee had the highest antioxidant activity and dark roasted coffee showed the lowest activity. (Da Silveira Duarte *et al*., 2005). The decrease of phenolic compounds is associated with the degradation of chlorogenic acid, which influences the antioxidant capacity of the roasted coffee. However, the antioxidant content and efficiency of roasted coffee can be maintained, or even enhanced, by the formation of compounds with antioxidant activity, such as Maillard reaction products (Nicoli *et al*., 1997b; Del Castillo *et al*., 2005). Some polyphenol derivatives, such as phenylindans are formed upon roasting, display high level of antioxidant activity (Guillot *et al*., 1996)

Table 5.18 Effect of roasting on DPPH radical scavenging activity of coffee from early germinated arabica coffee bean

Treatment		DPPH radical scavenging activity (µM Trolox/g DW)
Coffee bean type	Non-germinated	535.93 ± 24.83 ^{C}
	BS 0 mg/L 4th DAG	545.19 ± 20.75 ^{BC}
	BS 1.0 mg/L 4th DAG	625.17 ± 56.16 ^{A}
	BS 1.0 mg/L 6th DAG	571.41 ± 53.54 ^{B}
Roasting level	Light	642.82 ± 37.81 ^{a}
	Medium	553.16 ± 13.71 ^{b}
	Dark	512.29 ± 12.21 ^{c}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		*
C.V. (%)		11.26

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

Table 5.19 Interaction of effect of roasting on DPPH radical scavenging activity of coffee from early germinated arabica coffee bean

Treatment		DPPH radical scavenging activity
Coffee bean type	**Roasting level**	**(**μM Trolox/g DW**)**
Non-germinated	Light	580.74 ± 22.27 ^{CD}
	Medium	532.04 ± 13.26 ^{DEF}
	Dark	495.00 ± 11.89 ^{F}
BS 0 mg/L 4th DAG	Light	580.74 ± 22.27 ^{CD}
	Medium	545.93 ± 25.53 ^{CDE}
	Dark	508.89 ± 18.98 ^{EF}
BS 1.0 mg/L 4th DAG	Light	734.35 ± 16.20 ^{A}
	Medium	593.38 ± 19.99 ^{C}
	Dark	547.78 ± 13.01 ^{CDE}
BS 1.0 mg/L 6th DAG	Light	675.46 ± 17.48 ^{B}
	Medium	541.30 ± 16.69 ^{DEF}
	Dark	497.48 ± 9.59 ^{EF}
F-test		*
C.V. (%)		7.68

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

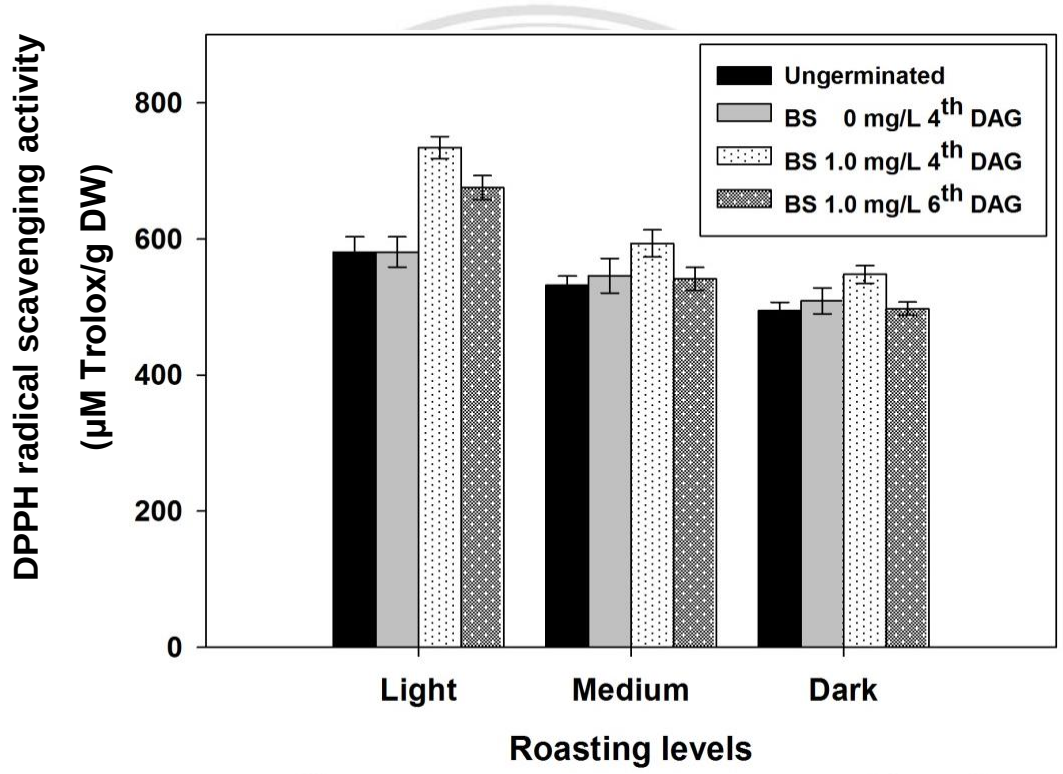

Figure 5.10 Effect of roasting on DPPH radical scavenging activity of coffee from early germinated arabica coffee bean

5.2.10 pH value

After roasting, a significant change in the pH value in roasted bean occurred in all coffee bean types. The non-germinated coffee bean had the lowest pH value (4.61), while the highest value was observed in 1.0 mg/L at 4th DAG and BS 1.0 mg/L at 6th DAG bean types (4.92). The pH value of roasted coffee significantly increased with an increased roasting (Table 5.20). The darker roasted coffee showed the greatest pH value and light roasted one showed the lowest value.

Both coffee bean type and roasting level significantly influence pH value. The interaction results showed that the highest pH value were obtained from 1.0 mg/L at 4th DAG and BS 1.0 mg/L at 6th DAG with dark roasted condition (Table 5.21, Figure 5.11). The increase in pH value was occurred by the destruction of organic acids, such as citric acid, malic acid and chlorogenic acids, especially when the coffee beans were undergone dark roasting (Ginz *et al*. 2000). This result was in agreement with Daglia *et al*. (2000), who reported that the pH value of coffee increased with roasting levels. The pH increasing could alter the degree of ionization of the chemical compounds and they could improve the taste of coffee beverages as well.

Table 5.20 Effect of roasting on pH of coffee from early germinated arabica coffee bean

Treatment		pH
Coffee bean type	Non-germinated	4.61 ± 0.11 ^{C}
	BS 0 mg/L 4th DAG	4.81 ± 0.09 ^{B}
	BS 1.0 mg/L 4th DAG	4.92 ± 0.11 ^{A}
	BS 1.0 mg/L 6th DAG	4.92 ± 0.10 ^{A}
Roasting level	Light	4.66 ± 0.05 ^{c}
	Medium	4.81 ± 0.08 ^{b}
	Dark	4.97 ± 0.09 ^{a}
Coffee bean type		*
Roasting level		*
Coffee bean type×*Roasting level*		*
C.V. (%)		1.79

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

Table 5.21 Interaction of effect of roasting on pH of coffee from early germinated arabica coffee bean

Treatment		pH
Coffee bean type	**Roasting level**	
Non-germinated	Light	4.53 ± 0.01 ^{G}
	Medium	4.60 ± 0.01 ^{FG}
	Dark	4.71 ± 0.05 ^{DE}
BS 0 mg/L 4th DAG	Light	4.65 ± 0.02 ^{EF}
	Medium	4.80 ± 0.03 ^{C}
	Dark	4.97 ± 0.01 ^{B}
BS 1.0 mg/L 4th DAG	Light	4.71 ± 0.02 ^{DE}
	Medium	4.95 ± 0.01 ^{B}
	Dark	5.11 ± 0.04 ^{A}
BS 1.0 mg/L 6th DAG	Light	4.76 ± 0.03 ^{CD}
	Medium	4.91 ± 0.01 ^{B}
	Dark	5.09 ± 0.03 ^{A}
F-test		*
C.V. (%)		1.79

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$. The results were expressed as mean value ± standard error.

(*) : significantly different between treatments

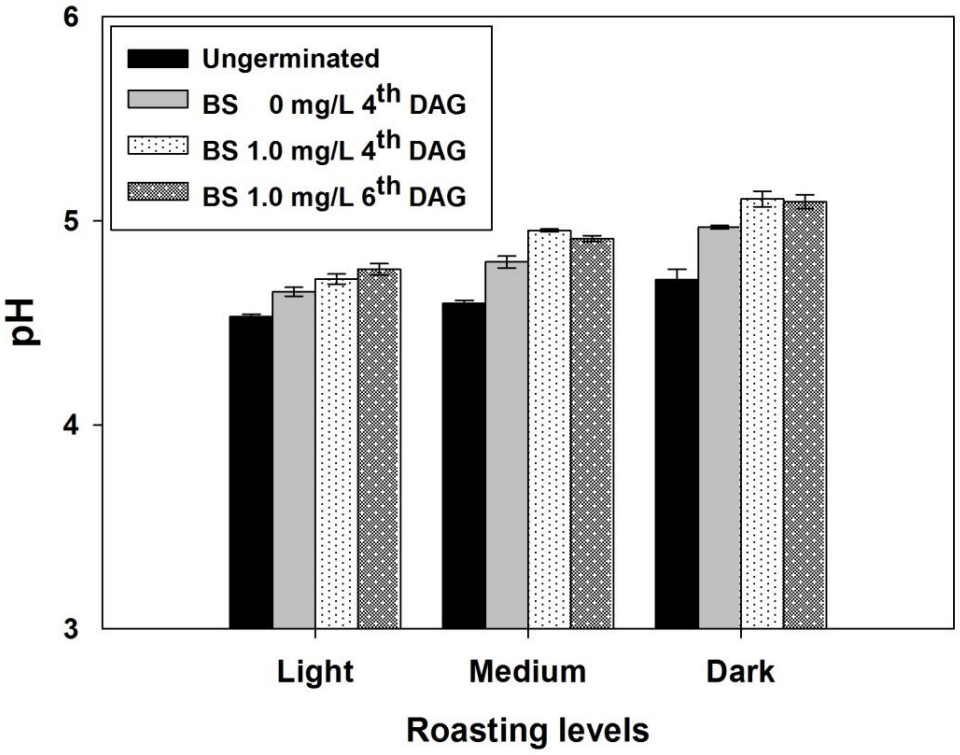

Figure 5.11 Effect of roasting on pH of coffee from early germinated arabica coffee bean

5.2.11 Cup qualities

Green coffee is lacking of the pleasant aroma and flavor that attract worldwide consumers. The desired aroma and flavor of coffee beans used for beverage are typically developed during the roasting. The beans undergo a series of reactions leading to the desired changes in biochemical and physical compositions (Dutra *et al*., 2001).

In this experiment, cupping test was evaluated based on scores ranging between 1 and 5 (5 hedonic scale).

These levels were:

Quality scales:

1.00 - Poor

2.00 - Good

3.00 - Very Good

4.00 - Fine

5.00 - Outstanding

The above scale was ranged from a minimum value of 1 to a maximum value of 5 points.

Aroma is the olfactory perception of the gases released from ground coffee and the vapors released from brewed coffee that are collectively combined into a single score. The aroma is experienced retro nasally through the back of the palate as the coffee is aerated in the mouth while it is slurped. Table 5.22 showed that type of germinated coffee bean had a significant affected on cup aroma. The highest score for aroma was observed in BS 1.0 mg/L at 4th DAG treatment. In addition, roasting levels had significant influence on aroma. The medium roasting level provided the highest score in aroma evaluation. There was significant interaction among type of germinated coffee bean and roasting level on aroma score. The BS 1.0 mg/L at 4th DAG treatment with medium roasted level had the highest aroma score (Table 5.23).

Grosch (2001) and Yeretzian *et al*. (2002) reported that the content of amino acids and sugars in the green coffee beans are important to the development of coffee flavor during roasting. Sucrose is the main contributor of reducing sugar which is implicated in Maillard reactions during the roasting process. Sucrose acts as aroma precursors that affect both taste and aroma of the coffee beverage (Maria *et al*., 1994).

Higher sucrose contents in green coffee beans have been shown to partly explain its better cup quality (Ky *et al*., 2001). The fat of green coffee bean also involved in aroma and flavor formation of roasted coffee (Russwurm, 1970). Fat in green coffee bean serves as flavor carrier and contributes to texture and mouth-feel in the coffee brew. High levels of trigonelline and lipids would improve the cup quality (Oestreich-Janzen, 2013). Decazy *et al*. (2003) also supported that preference is positively linked to fat concentration. The positive correlations were also observed between trigonelline and the cup quality traits. Similar observation was also supported by Farah *et al*. (2006a). Trigonelline give rise to flavor products, including furans, pyrazine, alkyl-pyridines and pyrroles (Ky *et al*., 2001; Dessalegn, 2005).

Body is the sensory perception of the mouth feel of the brew. It is a combination of fats, oils and sediment that swept off the surface of the freshly ground coffee particles and suspended in the unfiltered brew. Table 5.22 showed that type of germinated coffee bean had no significant effect on body score, while roasting level significantly influenced on brew's body. The medium roasted level had the highest score in body.

Flavor generally means the perceived combination of aroma and taste, with the modulation of the basic tastes: sweet, sour, bitter and salty, achieving a distinctive cup characteristic (Kerler *et al*., 2014) The brews from early germinated coffee of BS 1.0 mg/L at 4^{th} DAG and BS 1.0 mg/L at 6^{th} DAG treatments had better flavor scores than non-germinated coffee. Roasting level significantly influenced on flavor score. The medium roasted bean had the highest score of flavor, while the light roasted one had the lowest score rating. The interaction between type of germinated coffee bean and roasting level revealed non-significant difference to flavor evaluation (Table 5.23).

Acidity is the corporeal gustatory perception presented in the brew, an actual physical sensation on the tongue. Although the acidity can be measured quantitatively, its taste perception often varies between individuals, ranging from slightly sweet to slightly sour. The early germinated coffee from BS 1.0 mg/L at 6^{th} DAG treatment had lower acidity score than non-germinated coffee, BS 0 mg/L 4^{th} DAG, BS 1.0 mg/L at 4^{th} DAG and BS 1.0 mg/L at 6^{th} DAG treatments. Roasting level had a significant influence on acidity score. The light roasted level had provided the highest acidity score, while the dark roasted one provided the lowest score to the brew. There was no significant

interaction among type of germinated coffee bean and roasting level on acidity score. Acidity value is related to the total concentration of acids in the sample (Franca *et al*., 2005a), and it is inversely proportional to coffee quality since high acidity ratings are indicating low quality to coffee beverage (Carvalho *et al*., 1994). Degenhardt *et al*., (2006) reported that the acidity directly affected coffee bean quality and it might possibly led to changes in the final quality of processed coffee bean since the pH level was a major driver for flavor in roasted coffee. Variyar *et al*., (2003), Trugo and Macrae, (1984b) and Clifford and Wight, (1976) reported that chlorogenic acids played a great role in the formation of taste and flavor of coffee beans, which determined the quality acceptance of the beverages.

The results from previous experiment (Chapter 4) reported that germination time was one of the most important factors affecting the level of biochemical compositions and antioxidant activity. BS applications significantly increased the contents of protein, total sugars, fat and cholorogenic acid in coffee seed during germination. The content in biochemical compounds in green coffee directly related to cup quality. In cupping test, the overall score was based on cupper's preference in each of coffee characteristics. The overall scores in table 5.22 showed that type of germinated coffee bean has significant effects on aroma, flavor, acidity and total score of cup qualities. The chemical compositions of early germinated coffee bean that would affect the flavor and aroma were also determined in previous experiments. The overall scores of coffee from BS 1.0 mg/L at 4^{th} DAG and BS 1.0 mg/L at 6^{th} DAG treatments were higher than coffee from non-germinated coffee and BS 0 mg/L at 4^{th} DAG treatments. Roasting level also influenced on aroma, body, flavor and acidity. The medium roasted level had provided the highest overall score of the brew. Furthermore, there were significant interactions among type of germinated coffee bean and roasting level on overall score evaluation. The early germinated coffee from BS 1.0 mg/L at 4^{th} DAG and BS 1.0 mg/L at 6^{th} DAG treatments with medium roasting level gave higher overall scores than other treatments (Table 5.23).

Table 5.22 Effect of roasting on cup qualities of coffee from early germinated arabica coffee bean

Treatment		Cup qualities				
		Aroma	**Body**	**Flavor**	**Acidity**	**Overall**
Coffee bean type	Non-germinated	3.22 ± 0.17 ^{B}	2.58 ± 0.45	3.16 ± 0.31 ^{B}	3.49 ± 0.39 ^{A}	3.33 ± 0.12 ^{C}
	BS 0 mg/L 4th DAG	3.22 ± 0.08 ^{B}	2.71 ± 0.41	3.24 ± 0.29 ^{B}	3.42 ± 0.41^{AB}	3.60 ± 0.10 ^{B}
	BS 1.0 mg/L 4th DAG	3.67 ± 0.47 ^{A}	3.00 ± 0.41	3.71 ± 0.33 ^{A}	3.18 ± 0.72 ^{A}	4.04 ± 0.36 ^{A}
	BS 1.0 mg/L 6th DAG	3.13 ± 0.12 ^{B}	2.80 ± 0.38	3.53 ± 0.31 ^{A}	2.62 ± 0.96 ^{C}	4.09 ± 0.29 ^{A}
Roasting level	Light	3.28 ± 0.09 ^{b}	2.47 ± 0.12 ^{b}	2.95 ± 0.13 ^{c}	4.23 ± 0.18 ^{a}	3.50 ± 0.14 ^{b}
	Medium	3.68 ± 0.29 ^{a}	3.58 ± 0.07 ^{a}	4.00 ± 0.14 ^{a}	3.05 ± 0.48 ^{b}	4.18 ± 0.30 ^{a}
	Dark	2.97 ± 0.03 ^{c}	2.27 ± 0.08 ^{b}	3.28 ± 0.12 ^{b}	2.25 ± 0.26 ^{c}	3.62 ± 0.13 ^{b}
Coffee bean type		*	NS	*	*	*
Roasting level		*	*	*	*	*
Coffee bean type×*Roasting level*		*	NS	NS	NS	*
C.V. (%)		20.40	25.57	17.79	23.47	15.76

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.

The results were expressed as mean value ± standard error.

(*) : significantly different between treatments, (NS): not significantly different

Table 5.23 Interaction of effect of roasting on cup qualities of coffee from early germinated arabica coffee bean

Treatment		Cup qualities				
Coffee bean type	**Roasting level**	**Aroma**	**Body**	**Flavor**	**Acidity**	**Overall**
Non-germinated	Light	3.20 ± 0.17 ^{BC}	2.20 ± 0.14	2.67 ± 0.13	4.00 ± 0.22	3.13 ± 0.19 ^{C}
	Medium	3.53 ± 0.13 ^{B}	3.47 ± 0.13	3.73 ± 0.18	3.73 ± 0.15	3.53 ± 0.19 ^{BC}
	Dark	2.93 ± 0.18 ^{C}	2.07 ± 0.18	3.07 ± 0.12	2.73 ± 0.12	3.33 ± 0.13 ^{C}
BS 0 mg/L 4th DAG	Light	3.27 ± 0.23 ^{BC}	2.33 ± 0.13	2.80 ± 0.14	3.87 ± 0.24	3.53 ± 0.17 ^{BC}
	Medium	3.33 ± 0.16 ^{BC}	3.53 ± 0.19	3.80 ± 0.17	3.80 ± 0.14	3.80 ± 0.11 ^{B}
	Dark	3.07 ± 0.15 ^{BC}	2.27 ± 0.15	3.13 ± 0.13	2.60 ± 0.25	3.47 ± 0.17 ^{BC}
BS 1.0 mg/L 4th DAG	Light	3.53 ± 0.17 ^{B}	2.73 ± 0.21	3.20 ± 0.20	4.53 ± 0.13	3.53 ± 0.17 ^{BC}
	Medium	4.53 ± 0.13 ^{A}	3.80 ± 0.22	4.33 ± 0.13	2.93 ± 0.23	4.73 ± 0.12 ^{A}
	Dark	2.93 ± 0.18 ^{C}	2.47 ± 0.22	3.60 ± 0.19	2.07 ± 0.25	3.87 ± 0.17 ^{B}
BS 1.0 mg/L 6th DAG	Light	3.13 ± 0.24 ^{BC}	2.60 ± 0.19	3.13 ± 0.17	4.53 ± 0.13	3.80 ± 0.11 ^{B}
	Medium	3.33 ± 0.13 ^{BC}	3.53 ± 0.19	4.13 ± 0.17	1.73 ± 0.18	4.67 ± 0.16 ^{A}
	Dark	2.93 ± 0.18 ^{C}	2.27 ± 0.21	3.33 ± 0.13	1.60 ± 0.19	3.80 ± 0.14 ^{B}
F-test		*	NS	NS	NS	*
C.V. (%)		20.40	25.57	17.79	23.47	15.76

Means within the treatment in the same columns followed by different characters showed significantly different between treatments by Duncan's multiple range test (DMRT) at $p < 0.05$.The results were expressed as mean value ± standard error.

(*) : significantly different between treatments, (NS) : not significantly different

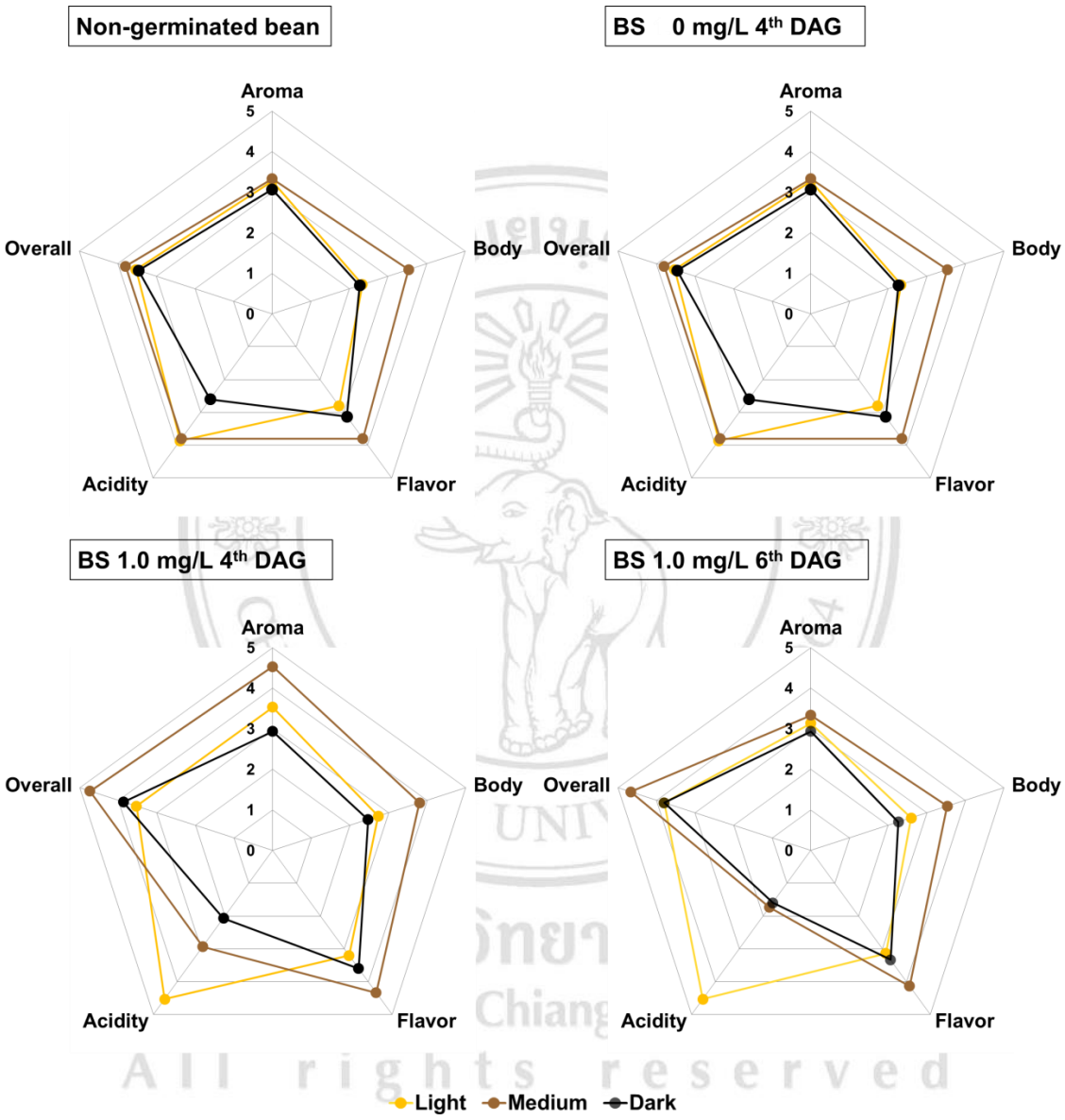

Figure 5.12 Effect of roasting on cup qualities of coffee from early germinated arabica coffee bean

5.3 Conclusion

An increase of roasting degree led to significant diminutions of protein, total sugars, caffeine, total phenols and chlorogenic acid content as well as DPPH radical scavenging activity. However, the higher roasting level increased the greater release of fat. Maximum cupping test score in term of overall acceptability was observed in seeds treated by BS 1.0 mg/L at 4th DAG when roasted moderately. Maximum cupping score was greatly influenced by high nutritional components in early germinated seeds.

CHAPTER 6

Overall Conclusion

According to three experimented results; (I) effect of brassin-like substance on seedling growth of arabica coffee, (II) effect of brassin-like substance and germination on changes of chemical composition of arabica coffee, and (III) Effect of roasting on changes of chemical composition of germinated arabica coffee, the concrete conclusions could be listed as follows:

1. Soaking the coffee seed with brassin-like substance for 24 hours at 1.0 mg/L before germination was the most effective for improving germination percentage and seedling vigor index, while lowering mean germination time.

2. Germination time was one of the most important factors affecting the level of biochemical compositions and antioxidant activity.

3. BS applications significantly increased the content of protein, total sugars, fat, and cholorogenic acid contents in coffee seed during germination.

4. Caffeine and total phenol contents in seed degraded by days of germination.

5. The increase of roasting degrees led to the decreases in protein, total sugars, caffeine, total phenols and chlorogenic acid contents as well as DPPH radical scavenging activity. But, the more degree of roasting increased the more releasing of fat and increased pH value.

6. Coffee bean color significantly changed upon roasting levels, however, the roasting of early germinated coffee bean had no effect on color analysis among coffee bean types.

7. Maximum cupping test in term of overall score was observed for early germinated bean treated by BS 1.0 mg/L at 4^{th} DAG when roasted at medium level.

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APPENDIX A

Determination of protein in arabica coffee seeds

Protein extraction and estimation

The analysis of protein in coffee extracts was determined by Lowry method (Lowry *et al*., 1951).

Chemicals

1. Sodium carbonate (Na_2CO_3)
2. Sodium hydroxide (NaOH)
3. Trichloro acetic acid; TCA (CCl_3-COOH)
4. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)
5. Bovine Serum Albumin (BSA)
6. Sodium potassium tartrate ($NaKC_4H_4O_6$)
7. Folin-Ciocalteu reagent

Preparation of protein extract

One gram of dry seed sample was homogenized with 10 mL of 20% trichloro acetic acid, and the extract was centrifuged at 6,000 rpm for 15 minutes. Then the supernatant was discarded. Five mL of 0.1 N NaOH was added to the residue, centrifuged again at 6,000 rpm for 15 minutes. The supernatant was stored and used for protein estimation.

Preparation of reagents

1. Reagent A : 2 g of Na_2CO_3 in 100 mL of 0.1 N NaOH solution
2. Reagent B : 0.5% of $CuSO_4 \cdot 5H_2O$ mixed with 1% of sodium potassium tartrate solution in the ratio of 1:1
3. Reagent C : Mixture of 50 mL of reagent A and 1 mL of Reagent B
4. Folin-Ciocalteu reagent was diluted at 1:1 ratio with distilled water
5. Bovine Serum Albumin (BSA) Standard: 1 mg/mL (0, 100, 500, 1,000, 2,500 and 5,000 µg/mL were used as standard curve.)

Method

Prepared 0.5 mL of sample or standard, add 5 mL of reagent C. Let the solution stand at room temperature for 10 min. Then added 0.5 mL of Folin-Ciocalteu reagent to a sample mixture, and strongly stirred using a vortex mixer prior to keeping in the dark condition for 20 minutes. Measured the absorbance at 660 nm, and plotted the standard graph.

APPENDIX B

Determination of total sugar in arabica coffee seeds

Determination of total sugars was described by James (1995).

Chemicals

1. Sulfuric acid (H_2SO_4) 1.5 M
2. 2% Sodium hydroxide (NaOH)
3. 10% Sodium hydroxide (NaOH)
4. Potassium sodium tartrate tetrahydrate ($C_4H_4KNaO_6$)
5. Glucose stock solution 15 mg/L
6. Dinitrosalicylic acid, DNS ($C_7H_4N_2O_7$)

Preparation of DNS

The DNS reagent was prepared by dissolving 10 g of DNS in 200 mL of NaOH at 80ºC. Dissolving 300 g of potassium sodium tartrate tetrahydrate in 500 mL of distilled water. Mixed thoroughly and adjusted to 1 L using distilled water.

Preparation of standard glucose

1. Prepared glucose standards ranging from 0, 0.1, 0.5, 1.0, and 5.0 mg/mL (total sample volume 1 mL).
2. Added 1 mL of DNS reagent and 2 mL of distilled water to 1 mL of glucose standard solutions in a test tube.
3. Kept all the test tubes in boiling water bath for 5 minutes.
4. After rapid cooling, added distilled water to bring the final volume up to 20 mL.

5. The absorbance was recorded at a wavelength of 540 nm using spectrophotometer.
6. Developed a standard graph of glucose by plotting glucose concentrations versus to the absorbance values.
7. Determined the value of glucose concentration of unknown samples using the standard calibration curve.

Analysis of total sugar

1. One gram of dried ground seed was added by 10 mL of 1.5 N H_2SO_4.
2. Boiled for 20 minutes in a water bath and immediately cooled afterward.
3. Twelve mL of 10% NaOH was added and mix well.
4. Filtered through Whatman no. 4 and adjusted final volume to 100 mL with distilled water.
5. The 1 mL filtrate was collected in a volumetric flask and adjusted final volume to 250 mL with distilled water.
6. Added 1 mL of DNS and 2 mL of distilled water to 1 mL of the sample solution, heated in a water bath at 100ºC for 5 minutes, removed the sample from water bath, and cooled rapidly in an ice bath. The volume was made up to 25 mL with distilled water.
7. Measured the color of each sample with a spectrophotometer at 540 nm (A540). The A540 was used to calculate total sugars using a standard calibration equation.

APPENDIX C

Determination of fat content in arabica coffee seeds

Most of the lipids or the coffee oils are located in the endosperm of green coffee beans (Wilson *et al*., 1997). Fat content of the samples were determined by the continuous solvent extraction method using a soxhlet apparatus.

Materials

Soxhlet extraction unit comprised of

1. A round bottom flask (150 mL)
2. Soxhlet extractor with 60 mL siphoning capacity
3. Condenser
4. Cellulose extraction thimbles (28×80 mm).

Preparation of sample

Two 2 gram of sample was put into the thimble. The thimble (with green coffee sample) was placed in a soxhlet extraction apparatus and the fat was extracted with 200 mL of dichloromethane solvent for 8 hours. The extract was then evaporated, dried and weighed. The weight of the fat (oil) extract was determined and calculated in percentage as follows:

$$\% \text{ Fat} = W2 - W1 \times 100\ W$$

Where,

W2 = Weight of flask and oil extract

W1 = Weight of empty extraction flask

W = Weight of sample

APPENDIX D

Determination of caffeine content of arabica coffee seeds

Chemicals

1. Methanol
2. Acetonitrile
3. Trifluoroacetic acid

Sample preparation

One gram of dried ground green coffee was dissolved in 10 mL methanol. The solution was shaken for 1 hour and stored overnight at room temperature. The supernatant was filtrated through a nylon filter 0.45 µm, 13 mm. Filtered solution was kept in a brown vial at -20ºC until analysis. The absorbance values of standards and samples were measured by a UV-Vis diode array detector (Shimadzu) at 210 nm. The caffeine levels of the samples were calculated from the standard regression.

An amount of 1.25 g of ground roasted coffee was dissolved in 50 mL of distilled water. The solution was stirred for 10 minutes at 80ºC in a water bath. Sample was cooled down to room temperature and filtered through Whatman no.1 filter paper. Filtrate was again filtered through a nylon filter 0.45 µm, 13 mm. Filtered solution was kept in a brown vial at -20ºC until analysis. The absorbance values of standards and samples were measured on a UV/Vis spectrophotometer (Shimadzu) at 210 nm. The caffeine levels of the samples were calculated from the standard regression.

HPLC condition

Mobile phase	:	Acetonitrile: Water (13:87) and Trifluoroacetic acid 0.5 mL/L
Detector	:	UV-Vis diode array detector (set 210 nm)
Flow rate	:	2.0 mL/min
Column	:	Platinum EPS C18 100A 3u (53 mm × 7 mm)

APPENDIX E

Determination of chlorogenic acid content in arabica coffee seeds

Chlorogenic acids are the major phenolic compounds in coffee. Coffee has one of the highest concentrations of chlorogenic acids of all plant constituents (Farah *et al*, 2005). The levels of chlorogenic acids in germinated coffee were determined using High Performance Liquid Chromatography (HPLC).

Chemicals and Reagents

1. 100% Methanol
2. Sodium carbonate (Na_2CO_3)
3. Zinc acetate ($Zn(O_2CCH_3)_2$)
4. Glacial acetic acid
5. Potassium hexacyanoferrate ($K_3Fe(CN)_6$)
6. Phosphoric acid (H_3PO_4)

Material

1. Column HPLC: Ultra C18 5 µm, 250 mm × 4.6 mm
2. Nilon filter 0.45 µm Ø 13 mm.
3. Syringe 1 mL
4. UV/Vis spectrophotometer (Shimadzu)

Extraction

1. One gram of dried ground seed sample was extracted by 70 mL of 100% methanol: 30 mL of distilled water and 0.5 mL of Na_2CO_3. (Ky *et al.*, 1997).
2. Samples were incubated at 4ºC under dark lighting condition and shaken at 125 rpm overnight (Colonna, 1997).
3. The extracts were filtered through cotton to eliminate powder.
4. Fifty mL of extract were added into 2 mL of Carrez reagent; which consisted of solution I and II. The solution I and II were prepared as follows;

   Solution I : dissolved 21.9 g of zinc acetate ($Zn(O_2CCH_3)_2$) and 3 mL of glacial acetic acid in 100 mL distilled water.

   Solution II : dissolved 10.6 g of potassium hexacyanoferrate ($K_3Fe(CN)_6$) in 100 mL of distilled water.
5. The extract solution was precipitated.
6. HPLC analysis

HPLC condition

Mobile phase	A	:	2 mM phosphoric acid, pH 2.7 in 5 % Methanol
	B	:	Methanol containing 5% of 2 mM phosphoric acid, pH 3.9
Detector		:	UV 325 nm
Flow rate		:	0.8 mL/min
Column		:	Ultra C18 5 um, 250 mm × 4.6 mm

APPENDIX F

Determination of total phenolic content in coffee seeds

Total phenolic compounds were measured by using Folin-Ciocalteu (1927) method and expressed as Gallic acid equivalent (GAE).

Chemicals and Reagents

1. 100% Methanol
2. Folin-Ciocalteu reagent
3. Sodium carbonate (Na_2CO_3)

Extraction of total phenolic content

One gram of dried ground seed sample was extracted for 24 hours with 10 mL of 100% ethanol at ambient temperature. The extracts were kept in the dark condition until further assays. One hundred microliters of supernatant was used for analysis.

Determination of total phenolic content

1. Standard gallic acid was prepared by dissolving 50 mg of gallic acid in 50 mL of distilled water. Final concentration was 1 mg/L.
2. 10% Na_2CO_3 (w/v) was prepared by dissolving 50 mg of Na_2CO_3 in 500 mL distilled water.
3. Phenol stock solution was prepared by diluting Folin-Ciocalteu reagent in distilled water with a ratio of 1:1.

Standard solution preparation

Gallic acid (mL)	Distilled water (mL)	Final concentration (µg/mL)
0	5	0
1	4	200
2	3	400
3	2	600
4	1	800
5	0	1,000

Analytical method

1. 100 µL of samples and/or standard were added with 1 mL of phenol stock solution.
2. One mL of 10% Na_2CO_3 and 4 mL of distilled water were added to the samples.
3. After incubation at room temperature for 45 minutes in the dark co ndition, the absorbance was measured at 760 nm using a spectrophometer. The blank for this experiment was distilled water. Total phenolic content was expressed as gallic acid equivalents (mg of GAE/g of extract).

APPENDIX G

Determination of antioxidant activity by DPPH scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical with an unpaired electron that is delocalized over the entire molecule and, thus, employed in the DPPH assay. DPPH possesses a purple color, with a maximum absorption at 519 nm in ethanol; hence, scavenging the DPPH radical by coffee antioxidants will result in a decrease in absorption readings over time. According to the principle of Blois (1958), the extent of decrease in DPPH absorption is a proportional to the concentration of radicals that are being scavenged.

The free radical scavenging activity of the germinated coffee seed was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to the method of Brand-Williams (1995).

Preparation of DPPH solution

1. *0.4 mM Trolox (standard)*

   A stock solution was prepared by diluting 10 mg of Trolox in 15 mL of 100% ethanol. Then the solution was adjusted its final volume to 100 mL with distilled water.

2. *0.8 mM DPPH (1,1-diphenyl-2-picrylhydrazyl)*

   A stock solution of DPPH was prepared by diluting 31.36 g of DPPH in 15 mL of ethanol and adjusted the final volume to 100 mL with distilled water.

3. *Trizma buffer (0.25 nM, pH = 7.4)*

   The buffer was prepared by dissolving Triss (hydroxymethyl) aminomethane 6.057 g and potassium chloride 17.184 g in 900 mL distilled

water. The mixture was adjusted to pH 7.4 with 5N HCN (Hydrogen cyanide) prior to making up final volume of 1,000 mL with distilled water.

Standard solution preparation

Trolox (µl)	15% Ethanol (µl)	Absolute concentration (n mol)
0	600	0
60	540	24
120	480	48
180	420	72
240	360	96
300	300	120
400	200	160
500	100	200
600	0	240

Method

1. DPPH solution, Tris buffer solution, and 85% ethanol were mixed in order to obtain a 1:1:1 ratio for 1.8 mL.
2. The plant extract (600 µL) or standard sample was added.
3. Incubated for 30 minutes under dark lighting condition.
4. The absorbance was read at 525 nm (50% Ethanol was used as blank).

APPENDIX H

Cupping test

1. The early germinated coffee beans were roasted at 240ºC. Three degrees of roasting were made by varying the length of the operation: light roast (6 min), medium roast (7 min) and dark roast (9 min) (Farah *et al*., 2006b).
2. The roasted samples were left for 24 hours and subsequently grounded at medium coarse level.
3. One hundred and fifty mL of hot water (94ºC) was poured thoroughly onto 20 grams of grounded coffee from the rim of the cup and left for 4 minutes prior to conducting cupping evaluation (Angasith and Warrit, 1999).
4. Five trained coffee cuppers randomly evaluated the cup samples from each treatment with three replications.
5. The scores were rated upon basic quality of coffee characteristics such as aroma, taste, body, acidity and overall acceptability. The overall score was based on the flavor experience of the individual cupper as a personal appraisal. Each character attribute was rated by using a numeric scale as follows;

   1.00 - Poor
   2.00 - Good
   3.00 - Very Good
   4.00 - Fine
   5.00 - Outstanding

   Theoretically the above scale ranges from a minimum value of 1 to a maximum value of 5 points.

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