UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF MATURITY STAGES, STORAGE TEMPERATURES AND DURATIONS ON CHILLING INJURY, ANTIOXIDANT RESPONSES AND QUALITY OF GINGER (Zingiber officinale Roscoe)

TAN XUE YI

FP 2017 20

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By

TAN XUE YI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

March 2017

DEDICATION

Dedicated to my beloved mother, aunty, sisters, supervisors and family for their endless love, support, understandings, sacrifices, motivation, advice and encouragement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

INFLUENCE OF MATURITY STAGES, STORAGE TEMPERATURES AND DURATIONS ON CHILLING INJURY, ANTIOXIDANT RESPONSES AND QUALITY OF GINGER (*Zingiber officinale* Roscoe)

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March 2017

Chairman: **Siti Hajar Ahmad, PhD**
Faculty: **Agriculture**

Ginger (*Zingiber officinale* Roscoe) harnesses an incredible healing power for a host of ailments and used as spice throughout the world. It contains bioactive compounds such as phenolics, terpenes, flavonoids and curcumin. However, storage life and usage of ginger is limited as it is susceptible to chilling injury (CI). Storage of rhizomes below 12 ºC causes CI and improper maturity stages at harvest caused reduction of ginger quality, decrease storage life and increase fiber and sprouting of ginger. Thus, the objective of the first experiment was to characterize CI of ginger as affected by maturity stages (7, 9 and 11-months after planting), storage temperatures (5, 15 and 25 ºC) and durations (0, 8, 16, 24 and 32 days). Weight loss due to loss of moisture content was significantly higher in the ginger stored at 25 ºC. Eleven-month ginger stored at 25 ºC showed 192% and 149% higher weight loss than 7 and 9-months ginger, respectively, after 32 days of storage. CI indices as measured by pulp translucency, skin peeling and browning on ginger were severe at 5 ºC, less at 15 ºC while no symptom occurred after 32 days storage at 25 ºC. Browning at 5 ºC storage caused ginger pulp colour changed from yellow to light brown with reduction in pulp firmness. However, the pulp firmness for ginger stored at 15 ºC still maintained after 32 days of storage. There was similar soluble solids concentration peak at storage day 24 and ascorbic acid contents dropped as storage durations were extended for all treatments. Thus, based on the results of the first experiment, 7 and 9-months ginger were selected for the second experiment.

The objective of the second experiment was to determine phytochemicals and chemical marker contents, antioxidant and browning enzyme activities of ginger under different maturity stages, storage temperatures and durations. Seven and nine months ginger rhizomes were stored at 5, 15 and 25 ºC for 24 days. The total phenolic contents (TPC), total flavonoid contents (TFC) and DPPH radical scavenging activity for 9-

months ginger was highest at 29.71 mg GAE/g FW, 44.94 mg QE/g FW and 74.63%, respectively, under 15 ºC storage at day 24. There were also significant interaction effects between storage temperatures x storage durations x maturity stages on 6-gingerol and 6-shogaol contents. Results showed a similar trend for all treatments whereby 6-gingerol and 6-shogaol increased sharply until day 16 followed by rapid reduction. Nine-month ginger at 15 ºC storage showed highest 6-gingerol and 6-shogaol contents, with 50.93 mg/g and 3.11 mg/g, respectively, after 24 days of storage. Also, the results indicated polyphenol oxidase and phenylalanine ammonia lyase enzyme activities were higher in 7-months ginger stored at 5 ºC and the activities increased with increasing storage durations compared to 9-months ginger. Thus, the 9-months ginger under 15 ºC storage were selected as optimum maturity stage and storage temperature since these ginger exhibited minimum CI with high phytochemical and chemical marker contents, and antioxidant activity after 24 days storage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGARUH PERINGKAT KEMATANGAN, SUHU DAN TEMPOH SIMPANAN TERHADAP KECEDERAAN DINGIN, TINDAKBALAS ANTIOKSIDAN DAN KUALITI HALIA (*Zingiber officinale* Roscoe)

Oleh

TAN XUE YI

Mac 2017

Pengerusi: Siti Hajar Ahmad, PhD
Fakulti: Pertanian

Halia (*Zingiber officinale* Roscoe) telah dikenal pasti sebagai salah satu herba yang berpotensi yang telah digunakan sebagai rempah dan ubat-ubatan tradisional di seluruh dunia. Halia mengandungi sebatian bioaktif dan antioksidan seperti fenolik, terpena, flavonoid dan kukumin. Tahap penyimpanan dan penggunaan halia adalah terhad kerana ia adalah mudah terdedah kepada kecederaan dingin. Penyimpanan rizom di bawah 12 ºC menyebabkan kecederaan dingin seperti lutcahaya pada isi, pengelupasan kulit rizom, tisu lembut dan pemerangan pulpa dan peringkat kematangan yang tidak sesuai pada musim menuai menyebabkan pengurangan kualiti rizom, tempoh penyimpanan dan meningkatkan serat dan percambahan rizom. Oleh itu, objektif eksperimen yang pertama adalah untuk mencirikan kecederaan dingin rizom halia yang dipengaruhi oleh peringkat kematangan (7, 9 dan 11 bulan selepas penanaman), suhu penyimpanan (5, 15 dan 25 ºC) dan tempoh penyimpanan (0, 8, 16, 24 dan 32 hari). Penurunan berat rizom halia akibat kehilangan kandungan lembapan adalah jauh lebih tinggi dalam rizom yang disimpan pada suhu 25 ºC. Halia yang berumur 11 bulan disimpan pada 25 ºC menujukkan penurunan berat rizom sebanyak 192% dan 149% lebih tinggi daripada halia 7 dan 9 bulan, selepas 32 hari penyimpanan. Indeks kecederaan dingin seperti lutcahaya pada isi, pengelupasan kulit dan pemerangan pada rizom adalah lebih teruk pada suhu 5 ºC, kurang pada 15 ºC manakala tiada gejala berlaku selepas 32 hari penyimpanan pada suhu 25 ºC. Indeks pemerangan pada suhu penyimpanan 5 ºC meningkat apabila jangka masa penyimpanan meningkat dan warna isi rizom berubah dari kuning ke perang muda dengan pengurangan kekerasan rizom. Pemanjangan jangka masa penyimpanan menyebabkan kelembutan dalam isi rizom. Manakala, penyimpanan halia pada 15 ºC, mengekalkan kekerasan isi halia selepas 32 hari penyimpanan untuk semua peringkat kematangan halia. Kepekatan pepejal terlarut didapati sama dalam penyimpanan hari 24 dan kandungan asid askorbik menurun apabila jangka masa penyimpanan telah dipanjangkan untuk semua rawatan.

Oleh itu, berdasarkan keputusan eksperimen pertama, 7 dan 9 bulan halia telah dipilih untuk eksperimen kedua.

Objektif eksperimen kedua adalah untuk menentukan peningkatan fitokimia dan kandungan penanda kimia, aktiviti antioksidan dan enzim pemerangan dalam rizom halia. Halia yang berumur 7 dan 9 bulan disimpan pada suhu 5, 15 dan 25 ºC selama 24 hari. Jumlah kandungan fenolik (TPC), flavonoid (TFC) dan aktiviti pemerangkapan radikal DPPH untuk halia 9 bulan adalah paling tinggi pada 29.71 mg GAE/g FW, 44,94 mg QE/g FW dan 74.63% masing-masing, di bawah suhu penyimpanan 15 ºC pada hari ke-24. Terdapat juga kesan interaksi yang signifikan antara suhu penyimpanan x jangka masa penyimpanan x peringkat kematangan halia untuk kandungan 6-gingerol dan 6-shogaol dalam halia. Keputusan menunjukkan trend yang sama untuk semua suhu penyimpanan dan tahap kematangan halia di mana 6-gingerol dan 6-shogaol meningkat dengan ketara sehingga penyimpanan ke-16 hari diikuti dengan pengurangan yang ketara. Halia berumur 9 bulan yang disimpan pada suhu 15 ºC menunjukkan kepekatan 6-gingerol dan 6-shogaol tertinggi, dengan 50.93 mg/g dan 3.11 mg/g, masing-masing, pada hari ke-24. Keputusan juga menunjukkan aktiviti enzim polifenol oksidase (PPO) dan fenilalanina amonia liase (PAL) adalah lebih tinggi pada halia berumur 7 bulan padasuhu penyimpanan 5 ºC dan aktiviti meningkat dengan peningkatan jangka masa penyimpanan berbanding halia pada 9 bulan. Oleh itu, halia 9 bulan di bawah suhu penyimpanan 15 ºC telah dipilih sebagai peringkat kematangan yang optimum dan suhu penyimpanan yang optimum kerana halia menunjukkan kecederaan dingin yang minimum dengan kandungan fitokimia dan kandungan penanda kimia, dan aktiviti antioksida yang tinggi selepas penyimpanan 24 hari.

ACKNOWLEDGEMENTS

First and foremost I would like to take this opportunity to express my utmost appreciation to my dedicated supervisor, Associate Professor Dr. Siti Hajar Ahmad. Thank you for giving me the opportunity to gain invaluable experiences in conducting my Master study. Also, thank you for the productive discussions, valuable teaching and shared a lot of her expertise, research insight and ideas for my research. I am truly grateful for your patience, encouragement, enthusiasm, advice, support and effort in guiding me to accomplish my Master study. I have been extremely lucky to have supervisor who cared so much about my research.

In addition, I owed my indebtedness and gratitude to Professor Nazamid Saari and Dr. Mohd Firdaus Ismail for their encouragement, insightful comments and give suggestions and feedbacks in improving my research. This thesis could not have been done without their strong supervision.

I would like to dedicate my heartfelt thanks to my beloved and wonderful family members, who deserve special attention for their unconditional loves, supports and encouragement that have kept me going on until the end. They have been there from the start until the finishing end of my Master study.

I would like to thank UPM for providing Graduate Research Fellowship (GRF) and research facilities to conduct my Master study. I consider it an honour to work with all the administrative and technical staffs of the Faculty Agriculture especially Mr Azhar Othman. I would also like to say thank you very much to Mrs. Nur Indah Shukor, Mrs. Nor Elliza Tajidin, Mrs. Bunga Raya Ketaren, Mrs. Munirah Mohamad, Miss Esther Yap, Miss Fatin Nabilah and all the juniors who helped me during my Master study duration. My master study would not success without the support of all these people.

I certify that a Thesis Examination Committee has met on 24 March 2017 to conduct the final examination of Tan Xue Yi on her thesis entitled "Influence of Maturity Stages, Storage Temperatures and Durations on Chilling Injury, Antioxidant Responses and Quality of Ginger (*Zingiber officinale* Roscoe)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Mohamad bin Osman, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Mahmud bin Tengku Muda Mohamed, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Zora Singh, PhD
Professor
Curtin University
Australia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 2 June 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree Master of Science. The members of Supervisory Committee were as follows:

Siti Hajar Binti Ahmad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Nazamid Saari, PhD
Professor
Faculty of Food Science
Universiti Putra Malaysia
(Member)

Mohd. Firdaus Ismail, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

____

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Name of Chairman of
Supervisory Committee: Siti Hajar Binti Ahmad, PhD

Signature: ________________________________
Name of Member of
Supervisory Committee: Nazamid Saari, PhD

Signature: ________________________________
Name of Member of
Supervisory Committee: Mohd.Firdaus Ismail, PhD

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LIST OF ABBREVIATIONS

°C	Degree Celsius
g	Gram
cm	Centimeter
rpm	Revolutions per minute
min	Minute
%	Percentage
MS	Maturity stage
ST	Storage temperature
SD	Storage duration
kg	Kilogram
mm	Millimetre
mm/min	Millimetre per minute
N	Newton
CI	Chilling injury
mL	Millilitre
uL	Microlitre
DOB	Degree of browning
L*	Lightness
C*	Chromaticity
h°	Hue angle
NaOH	Sodium hydroxide
TA	Titratable acidity
SSC	Soluble solids concentration
HPO_3	Metaphosphoric acid
ANOVA	Analysis of variance

LSD	Least significant differences
RCBD	Randomized complete block design
HSD	Honestly significant differences
FW	Fresh weight
FAO	Food and Agriculture Organization
ug/cm^2/hr/kPa	Microgram per centimetre square per hour per kilopascal
PPO	Polyphenol oxidase
PAL	Phenylalanine ammonia lyase
TPC	Total phenolic contents
TFC	Total flavonoid contents
DPPH	2,2-diphenyl-1-picrylhydrazyl
nm	Nanometre
GAE	Gallic acid equivalent
w/w	Weight over weight
FC	Folin-Ciocalteu
Na_2CO_3	Sodium carbonate
$NaNO_3$	Sodium nitrate
$AlCl_3$	Aluminium chloride
QE	Quecertin equivalent
HPLC	High performance liquid chromatography
ppm	Part per million
FW/min	Fresh weight per minute
PVP-30	Polyvinylpyrrolidone
FW/h	Fresh weight per hour
RH	Relative humidity
DOA	Department of Agriculture
mg/g FW	Milligram per gram fresh weight

MOA	Ministry of Agriculture
N	Nitrogen
P_2O_5	Phosphorus pentoxide
K_2O	Potassium oxide
TE	Trace element
$H_3PW_{12}O_{40}$	Phosphotungstic
$H_3PMo_{12}O_{40}$	Phosphomolybdic
W_8O_{23}	Tungstene
Mo_8O_{23}	Molybdene
MARDI	Malaysian Agricultural Research and Development Institute
AIP	2-aminoindan-2-phosphonic acid
AOA	a-aminooxyacetic acid
AOPP	a-aminooxi-bphenylpropionic acid

CHAPTER 1

GENERAL INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is one of the potential herbs under the 10th Malaysia Plan. It is one of the most profitable crops cultivated in Asia and India, which accounted for 40% of the world's ginger production. Malaysia is the 13th producer of ginger in the world, producing 10,775 tonnes of ginger in 2014. In Malaysia, ginger is mostly planted in Pahang, Sabah, Sarawak, Selangor and Johor. Pahang produced the largest amount of ginger (7,281MT) followed by Sarawak (2,599 MT) in 2014 (Anonymous, 2014). There are two main types of ginger, white and red. The 'Bentong' ginger, a white ginger with large rhizomes, juicy flesh, low fibre content, strong pungency, pale white pulp and light brown peel, is widely cultivated; whereas, the 'Bara' ginger, a red ginger with small rhizomes, yellow to the pinkish cross-section, small size and more pungency compared to other ginger (Mansor et al., 2005; DOA, 2015). A previous study reported that Bentong ginger has the highest gingerol contents (0.808 mg/g), which is an important constituent in ginger, compared to 'China', 'Tanjung Sepat' and 'Bara' gingers (DOA, 2015).

Ginger rhizomes are highly demanded in the local and international markets due to their excellent uses for medicinal purposes, food, health care and beauty. It is also used as a spice and flavouring agent for cooking due to its unique aroma. Ginger harnesses unusual and extraordinary healing power proven for a host of ailments such as arthritis, migraine, morning sickness, anticancer, anti-inflammatory, digestion and boosts immune system, headache and promotes healthy heart and cholesterol levels. Ginger contains high bioactive and antioxidant compounds such as terpenes, flavonoids and phenolics (Grzanna et al., 2005; Ghasemzadeh et al., 2010). The phenolic components which are 6-shagaol, 6-gingerol, 8-gingerol, 8-gingerol, 10-gingerol and curcumin have been identified as major antioxidant components in ginger (Yeh et al., 2014). Gingerol, a phenolic compound acts as a secondary metabolite and responsible for pungency and flavour in ginger. Gingerol has been reported to be an antioxidant, and possessed analgesic, antipyretic, chemopreventive and anti-inflammatory properties (Weiss, 2002). Shogaol, a phenolic compound with a pungent characteristic, is the degradation products of gingerol (Chen et al., 1986). Gingerol degradation to the shogaol occurs under high temperature and acidic conditions. Shogaol has been reported to possess anticancer, antioxidants, regulatory roles in cellular defense mechanism and impact the pungent characteristic to dried ginger (Afzal et al., 2001).

However, ginger is susceptible to chilling injury (CI). Maturity stages, varieties, environment and storage temperatures, and durations influence the occurrence of CI in the rhizome. Storage at ambient temperature leads to high moisture loss, shriveling and sprouting of rhizome during storage below 12 °C, causing CI on the rhizome. The CI caused pitting and sunken lesions on the ginger surface, browning, shriveling, softening, and postharvest decay. The shelf life is shortened and rhizomes degradation reduced the eating quality by the loss of flavour, aroma and pungency. Also, delaying harvest caused reduction of rhizome quality, decrease storage life and increase fibre and sprouting on rhizomes.

The high demand for ginger locally (18,600 MT) requires its importation from countries like China, India and Thailand. However, imported ginger is usually damaged and low quality due to the improper handling temperatures during transportation and storage in the wholesale market. Ginger rhizomes stored at temperatures below 5 °C during transportation and stockpiling at the wholesale market resulted in CI (browning, translucency, tissue softening), loss of flavour and odour, reduction in phytochemicals and reduction of shelf life. However, information on the maturity stages, and storage temperatures and durations on the postharvest quality and phytochemical contents of ginger are lacking. Therefore, the objectives of this study were to determine the main and interaction effects of maturity stages, and storage temperatures and durations on ginger rhizomes (i) postharvest quality and CI, and (ii) phytochemicals and chemical markers contents, antioxidant activity as well as the browning enzymes.

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