HOT-WATER DIPPING EFFECT ON POSTHARVEST QUALITY AND METABOLITE PROFILING OF BENTONG GINGER (*Zingiber officinale* Roscoe) STORED AT LOW TEMPERATURE

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2021

FP 2021 79

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Ginger is a tropical produce that is susceptible to chilling injury (CI). Storing ginger below 15 °C will defect its postharvest quality as it is susceptible to chilling injury (CI). Therefore, the objective of this experiment was to evaluate the effects of storage temperatures and storage durations on the physico-chemical qualities of rhizomes. The collected rhizomes from Bentong were stored at 5, 15, and 25 °C and were kept for 0, 8, 16, and 24 days. The experiment was laid out as a randomized complete block design (RCBD) and analysed as a two-way factorial with four replications. For physical attribute, storage under 15 °C showed that weight loss was concomitant with the firmness of rhizomes and was supported by a positive correlation between weight loss and firmness. For colours, changes of the values of h° were gradual when the rhizomes were stored at 15 °C contrary to chilling of rhizomes at 5 °C suggesting that the colour of the rhizomes was managed to be maintained at such condition. The phytochemical analysis resulted in a similar increment in total phenolic and flavonoid when stored at 5 and 15 °C up to 16 d of storage durations. It also showed that storage at 25 °C could retain 6-gingerol and 6-shogaol up to 16 d of storage. For DPPH activities, the antioxidant capacity became weaker, especially during storage at 25 °C as compared to 5 C of storage.

Hot water treatment is developed for disease control and provides an alternative to the application of synthetic chemicals. In conjunction to mitigate CI symptoms, a study on hot water dip at 45 °C for 0, 5, 10, and 15 min and immediately stored at 5 °C for 0, 8 and 16 d were assessed. Storage at 5 °C was chosen as it represents chilling temperature. The postharvest qualities were expected to be prolonged under chilling temperature after being treated in hot water treatment. The experiments were conducted using RCBD, analysed as a two-way factorial with four replications and evaluated for physico-chemical qualities. From the

results, the rhizome weight loss, increased proportionally with dipping durations and storage durations. For firmness, dipped rhizome for 5 min at 45 °C was found to maintain the rhizome firmness along storage as compared to other dipping durations. The hue (hº) colour value of undipped rhizome resulted in a significant decline after 16 d of storage. Exposed rhizome for 5, 10 and 15 min seemed to maintain the hº of the rhizome after storage at 5 °C. The lightness of colour (L*) also responded similarly. The decrease in hº from yellow to slightly brown also indicated rhizome browning. This was supported by a sharp increase of browning index in undipped rhizome within 16 d of storage at 5 °C. Rhizome held at 45 °C / 5 min managed to reduce browning as compared to other durations. Dipping for 15 min resulted in a significant increase in TPC, TFC and 6-gingerols, especially at 16 days of storage durations whilst DPPH activity was achieved when dipped for 5 min.

Metabolomic profiling on hot water dipped rhizomes has identified two secondary metabolites groups: gingerols-related compounds and diarylheptanoid group. The distinct separation between hot water dip treatment at 5, 10, and 15 min and control were observed via OPLS-DA. The heat map showed the distribution of the up and down regulation of the identified metabolites. Based on the distribution, heat map assists in suggesting the optimum durations of hot water treatment at 45 °C for 5 min to be the optimum duration in reducing browning index that could be an indicator for chilling injury. In conclusion, preconditioning of ginger rhizomes by hot water treatment at 45 °C increased chilling tolerance upon storage at chilling temperature.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN CELUPAN AIR PANAS TERHADAP KUALITI LEPASTUAI DAN PROFIL METABOLOMIK RIZOM HALIA (*Zingiber officinale* Roscoe) PADA SUHU PENYIMPANAN RENDAH

Oleh

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Halia merupakan hasil tanaman tropika yang retan kecederaan kedinginan (CI). Penyimpanan halia pada suhu dingin akan merosakkan kualiti lepas tuai akibat mengalami kecederaan kedinginan (CI). Oleh itu, objektif eksperimen ini adalah untuk menilai kesan suhu penyimpanan dan jangka masa penyimpanan terhadap kualiti fizik-kimia rizom. Rizom yang dikutip dari Bentong disimpan pada suhu 5, 15, dan 25 °C selama 0, 8, 16, dan 24 hari. Eksperimen ini disusun menggunakan reka bentuk rawak blok sepenuhnya (RCBD) dan dianalisis sebagai faktorial dua hala dengan empat replikasi. Untuk atribut fizikal, penyimpanan di bawah suhu 15 °C menunjukkan bahawa berlaku penurunan berat selari dengan ketegasan rizom dan disokong oleh korelasi positif antara penurunan berat dan ketegasan. Untuk warna, perubahan nilai h° berlaku secara beransur-ansur apabila rizom disimpan pada suhu 15 ℃ berbanding dengan penyejukan pada 5 °C. Hal ini menunjukkan bahawa warna rizom berjaya dikekalkan dalam keadaan suhu sedemikian. Analisis fitokimia menunjukkan peningkatan yang serupa bagi jumlah kandungan fenolik dan flavonoid apabila disimpan pada 5 dan 15 ℃ selama 16 hari tempoh penyimpanan. Ia menunjukkan bahawa penyimpanan pada suhu 25 °C dapat mengekalkan kandungan 6-gingerol dan 6-shogaol sehingga 16 hari tempoh penyimpanan. Untuk aktiviti DPPH, kapasiti antioksidan semakin berkurangan terutama semasa penyimpanan pada suhu 25 °C berbanding dengan penyimpananpada suhu 5 °C.

Rawatan air panas ialah rawatan, yang telah dikembangkan bagi tujuan kawalan penyakit, dan merupakan alternatif kepada penggunaan bahan kimia sintetik. Sehubungan itu, bagi mengurangkan simtom CI, kajian mengenai celupan air panas pada suhu 45 °C selama 0, 5, 10 dan 15 min dan disimpan pada 5 °C selama 0, 8 dan 16 hari dinilai. Penyimpanan pada suhu 5 °C telah ditentukan

sebagai suhu dingin. Kualiti lepas tuai dijangka dapat dikekalkan pada suhu dingin setelah dirawat dengan rawatan air panas.Eksperimen dilakukan menggunakan RCBD, dalam susunan factorial dua hala dengan empat replikasi dan dinilai untuk kualiti fizik-kimia. Hasil kajian menunjukkan penurunan berat rizom meningkat secara berkadar dengan jangka masa pencelupan. Untuk ketegasan, rizom dicelupkan selama 5 minit pada 45 °C didapati mengekalkan ketegasan rizom di sepanjang tempoh penyimpanan berbanding dengan jangka masa pencelupan yang lain. Nilai warna rizom (h°) yang tidak dicelup menunjukkan penurunan yang ketara setelah 16 hari tempoh penyimpanan. Rizom yang terdedah selama 5, 10 dan 15 minit dilihat mengekalkan nilai h° bagi rizom selepas penyimpanan pada suhu 5 °C. Kecerahan warna (L *) juga menunjukkan tindak balas yang sama. Penurunan h° daripada kuning hingga sedikit perang juga menunjukkan pemerangan rizom. Ini disokong oleh peningkatan ketara indeks pemerangan dalam rizom yang tidak dicelup dalam tempoh16 hari pada suhu 5 °C. Rizom yang dicelup pada 45 °C / 5 min berjaya mengurangkan pemerangan berbanding dengan jangka masa yang lain. Pencelupan selama 15 min menyebabkan peningkatan ketara bagi jumlah kandungan TPC, TFC dan 6-gingerol khususnya selepas 16 hari tempoh penyimpanan. Sementara itu, aktiviti antioksidan (DPPH) pula dapat dicapai pada jangka masa pencelupan 5 min.

Pencirian metabolomik terhadap rizom yang dirawat dengan celupan air panas telah mengenal pasti dua kumpulan metabolit sekunder iaitu gingerol dan diarilheptanoid. Pemerhatian terhadap pemisahan distingtif antara rawatan celupan air panas dalam tempoh 5, 10 dan 15 min dengan kawalan dilakukan menggunakan model OPLS-DA. Peta haba menunjukkan taburan peningkatan dan penurunan oleh metabolit yang dikenal pasti. Berdasarkan taburan tersebut, peta haba dapat membantu memberi cadangan jangka masa optimum 5 in bagi rawatan air panas pada suhu 45 °C sebagai tempoh optimum bagi mengurangkan indeks pemerangan yang menjadi tanda aras kepada kecederaan dingin. Berdasarkan penemuan tersebut, dapat dilihat bahawa metabolomik LCMS adalah pendekatan holistik dalam pengesahan rawatan pasca tuai.

Sebagai kesimpulan, rawatan air panas pada suhu 45 °C sebagai prakondisi ke atas rizom halia dapat dilihat mampu mencetuskan ketahanan sewaktu penyimpanan dalam suhu yang dingin.

ACKNOWLEDGEMENTS

Alhamdulillah that I have finally managed to complete this journey. I would like to express my gratitude to my beloved mother and my late father for supporting me throughout this journey, sending me back and forth to the faculty until I managed to drive on my own. Thank you for not pressuring me as I took many years to complete the entire story. May Allah bless them. To my first supervisor who are my mother's best friend, the ultimate Dr. Siti Hajar Ahmad for bringing me in to the Faculty of Agriculture and made myself to enrol as a phd student under her supervision. I was her last phd student. She made me believe in my capabilities even though I came from a different background. She wanted me badly to become a phd holder as a gift to my mom for not being able to complete her phd studies. Thank you for sharing your valuable knowledge and experiences in study and life. Thank you for those wonderful moments that we have spent together with the rest of the gang. Thank you for your endless support, courage, and guidance. To my last and current supervisor, Dr. Azizah Misran, thank you for adopting me joining your team. Thank you for the financial support and sharing of knowledge. To my first eating gang Kak Elly, Muni, Bunga, and Azimah, thank you guys for always being there for me. To all my little sisters Xue Yi and Elmira, the ginger gang, Aimi the parthenin gang, Tirah the Rawang fig, Xi Yun the peanut planter, Mai the corn breeder, Musalmah the snail collector, Sha my JPJ instructor, Anis the tissue culturist, Yus the nano emulsifier and Miss K the lovely Korean mint, thank you for the lovely friendship and for giving me a ride to anywhere we go. I pray for success and wish you all the best in everything that you do.

Al-fatihah to late Dr Bokhary Zainal.
Thank you.

I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of (student's name) on his (her) thesis entitled "Hot-Water Dipping Effect on Postharvest Quality and Metabolite Profiling of Bentong Ginger (*Zingiber officinale* Roscoe) Stored at Low Temperature" 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 Doctoral of Phisolophy.

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TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xv
LIST OF ABBREVIATIONS		xx

CHAPTER

		Page
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	4
	2.1 *Zingiber officinale* Roscoe	4
	2.2 Cultivation Characteristics and Harvesting	4
	2.3 Chemical Composition, Nutritional Values and Medicinal Values	5
	2.4 Storage of Rhizomes at Low Temperature	12
	2.5 Chilling Injury (CI)	12
	2.5.1 Symptoms of Chilling Injury	13
	2.5.2 Effects of Chilling Injury	14
	2.5.3 Mechanism of Chilling Injury	14
	2.6 Postharvest Treatment in Alleviation of Chilling Injury	16
	2.6.1 Heat Treatment	16
	2.6.2 Mechanism of Heat Treatment	17
	2.6.3 Quality of Evaluation	17
	2.7 Metabolomic Profiling	19
	2.7.1 Analytical Platform in Metabolomic Profiling using LC-MS	20
	2.7.2 Data Processing	21
	2.7.3 Chemometric in Metabolomics: PCA, PLS, Correlation and Heatmap	21
	2.7.4 Heatmaps	22
3	**THE CHANGES IN PHYSICO-CHEMICAL QUALITY OF BENTONG GINGER RHIZOMES STORED AT CHILLING TEMPERATURE**	24
	3.1 Introduction	24
	3.2 Material and Methods	25
	3.2.1 Rhizomes Materials and Treatment Conditions	25
	3.2.2 Physical Analysis	25
	3.2.3 Chemical Analysis	26
	3.2.4 Antioxidant Activity	28

3.2.5 Experimental Design and Statistical Analysis 29
3.3 Result and Discussion 29
3.3.1 Weight Loss (WL) 29
3.3.2 Firmness 32
3.3.3 Colour 34
3.3.4 Soluble Solid Concentrations (SSC) 39
3.3.5 Titratable Acidity (TA) 42
3.3.6 Browning Index (BI) 46
3.3.7 Total Phenolic Contents (TPC) 48
3.3.8 Total Flavonoid Contents (TFC) 50
3.3.9 Identification and Quantification of 6-gingerol and 6-shogaol 52
3.3.10 1,1-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assays 56
3.4 Correlation Analysis 59
3.5 Conclusion 60

4 EFFECTS OF HOT WATER TREATMENT ON POSTHARVEST QUALITIES OF BENTONG GINGER (*Zingiber officinale*) RHIZOMES 61
4.1 Introduction 61
4.2 Material and Methods 62
4.2.1 Rhizomes Materials and Treatment Conditions 62
4.2.2 Physical Analysis 62
4.2.3 Chemical Analysis 63
4.2.4 Antioxidant Activity 64
4.2.5 Experimental Design and Statistical Analysis 64
4.3 Result and Discussion 64
4.3.1 Weight Loss (WL) 64
4.3.2 Firmness 67
4.3.3 Colour 68
4.3.4 Soluble Solid Concentrations (SSC) 74
4.3.5 Browning Index (BI) 76
4.3.6 Total Phenolic Contents (TPC) 79
4.3.7 Total Flavonoid Contents (TFC) 81
4.3.8 Identification and Quantification of 6-gingerol and 6-shogoal 83
4.3.9 1,1-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Capacity Assays 85
4.4 Correlation Analysis 88
4.5 Conclusion 89

5 METABOLOMIC PROFILING ON THE EFFECT OF HOT WATER DIP TREATMENT IN GINGER RHIZOMES 90
5.1 Introduction 90
5.2 Material and Methods 90
5.2.1 Sample Preparation 90

	5.2.2	Extraction of Metabolites	91
	5.2.3	High Performance Liquid Chromatography using Mass Spectrometry	91
	5.2.4	Data Processing and Statistical Analysis	91
5.3	Results and Discussions		92
	5.3.1	Metabolite Profiling of Hot Water Dip Rhizomes using LC-HRESI-MS	92
	5.3.2	Discrimination of Secondary Metabolites as Affected by Dipping Durations of Hot Water Dip Treatment and Storage Durations of Ginger Rhizomes	96
5.4	Conclusion		101

6 CONCLUSION AND RECOMMENDATION 102

REFERENCES 104
APPENDICES 125
BIODATA OF STUDENT 137
LIST OF PUBLICATIONS 138

LIST OF TABLES

Table		Page
2.1	Chemical constituents isolated from rhizomes of *Zingiber officinale* by different solvent extraction	7
3.1	The main and interaction effects of storage temperatures (5, 15 and 25 °C) and storage durations (0, 8, 16 and 24 d) on weight loss and firmness of ginger	30
3.2	The main and interaction effects of storage temperatures (5, 15, and 25 °C) and storage durations (0, 8, 16, and 24 d) on hue, lightness, and chroma of ginger rhizomes	34
3.3	The main and interaction effects of storage temperatures (5, 15 and 25 °C) and storage durations (0, 8, 16 and 24 d) on soluble solids concentrations of ginger rhizomes	40
3.4	The main and interaction effects of storage temperatures (5, 15, and 25 °C) and storage durations (0, 8, 16, and 24 d) on titratable acidity of ginger rhizomes	43
3.5	The main and interaction effects of storage temperatures (5, 15, and 25 °C) and storage durations (0, 8, 16, and 24 d) on the browning index of Bentong ginger rhizomes	46
3.6	The main and interaction effects of storage temperatures (5, 15, and 25 °C) and storage durations (0, 8, 16, and 24 d) on total phenolic contents and total flavonoid contents of Bentong ginger rhizomes	48
3.7	The main and interaction effects of storage temperatures (5, 15 and 25 °C) and storage durations (0, 8, 16 and 24 d) on 6-gingerol and 6-shogaol contents of Bentong ginger rhizomes	53
3.8	The main and interaction effects of storage temperatures (5, 15, and 25 °C) and storage durations (0, 8, 16, and 24 d) on 1,1-diphenyl-1-picrylhydrazyl activities of Bentong ginger rhizomes	57
3.9	Pearson correlation coefficient (r) matrices among the responses of physical quality attributes of hot water dipped rhizomes for 0, 5, 10 and 15 min for 0, 8 and 16 days under storage of 5 °C	59
4.1	The main and interaction effects of dipping durations (0, 5, 10 and 15 min) and storage durations (0, 8, 16 and 24 d) on weight loss and firmness of ginger rhizomes	65

4.2 The main and interaction effects of dipping durations (0, 5, 10 and 15 min) and storage durations (0, 8, 16 and 24 d) on hue (h°), lightness (L*) and chroma (C*) of ginger rhizomes 69

4.3 Total colour changes ∆E of hot water dipped rhizomes (5, 10 and 15 min) at initial and day 16 after storage under 5 °C 73

4.4 The main and interaction effects of dipping durations (0, 5, 10 and 15 min) and storage durations (0, 8, and 16 d) on soluble solid concentrations (%) of ginger rhizomes 74

4.5 The main and interaction effects of dipping durations (0, 5, 10 and 15 min) and storage durations (0, 8, and 16 d) on browning index (abs) of ginger rhizomes 77

4.6 The main and interaction effects of dipping durations (0, 5, 10 and 15 min) and storage durations (0, 8, and 16 d) on total phenolic contents and total flavonoid contents of ginger rhizomes 79

4.7 The main and interaction effects of dipping durations (0, 5, 10 and 15 min) and storage durations (0, 8, and 16 d) on 6-gingerol and 6-shogaol contents of ginger rhizomes 83

4.8 The main and interaction effects of dipping durations (0, 5, 10 and 15 min) and storage durations (0, 8, and 16 d) on 1,1-diphenyl-1-picrylhydrazyl radical scavenging activities of ginger rhizomes 86

4.9 Pearson correlation coefficient (r) matrices among the responses of physical quality attributes of hot water dipped rhizomes for 0, 5, 10 and 15 min for 0, 8 and 16 days under storage of 5 °C 88

5.1 Chromatographic and mass spectral characteristics of metabolite contents in hot water dipped rhizomes by LC-ESI-MS from the Antimarin and DNP database 95

LIST OF FIGURES

Figure		Page
2.1	Chemical structures of common gingerols, shogaols, and paradols. (1) [6]-gingerol, (2) [8]-gingerol, (3) [10]-gingerol, (4) [6]-shogaol, (5) [8]-shogaol, (6) [10]-shogaol, (7) [6]-paradol, (8) [8]-paradol, and (9) [10]-paradol	10
2.2	Symptoms of chilling injury in ginger rhizome. (a) Water-soak appearance (b) Skin peeling (c) Shrivelling (d) Browning (e) Skin purple	13
3.1	The relationship between weight loss of ginger rhizome and storage duration (0, 8, 16 and 24 d) at different storage temperatures (5, 15 and 25 °C) of Bentong ginger rhizomes. Y (5) = - 1.87 x 10^{-14}+ 0.14x + 0.00031x^2 -0.00019x^3; R^2 = 0.99; Y (15) = -2.16 x 10^{-16} + 0.10x - 0.0037x^2 + 2.60 x $10^{-16}x^3$; R^2 = 0.99 and Y (25) = - 1.12 x 10^{-14} - 0.038x + 0.061x^2 – 0.0022x^3; R^2 = 0.99. Means with the different letters are significantly different at *P* =0.05. Vertical bars indicate the standard error (n = 4)	31
3.2	The relationship between firmness and storage duration (0, 8, 16 and 24 d) at different storage temperatures (5, 15 and 25 °C) of Bentong ginger rhizomes. Y (5) = 56.43 – 1.86x; R^2 = 0.76, Y (15) = 65.79 - 0.61x; R^2 = 0.89, Y (25) = 52.31 - 1.19x; R^2 = 0.43. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4)	33
3.3	The relationship between (a) hue (h°), (b) chroma (C*) and (c) lightness (L*) and storage duration (0, 8, 16 and 24 d) at different storage temperatures (5, 15 and 25 °C) of Bentong ginger rhizomes. h°: Y (5) = 98.26 - 0.58x + 0.02x2; R^2 = 0.77; Y (15) = 33.0 - 0.86x + 0.021x^2; R^2 = 0.73 and Y (25) = 31.99 - 1.94x - 0.097x^2; R^2 = 0.99. C* = Y(5) = 31.5 – 1.73x + 0.064x^2; R^2 = 0.88; Y (15) = 33.4 – 0.82x + 0.018x^2; R^2 = 0.70, and Y (25) = 32.9 – 2.07x + 0.10x^2; R^2 = 0.98. L*: Y (5) = 76.98 – 1.92x + 0.071x^2; R^2=0.98, Y (15) = 76.08 – 1.87x + 0.07x^2; R^2 = 0.75, and Y (25) = 76.49 – 2.16x + 0.096x^2; R^2 = 0.92. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4)	36
3.4	The relationship between soluble solids concentration and storage duration (0, 8, 16 and 24 d) at different storage temperatures (5, 15 and 25 °C) of Bentong ginger rhizomes. Y (5) = 3.4 - 0.23x + 0.019 x^2 -0.0007 x3; R^2 = 0.99, Y (15) = 3.4 - 0.21x + 0.024x^2 - 0.001x^3; R^2 = 0.99 and Y (25) = 3.4-0.45x + 0.075x^2 + 0.0019x^3; R^2 = 0.99. Means with the different letters	

are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 41

3.5 The relationship between titratable acidity and storage duration (0, 8, 16 and 24 d) at different storage temperatures (5, 15 and 25 °C) of Bentong ginger rhizomes. Y (5) = 0.36 + 0.072x - 0.058x2 + 0.00014x3, R^2 = 0.92; y(15) = 0.36 + 0.017x - $0.0023x^2 + 0.00009928x^3$, R^2 = 0.84 and Y(25) = 0.36 + 0.138x - $0.013x^2 + 0.00035x^3$, R^2 = 0.96. Means with the different letters are significantly different at *P* = 0.05 44

3.6 The relationship between browning index and storage duration (0, 8, 16 and 24 d) at different storage temperatures of ginger (5, 15 and 25 °C). Y (5) = 0.27 + 0.0026x + $0.0036x^2$ - $0.00010x^3$; R^2 = 0.99, Y(15) = 0.27 + 0.068x - $0.0050x^2$ + $0.00013x^3$; R^2 = 0.99 and Y(25) = 0.27 - 0.039x + $0.010x^2$ – 0.00032x3; R^2 = 0.99. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 47

3.7 The relationship between total phenolic contents and storage duration (0, 8, 16 and 24 d) at different storage temperatures of ginger (5, 15 and 25 °C). Y(5) = 30.04 – 6.09x + $0.88x^2$ - $0.026x^3$; R^2=0.99, Y(15) = 30.04 – 7.26x + $1.02x^2$ - $0.03x^3$; R^2 = 0.99 and Y(25) = 30.04 – 4.20x + $0.58x^2$ – $0.017x^3$; R^2 = 0.99. Means with the different letters are significantly different at *P* = 0.05 49

3.8 The relationship between total flavonoid contents and storage duration (0, 8, 16 and 24 d) at different storage temperatures (5, 15 and 25 °C) of Bentong ginger rhizomes. Y (5) = 30.78 – 2.44x + $0.71x^2$ - 0.023 x^3; R^2 = 0.99, Y(15) = 30.78 – 6.08x + $1.26x^2$ - $0.04x^3$; R^2 = 0.99 and Y(25) = 30.78 + 2.81x – $0.15x^2$ + $0.00034x^3$; R^2 = 0.99. Means with the different letters are significantly different at *P* = 0.05 51

3.9 The relationship between 6-gingerol and 6-shogaol contents and storage duration (0, 8, 16 and 24 d) at different storage temperatures (5, 15 and 25 °C) of Bentong ginger rhizomes. (a) Y(5) = 34.35 – 3.78x + $0.82x^2$ - 0.027 x3; R^2 = 0.99, Y(15) = 34.35 – 3.54x + $0.68x^2$ - $0.021x^3$; R^2 = 0.99 and Y(25) = 34.35 – 0.68x + $0.23x^2$ + $0.0083x^3$; R^2 = 0.99. (b) Y(5) = 1.52 – 0.22x + $0.03x^2$ - 0.012 x^3; R^2 = 0.99, Y(15) = 1.52 – 0.12x + $0.023x^2$ - $0.0007x^3$; R^2 = 0.99 and Y(25) = 1.52 + 0.13x - $0.0092x^2$ + $0.0002x^3$; R^2 = 0.99. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 54

3.10 Dehydration of 6-gingerol to 6-shogaol 55

3.11 HPLC profile of (1) 6-gingerol and (2) 6-shogaol in the juice of ginger rhizome 56

3.12 The relationship between percentage of DPPH radical scavenging activities and storage duration (0, 8, 16, and 24 d) at different storage temperature of Bentong ginger rhizomes (5, 15 and 25 ° C). Y(5) = 87.87 + 0.74x - $0.07x^2$; R^2 = 0.98, Y(15) = 85.96 + 7.94x - $0.96x^2$ + $0.023x^3$; R^2 = 0.82, and Y(25) = 90.95+ 1.77x - $0.65x^2$ + $0.020x^3$; R^2 = 0.59. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 57

4.1 The relationship between weight loss of ginger rhizome and storage duration (0, 8, 16 and 24 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger. Y (0) = 0.039+ 0.089x -$0.0027x^2$; R^2 = 0.89, Y(5) = 0.17 + 0.33x - $0.0049x^2$; R^2 = 0.96, Y (10) = 0.425 + 0.63x -$0.0068x^2$; R^2 = 0.94 and Y(15) = 0.91 + 0.99x - $0.025x^2$ + $0.0059x^3$; R^2 = 0.56. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 66

4.2 The relationship between firmness of ginger rhizome and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger. Y (15) = 49.36 – 1.41x + $0.096x^2$; R= 0.79. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 67

4.3 The relationship between hue (h°) of ginger rhizome and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger. h°: Y(0) = 95.85 – 1.31x; R^2 = 0.91; Y(5) = 97.66 – 0.18x; R^2 = 0.73. C* = Y (0) = 28.70 + 0.25x – 0.055 x^2; R^2 = 0.93; Y (5) = 26.84 + 0.56x – 0.04 x^2; R^2 =0.74, and Y (15) = 8.01 – 4.21x + $0.26x^2$; R^2 = 0.97. L*: Y (0) =65.14 + 0.38x – $0.078x^2$; R^2 = 97; Y(5) = 67.78 – 0.28x + $0.025x^2$; R^2 = 0.53; Y(10) = 69.35 + 0.41x – $0.026x^2$; R^2 = 0.54; Y(15)=75.87 – 0.34x + $0.024x^2$; R^2 = 0.62. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 70

4.4 The relationship between soluble solid concentrations (%) of ginger rhizomes and storage duration (0, 8, 16 and 24 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger. Y(0) = 5.01 - 0.07x; R^2 = 0.71; Y(10) = 2.95 – 0.13x; R^2= 0.99 and Y(15) = 4.75 + 0.06x; R^2= 0.92. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 75

4.5 The response for browning index ($abs_{480\ nm}$) of ginger rhizomes and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger. Vertical bars indicate the standard error (n = 4) 78

4.6 The relationship between total phenolic contents (g GAE kg^{-1} FW) of ginger rhizomes and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger rhizomes. Y (0) = 0.00126 - 8.4 x 10^{-5}x + 4.4 x $10^{-6}x^2$; R^2 = 0.86, Y (5) = 0.001 - 4.2 X 10^{-6}x – 1.9 x 10-6x^2; R^2 = 0.97, Y (10) =0.0013 – 5.5 x 10^{-5}x + 4.7 x 10^{-6} x^2; R^2 = 0.96, Y (15) = 0.0014 + 0.00013x – 4.2 x 10^{-6} x^2; R^2 = 0.97. Vertical bars indicate the standard error (n = 4) 80

4.7 The relationship between total flavonoids contents (g RUE kg-1 FW) and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger rhizomes. Y(0) = 0.032 – 0.00013 x 10^{-5}x – 1.2 x $10^{-5}x^2$; R^2 = 0.92, Y(5) = 0.022 – 0.0013x – 8.9 x $10^{-5}x^2$; R^2 = 0.54, Y(10) = 0.017 – 0.0025x – 0.00017x^2; R^2 = 0.89, Y(15) = 0.043 - 0.0052x + 0.00049x^2; R^2 = 0.99. Vertical bars indicate the standard error (n = 4) 82

4.8(a) The relationship between 6-gingerol contents (g kg^{-1} FW) and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger rhizomes. Y (0) = 3.09 x 10^{-5} + 1.1 x 10-6x – 6.4 x $10^{-8}x^2$; R^2 = 0.95, Y(5) = 0.00062 – 0.0001x + 6.4 x $10^{-6}x^2$; R^2 = 0.99, Y(10) = 0.0014 – 0.00022x + 9.5 x 10^{-6} x^2; R^2 = 0.99 and Y(15) = 0.0014 - 0.0002x + 8.5 x $10^{-6}x^2$; R^2 = 0.99. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 84

4.8(b) The relationship between 6-shogaol contents (g kg-1 FW) of ginger rhizomes and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger. Vertical bars indicate the standard error (n = 4) 84

4.9 The relationship between percentage of DPPH radical scavenging activities (%) and storage duration (0, 8, and 16 d) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger rhizomes. Y (0) = 14.01 + 4.04x – 0.21x^2; R^2 = 0.99, Y(5) = 77.3 + 0.07x + 0.0038x^2; R^2 = 0.80, Y(10) = 77.9 – 6.7x + 0.35x^2; R^2 =0.98, and Y(15) = 9.2 + 0.27x + 0.049x^2; R^2 = 0.98. Means with the different letters are significantly different at *P* = 0.05. Vertical bars indicate the standard error (n = 4) 87

5.1 Total ion chromatogram of hot water (45 °C) dipped rhizomes for 5, 10, and 15 min stored under for 0, 8 16 94

5.2 Secondary metabolites that were putatively identified using LCMS. 2,3,5-trihydroxy-1,7-bis(4-hydroxy- 3-methoxyphenyl) heptane (1), 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-

	3- methoxyphenyl)-3,5-heptanediol (2), Hexahydrocurcumin (3), 1,7-bis(4-hydroxyphenyl)-5-oxo-3-heptanyl B-D-glucopyranoside (4), 6-gingerdiol (5), 6-gingerol (6), 6-shogaol (7), 8-shogoal (8), and 6-gingerdione (9)	96
5.3	(a) Unsupervised PCA scores plot (R2 = 0.75, Q2= 0.42). (b) Supervised OPLS-DA score plot analysis	97
5.4	Heat map of secondary metabolites in treated and non-treated ginger rhizomes. The colours indicate the proportional content of each identified metabolites as determined by the average peak response area with *R* scale normalization. Three independent replicates were performed for each stage. Con = control; HT (5, 10, and 15) = hot treatment duration in min; D (0, 8, and 16) = storage durations	98

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
°C	Degree celsius
$AlCl_3$	Alumunium chloride
Aq	Aqueous
BI	Browning index
C*	Chroma
$CHCl_3$	Chloroform
CHS	Chalcone synthase
CI	Chilling injury
cm min^{-1}	Centimetre per minute
D	Days
DAD	Diode array detector
DCM	Dichloromethane
DD	Dipping duration
DNA	Deoxyribonucleic acid
DNP	Dictionary of Natural Products
DPPH	1, 1-diphenyl-2-picrylhydrazyl
ESI	Electron spray ionization
ESI	Electron spray ionization
EtOH	Ethanol
FW	Fresh weight
g	Gram
g kg^{-1}	Gram per kilogram
g L^{-1}	Gram per litre
GAE	Gallic acid equivalents
GC	Gas chromatography

h°	Hue
HPLC/EI-MS	High-performance liquid chromatography/electrospray ionization-mass spectrometry
HR-MS	High-resolution mass spectrometry
HSD	Tukey's honestly significant difference
HSP	Heat shock protein
IT	Ion trap
kg	Kilogram
kV	Kilovolt
L	Litre
L*	Lightness
m	Meter
MeOH	Methanol
mg	Milligram
min	Minute
mL	Millilitre
μm	Micro metre
MS	Mass spectrometry
MWA	Multivariate analysis
N	Force
Na_2CO_3	Sodium carbonate
NAD-MDH	NAD-dependent malate dehydrogenase
NADP-ME	NADP-malic enzyme
NADPH	
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
nd	Nonsignificant
nm	Nanometre

OPLS-DA	Orthogonal projection to latent structure
P	Probability
PAL	Phenylalanine ammonia lyase
PCA	Principal component analysis
PE	Petroleum ether
PEPC	phosphoenolpyruvate carboxylase
PLS-DA	Partial least squares-discriminant analysis
Ppm	Part per million
PPO	Polyphenol oxidase
PROC GLM	Procedure of the general linear model
PROC REG	Procedure of regression
RCBD	Randomized completely block design
RE	Rutin equivalent
ROS	Reactive oxidative species
SD	Storage duration
SSC	Soluble solid concentrations
ST	Storage temperature
TA	Titratable acidit
TFC	Total flavonoid content
TIC	Total ion chromatogram
TOF	Time of flight
TPC	Total phenolic content
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet detector
WL	Weight loss
ANOVA	Analysis of variance

CHAPTER 1

INTRODUCTION

Botanically, ginger, known as *Zingiber officinale* (Zingiberaceae), is a perennial herb grown as an annual crop. The thick tuberous rhizome is pale yellow in colour and aromatic in scent is filled with pungent taste (Gupta and Sharma, 2014). Ginger consumption as a medicinal herb has been practiced since ancient times in treating various illnesses such as nausea, menstruation disorder, inflammation, cough and cold, food poisoning, epilepsy, and even cancer (Shukla and Singh, 2007). The ability of ginger to reduce the symptoms of those illnesses is related to the therapeutic properties, including antimicrobial, antioxidant due to the presence of gingerol and shogaol and its derivatives (Kumar et al., 2011).

Up to 2017, the total number of ginger production has reached 3,038,120 tons worldwide with India is the top country contributing to 35.2 % of production. Malaysia ranked the 13th position with total ginger production of 14, 279 tons in 2017, equivalent to only 0.5 % of the world population (FAOSTAT, 2019). In Malaysia, Bentong, Bara, and Tanjung Sepat varieties have been cultivated locally. Bentong is the most popular ginger as the pungency is stronger, low in fibre contents and bigger in size as compared to other varieties (Mohd et al., 2015). The production of Bentong ginger has reached 7,382.399 kg in 2016, which is equivalent to RM 64 million of profits (Harian Metro, 2017).

Bentong rhizomes offered the best quality as compared to imported ginger from Thailand, China and Indonesia. Therefore, Bentong rhizomes have been exported mainly to Singapore followed by South Africa and Indonesia. However, improper temperature management of the rhizomes will reduce the postharvest qualities and marketability (IndexBox, 2021). It is known that ginger is a tropical produce which is prone to chilling injury when stored at a temperature lower than 15 °C (Kasamo et al., 2000). Storing the rhizomes under this temperature may develop physiological breakdown as it alters the physical properties of cell membranes, thus reducing the membrane elasticity (Kasamo et al., 2000).

Exported Bentong ginger follows a series of postharvest standards of procedure. Washed and cleaned rhizomes are treated with anti-fungal solutions at 500 ppm to prevent fungal growth. After the curing process, rhizomes are packed in corrugated fireboard and stored at 10-15 °C at relative humidity of 85-90% to extend its shelf life up to 4-5 months before being transported by ship (Mohd Ismail and Abd Shukor, 1990).

In common practice during transportation, the imported rhizomes were stored under refrigeration at low temperature around $\pm$2 °C. They deposited to a local wholesale market and stored in a cold room under the same condition, causing the rhizomes to manifest its CI symptoms such as translucency and shrivelling when returned to ambient temperature. Eventually, reducing its physical and chemical qualities such as the colour, sweetness, acidity, phytochemical contents, antioxidant, and enzyme activities. These deteriorating effects may imply that ginger rhizomes are intolerant to chilling temperatures and the means to avoid this deterioration needs to be addressed.

There are various ways to control chilling injury depending on the effectiveness of the techniques in achieving the target. Alleviation of chilling injury symptoms can be controlled by increasing the tolerance of commodities or retarding the development of chilling injury symptoms in the tropical and subtropical that are susceptible to chilling injury. Reducing chilling injury symptoms involves modifying the storage environment such as low and high temperature conditioning and intermittent warming (Wang, 2010). Previous studies showed that hot water treatment heat-treated by hot water dip at 45 °C for 4 min, and then stored at 2 °C for 90 days, the CI symptoms were slightly, but significantly reduced after being heat-treated (Mirdehghan et al., 2007). All tubers were treated with hot water (45, 50 and 55 °C), subjected to 10 min and 20 min immersion time was able to maintain the other postharvest qualities such as weight loss, firmness, titratable acidity and total soluble solid (TSS) of the treated sweet potatoes. Other findings on tuber such as potato that dipped into 45 °C hot water for 10 min effectively reduced the weight loss and the disease index of wounded potato tubers (Wang et al., 2020). In this study, hot water treatment was accessed to examine its mitigating effect to alleviate chilling injury symptoms.

Although hot water treatment could control the effect of chilling injury in many fruits (Wang et al., 2014), its effect on ginger rhizomes has yet to be tested, especially on ginger Bentong rhizome. Recently, the evaluation of the commodity reactions to the hot treatment has been widely studied at transcriptome, proteome and metabolome level (Lurie and Pedreschi, 2014). In this study, hot water dip treatment was implemented to control chilling injury symptoms on ginger rhizomes as it is more efficient than hot air (Lurie, 1998). Although hot water treatment is commonly used as pest control and prevention of fungal rot, research on hot water treatment on CI of postharvest qualities has been successful (Gonzales-Aguilar et al., 2000; Wang et al., 2014). The work on ginger rhizome, however, has not been carried out yet.

Evaluation of physico-chemicals postharvest qualities include weight loss, softening (Ketsa et al., 1997), ripening, colour (Wang et al., 2014), phytochemical contents, and antioxidant properties has been carried out. Under such treatment, ginger rhizome needs to be established. To facilitate an understanding between the relationship and correlation between the heat treatment and changes or alterations in metabolites, a metabolic profiling that covers primary and secondary metabolites was executed by high-performance liquid chromatography/electrospray ionization-mass spectrometry (HPLC/EI-MS). With the advance in data processing and analysis by multivariate analysis (MVA) using principal component analysis (PCA), a clear comprehension on the mechanism of hot water treatment in alleviating chilling injury in ginger rhizomes could be achieved. These metabolomic studies have been successfully adopted in some studies (Tanaka et al., 2015). Even though hot water treatments could pro-long the shelf-life of fresh-cut products or fruits, the literature on the effect of hot water treatments in reducing chilling injury symptoms on rhizomes is still not conclusive and limited.

It is hypothesized that optimum storage temperature would have significant interaction with storage duration in reducing the chilling injury symptoms of rhizomes. Secondly, the hot water treatment could reduce the chilling injury symptoms when the rhizomes are stored under chilling temperature. Therefore, to test the hypotheses, this study is initiated with the objectives:

1. To characterize chilling injury of Bentong ginger rhizome during storage at low temperature by physical and chemical mechanisms.
2. To evaluate the effect of hot water treatment at different dipping durations and storage durations on physical characteristics and chemical mechanisms on Bentong ginger rhizome.
3. To characterize the metabolic profiling of hot water-treated rhizome based on high-performance liquid chromatography coupled with electrospray ionization-mass spectrometry (HPLC-EI-MS).

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