

## Postharvest quality evaluation on the hot water-dipped *Zingiber officinale* rhizomes stored at low-temperature storage

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### Article history:

Received: 1 November 2022

Received in revised form: 5 December 2022

Accepted: 9 April 2023

Available Online: 25 August 2023

### Keywords:

Chilling injury,

Hot-water dip,

6-gingerol,

6-shogaol

### DOI:

[https://doi.org/10.26656/fr.2017.7\(4\).517](https://doi.org/10.26656/fr.2017.7(4).517)

### Abstract

Storing ginger at chilling temperature will reduce its postharvest quality as they are prone to chilling injury (CI). Therefore, a study on hot-water dip treatment in alleviating CI in 'Bentong' ginger rhizome on physical and chemical properties was conducted. Fresh ginger rhizomes were harvested in the 9<sup>th</sup> month after planting. The fresh and cleaned rhizomes were hot-water treated at 45°C for 0, 5, 10 and 15 mins before being packed in a box and stored at 5°C for 0, 8 and 16 days. There was a significant interaction effect between dipping and storage durations on the physical and chemical properties of the rhizome. Exposed rhizome for 5, 10 and 15 mins was observed to maintain the h<sup>o</sup> and L\* of the rhizome after storage at 5°C. The decreases in h<sup>o</sup> from yellow to slightly brown also indicated rhizome browning. Rhizome held at 45°C for 5 mins managed to reduce browning as compared to other durations. In addition, dipping for 15 mins resulted in a significant increase in total phenolic content, total flavonoid content and 6-gingerols. While 6-shogaols was maintained at 45°C for 5 mins. Preconditioning by the hot-water treatment could induce chilling tolerance upon storage at chilling temperature.

## 1. Introduction

Malaysia ranked 13<sup>th</sup> position with total ginger production of 14, 279 tons in 2017, equivalent to only 0.5% of the world population (FAOSTAT, 2017). Bentong, Bara and Tanjung Sepat varieties in Malaysia have been cultivated locally. Ginger (*Zingiber officinale* Roscoe) is tropical produce that is chilling sensitive. Commercially, during transportation, the imported rhizomes were stored under refrigeration at low temperatures around  $\pm 2^{\circ}\text{C}$ . They are deposited in a local wholesale market and stored in a cold room under the same condition, causing the rhizomes to manifest chilling injury (CI) symptoms such as translucency and shrivelling when returned to ambient temperature (FAMA, 2017). Eventually, reducing its physical and chemical qualities such as colour, sweetness, acidity, phytochemical contents, antioxidant and enzyme activities (Wang, 2013). These deteriorating effects may imply that ginger rhizomes are intolerant to chilling temperatures and the means to avoid this deterioration needs to be addressed. Previous reports supported that these rhizomes are sensitive when stored at a temperature below 10°C (Wang, 2013; Shukor *et al.*, 2020). Stored

rhizomes at chilling temperature may alter their physiology and biochemical properties, leading to enzyme inactivation, membrane dysfunction, and an increase in permeability and alteration to cellular structure. These alterations will eventually be manifested as shrivelling and translucency in ginger rhizomes (Paull, 1990).

Chilling injury is related to a syndrome that involves several physiological events and is an indicator of cold-stored produces. The occurrence of browning is caused by the oxidation of phenolic compounds, which is catalysed by polyphenol oxidase enzyme, which seems to depend on low temperature (Tomás-Barberán *et al.*, 2001). For example, eggplant expresses CI as the browning of the pulp (Massolo *et al.*, 2011).

To alleviate the problem of CI symptoms, various methods have been applied to other produce such as low-temperature conditioning, intermittent warming, controlled atmospheres, growth regulators and natural products, application of calcium and other chemicals, packaging, waxing and coatings and even genetic modification. However, in this experiment, hot-water dip

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treatment was applied to ginger rhizomes in reducing CI. Hot treatment was developed for disease control, disinfestation of insects and disinfection of fruit flies (Lurie, 1998) and provided an alternative to the application of synthetic chemicals. Most fruits and vegetables could tolerate exposure to water temperatures of 45 – 60°C up to 10 mins. Higher temperatures and longer durations are commonly used to control either insects or microorganisms (Lurie and Pedreschi, 2014). Moreover, hot-water treatments is a non-damaging physical treatment that has been effectively applied to various other fresh produce such as bell pepper (Gonzales-Aguilar *et al.*, 2000), 'Valencia' oranges (Erkan *et al.*, 2005), Jerusalem artichoke (Wang *et al.*, 2014), date palm (Hazbavi *et al.*, 2015), tomatoes (Mama *et al.*, 2016) and banana (Chopsri *et al.*, 2018) in the prevention of postharvest storage disorders and extending the shelf life of the produces (Lurie and Pedreschi, 2014).

Nevertheless, hot-water treatment has never been experimented on rhizomes in treating CI, especially ginger. It could be hypothesized that there is a significant effect on postharvest qualities as affected by hot-water dip treatment. Herein, the present work was initiated to assess the effectiveness of hot water dip and storage durations on postharvest qualities for the first time on ginger rhizomes. A moderate temperature at 45°C was chosen as it is applied in a quarantine treatment before the produce is shipped. Harvested ginger rhizomes were hot water dipped at 45°C for 0, 5, 10 and 15 mins and stored at 5°C for 0, 8 and 16 days. The treated rhizomes were characterized for physicochemical attributes, including weight loss, firmness, colour, phytochemical contents, pungency level, and their potential in scavenging DPPH free radicals.

## 2. Materials and methods

### 2.1 Plant material and sample preparation

Nine months old rhizomes were collected from Bentong, Pahang. The rhizomes were cleaned from debris, washed, and air-dried before being subjected to hot-water treatment. About 200-300 g of rhizomes were dipped in a temperature-controlled water bath (Stuart SWB24D, 300 × 500 × 200 mm) at 45°C for 0, 5, 10 and 15 mins. At 0 min, the rhizomes were not hot-water dipped. The bath temperature was constantly maintained during each treatment within ±0.5°C of the required temperature by using an electronic thermostat. Following hot-water treatment, the rhizomes were air-dried and packed in a box (24 × 15 × 30 cm) lined with polyethylene plastic with a standard thickness of 0.25 mm before being transferred to 5°C for 0, 8 and 16 days. Storage durations were shortened to 16 days due to the

previous experiment; all postharvest qualities showed a decline after day 16, indicating that the shelf life of the rhizomes had reached an end.

### 2.2 Weight loss

The initial weight of the rhizome of about 100 g per replicate was weighed. Then, the rhizome was weighed again at 8, 16 and 24 days to get the final weight. Weight loss was calculated according to the formula and expressed as a weight loss percentage.

$$\% \text{ Weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

### 2.3 Firmness

Each rhizome was sliced into 1 cm thick. The firmness of the rhizome was analysed by a texture analyzer (model 5543; Instron Inc., Canton, Mass.) fitted with a 9-cm-diameter, round, flat-faced anvil. The amount of force (N) required to compress the radial pericarp surface 3 mm at a constant speed of 5 cm·min<sup>-1</sup> was recorded.

### 2.4 Colour ( $h^\circ$ , $L^*$ and $C^*$ )

The colour of the peeled rhizome surface was measured quantitatively using a chromameter (model CR-300; Minolta, Ramsey, New Jersey, USA) to obtain three parameters which were the value of lightness ( $L^*$ ), chroma ( $C^*$ ), and hue ( $h^\circ$ ). The reading was taken in three replicates.

### 2.5 Soluble solids concentrations

The soluble solids concentrations (SSC) of ginger rhizome juice was determined by a digital refractometer (ATAGO RX-5000, ATAGO, Japan) calibrated with distilled water. The results were expressed as percentage.

### 2.6 Browning index

A volume of 10 mL of rhizome juice was added to 290 mL of distilled water. The solution absorbance was measured immediately using a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific 1510, Waltham, MA, USA) at 420 nm to determine the browning index (Rocha and Morais, 2002).

### 2.7 Total phenolic contents and total flavonoid contents

The total phenolic contents (TPC) was determined using Folin–Ciocalteu reagents (Policegoudra *et al.*, 2007) with slight modification. An aliquot (0.1 mL) of extracts was added to 2 mL of 2% sodium carbonate and incubated for three mins. Then, 0.1 mL of 50% Folin–Ciocalteu reagent was added and incubated for 30 mins at room temperature. Absorbance was measured at 750 nm using a Multiskan GO microplate spectrophotometer

(Thermo Fisher Scientific 1510, Waltham, MA USA). Amounts of TPC will be calculated using a gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE)  $\text{g kg}^{-1}$  of fresh weight (FW).

The total flavanoid content (TFC) was measured by the aluminium chloride colorimetric assay (Singh *et al.*, 2012). An aliquot (1 mL) of extracts or standard solutions of rutin (0.05, 0.1, 0.15, 0.2 and 0.25  $\text{g L}^{-1}$ ) was added to 10 mL volumetric flask containing 4 mL of distilled water. First, 0.3 mL of 5% sodium nitrate was added to 0.2 mL of the ginger juice. Then, 0.3 mL 10% aluminium chloride was added after 5 min. A volume of 2 mL of 1 M sodium hydroxide were added after 6 mins, and the samples were measured against the blank at 510 nm using a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific 1510, Waltham, MA, USA). The total flavonoid content was expressed as rutin equivalents (RE)  $\text{g kg}^{-1}$  of fresh weight (FW).

### 2.8 1,1- diphenylpicrylhydrazyl free radical scavenging activity

The antioxidant activity of the ginger extracts was determined (Maizura *et al.*, 2011) using DPPH radical base on the electron transfer reaction between the DPPH reagent and the ginger extracts. Firstly, 0.4 mL of ethanol was added to 100  $\mu\text{L}$  of ginger extract. Then, 1 mL of 0.1 mM DPPH was added to the samples and incubated at 37°C for an hour. The absorbance value was measured by a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific 1510, Waltham, MA, USA) at 517 nm. The percent of inhibition of DPPH was calculated by  $((\text{abs control} - \text{abs sample}) / \text{abs control}) \times 100$ .

### 2.9 Experimental design and statistical analysis

The experiment was laid out as a randomized completely block design (RCBD), analysed as a two-way factorial comparison of dipping duration (0, 5, 15 and 15 mins) versus storage durations (0, 8 and 16 days) with four replications. Data were subjected to analysis of variance (ANOVA) using the procedure of the general linear model (PROC GLM). The main and interaction effects between the two factors were included in the model. Orthogonal regression partitions were carried out into linear or quadratic degree of polynomial. Multiple regression analysis (PROC REG) with two variables was used to determine the estimation of regression coefficients for best-fit regression.

## 3. Results and discussion

### 3.1 Postharvest analysis on ginger rhizomes as affected by hot-water dip treatment

#### 3.1.1 Weight loss, firmness and SSC

The influence of heat treatment was evaluated on the weight loss of Bentong ginger rhizomes. There was a significant interaction between dipping durations (0, 5, 10 and 15 mins) and storage durations (0, 8, 16 and 24 days) on the rhizome's WL (Figure 1a), firmness (Figure

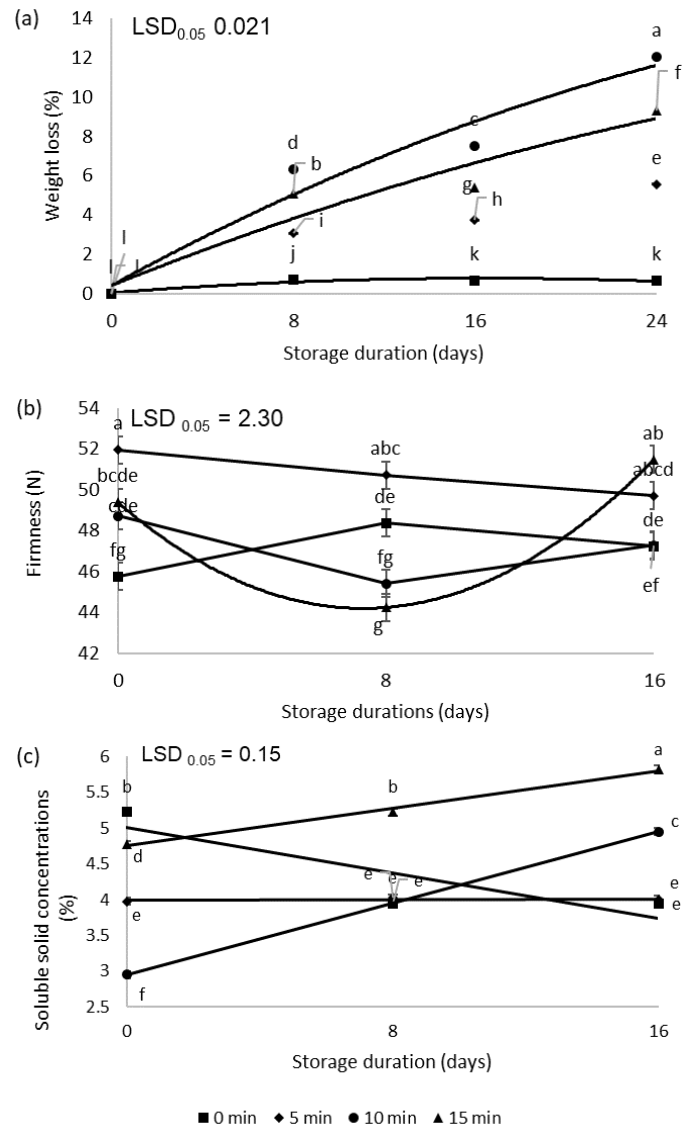


Figure 1. The relationship between (a) weight loss (b) firmness and (c) soluble solids concentrations and storage duration (0, 8, 16 and 24 days) at different dipping duration (0, 5, 10 and 15 mins) of Bentong ginger. Means with the different letters are statistically significantly different at  $P = 0.05$ . Vertical bars indicate the standard error ( $n = 4$ ).

(a) Weight loss:  $Y(0) = 0.039 + 0.089x - 0.0027x^2$ ;  $R^2 = 0.89$ ;  $Y(5) = 0.17 + 0.33x - 0.0049x^2$ ;  $R^2 = 0.96$ ;  $Y(10) = 0.425 + 0.63x - 0.0068x^2$ ;  $R^2 = 0.94$ ;  $Y(15) = 0.91 + 0.99x - 0.025x^2 + 0.0059x^3$ ;  $R^2 = 0.56$ .

(b) Firmness:  $Y(15) = 49.36 - 1.41x + 0.096x^2$ ;  $R = 0.79$ .

(c) Soluble solids concentration:  $Y(0) = 5.01 - 0.07x$ ;  $R^2 = 0.71$ ;  $Y(10) = 2.95 - 0.13x$ ;  $R = 0.99$ ;  $Y(15) = 4.75 + 0.06x$ ;  $R = 0.92$ .

1b) and SSC (Figure 1c). The storage durations were partitioned into significant linear and quadratic responses. The WL resulted in the lowest changes among the rest of the dipping durations for unheated rhizomes. However, the decrease was not significant. Furthermore, rhizomes dipped for 15 mins at 45°C have lower WL than at 10 min by 9%.

Weight loss is one of the symptoms of deterioration, degrading the quality and losing the quality (Ullah, 2009). This result indicated that hot-water treatment was ineffective in reducing WL in rhizome compared to control, as the treatment has increased the WL significantly and led to wilting and shrivelling of the rhizome (Wills *et al.*, 2007). A previous study on the hot-water dip of Big Top nectarine showed that dipping at 45°C for 2 min reduced WL and CI incidence compared to control. However, as the temperature was increased to 50 and 55°C, the percentage of WL increased significantly compared to dipping at 45°C for 2 mins (Çandir *et al.*, 2009). Meanwhile, Angasu *et al.* (2014) revealed that hot-water treatment on mango fruit resulted in the least WL and the highest WL was recorded in fruits that were not treated by hot water. In this case, the hot-water treatment effectively keeps a higher percentage of marketability of mangoes.

The acceleration of WL increased proportionally during storage with the dipping duration when the dipping duration was increased. The longer it takes for the rhizome to be immersed in the water, the higher the evaporation rate from the rhizome as more heat in the rhizome was retained (Khalil *et al.*, 2012). In a recent study of the hot-water treatment of tomatoes, Mama *et al.* (2016) postulated that as the dipping duration increases, transpiration and respiration could also be the mechanism involved in water loss.

The firmness of Bentong rhizomes was affected by hot-water dip treatment. The flesh firmness of the unheated rhizome responded in a positive quadratic when dipped for 0, 5 and 15 mins. Initially, the firmness for untreated rhizomes was significantly the lowest compared to hot dipped rhizomes for 5, 10 and 15 mins. From this experiment, the insignificant reduction in firmness showed that exposure to rhizome at 45°C for 5 mins could delay the rhizome's softness during storage compared to dipped rhizomes at 45°C for 10 and 45°C for 15 mins. It was clearly observed that the hot-water dip could maintain firmness compared to non-heated rhizomes. Similar findings on hot-water treated San Jose apples reported that exposure at 46°C caused apples to be firmer than control apples (Lurie *et al.*, 1998), nectarines and peaches (Malakou and Nanos, 2005).

Fruit softening is related to the modification of cell wall polysaccharides which undergo enzyme hydrolysis involving polygalacturonase, pectinesterase,  $\beta$ -1,4-glucanase and  $\beta$ -galactosidase. It was found that heat treatment reduced endo-1,4- $\beta$ -D-glucanase,  $\beta$ -xylosidase activity and delayed hemicellulose degradation which is involved in cellular structural integrity (Duan *et al.*, 2016). Polygalacturonase and  $\beta$ -galactosidase also were inhibited by this treatment. The inactivation of these enzymes leads to the delay of firmness (Malakou and Nanos, 2005; Vicente *et al.*, 2005).

For SSC, initially, hot-water treated rhizomes at 45°C for 10 mins showed the lowest SSC followed by dipping duration for 5, 15 mins and non-heated rhizomes by 34%, 66% and 71%, respectively. SSC decrease in undipped rhizome was observed when a produce has reached senescence as storage duration increased. The linear decline of SSC in the undipped rhizome is explained by the decrease of starch degradation in the formation of hexose sugar as the sugar is used as a precursor in a respiration process (Wills *et al.*, 2007). Hence, the level of sweetness is decreased. Based on this analysis, when the rhizome was held at 45°C for 5 mins, it did not alter the SSC percentage throughout storage as compared to the control due to the rate of starch conversion that did not change significantly. Therefore, extends its shelf life. Previous studies also reported similar findings which reported that there was no difference in the SSC between heated and unheated at various temperatures (Çandir *et al.*, 2009; Khalil *et al.*, 2012).

The increase of SSC in hot water at 45°C for 10 and 15 mins suggested that the degradation of starch to sugar increased linearly within 16 days of storage. Nevertheless, when the rhizome is exposed for 10 mins, the increase of SSC was markedly more rapid than 15 mins of dipping, which showed a proportionate increment for 16 days of storage. The ascending of SSC contributed to the relatively sweeter taste of rhizome. These results may be linked to the increasing rate of starch conversion to hexose sugar as more heat was accumulated during a long exposure in the water bath. Therefore, the rhizome is suitable to be produced as candy for its sweeter taste. However, the increase of SSC during 16 days of storage might be declined after prolonged storage due to the deceleration of starch degradation that may limit respiration (Kays and Paull, 2004). This agrees with Verlinden *et al.* (2014), who reported a strong correlation between respiration rate and sugar contents. Eventually, the depletion of carbohydrates decreases the tissue's ability to produce sufficient energy for some metabolic processes. The

inability to maintain metabolic homeostasis is suggested to promote senescence (Verlinden *et al.*, 2014).

### 3.1.2 Colour properties $h^\circ$ , $L^*$ and $C^*$

The colour of hot-water dip has affected dipping durations and storage durations on the rhizome  $h^\circ$ ,  $L^*$  and  $C^*$  of ginger rhizomes as proven by a significant interaction between dipping durations and storage durations (Figure 2 (a-c)). Based on the observations, dipped rhizomes at 45°C for 5, 10 and 15 mins could maintain the  $h^\circ$  of rhizomes for 16 days of storage durations. At day 0, the range of  $h^\circ$  was between 94° to 97° indicating the colour of the rhizomes was in the yellow region of the colour chart. However, a major reduction on  $h^\circ$  was observed on undipped rhizomes during 16 days of storage at 5°C. Similar findings were also reported on Valencia oranges (Erkan *et al.*, 2005) and persimmons (Khademi *et al.*, 2013) which support the results. However, a contrast study on date palms by Hazvabi *et al.* (2015) showed that hot treatment at 50, 60 and 70°C reduced the date palm's hue significantly compared to non-heated date, which showed higher retention of the  $h^\circ$  than that heated produce.

Chroma is related to the dullness or vividness of the colour, which is valued from 0 - 60°. The vividness of a produce will increase as the  $C^*$  increases. From this experiment, hot-water treatment reduced  $C^*$  regardless of the dipping durations. Initially, the degree of  $C^*$  was 38° which was the highest when exposing rhizomes for 15 mins at 45°C of water bath followed by control and 5 mins. Whereas dipping the rhizomes for 10 mins recorded the lowest  $C^*$ . These observations indicated that extending dipping durations for 15 mins could improve the vividness of the yellow colour of the rhizomes immediately after treatment as indicated by the highest  $C^*$  value compared to dipping for 5, 10 mins and non-dipped rhizomes. Nevertheless, the  $C^*$  reduced sharply after 8 days of storage, indicating that the vividness has reduced to become duller. Even though immersing the rhizomes for 10 mins at 45°C of water bath resulted in the lowest  $C^*$  which described the hue as dull yellow, however, the dullness was maintained throughout 16 days under 5°C of storage. Previous studies on tomatoes showed that heat treatment at 40°C for 20 mins increased the values of  $C^*$  more than treated tomatoes at 50°C and control that indicate the improvement in vividness throughout 16 days of storage at 20°C (Tadesse *et al.*, 2016).

The  $L^*$  values indicate the luminescence of the colour. The higher the angle of  $L^*$ , the brighter the hue will be (Schreiner *et al.*, 2003). At day 0, the recorded initial of  $L^*$  was 67°, 69° to 76° for dipped rhizomes in 45°C of water bath for 5, 10 and 15 mins, respectively,

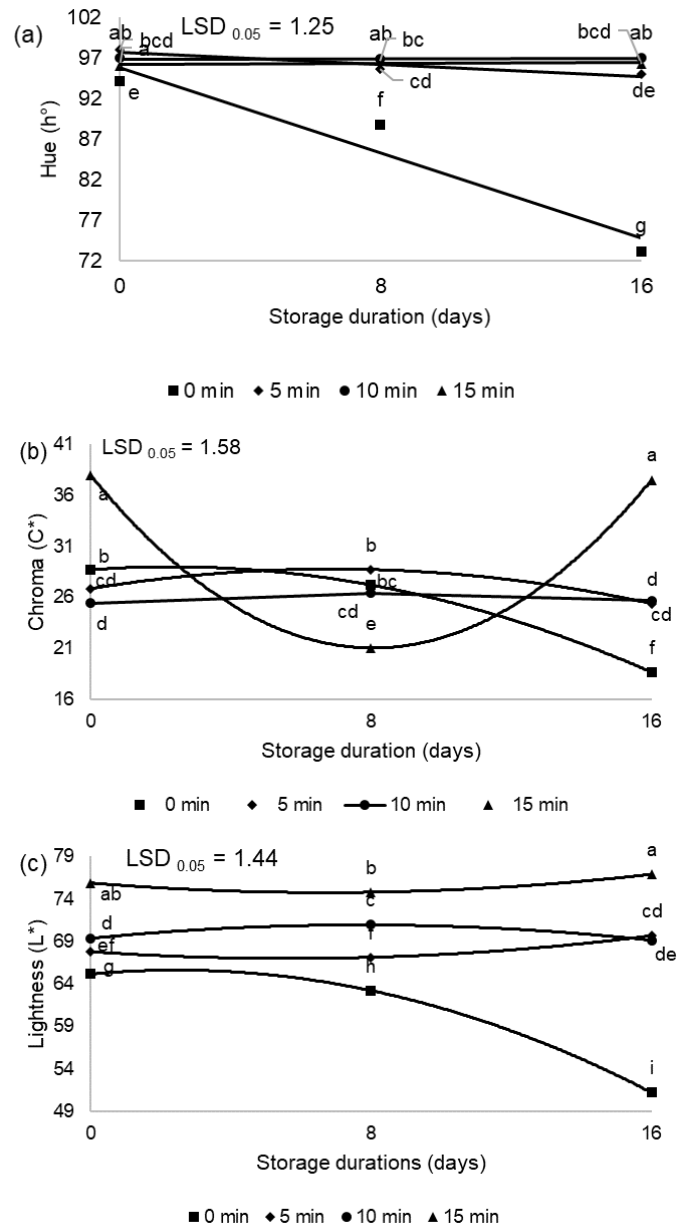


Figure 2. The relationship between (a) hue ( $h^\circ$ ), (b) chroma ( $C^*$ ) and (c) lightness ( $L^*$ ) of ginger rhizome and storage duration (0, 8, and 16 days) at different dipping duration (0, 5, 10 and 15 mins) of Bentong ginger. Means with the different letters are statistically significantly different at  $P = 0.05$ . Vertical bars indicate the standard error ( $n = 4$ ).

$h^\circ$ :  $Y(0) = 95.85 - 1.31x$ ;  $R^2 = 0.91$ ;  $Y(5) = 97.66 - 0.18x$ ;  $R^2 = 0.73$ .

$C^*$ :  $Y(0) = 28.7 + 0.25x - 0.055x^2$ ;  $R^2 = 0.93$ ;  $Y(5) = 26.84 + 0.56x - 0.04x^2$ ;  $R^2 = 0.74$  and  $Y(15) = 8.01 - 4.21x + 0.26x^2$ ;  $R^2 = 0.97$ .

$L^*$ :  $Y(0) = 65.14 + 0.38x - 0.078x^2$ ;  $R^2 = 0.97$ ;  $Y(5) = 67.78 - 0.28x + 0.025x^2$ ;  $R^2 = 0.53$ ;  $Y(10) = 69.35 + 0.41x - 0.026x^2$ ;  $R^2 = 0.54$ ;  $Y(15) = 75.87 - 0.34x + 0.024x^2$ ;  $R^2 = 0.62$ .

while the  $L^*$  for undipped rhizomes was 65° which was significantly the lowest as compared to treated rhizomes. This observation described that the yellow  $h^\circ$  of undipped rhizomes became darker than those dipped in hot-water bath. An extension of storage durations for 16 days, has reduced the  $L^*$  significantly by 21%. As

supported by Çandır *et al.* (2009), a lower L\* value signified the rhizome colour changes to a darker tone related to browning (Burdurlu *et al.*, 2003). This finding was reinforced by reducing h° in unheated rhizome as depicted in Figure 2a.

An extension of storage durations to 16 days under 5°C has worsened the colour of rhizomes which turned them from dark dull yellow into dark and dull brownish yellow. This observation suggested that non-dipped rhizomes could not maintain the colour of the rhizomes during storage. Meanwhile, the colour of hot-water dipped rhizomes at 45°C of water bath for 15 mins could be deduced as bright and vivid yellow. Among other dipping durations, hot water dip at 15 mins could maintain acceptable colour by achieving the highest brightness and vividness compared to dipping for 5 and 10 mins.

Colour could be an indicator of CI due to the changes of hue, and chroma of the ginger rhizomes. This could be related to higher temperature induced by heat treatment resulting in the accumulation of heat shock protein which inhibits browning reaction due to the inactivation of browning enzymes such as phenyl alanine ammonia-lyase (PAL) or may be due to general diversion of protein synthesis (Saltveit, 1998). PAL is said to be the main enzyme related to browning as it was found to decrease due to heat shock protein. Moreover, Fukumoto *et al.* (2002) found that heat shock also affects POD and PPO by inactivation or inhibition of their synthesis during storage.

### 3.1.3 Browning index

An interaction between storage duration and dipping duration on the browning index (BI) of the rhizome was displayed. However, regression partitioned resulted in neither an insignificant linear nor quadratic relationship (Figure 3). For unheated rhizomes, initial absorbance was significantly higher than heated rhizomes for 5 and 15 mins, which indicated a higher browning index. The BI increased sharply, which explained that the browning was intensified after extended after being stored at 5°C for 8 days. The BI for rhizome that was held at 45°C for 15 mins showed a similar trend of changes but lower acceleration. Fifteen mins of dipping showed that the initial BI was lower than other treatments and unheated rhizomes, which indicated that the browning of the rhizomes was improved immediately after dipping but continued to increase in BI when stored longer at 5°C. However, immersing the rhizome at 45°C for 5 mins showed a gradual decrease of BI by 56%, lower than 10 mins of dipping, which showed a drastic decrease of 82% throughout 16 days of storage. This indicated that this treatment could alleviate the increase of BI as

compared to the rhizome that was held at 45°C for 15 mins and unheated rhizome.

The increase of BI in fruits and vegetables indicates an enzymatic browning reaction. This reaction involves polyphenol oxidase (PPO) and peroxidase (POD) contained in the cytoplasm (Ioannou and Ghoul, 2013). This enzyme is released during cutting, shock, loss of firmness and food oxidation, which induce losses or changes of flavour, odour and nutritional value (Toivonen and Brummell, 2008). The mechanism could explain a high correlation between enzymatic browning and PPO activity in previous studies on apples and potatoes (Rocha and Morais 2002). A study on the chilling injury of bananas revealed that when hot-water treatment was applied at 38°C for 3 days before storage at 8°C, the chilling injury was alleviated and enhanced PAL activity, protein amount and MaPAL1 and MaPAL2 transcript levels. The increases in parameters of PAL upon heat pre-treatment were all inhibited by 2-aminoindan-2-phosphonic acid (AIP) treatment, worsening the CI. Thus, it could be concluded that the induction of PAL by heat pre-treatment was regulated at both the transcriptional and the translational levels and that PAL may play a role in heat pre-treatment-induced chilling tolerance of banana fruit (Chen *et al.*, 2008). Loaiza-Velarde and Saltveit (2001) and Viñã and Chaves (2008) also conclude that heat treatments could prevent the wound-induced synthesis of phenols by inactivating PAL activity and, thus, reducing browning development in fresh-cut vegetables such as celery and lettuce. Nevertheless, previous findings stated that browning evaluation showed that the discolouration was mainly affected by storage (by 99%) and less by hot-water treatment and pre-processing duration (Tsouvaltzis *et al.*, 2011). Generally, browning was promoted by a high content of phenolics and sugar (Jung and Watkins, 2011).

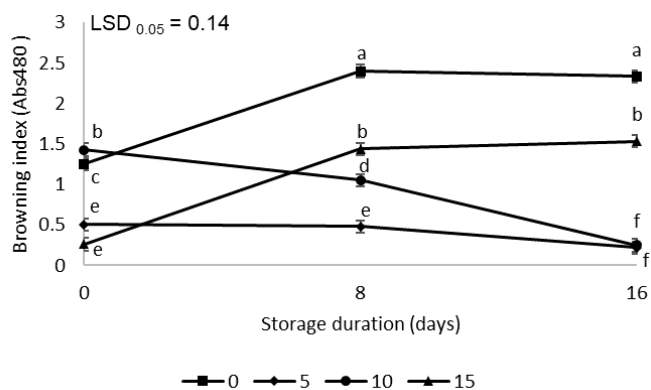


Figure 3. The relationship between browning index (abs 480 nm) of ginger rhizomes and storage duration (0, 8 and 16 days) at different dipping duration (0, 5, 10 and 15 mins) of Bentong ginger. Different notations above the data points are statistically significantly different at P = 0.05. Vertical bars indicate the standard error (n = 4).



### 3.1.4 Total phenolic contents and total flavonoid contents

In this experiment, the hot-water treatment showed a significant interaction between storage duration and dipping duration at  $P = 0.05$  on TPC and TFC (Figure 4 (a and b)). The TPC of unheated rhizomes declined gradually by 31% after 8 days and levelled off by 16 days of storage. In accordance with a study of hot water treatment on dates, Mustafa and Ghalem (2007) also reported that TPC in unheated dates decreased drastically compared to hot-water-treated dates.

The TPC for treated rhizome at 45°C for 5 mins ranging from  $5.6 \times 10^{-5}$  to  $1.1 \times 10^{-4}$  g GA kg<sup>-1</sup> FW decreased after 16 days of storage by 49%. In contrast, previous studies on pomegranates revealed that hot water dipping at 45°C for 4 mins induced higher levels of total phenolic compounds, ascorbic acid and total anthocyanins, as well as antioxidant activity during storage (Mirdehghan *et al.*, 2007). However, exposure to 10 mins of immersion resulted in a slight reduction of 11% in TPC from the initial to the 8<sup>th</sup> day of storage and gave rise to day-16 by 42%. A quadratic and sharp increase of TPC by 70% for rhizome held at 45°C for 15 mins was markedly observed throughout 16 days of storage. A previous study on hot water treatment in Navel and Valencia orange fruit at 41°C for 20 mins was found to be more effective than at 50°C for 5 mins. This treatment increased the free phenolic content during storage which was related to alleviating chilling injury (Bassal and El-Hamahmy, 2011).

Generally, hot water treatment has many roles in the improvement of wounds and micro cleaves on the skin

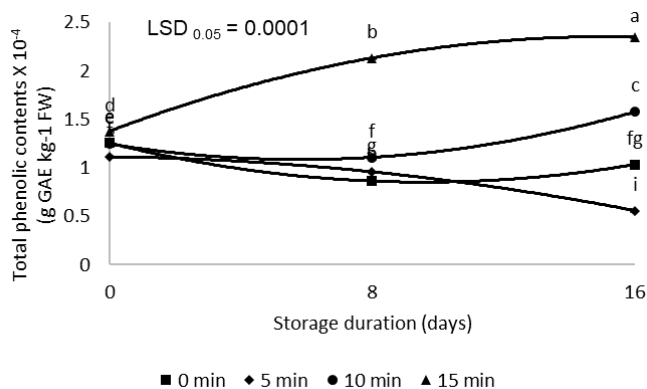


Figure 4 (a). The relationship between total phenolic contents (g GAE kg<sup>-1</sup> FW) of ginger rhizomes and storage duration (0, 8, and 16 days) at different dipping duration (0, 5, 10 and 15 mins) of Bentong ginger.  $Y(0) = 0.00126 - 8.4 \times 10^{-5}x + 4.4 \times 10^{-6}x^2$ ;  $R^2 = 0.86$ ,  $Y(5) = 0.001 - 4.2 \times 10^{-6}x - 1.9 \times 10^{-6}x^2$ ;  $R^2 = 0.97$ ,  $Y(10) = 0.0013 - 5.5 \times 10^{-5}x + 4.7 \times 10^{-6}x^2$ ;  $R^2 = 0.96$ ,  $Y(15) = 0.0014 + 0.00013x - 4.2 \times 10^{-6}x^2$ ;  $R^2 = 0.97$ . Different notations above the data points are statistically significantly different at  $P = 0.05$ . Vertical bars indicate the standard error ( $n = 4$ ).

surface of the fruit and the production of lignin and free phenols compounds in tissues. Heat treatment seems to act by a stabilisation of the phenolics content (Mustafa and Ghalem, 2007). The biosynthesis of phenolic compounds is induced by phenylpropanoid metabolism. PAL is the enzyme that contributes towards the pathway of polyphenol synthesis. In a previous study on sugarcane, heat treatment retarded the accumulation of total phenolics and PAL activity was significantly suppressed in the heat-treated fresh-cut sugarcane (Luo *et al.*, 2014). However, there is always a reduction in the phenolic compounds during storage due to the oxidation of phenolic acids to quinone which leads to browning.

It is known that cell wall dietary fibre will thermally decompose during heating (Xu and Chang, 2008). Therefore, the accumulation of phenolic contents during hot water treatment at 45°C for 15 mins could be due to some bound phenolics that may release from dietary fibres to increase the contents of free phenolics in the OD sample. Bound phenolics are bound to cell wall materials, especially to the cell wall dietary fibre. According to Xu and Chang (2008), the thermal treatment applied to foods of plant origin by heating or roasting causes evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promotes protein denaturation, which might result in greater availability of plant phenolic compounds in the matrix.

Generally, the type of flavonoids contained in most fruits and vegetables are with C-glycoside bonds and exist as dimers and oligomers. However, industrial processing such as heating or boiling results in the

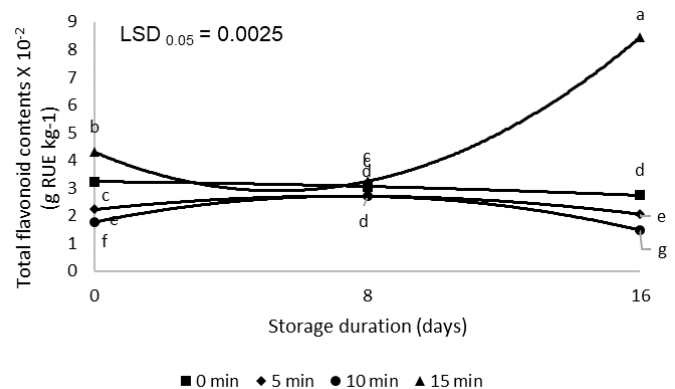


Figure 4 (b). The relationship between total flavonoid contents (g RUE kg<sup>-1</sup> FW) of ginger rhizomes and storage duration (0, 8, and 16 days) at different dipping duration (0, 5, 10 and 15 mins) of Bentong ginger.  $Y(0) = 0.032 - 0.00013x + 1.2 \times 10^{-5}x^2$ ;  $R^2 = 0.92$ ,  $Y(5) = 0.022 - 0.0013x - 8.9 \times 10^{-5}x^2$ ;  $R^2 = 0.54$ ,  $Y(10) = 0.017 - 0.0025x - 0.00017x^2$ ;  $R^2 = 0.89$ ,  $Y(15) = 0.043 - 0.0052x + 0.00049x^2$ ;  $R^2 = 0.99$ . Different notations above the data points are statistically significantly different at  $P = 0.05$ . Vertical bars indicate the standard error ( $n = 4$ ).

formation of monomers by the hydrolysis of C–glycosides bonds (Manach *et al.*, 2004). Therefore, reducing the TFC over-storage due thermal instability of particular flavonoids (Sharma *et al.*, 2015). The TFC ranged from 0.0025 to 0.0084 g RUE kg<sup>-1</sup> FW after being subjected to hot water treatment at 45°C. The increase of TFC when dipped for 15 mins agreed with Shen *et al.* (2013) who reported that hot water treatment at a temperature of 50°C for 3 mins resulted in a significant increase in TFC of Satsuma mandarin during 15 days of storage. Ioku *et al.* (2001) also found that TFC increased after heating at a certain temperature and magnitude of time.

A hypothesis that may explain the increase in the level of some phenolic compounds in plant food after heat treatment is that the heat alters the structure of some molecules, including proteins that are associated with the phenolic compounds resulting in increased levels of free phenolic compounds. This hypothesis could explain the different effects of the roasting process on the content of individual phenolic compounds and the antioxidant activity found in the present study (Hii *et al.*, 2009).

### 3.1.5 DPPH scavenging activities

A significant interaction between storage duration and dipping duration on the inhibition of DPPH free radical ( $P = 0.05$ ) was observed as affected by hot water dip treatment on ginger rhizomes. Further regression analysis showed that there was a significant quadratic response of SD in the DD × SD interaction (Figure 5). The percentage of DPPH inhibition by unheated rhizomes showed a higher initial percentage of DPPH inhibition at day-0 as compared to hot water-dipped rhizomes. In this study, an exposure of 5 min of dipping duration of rhizomes has maintained the inhibition of DPPH radical throughout storage durations. As the dipping duration increased, to 10 and 15 mins, the DPPH radical scavenging activities were lower as compared to 5 min of dipping.

In a previous study of heat treatment on sweet potatoes, the DPPH radical scavenging activities also showed a reduction when thermally treated (Tang *et al.*, 2015). The decreased antioxidant activity may be due to the loss of antioxidants or the formation of compounds having pro-oxidant action. Structure modification of the existing antioxidants and the formation of novel antioxidant components may enhance the initial antioxidant status (Jimenez-Monreal *et al.*, 2009). Furthermore, the antioxidant activities are because of the combination of different compounds, acting either synergistically or antagonistically. Oxidation system, degree of glycosylation, partition coefficient and concentration are the factors that influence the

antioxidant activity (Hassimotto *et al.*, 2005). Manzocco *et al.* (2001) reported that the heating process could enhance antioxidant activity in fruits and vegetables because of the enhancement of the antioxidant properties of naturally occurring compounds or the formation of novel compounds such as Maillard reaction products that have antioxidant activity.

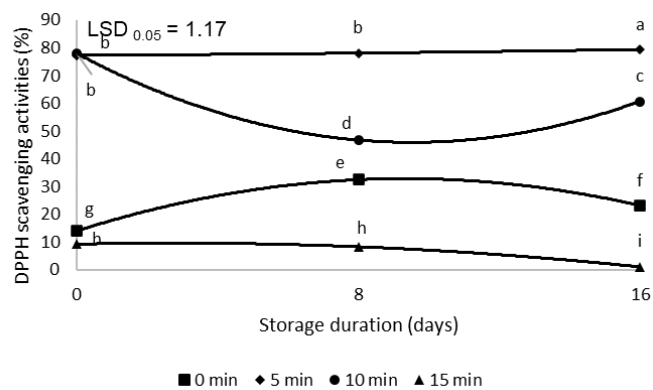


Figure 5. The relationship between DPPH radical scavenging activities (%) of ginger rhizomes and storage duration (0, 8, and 16 days) at different dipping duration (0, 5, 10 and 15 min) of Bentong ginger.  $Y(0) = 14.01 + 4.04x - 0.21x^2$ ;  $R^2 = 0.99$ ,  $Y(5) = 77.3 + 0.07x + 0.0038x^2$ ;  $R^2 = 0.80$ ,  $Y(10) = 77.9 - 6.7x + 0.35x^2$ ;  $R^2 = 0.98$ , and  $Y(15) = 9.2 + 0.27x + 0.049x^2$ ;  $R^2 = 0.98$ . Different notations above the data points are statistically significantly different at  $P = 0.05$ . Vertical bars indicate the standard error ( $n = 4$ ).

## 4. Conclusion

The treatment of hot water dip of rhizomes at 45°C held for 5, 10 and 15 mins is a promising method in controlling chilling injury during storage at a chilling temperature under 10°C. Based on the findings, hot water dip treatments could not control the weight loss of ginger. Untreated rhizomes were observed to maintain weight loss better than treated rhizomes at different dipping durations. Dipped rhizomes at 45°C for 5 mins could retain the firmness and browning index of rhizomes after storage at 5°C. The TPC and TFC could be retained when rhizomes were held at 45°C for 15 mins by showing an increase of total contents as storage duration prolonged up to 16 days. Meanwhile, 6-gingerols contents were the highest in the ginger when treated at 45°C for 15 min at initial. Whereas, 6-shogaols contents were maintained for 8 d when exposed for 5 min at 45°C. For its antioxidant scavenging assay, the strongest inhibition of DPPH free radical was at 5 min of dipping duration in 45°C water bath. Previously, hot water dip treatment has been widely applied in disease management. However, this treatment also has been widely subjected to increasing postharvest qualities of chilling-induced fruits. Herein we report for the first time the effect of hot water dip treatment in rhizomes.



## Acknowledgements

This work was supported by the IPS grant of the University of Putra Malaysia (Grant No. 9483200).

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