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Effect of blanching treatments on antioxidant activity of frozen green Capsicum (*Capsicum annuum* L. var bell pepper)

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Abstract

Antioxidant activities were evaluated in steaming, hot water, and microwave blanching's at different temperature, time and microwave power level on frozen green capsicum. Results showed frozen green capsicum blanched using microwave at high level/90 seconds (sample J) contain higher level of Ferric Reducing Antioxidant Potential (FRAP) compared to fresh green capsicum. Sample J and fresh green capsicum were significantly higher ($p \leq 0.05$) compared to other treatments for Total Phenolic Content (TPC), Radical Scavenging Activity (DPPH), and FRAP from 0 to 3rd month frozen storage. Overall, the sequences from highest to lowest in blanching treatments for TPC, DPPH, and FRAP were J (microwave high level/90 seconds)>A (Fresh)>H (Microwave Medium Level/120 seconds)>D (Hot Water 80°C/150 seconds)>K (Microwave High Level/120 seconds)>I (Microwave Medium Level/150 seconds)>F (Microwave Low Level/150 seconds)>B (Steam 100°C/150 seconds)>E (Boiling Water 100°C/120 seconds)>G (Microwave Low Level/180 seconds)>C (Steam 100°C/180 seconds). Frozen storage for 0 and 3rd months showed no significant difference ($p > 0.05$) which indicated no changes on antioxidant activity during frozen storage at -18°C.

Introduction

Freezing is one of the food preservation processes as the shelf life of fruits and vegetables are longer compared to fresh which it can be kept more than 6 months (Man and Jones, 2000). Freezing technology can give various benefits such as it can overcome glut fruits and vegetables problem. Fruits and vegetables that usually were thrown away because of short shelf life can be kept longer with freezing process. Consumers nowadays with a hectic and busy lifestyles demands healthier products that are fast to prepare with high nutritional content such as sliced and diced frozen raw peppers that can be eaten directly after washing or thawing. Consumer's willingness to eat raw, and minimally processed vegetable products, as part of healthier food habit created a high demand of these food products in frozen food market.

Sweet bell pepper is a Solanaceous fruit belonging to the *Capsicum annuum* L. species, and native from America. Bell pepper has a wide variety of colours (ranging from green, yellow, orange, red, and purple), shapes, and sizes differentiates by different maturity states (Lucier and Lin, 2001). Capsicum is known to have antioxidant properties (Marin *et al.*, 2004). The bell pepper (*Capsicum annum*. L) is well known for its high content in bioactive compounds and strong antioxidant capacity (Blanco *et al.*, 2013). In fruits and vegetables, phytochemicals can be bound in the plant cell membranes or exist as free compounds. Food processing, such as heating or freezing can disrupt the cell membrane leading to the release of membrane-bound phytochemicals, which implies higher bioaccessibility (Lemmens *et al.*, 2009). The amount of phytochemicals retained in fruits and vegetables depends on their stability during food preparation and processing before consumption, and it is related with oxidation, and the environmental conditions. Large quantities of neutral phenolic compounds or flavonoids called quercetin, luteolin, and capsaicinoids were found in peppers (Hasler, 1998). These bioactive compounds provide beneficial effects in human health due to their antioxidant properties, which protect against the oxidative damage to cells and thus prevent the development of common degenerative diseases such as cancer, cardiovascular diseases, cataracts, diabetes, Alzheimer's, and Parkinson's (Blanco *et*

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al., 2013). Different colours of capsicums indicated different maturity index, where green capsicum was harvested before fully ripe.

Among the phytochemical compounds, polyphenols get the most attention in most research studies due to their property of scavenging free radicals in vivo (Celia *et al*., 2015). Studies have shown a possible association between the consumption of polyphenols and a lower risk of coronary disease and cancer (Segura *et al*., 2013). The polyphenols have the strong capacity to scavenge free radicals which are found in high quantities in bell peppers, whose levels vary strongly during growth and ripening (Nadeem *et al*., 2011).

Blanching is a pre treatment before vegetables undergo freezing process. Blanching inactivate enzyme that caused color and textural changes in vegetables. Deactivation of peroxidase enzyme is important in frozen vegetables, due to this enzyme is more heat resistant (United States Department of Agriculture, 2013) thus longer time is needed to deactivate this enzyme. However, treatment using heat can cause quality changes in products such as texture, taste, color and nutritional content e.g. reduction of ascorbic acid content (Howard *et al*., 1994). The correct blanching treatments with precise time and temperature used can reduce these quality changes in frozen vegetables. It is desirable to keep blanching treatment conditions at a level strictly sufficient to cause inactivation of the enzymes. It is necessary to minimize quality losses especially for frozen sweet bell peppers which intended to be stored frozen and eaten raw after thawing, to preserve the texture properties, namely firmness and crispness (Sonia *et al*., 2007).

They were studies showed that thermally cooked foods had lower nutritional value than fresh foods because of the loss of vitamins and loss of physiochemical characteristics such as in a study by Barros *et al*. (2011). However, in contrast, they were reports suggest that cooking and thermal treatment, increased antioxidant activities by liberating antioxidant compounds from insoluble portions of foods (Dewanto *et al*., 2002; Turkmen *et al*., 2005). A study to investigate the effect of different cooking methods (boiling, steaming, stir-frying, and roasting) at different cooking times on the antioxidant properties of red pepper showed dry-heat cooking methods such as stir-frying and roasting maybe preferred to retain the nutrient compositions and antioxidant properties of red pepper (In Guk Hwang *et al.,*2012). Previous studies with different contrast results about the effect of heat treatment on antioxidant activity of vegetables showed that the effect of thermal processes on antioxidant compounds such as polyphenols, carotenoids, and vitamin C in fruits and vegetables are inconclusive.

The objective of this study was to evaluate the effect of different blanching treatments using steaming, boiling water and microwaves at different temperature, time and microwave power level on antioxidant activity of frozen green capsicum at 0, and 3rd months frozen storage.

Materials and Methods

Raw materials and chemicals

Capsicum (*Capsicum annuum* L.var Bell Pepper) in green maturity were bought from supplier of wet market in Selangor where the vegetable supplies were collected from Cameron Highland and were kept chilled at temperature 4±1°C until further processing and analysis conducted. The packaging material used is Oriented Nylon/Linear Low Density Polyethylene (Ony/LLDPE) which is suitable for low temperature processing. Folin-Ciocalteu phenol reagent, ferric chloride ($FeCl_3$•$6H_2O$), and HCl were obtained from Merk, (Darmstadt, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), sodium acetate trihydrate, $K_2S_2O_8$, $AlCl_3$•$6H_2O$, $NaNO_2$, NaOH, gallic acid,Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), methanol were purchased from Sigma (USA). Sodium carbonate was purchased from RDH (Germany). Chemicals and reagents used for antioxidant analysis were analytical grade and chemical food grade was used for calcium chloride.

Sample preparation and blanching treatments

Capsicum were soaked in 50 ppm chlorinated water for 1 minute and rinsed using filtered water. Green capsicums were cut approximately 2 cm cube manually using clean knife and were soaked in 1.5% (w/v) food grade calcium chloride for 30 minutes to preserve color and texture.

For all blanching treatments, 200 grams of set capsicum were used per batch and after each blanching treatments, capsicum were soaked in ice water, approximately 3-4°C to stop the blanching process and time was set same according to each blanching treatment time before capsicum were packed .

Green capsicum were placed in steamer (Tefal S02 series, China) power 650W-230V~50/60 Hz, for steam blanching and time were set at 150 and 180 seconds respectively. Capsicum were blanched in hot water using cooking pot and temperatures were set at 80°C for 150 seconds and 100°C for 150 seconds.

Microwave used (National, Malaysia) with frequency 2450 MHz, and output power 1000 W. The capsicums were arranged in round shape microwavable glass dish. The powers level used were low power at 150 and 180 seconds, medium power level at 120 and 150 seconds, and high level 90 and 120 seconds. The blanching treatments used were indicated in Table 1.

Inactivation of peroxidase enzyme

Peroxidase test was used to estimate the sufficient time for inactivation of peroxidase enzyme of frozen vegetables. Approximately 200 gram samples were weight and macerate in blender with 600 mL of water for 1 minute at high speed. A blank was prepared by adding 21 mL of distilled water to 2 mL of filtered samples in test tube and 1 mL of 50% of guaicol solution were added without mixing. The same step applied for second test tube with addition of 1 ml of 0.08% hydrogen peroxide without mixing. Both tubes were mix thoroughly by inverting each 3 times. Any color change contrast to the blank is considered positive test. If no such color contrast develops in 3 ½ minutes the test consider negative and the product adequately blanched (United States Department of Agriculture, 2003).

Freezing process

Fast freezing process was conducted using commercial blast freezer (Technomac AX8, Italy). Packed green capsicums were placed on stainless steel tray and a probe was put in the middle of selected capsicum sample to measure the completeness of freezing process until the temperature reached -18°C in the middle of product. The fast freezing process took 45 minutes to 1 hour for capacity of 14 trays with 6 packs weight 150-200 grams each pack. Frozen green capsicums were kept in chest freezer and the temperature maintained at -18°C for 0, and 3rd month storage.

Sample extraction

The green capsicum was placed in a food processor to form uniform slurries using a glass blender (Faber, Malaysia) speed 1 for 1 minute. Approximately 1 gram of capsicum slurries were weighed into a universal bottles containing 10 mL methanol solvent. Capsicum slurries were mixed thoroughly using stirer (IkaWerke RO 5, Germany) speed 5, for 1 hour. Slurries further centrifuged using a centrifuge (Rotina 380, Germany) at 5000 x g for 10 min. The supernatants were collected for further analysis.

Total phenolic content (TPC)

Table 1. Blanching treatments

Samples
A (Fresh)
B (Steam 100°C/150 seconds)
C (Steam 100°C/180 seconds)
D (Hot water 80°C/150 seconds)
E (Boiling water 100°C/120 seconds)
F (Microwave low level/150 seconds)
G (Microwave low level /180 seconds)
H (Microwave medium level/120 seconds)
I (Microwave medium Level/150 seconds)
J (Microwave high level/90 seconds)
K (Microwave high level /120 seconds).

Capital letters (A-K) indicate different types of blanching treatments

Antioxidant activity was determined using TPC based on the method of Musa *et al.* (2010). Approximately 0.4 mL distilled water and 0.5 mL diluted Folin–Ciocalteureagent was added to 100 µL capsicum extracts. The samples (capsicum extracts with Folin–Ciocalteu reagent) were set aside for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was taken at 765 nm wavelength using a spectrophotometer after 2 h. The calibration curve of gallic acid (GA) was used for the estimation of sample activity capacity. The result was recorded in terms of mg of GA equivalents per 100 g of fresh sample (mg GAE/100 g of

Ferric reducing antioxidant power (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method modified by Zuhair *et al.* (2013). FRAP reagent was prepared fresh using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), in 40 mMHCl and 20 mM $FeCl_3$•$6H_2O$ in the ratio of 10:1:1 to give the working reagent. About 1 ml FRAP reagent was added to 100 µL sample extracts and the absorbance were taken at 595 nm wavelength with spectrophotometer after 30 minutes. Calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of dry sample (mg TE/100 g of FW).

DPPH radical scavenging activity

The determination of antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of Musa *et al.* (2010). Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solutions were mixed with 350 ml methanol to obtain the absorbance of 0.01 units at 516 nm wavelengths by using spectrophotometer (Epoch, Biotek, USA). About 100 µL sample extracts with 1 ml methanol DPPH solution prepared were kept for 30 min for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow, DPPH scavenging activity (%) = [(A blank –A sample) / A blank] × 100, where A is the absorbance.

Statistical analysis

Analysis of variance (ANOVA) was used to compare mean of minimum 3 measurements. Significant differences between means were determined by Duncan ($p \leq 0.05$). The software used was SPSS 16.0.

Results and Discussion

Total phenolic content (TPC)

The effect of blanching treatments on Total phenolic content (TPC) for 0, and 3rd month storage of frozen green capsicum was presented in Figure 1.Frozen green capsicum treated with microwave at high power level for 90 seconds (sample J) contain the highest TPC for 0 month storage at 88.95 mg GAE/100 g sample compared to untreated fresh green capsicum (sample A) at 83.59 mg GAE/100 g and other blanching treatments. There were significant difference ($p \leq 0.05$) between sample J and other samples (samples A, B, C, D, E, F, G, H, I, and K) at 0 month storage. However, there were no significant difference ($p > 0.05$) for 3rd month storage between sample J and A. A study by Chen *et al*. (2011), observed that when the citrus fruit (*Citrus sinensis* (L.) Osbeck) peels were dried at 50°C and 60°C, the total phenolic contents (TPC) were significantly lower than those of fresh peels. However, the phenolic content gradually increased as drying temperature increased and TPC content was increased around two-fold at peel dried at 100°C compared to fresh peel. Fresh green capsicum contains high TPC at 83.59 mg GAE/100 g sample, higher than other treatments except for sample J. According to Miller *et al.* (2005), fresh fruit and vegetables are expected to have higher health protecting capacity than processed products. However phenolic can increase due to heat which can break supramolecular structures, releasing the bound phenolics which react better with Folin- Ciocalteau reagent (Bunea *et al*., 2008).

A study suggested that an appropriate temperature maintained a high antioxidant activity of phenolic

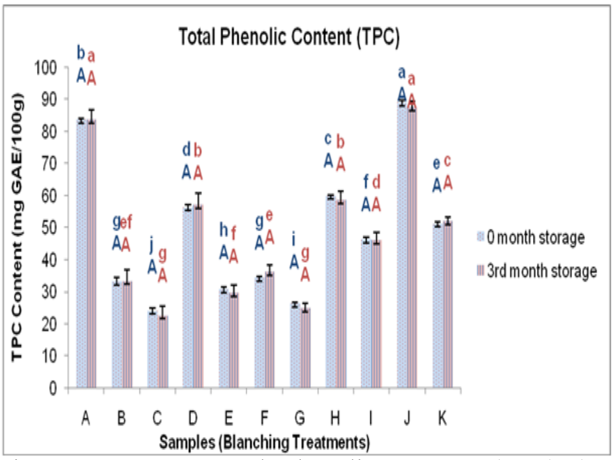

Figure 1. Mean Total Phenolic Content (TPC) (mg GAE/100 g sample) in Frozen Green Capsicum for 0, and 3rd Month Frozen Storage

Small letters (a-j). Different letters between different samples within the same storage month indicate significant differences ($p \leq 0.05$).

Capital letters (A) indicate no significant differences ($p > 0.05$)

*Sample A is a fresh green capsicum, without frozen storage

compound, which could be due to the combined effect of nonenzymatic reaction and phenolic compound stability (Reyes *et al*., 2007). Blanching using microwave can reduced heating time and reduced the loss of water soluble nutrients during steaming and boiling water blanching (Dorantes-Alvarez *et al*., 2011).

Results showed, blanching time effect the antioxidant activity where capsicum exposed to longer heat treatment showed reduction in TPC. This can be seen in microwave treated samples at different power level and time as high power level but shorter time exposure showed higher antioxidant activity level.

Sample D (Hot Water 80°C/150 seconds) showed higher level of TPC at 0, and 3rd month storage compared to other treatments, sample K,I F,B,E,G, and C. This may be due to decreasing temperature at suitable minimal blanching time preserves the antioxidant content. A study by Roy *et al*. (2007), found decreasing temperature of processing was also found to preserve 80-100% of phenolic content in some vegetables.

In most studies on the effects of heat treatment on the total phenolic content, the results are contradicting. Some researchers reported an increased in the phenolic content whilst others observed a decreased (Chipurura *et al*., 2010). Lima *et al.* (2009) observed a significant loss of phenolic content in edible vegetables when heat treated. Lopez *et al.* (2010) observed that an increased in drying temperature had impact on TPC of blueberry varieties compared to the fresh sample. Other study showed that different temperatures, affect the antioxidant activity of sweet bell pepper phenolic extracts. The extracts from sweet bell pepper contain different antioxidant and antiradical activity value which could vary in different varieties, and temperatures (Narmin *et al*., 2013). Phenol compounds showed good antioxidant ability (Duan *et al.,* 2007), but relatively unstable (Zhang *et al*., 2000). The stability of phenol compounds depends on various factors, such as pH value and temperature (Zhang *et al*., 2001).

Sample C (Steam 100°C/180 seconds) had the lowest TPC content. This may be due to leaching of antioxidant and other nutritional content as excessive long time exposure to heat. There were no significance difference ($p>0.05$) between each sample throughout frozen storage from 0 to 3rd month.

DPPH radical scavenging activity (DPPH)

Sample A (fresh capsicum) and sample J (microwave at high power level for 90 seconds), contained the highest DPPH radical scavenging activity as showed in Figure 2 for 0, and 3rd month storage. Both samples were significantly higher ($p\leq0.05$) than the other treated samples. Sample A (fresh) showed high percentage, 77.7% DPPH radical scavenging activity. There were no significant differences ($p>0.05$) between sample A and J. The DPPH percentage range of sample J was approximately 77-78% for 0 and 3rd month frozen storage.

Figure 2 showed, sample H, K and I blanched using microwave medium, and high level and also sample D blanched in hot water at 80°C/150 seconds showed no significant difference ($p>0.05$) between these samples for 0 and 3rd month storage. Samples treated using microwave at medium and high level showed high percentage DPPH radical scavenging activity with more than 70% from 0 to 3rd month storage. The DPPH value for water boil at 80°C/150 seconds (sample D) was 72-74% inhibition. A previous study showed high DPPH value in bitter gourd in boiling, steaming and microwave cooking method with inhibition percentage more than 80% (Aminah and Anna, 2013). However, from other study, it showed boiling treated samples produced low DPPH radical scavenging activity in most vegetables but it stated vegetables which increased their scavenging activity were eggplant, maize, pepper and swiss chard (Jimenez-Monreal *et al*., 2009).

Sample F were higher than sample B and sample B was higher than sample E in DPPH (Figure 2), could be due to less leaching in antioxidant compound in dry heating by microwave compared to wet heating

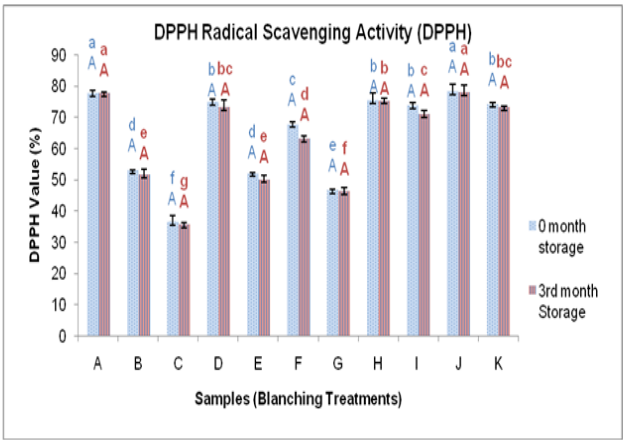

Figure 2. Mean DPPH Radical Scavenging Activity (%) in Frozen Green Capsicum for 0, and 3rd Month Frozen Storage

Small letters (a-g). Different letters between different samples within the same storage month indicate significant differences ($p\leq0.05$).
Capital letters (A) indicate no significant differences ($p>0.05$)
*Sample A is a fresh green capsicum, without frozen storage

by steaming and water boil. Microwave treated samples extracted using methanol, showed significant increase in DPPH scavenging activity compared to distilled water and distilled boiling water extracted samples(Sathiskumar *et al*., 2005). A study observed that cooked peppers showed marked differences ($p \leq 0.05$) in the radical scavenging activity (RSA), when cooked for 5 min in boiling water with further reduction observed after boiling for 30 min. This may be due to the leaching of antioxidant compounds from the pepper into the cooking water during the prolonged exposure to water and heat. It was concluded, less water and cooking time were important to obtain the optimum benefits of bioactive compounds present in peppers (Ai Mey *et al*., 2008).

Steaming at 100°C/150 seconds (sample B) showed moderate DPPH inhibition percentage around 49% to 52% throughout frozen storage (Figure 2). However, with longer steaming time in sample C (steam 100°C/180 seconds) the value drops significantly ($p\leq0.05$). This could be due to more leaching in antioxidant compounds. Sample C had the lowest radical scavenging percentage approximately only 30% which was significantly ($p\leq0.05$) lower than other treatments.

Ferric reducing antioxidant power (FRAP)

In Figure 3, frozen green capsicum treated with microwave at high power level for 90 seconds (sample J) contain the highest FRAP value for 0 to 3rd month storage, higher significantly ($p\leq0.05$) compared to fresh capsicum and other treated samples. FRAP value for fresh capsicum (sample A) is 134.03 mg TE/100 g sample, significantly lower ($p\leq0.05$) than

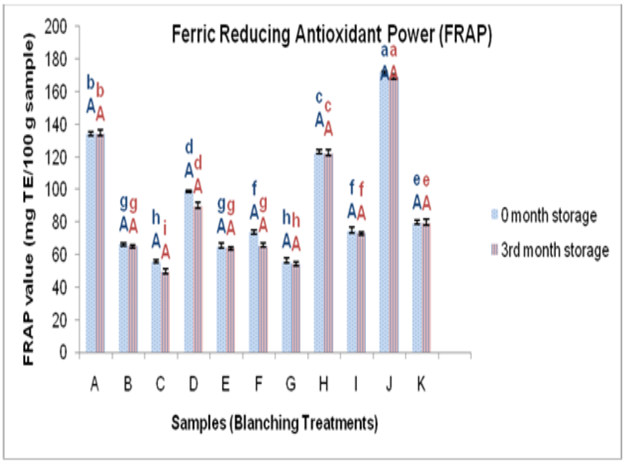

Figure 3. Mean Ferric Reducing Antioxidant Power (FRAP) (mg TE/100g sample) in Frozen Green Capsicum for 0, and 3rd Month Frozen Storage

Small letters (a-h). Different letters between different samples within the same storage month indicate significant differences ($p \leq 0.05$).

Capital letters (A) indicate no significant differences ($p > 0.05$)

*Sample A is a fresh green capsicum, without frozen storage

sample J for 0 and 3rd month at 171.73 mg TE/100 g sample and 168.82 mg TE/100 g sample respectively. However, sample A was significantly higher ($p \leq 0.05$) than other treatments. The increasing value in FRAP for vegetables treated with heat treatment compared to raw vegetable may be due to the conversion of antioxidants to higher amounts of antioxidant chemical species (Miglio *et al.*, 2008).

Sample H (microwave medium level/120 seconds) contain higher FRAP value compared to sample D (hot water 80°C/150 seconds). This could be due to longer time exposure in boiling water cause leaching of antioxidant compound in water. However, a study reported that the FRAP value were higher when exposed to water (Andlaur *et al.*, 2003). Sample K (microwave high level/120 seconds) showed higher FRAP value compared to sample I (microwave medium level/150 seconds) and F (microwave low level/150 seconds) because of shorter time exposure to heat.

The lowest FRAP value can be observes in sample C (steam 100°C/180 seconds) with FRAP range 47 to 55 mg TE/100g sample. At steam temperature, with longer exposure to heat may cause leaching and is not a suitable combination parameter for blanching treatment that causes higher loss in antioxidant properties. It is important to minimize heating treatment and time exposure to heat to preserve antioxidant properties, nutrient content and vegetables texture.

Overall, for antioxidant activity the order from the highest antioxidant activity to the lowest, between different blanching treatments were; J (microwave high level/90 seconds) >A (fresh) > H (microwave medium level/120 seconds) > D (hot water 80°C/150 seconds) >K (microwave high level /120 seconds) > I (microwave medium level/150 seconds) > F (microwave low level/150 seconds) > B (steam 100°C/150 seconds) > E (boiling water 100°C/120 seconds) > G (microwave low level/180 seconds) > C (steam 100°C/180 seconds).

There were no significant difference in TPC, DPPH, and FRAP for 0, and 3rd month frozen storage. This indicated low freezing temperature can preserves antioxidant compound and stabilized antioxidant activity.

Conclusion

As a conclusion, microwave heating without using water are more suitable cooking methods for pepper, to ensure the maximum retention of antioxidant molecules. Microwave blanching at high power level for 90 seconds is the best blanching method for frozen green capsicum to retain high antioxidant activity. It is important to minimize heating treatment and time and a suitable combination of suitable temperature and time are important in establishing a good blanching method that can preserve antioxidant activity of frozen green capsicum.

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