Comparison between Superheated Steam and Convectional Roasting on Changes in the Phenolic Compound and Antioxidant Activity of Cocoa Beans

Wahidu ZZamaN\textsuperscript{1,2}, R. Bhat\textsuperscript{2}, Md. Zainul Abedin\textsuperscript{2} and Tajul A. Yang\textsuperscript{2*}

\textsuperscript{1} Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh
\textsuperscript{2} Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia

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Cocoa beans (\textit{Theobroma cacao}) are rich in phenolic compounds which show antioxidant properties. Roasting is one of the most important unit operations in the cocoa base industries which reduces the antioxidant activity. Cocoa beans were subjected to roast at 150, 200 and 250\textdegree C for 10 – 50 min using conventional and superheated steam methods on changes in the total phenol content (TPC), total flavonoid content (TFC), free radical scavenging activity and antioxidant properties. The total phenols and total flavonoid were significantly (p < 0.05) higher using superheated steam than conventional roasting method. The cocoa beans treated by conventional method showed significantly (p < 0.05) lower the free radical scavenging activity and antioxidant properties than superheated steam roasting method.

Keywords: cocoa bean, superheated steam roasting, conventional roasting, phenolic compound, antioxidant properties

Introduction

The phenolic compounds present in the food that can prevent or delay lipid oxidation through the inhibiting of oxidizing reactions in our body. It has been reported that food such as fruit, vegetable and seeds contain a significant amount of natural antioxidant components including phenolics (Mandic \textit{et al.}, 2008; Katalinic \textit{et al.}, 2004). Studies reported that there is a negative correlation found between dietary intake phenolics and coronary heart diseases, stroke and cancer (Sun \textit{et al.}, 2002).

Cocoa beans are the seed from the fruit \textit{Theobroma cacao} tree which is the essential ingredient of chocolate and chocolate base products. The cocoa beans are rich in phenolic and other antioxidant properties. Approximately 12 – 18% of phenolic compounds are reported in unfermented cocoa beans (dry weight). Almost 60% of the total phenolic compounds are procyanidin (oligomers), epicatechin and catechin (flavanol monomers) in raw cocoa beans (Dreosti, 2002). The study described that the cocoa beans contain higher amount of flavan-3-ol. The flavan-3-ol (catechin and epicatechin) are the main monomeric compound of polyphenol found in cocoa (Lamuela-Raventós \textit{et al.}, 2005). These compound have the highest bioavailability among cocoa phenolic compound because the absorption is depends on the molecular size. The monomeric compounds are biologically more active than oligomers and polymers and found in high concentration that can interact with the target compound easily especially free radicals (Cooper \textit{et al.}, 2009). These compounds have a potential contestant to inhibit or delay the oxidative damages in our body (Adamson \textit{et al.}, 1999). The studies revealed that these compounds are capability to reduce lipid peroxidation, inhibit peroxyl radicals and scavenge superoxide radicals and hydroxyl radicals (Salah \textit{et al.}, 1995; Vinson and Hontz, 1995; Kanner \textit{et al.}, 1994). Furthermore, the study demonstrated that the antihyperglycaemic effects on streptozotocin induced diabetic rats were found using the extracts prepared from cocoa (Ruzaidi \textit{et al.}, 2005). Antioxidant properties of cocoa base products are most important consideration factors to promote the consumption of these products. It has been reported that the polyphenol content in cocoa-derived products is much lower than the raw materials used for production. Thus, the antioxidant property depends mostly on the production steps used in industry (Kim and Keeney, 1984).

Roasting is one of the most important unit operation in cocoa base food industries. Convectional roasting (hot air roasting) method is the most commonly used for roasting of cocoa beans with different time and temperature vary-
ing from 150°C to 250°C for 30 to 120 min (Raml et al., 2006). This conventional roasting method has some disadvantages because of traditional ways of heat and energy transfer. About 70% of phenolic compounds and most of the antioxidant properties of the products are destroyed during that step due to prolong heating (Zzaman and Yang, 2013a; Świechowski, 1996). There are no acceptable processing techniques to avoid polyphenol degradation in the conventional cocoa industry.

In recent years many research has been focused and succeeded for the application of superheated steam in food industry as a new method. It is obtained by reheating saturation steam exceeding the boiling temperature of H₂O that is a colorless and transparent gas. Superheated steam has many interesting properties although it performs as a hot dry gas (Idrus and Yang, 2012; Zzaman and Yang, 2013b). It also does not increase the moisture of the sample during heat treatment and has also low risk of fire and explosion due to lack of air. Most important characteristics of the steam are that the products are not oxidized because the air in the system is replaced by superheated steam and is thus, the samples can be heated or dried under non-oxygen environments (Amatsubo et al., 2005). Research on thermal treatment such as cooking, baking, drying and sterilization has been enabled by the application of superheated steam (Wang, 2012; Hosaka, 1999). It has been reported that the drying of sliced raw potatoes and shrimps using superheated steam was better than hot air drying system (Prachayawarakorn et al., 2002; Iyota et al., 2001). The quality of the boiled and dried adductor muscle of the scallop (shiraboshi) have been investigated and described that superheated steam made a product of high quality (Nishioka et al., 2004). Many researches have been done on the quality of agricultural products and the most of these studies have reported the better quality using superheated steam than hot air drying treatment (Hamada et al., 2003). Superheated steam as drying medium is an energy efficient process compared to conventional hot air because of possibly reuse of the latent heat of evaporation (Berghel and Renström, 2003; Fitzpatrick, 1998). It can be applied to a wide range of fields including food processing such as roasting of cocoa beans because of its high heat transfer capabilities. It has been claimed that this technology heated foods while retaining antioxidants, vitamins and other essential nutrients due to absence of oxygen (Head et al., 2011; Mujumdar, 2007; Pronyk et al., 2004; Chen et al., 1992). The aim of this present study was to investigate a comparison between roasting by superheated steam and convection mode with regard to changes in the phenolic and antioxidant properties of roasted cocoa bean (*Theobroma cacao*).

### Materials and Methods

**Cocoa samples** The raw cocoa beans were collected from Cocoa Research and Development Center, Hilir Perak, Malaysian Cocoa Board, Malaysia. The samples were stored in a chiller (7°C) until further use.

**Chemicals** Gallic acid, Folin-Ciocalteu reagent and Sodium bicarbonate from Merck (Darmstadt, Germany); sodium nitrite, aluminum chloride, sodium hydroxide, catechin, n-hexane and Methanol (99.8%) were obtained from Fisher Scientific (UK). Sodium acetate, hydrogen chloride, ferric chloride, ferrous sulphate, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) were purchased from by Sigma-Aldrich (USA) and all chemicals and reagents used were analytical grade.

**Roasting of cocoa beans** The collected cocoa beans were allowed to equilibrate before roasting at room temperature overnight. Approximately 250 g portions of cleaned medium size raw beans with a diameter of 18 − 24 mm (moisture content: 6.44 ± 0.13%/weight) were used in this study for roasting. The beans were distributed in a single layer on a plate regardless of the roasting method. A superheated steam oven (Healsio, AX-1500, SHARP) were used in conventional and superheated steam mode that was preheated to the appropriate roasting temperature. The roasting was carried out at 150, 200 and 250°C for 10, 20, 30, 40 and 50 min. The oven door was only opened once to remove the beans after roasting. During roasting process samples were taken at different time intervals and immediately equilibrated to room temperature. The different roasted sample beans were packed in polyethylene plastic bags and stored for further experiment. For each roasting time and temperatures experiments were done in triplicate for a total of three replications.

**Preparation of sample extracts** After roasting raw and roasted sample of cocoa beans were manually deshelled and grinded with blender and defatted by refluxing in a Soxhlet apparatus with petroleum ether (bp. 40 − 60°C) for 16 h (AOAC, 1990). The defatted powder obtained was dried in an oven Memmert ULM 500 (Schwabach FRG, Germany) at 60°C for 16 − 18 h. and 2.5 g of each resulting sample were used to prepare methanol extracts. The extracts were obtained by dissolving with 5 mL n-hexane and 5 mL methanol: water mixture (70:30 v/v) and then mixed well using vortex for 1 min. The mixtures were then centrifuged at 3500 rpm for 15 min at 4°C. The mixtures were filtered through filter paper with Whatman No. 1 using Bucher funnel and the filtrates were considered as cocoa extract. The resulting extracts were transferred into glass bottles. Each sample was extracted in triplicate and the extracts were stored in a deep freezer for further analysis.

**Total phenolic content** Total phenolic content was
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The total phenol content was determined according to method with slight modification described by Szydłowska-Czerniak et al. (2008). The methanol extract (0.2 mL) was taken into graduated flask and then added with 0.5 mL of Folin-Ciocalteu reagent in the same flask and shaken for 3 min. After that one mL of saturated aqueous solution of sodium carbonate (Na₂CO₃) was transferred to the flask and then the solution was made up to 10 mL with adding distilled water. The solution was incubated at room temperature for 60 min. After incubation the absorbance was read at 725 nm against reagent blank using UV-160A spectrophotometer (Shimadzu Corp., Nagakyo-ku, Kyoto, Japan). A blank sample was prepared with reagent and distilled water. Gallic acid was used as standard in calibration curve preparation (10–60 mg/100 mL). Quantification (mg/g of cocoa bean) was obtained by reporting the absorbance in the calibration curve.

Total flavonoid content  Total flavonoid content (TFC) of the cocoa beans was determined according to the method described by Lee et al. (2003). For sample preparation, 1 mL of the cocoa bean extract was added with 4 mL of distilled water into 10 mL of measuring flask and after that 0.3 mL of sodium nitrite solution (5%, w/v) was transferred to the same flask. Then 0.3 mL of aluminum chloride solution (10%, w/v) and 2 mL of sodium hydroxide (1M) were added after 5 and 6 min respectively. The volume of the flask was made with distilled water and the mixture was mixed well. Subsequently the absorbance of the mixture was measured against blank at 510 nm. The total flavonoid content in each extract was measured using standard curve made using (−)-Epicatechin (10–90 mg/L) and the results were expressed as mg (−)-epicatechin equivalent (EEQ)/g of cocoa bean.

DPPH radical scavenging activity  The radical scavenging activity of the cocoa samples was estimated according to the method of Kalantzakis et al. (2006). The analysis was performed based on the activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH is a commercially available free radical which is soluble and stable in methanol. An aliquot of methanol extract (1 mL) was placed into test tube and mixed with 4 mL of DPPH solution (0.1 mM in methanol) then shaken vigorously. The mixture was left to stand for 30 min at room temperature in dark place. After incubation the absorbance was read at 517 nm using UV-160A spectrophotometer (Shimadzu Corp., Nakagyo-ku, Kyoto, Japan). The scavenging effect was determined based on the percentage of DPPH radical scavenging activity. Triplicate measurements were carried out for each sample. The percentage of DPPH radical inhibition was measured from the following equation where the absorbance of DPPH solution without extract was used as control:

% inhibition of DPPH =

\[
\frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100
\]

Ferric reducing antioxidant power assay  The total antioxidant activity of the cocoa extract was determined based on the reduction of Fe²⁺(iron(III))-TPTZ (tripyridyl triazine) to a blue coloured of Fe²⁺(iron(II))-TPTZ according to a modified method described by Benzie and Strain (1996). 1 mL of methanol extract was added with 3 mL of FRAP reagent and mixed well. Then the mixture is incubated at room temperature for 30 min and the absorbance was read at 593 nm against a blank r. FRAP reagent was prepared in the ratio of 1:1:10 of 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mM HCl, 20 mM FeCl₃·6H₂O and 300 mM sodium acetate buffer (pH 3.6). Ferric reducing antioxidant power (FRAP) values were expressed as ferrous equivalent (μmole/g of sample). A standard curve was made using FeSO₄·7H₂O solution (200–1000 μM).

Statistical Analysis  The data obtained were presented as means ± standard deviation (SD) and differences among treatments were determined using a paired t test. All measurements were performed in triplicate and the data analyzed by ANOVA using SPSS 17.0. The significant difference was considered at the level of p < 0.05.

Results and Discussion

Total phenolic content  Polyphenols are secondary metabolism present in many plants and play an important role in the defense mechanism in our body. Much attention have been given on polyphenol research recently because of antioxidant activity and other beneficial effect on human health such as in the prevention and treatment of cardiovascular diseases, cancer and other antimicrobial activities. The high concentration of polyphenol present in cocoa bean has been reported that found in the pigment cells of the cotyledons (Nazarudin et al., 2006). The existence of polyphenols in the cocoa beans is depends on the roasting condition used. Roasting treatment with high temperature and time develops suitable flavors and varies the total polyphenolic content. The polyphenolic compound can be changed due to thermal treatment including roasting (Wollgast and Anklam, 2000; Voigt and Biehl, 1995).

In this study we examined the effect of roasting condition using two methods with different temperature and time on total polyphenols content in cocoa beans. The total phenol content of cocoa beans during convectional roasting and superheated steam roasting at different temperatures (150°C, 200°C and 250°C) for 10–50 min is shown in Fig. 1. The study found that the total phenol content gradually decreased during superheated steam and convectional roasting with...
temperatures severely affected and decreased not only the water content but also the polyphenolic content in all roasted samples during convectional roasting. It was reported that the phenol content was decreased from 32.63% (cocoa beans Arriba) to 54.74% (cocoa beans Ghana) during conventional roasting. Polyphenols have been the subject of numerous investigations for their several properties and antioxidant capacity and cocoa has been shown to be rich in polyphenols, especially catechins, epicatechins and proanthocyanidins but significant loss observed during thermal process of roasting (Wollgast and Anklam, 2000). The similar decreasing trend was reported during roasting of pistachio beans compared with different roasting condition with chemical composition (Gentile et al., 2007). A noticeable decreased was recorded on the phenolic content during convectional roasting of cocoa bean model system (Oliviero et al., 2009). It is also important that the efficiency of polyphenol extraction depends on many factors such as cocoa variety, drying, fermentation method of extraction and solvent used in extraction process (Azizah et al., 1999).

**Total flavonoid content**

The flavonoids content in cocoa are higher than red wine or green teas per serving. Flavonoids include flavanols, flavonols, flavones and anthocyanins present in cocoa power (the product obtained after grinding of cocoa beans) (Wollgast and Anklam, 2000). The most abundant flavonoids are flavanols in cocoa, contain the monomeric flavanols, (+)- catechin, (+)- epicatechin and their oligomeric and polymeric forms (Procyanidins). Anthocyanins comprise the -3-galactoside and -3-arabinoside derivatives of cyaniding (Cooper et al., 2007). The cocoa nib was ground after roasting known as the cocoa liquor (Baba et al., 2001).

The total flavonoid content of cocoa beans during convection roasting and superheated steam roasting at different temperatures for 10 – 50 min is shown in Fig. 2. The study identified the mean total flavonoid content was 8.33 mg/g of raw cocoa power. The study observed that the total flavonoid content gradually decreased with increasing time and temperatures in both methods. The total flavonoid content was significantly (p < 0.05) higher in all samples during convectional than superheated steam roasting method. The authors conclude that the higher loss in convectional roasting occurred because of roasting method and thermal degradation. Previous studies suggested that superheated steam heated foods while retaining antioxidants, vitamins and other essential nutrients due to absence of oxygen (Head et al., 2011; Pronyk et al., 2004).

The decreased of polyphenolic compounds in cocoa beans are strictly correlated to the oxidation and thermal degradation of these compounds. It has been reported that high temperatures severely affected and decreased not only the water content but also the polyphenolic content in all roasted samples during convectional roasting. It was reported that the phenol content was decreased from 32.63% (cocoa beans Arriba) to 54.74% (cocoa beans Ghana) during conventional roasting. Polyphenols have been the subject of numerous investigations for their several properties and antioxidant capacity and cocoa has been shown to be rich in polyphenols, especially catechins, epicatechins and proanthocyanidins but significant loss observed during thermal process of roasting (Wollgast and Anklam, 2000). The similar decreasing trend was reported during roasting of pistachio beans compared with different roasting condition with chemical composition (Gentile et al., 2007). A noticeable decreased was recorded on the phenolic content during convectional roasting of cocoa bean model system (Oliviero et al., 2009). It is also important that the efficiency of polyphenol extraction depends on many factors such as cocoa variety, drying, fermentation method of extraction and solvent used in extraction process (Azizah et al., 1999).

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Steam roasting. The study found that convectional roasting sample shown significantly (p > 0.05) lower activity than superheated steam roasting sample at the same temperature and time. The highest antioxidants were observed in roasted during superheated steam at 150°C for 10 min and lowest at 250°C for 50 min during convectional roasting. The optimum roasting period was 10 min which showed the highest radical scavenging activity at each temperature in both roasting methods however superheated steam roasting exhibited higher scavenging activity at each temperature and time than convection heating. Due to absence of oxygen in superheated steam roasting system there were less oxidation occurred and shown higher antioxidant properties while more oxidation during convectional roasting because of hot air present in the system. The radical scavenging effect was decreased with increased time and temperature in both methods. The study observed that more decreased trend found in convectional roasting than superheated steam roasting.

Research has already been reported that foods treated by superheated steam while retaining antioxidants, vitamins and other essential nutrients due to absence of oxygen (Wang, 2012; Pronyk et al., 2004). It has been reported that lower antioxidant properties observed after convectional roasted than unroasted cocoa beans (Hosaka, 1999). The reduced of antioxidant activity during convectional roasting of cocoa beans was already described by Arlorio et al. (2008) who determined the antioxi-

DPPH radical scavenging activity The DPPH radical scavenging activity is one of the known methods to measure antioxidant activity in food sample. DPPH contains free radical and shows a maximum absorption at 517 nm and its purple colour fade rapidly when DPPH encounters radical scavengers. The DPPH assay measures the scavenging of stable radical species of DPPH by antioxidants. The results obtained measuring the DPPH radical scavenging activity using two roasting methods with different temperature and time. The percent radical scavenging effect of convectional and superheated steam roasting is shown in Fig. 3.

DPPH radical activity decreased with the increased of time and temperature in both conventional and superheated steam roasting. The study found that conventional roasting sample shown significantly (p > 0.05) lower activity than superheated steam roasting sample at the same temperature and time. The highest antioxidants were observed in roasted during superheated steam at 150°C for 10 min and lowest at 250°C for 50 min during convectional roasting. The optimum roasting period was 10 min which showed the highest radical scavenging activity at each temperature in both roasting methods however superheated steam roasting exhibited higher scavenging activity at each temperature and time than convection heating. Due to absence of oxygen in superheated steam roasting system there were less oxidation occurred and shown higher antioxidant properties while more oxidation during convectional roasting because of hot air present in the system. The radical scavenging effect was decreased with increased time and temperature in both methods. The study observed that more decreased trend found in convectional roasting than superheated steam roasting.

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Fig. 2. Changes in the total flavonoid content (TFC) of cocoa beans during convection and superheated steam roasting at different temperatures (150°C, 200°C and 250°C) for 10 – 50 min.

Fig. 3. Changes in the Radical scavenging activity (DPPH) of cocoa beans during convection and superheated steam roasting at different temperatures (150°C, 200°C and 250°C) for 10 – 50 min.
dant activity of roasted cocoa beans using methanol solvent. During convectional roasting of pistachio beans considering chemical composition and roasting conditions, a similar decreased of antioxidant activity observed by Gentile et al. (2007). A decreased trend of antioxidant activity was reported during convectional roasting of complete cocoa beans model system with increased roasting time (Oliviero et al., 2009).

**Ferric reducing antioxidant power** Ferric reducing antioxidant power assay is normally used to determine the antioxidant capacity of natural products. The antioxidant capacity is measured by the ability of antioxidants compound present in the cocoa powder to reduce ferric iron to ferrous in the presence of FRAP reagent. A color product (ferrous-TPTZ complex) was formed by the reduction of ferric iron in FRAP reagent. The FRAP value expressed as μmol Fe(II)/g of cocoa samples during convection roasting and superheated steam roasting at different temperatures (150°C, 200°C and 250°C) for 10 – 50 min is shown in Fig. 4.

In both methods of roasting the FRAP value gradually decreased with increasing time and temperature. The FRAP value were significantly (p < 0.05) higher at the same temperature and time in all samples during roasting by superheated steam. The highest FRAP value observed at 150°C for 10 min during superheated steam roasting whereas the lowest at 250°C for 50 min during convectional roasting. There were decreases of the FRAP value from 17.6 to 35.8% during superheated steam method whereas it was from 45.2 to 63.6% during conventional method of roasting for 10 – 50 min. The optimum roasting period was 10 min which exhibited the highest phenolic antioxidant compound at each temperature in both roasting methods however superheated steam roasting showed higher phenolic antioxidant compound at the same time and temperature than convection heating.

The height loss also occurred in conventional method may due to roasting method whereas the lowest loss may due to absence of oxygen when roasted by superheated steam method. Studies indicated that the antioxidant capacity depends on the method of roasting of cocoa beans. The similar decreasing trends were observed during conventional and superheated steam process in food samples for ferric reducing antioxidant properties (Hosaka, 1999).

**Conclusion**

In this study, the roasting of cocoa beans with superheated steam and conventional methods were evaluated. The phenol content and antioxidant properties decreased during both methods of roasting. The lowest loss of total phenol and flavonoid content were found using superheated steam method in comparison with conventional hot air method. This is because of low oxygen environment and even degree of heating during superheated steam roasting process. The results have obtained in a limited condition of superheated steam mode of a commercially available domestic oven used. Therefore the authors conclude that superheated steam roasting method can improve the quality of cocoa beans as a raw material of cocoa base industries. Superheated steam roasting could be more appropriate and flexible than conventional method because the higher total phenol and antioxidant properties are preserved using the same temperature and time. This method takes short time to achieve the optimum roasting characteristics whereas the conventional method takes too long that may contribute loss of antioxidant properties of the products.

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