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Original article

Impact of superheated steam roasting on changes in antioxidant and microstructure properties of raw and processed cocoa cotyledon

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ABSTRACT

This research focused on the roasting of cocoa beans at 184 °C for 16 min duration in a superheated steam oven using two separate modes of heating: convection mode and superheated steam mode. After roasting, the antioxidant properties of the cooked cocoa were assessed as ferric reducing antioxidant power activity (FRAP), DPPH radical scavenging activity, total flavonoid content (TFC) and total phenol content (TPC). The micro structural properties of raw and processed cocoa beans were observed using scanning electron microscopy (SEM). As discovered in the scan, conventional roasting showed a nearly complete rapture of the cytoplasmic network system and the destruction of the organelles, whereas superheated steam mode showed satisfactory images. Studies indicated that superheated steam roasting preserved significantly ($p < 0.05$) greater antioxidant properties as opposed to conventional method of roasting.

1. Introduction

Dry heat treatment is commonly employed to roast cocoa beans which target to the development of characteristics cocoa flavour (N'Zi et al., 2015). Plus, the roasting of the beans fabricates favourable structural changes for grounding process. Roasted cocoa beans are used for the production of both cocoa powder and cocoa liquor after deshelling and grounding the cocoa nib (Caporaso et al., 2021; Krysiak, 2006).

The conventional roasting for cooking the cocoa is a popular method where the raw cocoa beans are roasted using hot air flow at different time and temperature (Lemarcq et al., 2022).Previous research on conventional roasting has reported a number of drawbacks including extended heating and cooking duration causing to occur unappealing colour changes, brunt flavour, loss of valuable antioxidant properties, and also producing harmful substance such as acrylamide (Devos et al., 2020; Nebesny&Rutkowski 1998). Cocoa beans are proven to carry high concentration of polyphenols in the cotyledons pigment cells which are powerful antioxidant that prevents the deleterious effects of the free radicals (Fanget al., 2020). Quantity and quality of the cocoa bean polyphenols relies on the method and condition implemented during roasting (Siow et al., 2022). As the polyphenols tends to have a bitter tasteand the compound gets modified due to heat treatments such as roasting, treating them with high temperature and time duration are reported to have marked fluctuations in taste, flavour and variation in total polyphenol contents (Pirouzian et al., 2020; Wollgast&Anklam 2000).

Superheated steam is a novel technology in which steam is heated above its boiling point.Implementing superheated steam technology on drying and heating foods provides numerous advantages in the food industry due to its high heat transfer and low energy consumption(Fang et al., 2022).Previous studies have shown that utilization of superheated steam in cocoa roasting can drastically enhance its quality attributes such as texture, colour, aroma and also the microstructural properties (Idrus& Yang 2012; Zzamam& Yang 2017).Microstructural properties are crucial in determining the textural and quality parameters of cocoa beans. In microstructural analysis, the superheated-steam-roasted cocoa beans had smoother, clearer crystal formation than the conventionally roasted sample(Zzamam& Yang 2017).Scanning electron microscopy (SEM) was used to examine the micro-anatomy of the roasted cocoa beans, and the findings showed changes in structure as a result of heat treatment as the SEM analysis is a common method for evaluating qualitative attributes and changes in roasting degree which was used in previous studies (Martini et al.,

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2008; Zzamam& Yang 2013). Following a review of the literature, the authors hypothesized that superheated steam cooking would result in higher phenolic and textural quality cocoa beans than traditional conventional roasting.The effect of superheated steam and convectional roasting on physical properties such as colour, texture (hardness and crunchiness), and moisture content has been studied (Zzamam & Yang 2013). However, the antioxidant and microstructural properties before and after roasting using superheated steam and convectional mode has not been studied before.Therefore, the goal of the research was to compare the antioxidant activity and micro structural properties of cocoa beans after superheated steam roasting versus conventional roasting.

2. Materials and methods

2.1. Cocoa sample collection

To acquire the highest quality cocoa beans available in Malaysia, we collected Grade SMC1A Malaysian cocoa beans from the Malaysian Cocoa Board's Cocoa Research and Development Center in Hilir Perak. To conduct the analysis, we used cocoa beans of standard grade (SMC1A) that were free of noticeable defects such as spots or blemishes.

2.2. Cocoa bean roasting and sample preparation

For roasting, 200 g of the sample cocoa beans with a diameter of 18–24 mm were used for roasting which had an initial moisture content of 6.48 ± 0.13 % (wet basis) were used. Using convection mode (normal without steam) and also superheated steam mode, respectively, sample cocoa beans were roasted for 16 min at 184 °C temperature in a superheated steam oven (Healsio, AX-1500 V, Sharp, Japan). The oven is made up of a steam engine heater with a 900 W power output, a 16 cc/min steam generation rate, a 31 L oven capacity, and a centrifugal fan with backward-curving blades. The steam produced by the boiler at a pressure of roughly 1 bar was heated by the electric heater until it reached the superheated state at the start of the drying process. Superheated steam was introduced into the drying chamber and routed through a 359 (W) × 256(H) × 339(D) mm-diameter tray. According to previous studies, a moisture content of 2 % in oven cooked cocoa beans gives ideal condition for texture, colour, taste, and fat extraction. Therefore, roasting was stopped when the moisture content reached to 2 % (Zzaman& Yang 2013; Krysiak, 2006; Nebesny & Rutkowski 1998). The steam oven used is equipped with a 900 W steam engine heater, a 31 L oven size, a 16 cc/min steam production capacity, and a centrifugal fan with backward-curved blades. Until the steam produced by the boiler reached the superheated condition, it was heated by the electric heater at a pressure of about 1 bar. The drying chamber was pumped with superheated steam which passed through a tray measuring 359 (W) × 256 (H) × 339 (L).

2.3. Sample preparation for antioxidant activity for the roasted cocoa beans

Both fresh and roasted cocoa bean samples were manually deshelled and ground with a blender after roasting. Methanolic extracts were made from 2.5 g of each of the samples. 5 mL of *n*-hexane and 5 mL methanol were taken and mixed with water to obtain a 60 % organic solvent solution (60:40 v/v). The solvent was supplemented with samples, and the extracted solution was vortexed for 2–3 min. The formulations were then centrifuged for 15 min at 3500 rpm in an Eppendorf Model-5804 centrifuge at 4 °C temperature. After filtering the solutions into Whatman No. 1 filter paper using a Bucher funnel, the supernatant was considered cocoa extract. The residue was taken and the same procedure was followed twice more. The resulting extracts were placed in glass containers and deposited in a deep freezer for further study (Zzaman et al., 2014).

2.4. Antioxidant properties determination

2.4.1. Total phenolic content determination (TPC)

Total phenolic content (TPC) was analysedspectrophotometrically at 480 nm utilizingFolin and Ciocalteu'sreagent according to method of Szydlowska- Czerniak, (2008) with slight modifications. A standard Gallic acid curve (10–60 mg/100 mL) was prepared for the quantification of TPC of the cocoa beans in mg/g unit.

2.4.2. Total flavonoid content determination (TFC)

The total flavonoid content (TFC) of the extract samples was determined using the technique of Lee et al. (2003). A spectrophotometer was used to determine the final absorbance at 510mn. The standard curve for (−)-Epicatechin was constructed using concentrations varying from 10 to 90 mg/L, and the findings were represented as mg (−)-Epicatechin equivalent (EEQ)/g cocoa bean.

2.4.3. Determination of DPPH radical scavenging activity

The DPPH radical scavenging capacity of cocoa extract samples was assessed using the process of Kalantzakis et al. (2006). At 517 nm, the scavenging effect was measured spectrophotometrically, and the percentage of DPPH radical inhibition was calculated using the equation given as below:

$$percentagescavengingactivity = [(Ac - As)/Ac] \times 100$$

Where Ac is the absorbance of control and As is the absorbance of sample.

2.4.4. Determination of ferric reducing antioxidant power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay was used to determine the total antioxidant potential of the sample extract. This was accomplished by reducing Fe^{+3} ferric-TPTZ (tripyridyl triazine) to a blue-colored Fe^{+2} ferric-TPTZ using the technique described by Benzie and Strain (1999) with slight modifications. The ferrous counterpart was used to convey the antioxidant ability of ferric (FRAP). A standard curve was constructed using $FeSO4{\cdot}7H_2O$ solution (100–1000 μM) and the findings were represented as ferrous equivalent μM/g of dry sample using the curve.

2.5. Formulation of samples for scanning electron microscopy (SEM)

The cocoa samples were specifically formulated for SEM specimen usinghexamethyldisilazane (HMDS) as per the method outlined by Braet et al. (1997).To begin the heating process, the samples were cut into 3 mm^2 cubes. A Mc-Dowell-Trump fixative was generated using a 0.1 M phosphate buffer with a pH of 7.0. The sample cubes were then treated with the fixative for 24 h at 4 °C. Following that, the sample cubes were rinsed three times with 0.1 M sodium phosphate buffer, with a gap of 10 min between each rinse. After rinsing, they were fixed with 1.0 percent osmium tetroxide PBS buffer for 1 h at 4 °C and washed three times with di-ionized water after.The fixated samples were dehydrated at room temperature using a sequence of ethanol concentrations of 50 %, 75 %, and 95 % with a 15-minute period. The final dehydration was performed three times with absolute ethanol (100 %), with a 20-minute break between each. The final dehydrated samples were then soaked in 2 mL of hexamethyldisilazane (HMDS) and gradually poured from one container into another after 10 min to separate out the sediment sample. The samples were dried to a critical

point in a SamDri 805 CPD (Tousimis), then mounted on aluminum specimen stubs and covered with a 20 nm gold palladium coating. The cocoa tissues were then examined using a Leo Supra 50 Vp SEM (Model - Carl-Zeiss SMT, Oberkochen, Germany).

2.6. Statistical analysis

These findings have been represented as means with standard deviations (SD).The paired *t*-test was used to distinguish differences between the variables, and the Pearson correlation was applied to determine whether or not there is a relationship between the activity of TFC and DPPH. The sample measurements were duplicated three times, and statistical analysis was done using the computer program ANOVA of SPSS, version 20.0 for windows. The Duncan test was employed to find sizeable differences between various varieties by looking for differences in which results $p < 0.05$ were deemed significant.

3. Results

3.1. Changes in cocoa bean antioxidant properties as a result of roasting methods

The total phenol content (TPC), the total flavonoid content (TFC), the DPPH radical scavenging assay, and the ferric reducing antioxidant power assay (FRAP) of untreated, superheated steam roasted, and conventionally roasted cocoa beans are shown in Table 1.

According to the study, both superheated steam and convectional roasting processes decreased the total phenol content. The convectional roasting process had a considerably faster reducing rate ($p < 0.05$) than the superheated steam roasting method. TPC concentration was 47.95 mg/100 g in untreated, 42.86 mg/100 g in superheated steam, and 31.42 mg/100 g in standard roasted samples, respectively. Additionally, the research found that superheated steam roasted beans contained 7.14 mg/100 g total flavonoid content (TFC) compared to 5.86 mg/100 g conventional roasted beans, which was significantly ($p < 0.05$) higher than convectional roasting beans. The DPPH radical scavenging activity of superheated steam treated cocoa extract was significantly ($p < 0.05$) greater (58.37 %) compared to that of convectional roasting beans (29.65 %). The ferric reducing antioxidant power assay (FRAP) quantifies biological materials' accumulated antioxidant function. Convectional roasting beans had a lower FRAP value (15.76 μM/g) than superheated steam beans (25.83 μM/g). Both methods depleted the antioxidant properties of roasted beans, but the rate of degradation of TPC, TFC, FRAP, and DPPH radical scavenging activitywas significantly ($p < 0.05$) greater in convectional roasting than in superheated steam roasting.

Polyphenolic compound depletion of the cocoa bean is inversely proportional to thermal degradation and oxidation. It has been shown that high temperatures used in convectional roasting decrease not just the water content, but also the polyphenol content of the roasted cocoa samples shown in Fig. 1. Roasting cocoa beans traditionally lowered their phenol content by 32.63 percent (Arriba cocoa beans) to 54.74 percent (Ghana cocoa beans) (Arlorio et al. 2008). Numerous studies on polyphenols have been performed to ascertain their numerous properties and antioxidant capacity, and although cocoa is high in polyphenols, especially epicatechins, catechins, and proanthocyanidins, significant loss occurs during the thermal roasting process (Zzaman et al., 2014; Wollgast and Anklam 2000). When pistachio beans were roasted under a variety of roasting conditions with varying chemical compositions, a typical decreasing trend was seen(Gentile et al. 2007).

Table 1
The total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) of raw, superheated steam roasted, and conventionally roasted cocoa beans.

Properties	Raw	Superheated steam	Conventional
TPC(mg/100 g)	47.95 ± 07[a]	42.86 ± 21[b]	31.42 ± 43[c]
TFC(mg/100 g)	8.33 ± 12 [a]	7.14 ± 09[b]	5.86 ± 21[c]
DPPH(% inhibition)	68.65 ± 03 [a]	58.37 ± 11[b]	29.65 ± 17[c]
FRAP(μM/g)	32.52 ± 06 [a]	25.83 ± 13[b]	15.76 ± 15[c]

[a–c]In a row of distinct characters, means are significantly different ($p < 0.05$).
[α]Means value ± standard deviation of three replications.

3.2. Changes on micro structural properties of cocoa beans

The chipped surfaces of raw and roasted cocoa beans were examined. At magnifications of 250X, 500X, 1000X, 2000X, and 5000X, the microstructure of the mid-region of cocoa bean samples was analyzed in cross-section for both the conventional and superheated steam roasted methods. Figs. 2 and 3 display the monograms for the different magnifications. As obvious, the bulk of the nib seemed to be made up of parenchymal cells. Starch grains and protein bodies made up the majority of the cytoplasmic network that surrounded the subcellular organelles. After extracting the lipid portion of the sample through alcoholic dehydration, a

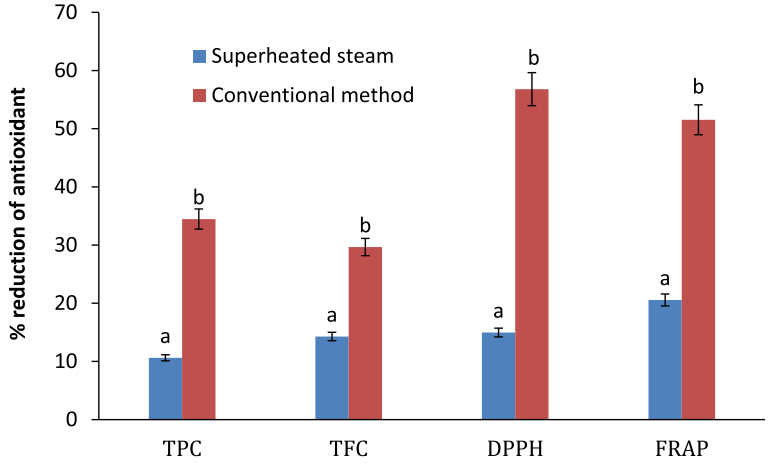

Fig. 1. Percentage reduction of antioxidant propertiess during superheated and conventional roasting of cocoa beans.

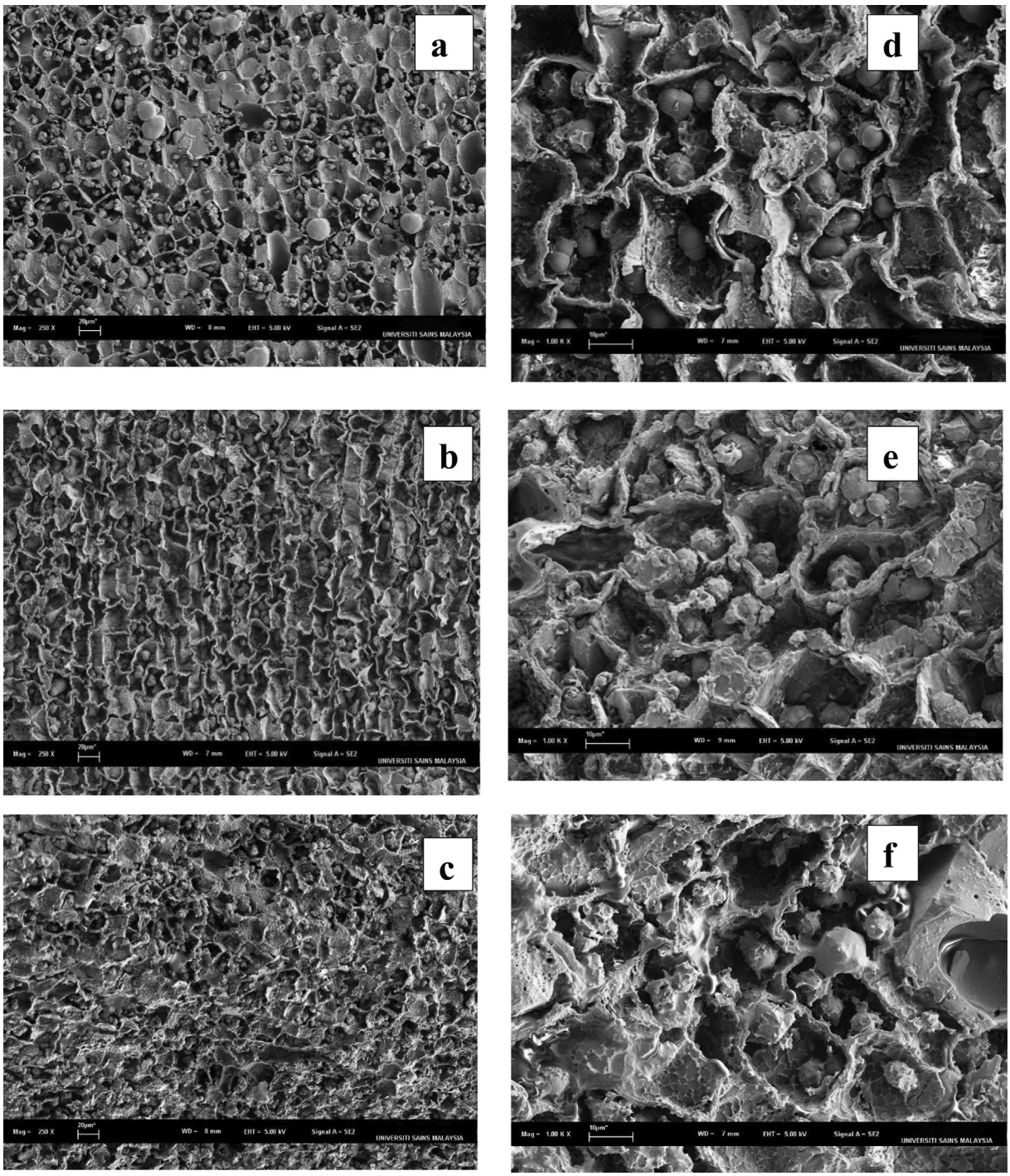

Fig. 2. At 250X magnification, scanning electron micrographs of unroasted cocoa (a), superheated steam-roasted cocoa (b), and conventionally roasted cocoa (c), and, at 1000X, SEM of unroasted cocoa (d), superheated steam-roasted cocoa (e), and conventionally roasted cocoa (f).

hollow filled by lipid bodies was apparent. Additionally, the empty spaces left by lipid bodies following lipid separation during alcohol dehydration were visible. In the SEM picture, protein and starch grains appeared as distinct spherical bodies that were difficult to discern from one another. Fig. 4 depicts pigment cells that are - shapes as a bud inside a vacant room.

Almost every cotyledon is made up of parenchymal cells with thin walls (1–4 mm thick) and a 15–45 mm diameter. A cytoplasmic network encircled angular protein objects with diameters ranging from 2 to 7 mm, as well as the spaces previously filled by lipid bodies with widths of up to 4 mm in parenchymal cells. Polyphenolic pigment cells are found in the parenchymal cells. Such cells were angular in shape, with lengths varying from 10 to 25 m and widths ranging from 5 to 10 μm, as seen in Fig. 4.

Not only does roasting decrease moisture content, but it also speeds up shell removal during subsequent processing. Both superheated steam and conventional roasting methods, the microstructure were contrasted in terms of roasting effects. Roasting resulted in swollen cellular organelles. Subcellular organelles were visible during thermal processing as moisture escaped and oil was

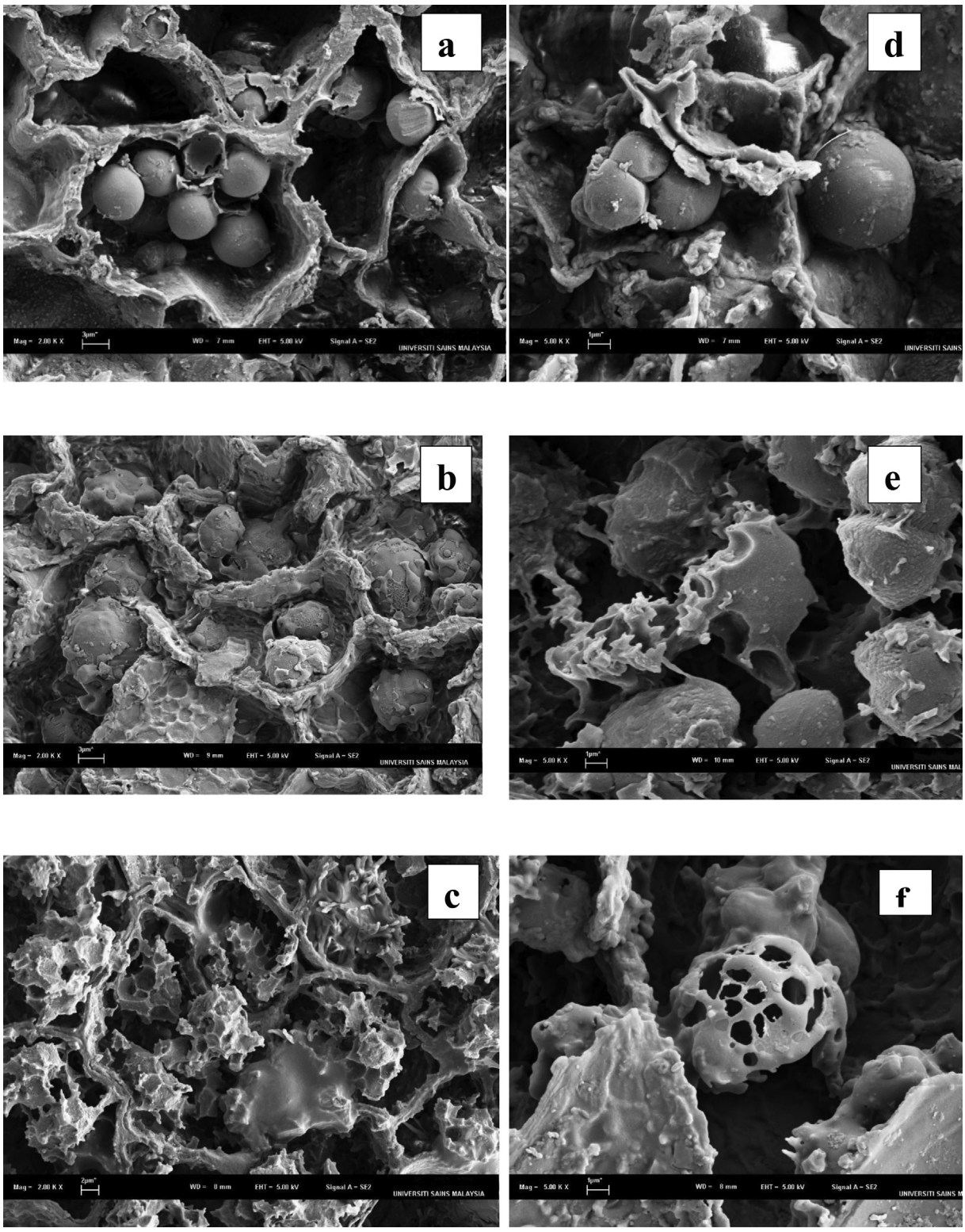

Fig. 3. SEM images of unroasted cocoa bean (a), superheated steam roasted (b), and roasted with conventional method (c) at 2000X magnification, whereas unroasted cocoa bean (d), superheated steam roasted (e), and roasted with conventional sample (f) at 5000X magnification.

released from lipid bodies. Thermal processing of subcellular organelles disturbs them in all roasting methods.

Fig. 3**a and 3dillustrates** the intact subcellular organelles in the raw unroasted sample's cytoplasmic network. In contrast to typical roasted samples, the cytoplasmic network surrounding subcellular organelles, especially buddy pigment organelles, was comparatively more intact in superheated steam roasted samples, as seen in Fig. 3**b and 3e**. Traditional roasting virtually entirely disrupted

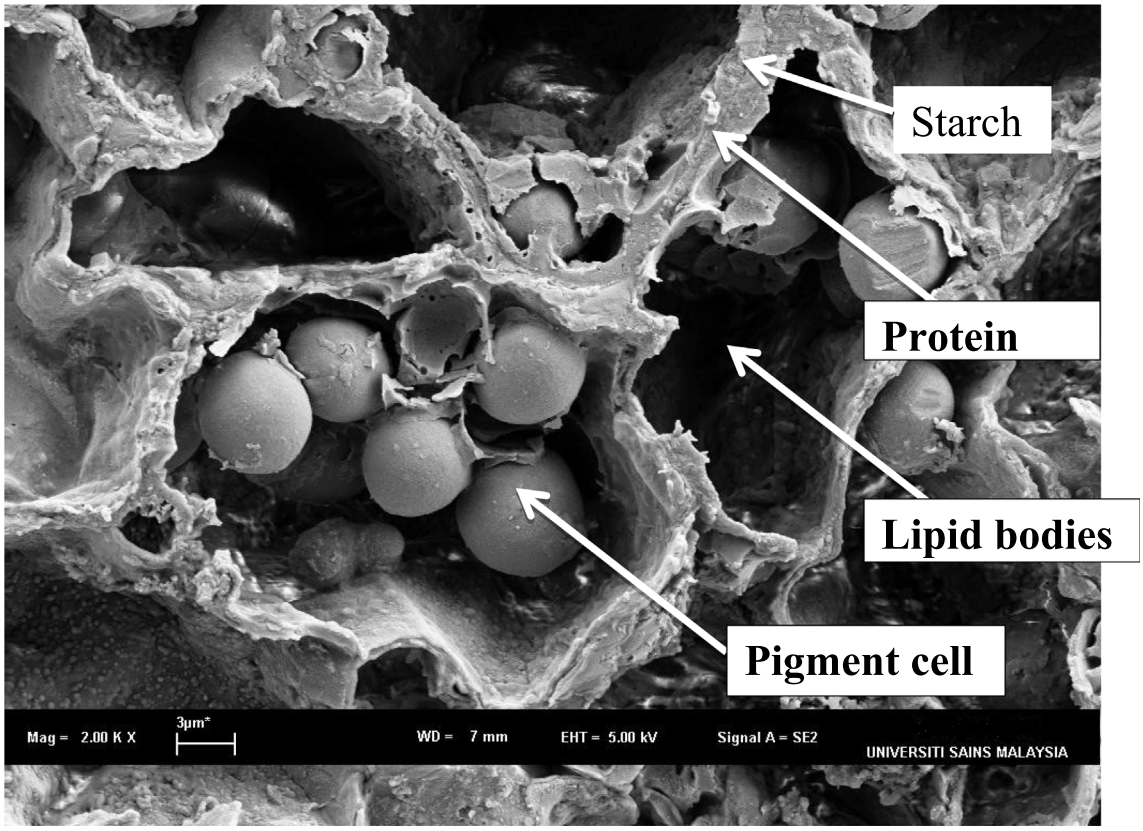

Fig. 4. A scanning electron micrograph of an unroasted cocoa sample; showing the starch, carbohydrate, lipid bodies, and pigment cell at 2000X magnification.

the cytoplasmic network and destroyed the organelles, while superheated steam roasting left them bloated and loosely packed, as seen in Fig. 3**c and 3f**.

4. Discussion

During convectional roasting, the phenolic content of cocoa beans was observed to decrease dramatically (Oliviero et al. 2009). According to previous research, superheated steam cooked foods preserved carbohydrates, minerals, and other essential nutrients due to the lack of oxygen (Pronyk et al. 2004; Head et al. 2011). Furthermore, a critical aspect to remember is that the efficiency of polyphenol extraction is influenced by a number of variables, including the type of cocoa used, the drying process, the method of extraction through fermentation, and the solvent used during process of extraction (Azizah et al. 1999; Manga et al., 2020). Relatable diminishing patterns in cocoa quality have been found, with conventional roasting accounting for up to 67 % of overall flavonoid reduction (Donovan et al. 2006). In conventionally prepared food samples, a similar diminishing phenomenon in ferric reducing antioxidant properties was discovered (Hosaka 1999). Therefore, according to research, the antioxidant potential differs depending on the roasting process.

Young et al. (2004) previously demonstrated the almond microstructure and discovered that the parenchymal cytoplasmic network covers the protein bodies and the area previously filled by lipid bodies. As roasting times were increased in both superheated and conventional roasting, certain cytoplasmic networks became rapidly disrupted (Idrus and Yang 2012). Polyphenolic compounds found in parenchymal cells provide protection during the formation of cocoa flavour, as well as in the production of cocoa butter, aleurone beans, and starch granules (Gregersen et al., 2015). The morphological structure of roasted Theobroma species - beans showed pores or holes in the cotyledon's surface (Martini et al., 2008). Such surface modifications can account for the shell's fragility and the ease with which cocoa beans grind into cocoa liquor (Schenker et al., 2000).

5. Conclusions

Roasting is critical technical phase in the manufacture of high-quality chocolate and cocoa goods. Roasting methods were established as major variables influencing the antioxidant and microstructural properties of superheated steam and typical roasting cocoa beans. While both methods reduced the antioxidant properties of roasted cocoa beans, the rate of decreases in total flavonoid content (TFC), phenol content (TPC),ferric reducing antioxidant potential (FRAP), and DPPH radical scavenging activity was significantly ($p < 0.05$) greater with convectional roasting than with superheated steam roasting. During conventional roasting, the cytoplasmic network was nearly entirely destroyed, and the organelles were broken, while during superheated steam roasting, they were bloated and loosely packed, affecting the development of high-quality roasted cocoa beans. The adoption of this novel approach is projected to increase demand for superheated steam roasting of cocoa beans in industrial level cocoa processing and manufacturing. As a result of the findings, the authors suggest that superheated steam roasting enhances the quality and consistency of roasted cocoa beans. In order to implement the intervention in the chocolate industry, more research on the development of parameters such as time and temperature that will produce the highest quality of roasted cocoa is recommended.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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