

## Plant polyphenols to enhance the nutritional and sensory properties of chocolates

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### Abstract

A relatively unexplored method to enhance the sensory and nutritional properties of chocolate is to use plant polyphenols. In this study, a low cost agricultural waste product – mangosteen (*Garcinia mangostana* Linn.) pericarp - was added as powder in graded amounts (1 %, 2% and 3 % w/w) to dark and compound chocolates during the mixing stage and evaluated. The particle size distributions of the chocolates were mostly within 30 µm and the chocolates displayed a homogeneous morphology. The polyphenols (procyanidins and xanthenes) in mangosteen pericarp powder were also stable to simulated chocolate processing. The 3 % pericarp powder concentration significantly expanded the bioactive profile and

total phenolic content (13 % in dark chocolates and 50 % in compound chocolates) compared to their plain counterparts without affecting sensory qualities. Such low cost plant polyphenols could enhance the bioactive and flavor profile of chocolates, especially in low cocoa content compound chocolates.

*Keywords:* Plant polyphenols, Mangosteen pericarp, Compound chocolates, Xanthones

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## 1. Introduction

Polyphenols are the most abundant antioxidants found in foods and have been extensively studied in the last 20 years (Scalbert, Johnson, & Saltmarsh, 2005; Tomas-Barberan & Andres-Lacueva, 2012). Cocoa in particular is rich in flavonoids that mainly consists the monomeric (–)-epicatechin and catechins, and polymeric procyanidins (Steinberg, Bearden, & Keen, 2003). Procyanidins form the most abundant phytochemicals in cocoa and chocolate products (Kim, Kim, Shim, Lee, Lee, & Lee, 2014; Wollgast & Anklam, 2000). Besides anti-oxidative and anti-inflammatory properties, cocoa polyphenols have demonstrated beneficial cardiovascular, metabolic, antiradical and anti-skin ageing effects (Andújar, Recio, Giner, & Ríos, 2012; Hooper, Kay, Abdelhamid, Kroon, Cohn, Rimm, et al., 2012; Kim, Kim, Shim, Lee, Lee, & Lee, 2014).

The cocoa phenolic content is largely dependent upon the cultivar, origin, agricultural and postharvest practices, with major losses during processing (Andres-Lacueva, Monagas, Khan, Izquierdo-Pulido, Urpi-Sarda, Permanyer, et al., 2008; Wollgast & Anklam, 2000). A low polyphenol concentration was found to be crucial for flavor development during the roasting stage (Misnawi, Jinap, Jamilah, & Nazamid, 2004; Noor-Soffalina, Jinap, Nazamid, & Nazimah, 2009), yet

paradoxically, a high phenolic content is desirable in the final product for its nutritional benefits. There have been some approaches to balance and maximize both flavor development and phenolic content (E. Afoakwa, Ofoosu-Ansah, Budu, Mensah-Brown, & Takrama, 2015; Bordiga, Locatelli, Travaglia, Coisson, Mazza, & Arlorio, 2015; Ioannone, Di Mattia, De Gregorio, Sergi, Serafini, & Sacchetti, 2015; Payne, Hurst, Miller, Rank, & Stuart, 2010), but they are limited to the scope of cocoa cultivation and processing. A relatively less explored approach to expand both flavor and phenolic profile of chocolates is to add naturally derived polyphenolic compounds from plant sources. This includes plant polyphenols from red raspberry leaves (Belščak-Cvitanović, Komes, Benković, Karlović, Hećimović, Ježek, et al., 2012), ginseng (Chung, Lee, Kyung Rhee, & Lee, 2011), green tea and fruit extracts such as red grape and acai berries. This approach is restricted, however, by cost and must not be detrimental to taste and consumer acceptability.

Mangosteen (*Garcinia mangostana* Linn.), often considered “the queen of fruits” for its pleasant flavor, is a tropical fruit from the Guttiferae family (Morton, 1987). It is commonly grown in Thailand, Malaysia and Indonesia and has a worldwide production of about 150,000 tons per year (Ramage, Sando, Peace, Carroll, & Drew, 2004). It consists of a sweet

edible milky white pulp and a dark red pericarp. The pericarp makes up about two-thirds the whole fruit weight and is often discarded as agricultural waste. The pericarp however is rich in bioactive compounds such as anthocyanins, xanthenes and procyanidins (Du & Francis, 1977; Fu, Loo, Chia, & Huang, 2007) and has been long used in traditional Thai medicine for treating diarrhea, wounds and skin infections (Peres & Nagem, 1997). There are also health benefits associated with consuming the pericarp (Ohno, Moroishi, Sugawa, Maejima, Saigusa, Yamanaka, et al., 2015; Xie, Sintara, Chang, & Ou, 2015). The incorporation of this low cost agricultural waste product could potentially expand the bioactive profile of chocolates.

Compound chocolate differs from regular chocolates in that the cocoa butter fat source is partially or fully replaced with cheaper vegetable and tropical fats of similar melting characteristics, such as palm kernel oil (Beckett, 2000). The lower cocoa content in compound chocolate also results in lower polyphenol content and a poorer flavor profile. Due to strict EU regulations on what constitutes chocolate (2000/36/EC directive), compound chocolates finds itself mainly in confectionery use such as ice cream coatings. The recent surge in chocolate demand and cocoa butter prices have led to a boom in compound chocolate production and are

especially fueled by Asian and Eastern European markets (Nieburg, 2014). As more people consume compound chocolates, the addition of plant polyphenols presents an opportunity to increase the nutritional and organoleptic qualities of compound chocolates.

Conventional chocolate processing involves roasting, nib grinding, mixing, refining, conching, tempering and molding (Wollgast & Anklam, 2000). In previous studies, the polyphenols were added either as powder to melted chocolate, or during the molding stage of chocolate processing. While high temperatures and long roasting time may be detrimental to the quantity and quality of these polyphenols (Bordiga, Locatelli, Travaglia, Coisson, Mazza, & Arlorio, 2015; Ioannone, Di Mattia, De Gregorio, Sergi, Serafini, & Sacchetti, 2015; Wollgast & Anklam, 2000), it was found that total polyphenol content was not significantly reduced with conching time (Bordin Schumacher, Brandelli, Schumacher, Carrion Macedo, Pieta, Venzke Klug, et al., 2009). Polyphenols could therefore be added somewhere in between these two stages, such as during the mixing stage.

This study therefore aims to explore incorporating a low cost agricultural waste product with high bioactive content such as mangosteen pericarp powder, as a plant polyphenol source, in

graded amounts to dark and compound dark chocolates and to determine the resultant physical, bioactive and sensory properties.

## 2. Materials and methods

### 2.1. Chemicals

Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Anhydrous sodium carbonate was purchased from Thermo Fisher (Singapore). Gallic acid was purchased from Acros Organics (Singapore). HPLC grade hexane was purchased from JT Baker (Pennsylvania, USA).

### 2.2. Preparation of mangosteen pericarp powder

Mangosteen pericarp powder (*Garcinia mangostana* Linn.) was obtained from a global supplier of botanical ingredients, NP Nutra. The mangosteens were grown and harvested in Thailand. Fresh mangosteens were cleaned, sun dried or tray dried at 68 °C for 8 hours, milled to powder, sieved (with greater than 95 % through 80 mesh size) and packaged. Quality control checks were made during the processing to ensure the appearance, taste, odor and safety aspects conform to standards. The

powder physical and nutritional information is shown in Supplementary data.

### *2.3. Formulation of pericarp powder enriched chocolates*

The chocolates were made using a classical technological process in a confectionery factory (Aalst Chocolate, Singapore). 1 %, 2 % and 3 % (w/w) of mangosteen pericarp powder were added to both dark chocolate (DC) and compound chocolate (CC) to give mangosteen pericarp powder enriched chocolates (DC<sub>1%</sub> - DC<sub>3%</sub> and CC<sub>1%</sub> - CC<sub>3%</sub>). Plain unenriched chocolates (DC<sub>0%</sub> and CC<sub>0%</sub>) were also made. The powder concentrations were chosen based on sensory differences found in 3 % samples during preliminary bench tasting. In addition, the concentrations are kept sufficiently low such that any sensory differences are less likely to be due to the reduction of the other ingredients. The formulations used for the production of chocolates are shown in Supplementary data.

For the dark chocolates, cocoa butter was pre-melted by heating between 40-50 °C and added to the cocoa liquor. Sugar, cocoa powder and mangosteen pericarp powder (for enriched chocolates) were added and mixed (Hobart, USA). For the compound chocolates, hydrogenated palm kernel oil was pre-melted between 40-50 °C and added to cocoa powder, sugar and mangosteen pericarp powder (for enriched chocolates) and



mixed (Hobart, USA). In both cases, a minimum total fat content of 25 % was achieved. The respective mixtures were then refined in a five-roll press (Buhler, Switzerland) and conched (BSA, Germany). The dark chocolates were conched for 8 h (6 h dry conche at 90 °C, and 2 h liquid conche at 45 °C) while the compound chocolates were conched for 4 h (2 h dry conche at 60 °C, and 2 h liquid conche at 45 °C). Emulsifier (lecithin E322), vanilla flavor and the remaining fat (natural vanilla and cocoa butter for dark chocolate, vanillin and hydrogenated palm kernel oil for compound chocolate) were added prior to liquid conching. The conched samples were kept in a dry cool room (18-23 °C) until analysis, where the dark chocolates were then hand tempered and both types of chocolates molded using plastic molds to form 4 x 3 cm neapolitans. The chocolates were transported in dry cardboard boxes.

#### *2.4. Determination of physical properties of chocolate*

##### *2.4.1. Determination of particle size distribution*

The particle size distribution (PSD) of the plain and enriched chocolate samples was determined using laser diffraction technique (Mastersizer 2000, Malvern Instruments Ltd., UK). The analysis was conducted in a wet dispersion mode (Hydro 2000S wet dispersion unit, Malvern Instruments Ltd., UK).

About 5 g of each chocolate sample was dispersed in 50 mL of HPLC grade hexane at room temperature (25.2 °C). The samples were placed under ultrasonic dispersion for 2 min to ensure the particles were independently dispersed. All samples were measured in triplicate and the size distribution was quantified. PSD parameters (in  $\mu\text{m}$ ) obtained included the largest particle size  $d(0.9)$ , mean particle volume  $d(0.5)$ , smallest particle size  $d(0.1)$ , Sauter mean diameter ( $D [3,2]$ ) and mean particle diameter ( $D [4,3]$ ).

#### *2.4.2. Microstructure visualization*

The morphology of defatted and non-defatted chocolate samples were observed under JEOL JSM-6700F Field Emission Scanning Electron Microscope (FESEM) (JEOL Ltd, Japan) with a Gatan Alto 2500 cryotransfer system and a Gatan C1002 liquid nitrogen cold stage (*Gatan Inc.*, USA). The chocolate were placed on a brass holder and frozen in liquid nitrogen prior to scalpel fracturing to expose an internal structure. The sample was heated to -90 °C for 5 min to remove ice crystals through sublimation followed by a platinum sputter target coating in an argon atmosphere (60 sec, 10 mA) at about -120 °C. Imaging was carried out at about -160 °C using an accelerating voltage of 5 kV under secondary electron imaging (SEI) mode.

*2.5. Determination of bioactive properties of mangosteen pericarp powder and chocolates*

*2.5.1. Determination of total phenolic content (TPC)*

*2.5.1.1. Preparation of mangosteen pericarp powder*

Mangosteen pericarp powder (1.0 g) was extracted with 60 % ethanol (10 mL) containing 0.1 % HCl and placed on an orbital platform shaker for 1 h at 300 rpm. The mixture was centrifuged, filtered and the above process repeated twice to obtain 30 mL extract.

*2.5.1.2. Preparation of chocolate powder*

Chocolate and celite (w/w, 1:1) were mixed and homogenized to powder and 4 g powders were extracted with 60 % ethanol containing 0.1 % HCl (80 mL).

*2.5.1.3. TPC of mangosteen pericarp powder and chocolates*

Total phenolic content was determined using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The assay was carried out on a microplate reader; the respective pericarp powder and chocolate extracts (20  $\mu$ L) were mixed with Folin reagents (100  $\mu$ L, diluted ten times from the original reagent) and 7.5% Na<sub>2</sub>CO<sub>3</sub> (80  $\mu$ L). The absorbance was measured at 765 nm after standing at 30 °C for 30 min. Gallic acid was used as a standard with concentrations ranging

from 0.0025 to 0.6 mg / mL. The total phenolic content is expressed as milligram gallic acid equivalents per gram of pericarp powder or chocolate. The measurements were carried out in quadruplicate.

### *2.5.2. Heat treatment of mangosteen pericarp powder*

To investigate how mangosteen polyphenols would behave under the chocolate processing conditions, mangosteen pericarp powder was heated at 80 °C in a pre-heated oven for up till 7 h, with samples removed hourly that were chilled immediately in an ice water bath and kept in a -30 °C freezer. The pericarp powders (0.4 g) from different time points were extracted with 60 % ethanol (5 mL) containing 0.1 % HCl, sonicated for 30 min and subsequently placed on the orbital shaker for 1 h at 300 rpm. The samples were then centrifuged at 2000 g for 10 min at 20 °C, with the supernatant collected and concentrated under reduced pressure to remove the ethanolic solvent. The resultant aqueous portion was frozen under liquid nitrogen and freeze dried for 24 h. The dried extract powders (10 mg) were re-dissolved in the extraction solvent (1 mL) and diluted to give 0.1 mg / mL and 1 mg / mL concentrations.

### *2.5.3. HPLC analyses for procyanidins and xanthenes*

#### *2.5.3.1. Chocolate sample preparation*

It was found that the lipophilic xanthenes were washed away during the chocolate defatting stage. Hence the procyanidins were measured from defat chocolate extract while the xanthenes from the non-defat chocolate extract. The extraction procedure is as follows: chocolate and celite (w/w, 1:1) were first mixed and homogenized to powder. To create the non-defat extract, the powder (2 g) was extracted with 60 % ethanol (10 mL) containing 0.1 % HCl and placed on the orbital shaker for 1 h at 300 rpm. The mixture was centrifuged at 2000 g for 5 min at 4 °C and the supernatant filtered. The process was repeated twice to obtain 30 mL extract. The extract was then filtered with 0.20 µm nylon filter before 20 µL extract was injected in HPLC. For the defat extract, the powder (4 g) was defatted with diethyl ether (100 mL) and extracted with 60 % ethanol (10 mL) containing 0.1 % HCl and placed on the orbital shaker for 1 h at 300 rpm. The mixture was centrifuged at 2000 g for 5 min at 4 °C and the supernatant filtered. The process was repeated twice and the collective extracts concentrated under reduced pressure to remove the ethanolic solvent. The resultant aqueous portion was frozen under liquid nitrogen and freeze dried for 4 days. The dried extract powders (10 mg) were re-dissolved in the extraction solvent (5 mL) to give 2 mg / mL concentration.

#### 2.5.3.2. HPLC for xanthenes

The pericarp powder and chocolate extracts were separated on an analytical C18 column (250 mm x 4.6 mm i.d., 5  $\mu$ m) using a Waters (Milford, MA) HPLC system connected with a diode array detector (DAD). The flow rate and oven temperature were maintained at 1 mL/min and 25  $^{\circ}$ C, respectively. Elute A consisted of 5 % formic acid (v/v) in DI water. Elute B was 100 % acetonitrile. Extracts were filtered with 0.45  $\mu$ m nylon filter before injection into the HPLC. The injection volume was 10  $\mu$ L. A gradient elution process was applied, starting with 0 % B for 5 min, ramping up to 10 % B at 20 min, 13 % at 40 min, 20 % at 44 min, 25 % at 50 min and 100 % at 55-100 min (Sadilova, Stintzing, & Carle, 2006). Monitoring of eluted xanthenes was performed at 320 nm. The measurements were done in triplicate for the chocolate extracts.

#### 2.5.3.3. HPLC for procyanidins

This was performed following a reported method (Wang, Liu, Song, & Huang, 2012). The pericarp powder and chocolate extracts were filtered with RC 0.45  $\mu$ m membrane before 20  $\mu$ L was injected for HPLC analysis (250 mm x 4.6 mm i.d., 5  $\mu$ m, Develosil diol with a 4 mm x 4mm i.d. guard column of the same materials Seto, Japan). The elution conditions were as follows: flow rate, 1.0 mL/min; column temperature, 35  $^{\circ}$ C; mobile phase A, 2 % acetic acid in acetonitrile; mobile phase B, acidic aqueous methanol (CH<sub>3</sub>OH: H<sub>2</sub>O: HOAc, 95:3:2

v/v/v). The starting mobile phase condition was 7 % B holding isocratic for 3 min before ramping solvent B to 37.6 % over 57 min and then to 100 % B 3 min thereafter. B was held at 100 % for 7 min prior to returning to starting conditions (7 % B) in 6 min. A fluorescence detector was used with the following parameters: x- $\lambda$ : 230, e- $\lambda$ : 321, gain 100 and EUFS: 5000. The measurements were done in triplicate for the chocolate extracts.

#### *2.6. Sensory evaluation*

Chocolates were evaluated via quantitative descriptive analysis with a trained panel and according to ISO 8586:2012 standards (ISO, 2012). 5 male and 5 female participants were recruited from the Clinical Nutrition Research Centre and from the public. All participants gave written informed consent before starting, and the study was initiated after ethical approval by the Domain Specific Review Board (DSRB) of National Healthcare Group. They were selected according to the following criteria: a) the absence of allergies, aversions or intolerance against chocolate and mangosteen fruit and/or peel, b) non-smokers, c) healthy and between the ages of 21-50, d) normal perceptual abilities, e) not pregnant for women, f) not taking medications known to alter taste function and g) interested and available for all sessions. All panelists scored

above 70 % correct (except for one who scored above 60 %) for a taste ranking test before being qualified.

The panelists were first trained and calibrated during three 2 h sessions where they discussed and familiarized with a set of test samples and with the scale until reaching a consensus of the major sensory attributes and their quantification. The sensory attributes were presented on a 100 mm structured continuous line scale with word anchors “low” and “high” placed at the 5 mm and 95 mm mark respectively. A list of definitions and reference intensity ratings were developed during the training sessions and were available for all subsequent test sessions. The samples were presented as 4 x 3 cm Neapolitan pieces, with “chocolate” worded on it and weighing 5 g apiece. They were equilibrated to room temperature (22 °C) and served in 35 mL tasting cups encoded with random 3-digit numerical labels.

A further four sessions spanning two weeks was held for the product evaluation phase. All samples were evaluated in partitioned sensory booths under white light illumination and at room temperature. During each session, panelists first perform a standardized warm-up, evaluating one compound (CC<sub>2%</sub>) and one dark chocolate (DC<sub>2%</sub>) in this order. They then evaluate four compound (CC<sub>0%</sub> - CC<sub>3%</sub>) and four dark chocolates (DC<sub>0%</sub> - DC<sub>3%</sub>) in a randomized and counterbalanced order, with half the participants starting with compound chocolates and the other half starting with dark chocolates. Six attributes were



evaluated for dark chocolates (sweetness, bitterness, sourness, pastiness, graininess and aftertaste) and seven attributes were evaluated for compound chocolates (sweetness, bitterness, chocolate flavor, milky-ness, pastiness, graininess and aftertaste). Water and dry crackers were provided for rinsing between samples. For each attribute, the intensity ratings marked on the 100 mm line scale was measured and scaled to a range between 0 and 10 (with 0 and 10 corresponding to the ends of the line scale) and the mean values presented on spider charts.

### *2.7. Statistical analysis*

Statistical analysis was performed using the SPSS v. 16.0 (SPSS Inc., Chicago, IL, USA).

A one-way analysis of variance (ANOVA) was performed to determine if the mean values differ significantly from each group of varying mangosteen pericarp powder concentration.

The significance was established using Bonferroni post-hoc test. A probability level of  $p < 0.05$  was considered significant.

All data are expressed as means  $\pm$  standard deviations (SD) of at least three independent measurements.

## **3. Results**

### 3.1. Particle size distribution

As shown in table 1, the particle size for compound chocolates increased with pericarp powder concentrations, while DC<sub>1%</sub> and DC<sub>2%</sub> had the smallest particle sizes among the dark chocolates. Dark chocolates also had larger particle size distributions compared to compound chocolates. Nonetheless, the deviations between pericarp powder concentrations are small and with the exception of DC<sub>3%</sub>, the chocolate particle sizes are mainly under the acceptable limit of 30  $\mu\text{m}$  needed for a smooth sensation. A longer refining time should enable DC<sub>3%</sub> to achieve a similar particle size to the other chocolates. As the particle size distributions of the naturally obtained plant polyphenol extracts may vary largely, one could capitalize on the chocolate refining stage to effectively control the particle sizes of both chocolate and plant particles at the same time.

### 3.2. Microstructure Analysis

Cryo-SEM micrographs of the defatted chocolates show irregular cocoa and pericarp powders dispersed among larger faceted sugar crystals (Fig. 1A-D). The micrograph of defatted CC<sub>3%</sub> is similar to defatted CC<sub>0%</sub> except for a higher concentration of powders. Fig. 1C-D show that the bigger sugar crystals are responsible for the larger particle size distributions

of dark chocolate compared to compound chocolates. The micrograph of defatted DC<sub>3%</sub> is also similar to defatted DC<sub>0%</sub>.

Fig. 1E-H show the micrographs of the non-defatted chocolates. The compound and dark chocolate microstructure is different due to the different fat types, but there are no significant microstructural differences between the plain and enriched chocolates. The morphologies are also relatively homogeneous and the sugar crystals and powders are well incorporated within the fat matrix. This could have been facilitated during the conching phase since conching enables the solid particles to be coated with fat. Such a homogeneous fat matrix is ideal for visual and textural properties.

### *3.3. Heat treatment of mangosteen pericarp powder*

The mangosteen pericarp powder was heated at 80 °C for 7 h to simulate chocolate processing conditions and to test the stability of its polyphenols. The HPLC chromatograms show little variation in xanthenes peaks after 7 h of heating. The two main peaks (Fig. 2A) were identified to be  $\alpha$ -mangostin ( $m/z$  [M-H]<sup>-</sup> 409, max UV absorbance at 324 nm) and  $\beta$ -mangostin ( $m/z$  [M-H]<sup>-</sup> 423, max UV absorbance at 316 nm) respectively. There were insignificant changes to both the  $\alpha$ - and  $\beta$ -

mangostin peak areas (Fig. 3A) over 7 h, suggesting the xanthones would be stable under the processing conditions.

As seen in Fig. 2B, mangosteen pericarp powder contains mainly monomeric flavan-3-ol (peak a) and a relatively smaller amount of polymeric procyanidin (peak e). There was a sharp decrease in peak a accompanied by the introduction of higher order peaks after the first hour of heating (Fig. 2C). Based on work done by (Fu, Loo, Chia, & Huang, 2007) and (Yoshimura, Ninomiya, Tagashira, Maejima, Yoshida, & Amakura, 2015), peak a could be the epicatechin monomer, peak b the B2 dimer and peaks c to e could be higher oligomers. The formation of these higher order peaks after the first hour of heating could be due to the condensation of the monomeric flavan-3-ols to form oligomeric and polymeric procyanidins. The sharp drop could also have been exacerbated by condensation between anthocyanins with the flavan-3-ols as the loss of peak a is not equitably compensated by the total increase of higher order peaks. The remaining procyanidins were however stable to subsequent heating after the first hour (Fig. 3B), possibly suggesting a dynamic equilibrium between the condensation of flavan-3-ols and hydrolytic reactions of polymeric procyanidins. Overall, the heat treatment suggests that the xanthones and procyanidins would be stable under the chocolate processing conditions.

### 3.4. Total phenolic content

Table 2 shows the total phenolic content of mangosteen pericarp powder, plain, 1 % and 3 % pericarp powder-enriched dark and compound chocolates. There is a general increase in TPC with more pericarp powder added, and with the exception DC<sub>1%</sub>, the increase is within the expected range calculated using TPC of mangosteen pericarp powder. This further reflects the stability of the polyphenols to oxidation under the chocolate processing conditions.

Plain compound chocolates have about three times less TPC than plain dark chocolates. This is due to the lower cocoa content of compound chocolates. With the pericarp powder enrichment, there was a 13 % and 50 % increase in TPC for DC<sub>3%</sub> and CC<sub>3%</sub> compared to their plain counterparts respectively. Using only 3 wt% powder concentration increased the TPC of CC<sub>3%</sub> to almost half that of plain dark chocolates. This highlights the use of such low cost waste product as an effective method to significantly increase polyphenolic content especially in compound chocolates.

As the xanthones could not be detected in the defatted extracts under HPLC, they could have possibly been solubilized into the

lipid fraction during chocolate defatting with diethyl ether. The TPC of the defatted lipid fraction was found to be small ( $< 1$  mg GAE / g chocolate), suggesting that most of the TPC is contributed by flavonoids.

### 3.5. HPLC of chocolate samples

We investigated the changes in procyanidins and xanthones in the enriched chocolates. Fig. 3C shows the procyanidin peak areas for plain, 1 % and 3 % pericarp powder enriched dark and compound chocolates. Compared to plain dark chocolates, there was no significant ( $p > 0.05$ ) increase in procyanidins for DC<sub>1%</sub> except for peaks a and e, while there was an almost two-fold increase in procyanidins of all orders for DC<sub>3%</sub>. The insignificant increase in DC<sub>1%</sub> could be related to the much smaller than expected increase in TPC for DC<sub>1%</sub>. Plain compound chocolates had a much lower amount of procyanidins and the higher order oligomers could not be detected. This was significantly boosted in CC<sub>3%</sub> with an almost ten-fold increase in monomeric flavan-3-ols.

One might notice the comparatively smaller flavan-3-ol contribution of 3 % pericarp powder to compound chocolate than to dark chocolate. This could be attributed to the matrix effect differences of palm oil and cocoa butter on the solvent

extraction propensity. Nevertheless, we would expect similar procyanidin contributions to both dark and compound chocolates.

Comparing dark and compound chocolates enriched with the same concentration of pericarp powder, we do however find similar amounts of  $\alpha$ -mangostin or  $\beta$ -mangostin (Fig. 3D). There is also a linear relationship between the amount of pericarp powder added and the amount of xanthenes found in the chocolates. Interestingly, while there was more  $\beta$ -mangostin than  $\alpha$ -mangostin in the pericarp powder (Fig. 3A), the reverse is true here. Overall, the pericarp powder is able to expand the bioactive profile of the chocolates, and especially so in compound chocolates.

### 3.6. Sensory evaluation

Fig. 4A-B shows the sensory evaluation of the compound and dark chocolates. Except for graininess, the sensory attributes did not differ significantly ( $p > 0.05$ ) between the plain and pericarp powder enriched chocolates for both the compound and dark chocolates. And only the chocolates containing 3 % powder were significantly grainier than their plain counterparts. The increasing graininess with pericarp powder concentration could be attributed to the increasing particle size (Table 1).

(Beckett, 2000) concluded that large particle sizes would lead to a gritty mouthfeel while small particle sizes lead to higher viscosity due to increased surface area of particles in contact with fat. Such an inverse relationship is seen between the graininess and pastiness of the chocolates. An ideal particle size range is between 15-30  $\mu\text{m}$  for a desirable mouthfeel (E. O. Afoakwa, Paterson, & Fowler, 2007), and a longer refining time for the higher pericarp powder concentration chocolates would likely achieve this.

#### 4. Discussion

Overall, the particle size distributions of the chocolates were mostly within the acceptable range ( $< 30 \mu\text{m}$ ) and the chocolates displayed a homogeneous microstructure. The polyphenols (procyanidins and xanthenes) in mangosteen pericarp powder were also stable to simulated chocolate processing. Chocolate makers could potentially add the plant polyphenols during the mixing stage, since this would take advantage of the refining and conching phases while preserving the added polyphenols. In addition, the bioactive profile and total phenolic content of the enriched chocolates were expanded without significantly affecting the sensory qualities. (Larrauri, 1999) suggested that the 'ideal dietary fibre' should be bland in taste, colour, texture and odour, and is compatible



with food processing. In these respects, mangosteen pericarp powder presents itself as a suitable candidate.

While cocoa is rich in healthful flavonoids, such benefits can only be derived through the consumption of high cocoa content chocolates. A large number of chocolate confectionery products are unfortunately made using low cocoa content compound chocolates and consumers do not maximally benefit from these cocoa polyphenols. Cost and sensory qualities are among the most important factors to chocolate makers. With such considerations, mangosteen pericarp powder, being a low cost agricultural waste product acts as a model for other plant polyphenol sources to enhance the nutritional content of chocolate confectionery products made with compound chocolates. Other possible low cost agricultural waste products with high bioactive properties could include fruit and vegetable peels like banana, mango and potato (Faller & Fialho, 2010). The bioactive compounds must similarly be stable under chocolate processing and not significantly affect the sensory qualities. Furthermore, some fruit peels such as citrus fruit peels may have subtle aromatic properties that could enhance the natural flavor palette of the chocolates, especially for compound chocolates with a limited flavor profile. It would be interesting if the flavor and nutritional properties of these

enriched compound chocolates can match that of high quality dark chocolates but at a significantly lower cost.

As a low polyphenol concentration is crucial to flavor development in the roasting stage (Misnawi, Jinap, Jamilah, & Nazamid, 2004; Noor-Soffalina, Jinap, Nazamid, & Nazimah, 2009), a potential strategy to maximize both flavor and nutritional properties is to reduce the initial polyphenol content before roasting and to supplement the loss using plant polyphenols at a later processing stage. Additionally, non-cocoa polyphenols introduced to pre-roasted cocoa liquor might lead to novel flavor products during roasting. Free amino acids, peptides and reducing sugars are central to cocoa flavor development via the Maillard reactions (E. O. Afoakwa, Paterson, Fowler, & Ryan, 2008) . In particular, hydrophobic amino acids such as alanine, tyrosine, leucine and phenylalanine are some important contributors. While epicatechins, catechins and procyanidins in cocoa are mainly hydrophilic, foreign xanthenes from mangosteen pericarp are hydrophobic and may bind to these hydrophobic amino acids, impacting the subsequent flavor development. Moreover, flavor precursors from these plant sources may lead to interesting cross-reactions during roasting. The potential of using these plant polyphenols for novel flavor development may be limited only by imagination.

## 5. Conclusion

In this study, a low cost agricultural waste product – mangosteen pericarp - was added as a source of plant polyphenols to dark and compound chocolates during the mixing stage and evaluated. The particle size distributions of the chocolates were mostly within 30  $\mu\text{m}$  and the chocolates displayed a homogeneous morphology. The polyphenols (procyanidins and xanthenes) in mangosteen pericarp powder were also stable to simulated chocolate processing. The 3 % pericarp powder concentration significantly expanded the bioactive profile and total phenolic content of the chocolates without affecting the sensory qualities. This serves as a model for the use of plant polyphenols to be low cost solutions to enhance the nutritional and sensory properties of chocolates, especially in low cocoa content compound chocolates.

## Conflict of interest

None of the authors declare any conflict of interest.

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**Table 1**

Particle size distribution of plain and enriched chocolates

	Particle size distribution [ $\mu\text{m}$ ]			Surface weighted mean D[3,2]	Volume weighted mean D[4,3]
	d(0.1)	d(0.5)	d(0.9)		
<i>Dark Chocolate</i>					
DC <sub>0%</sub>	1.63 $\pm$ 0.05 <sup>a</sup>	8.77 $\pm$ 0.20 <sup>a</sup>	30.78 $\pm$ 0.28	2.97 $\pm$ 0.09	13.06 $\pm$ 0.14
DC <sub>1%</sub>	1.47 $\pm$ 0.04 <sup>b</sup>	8.22 $\pm$ 0.49 <sup>a</sup>	24.85 $\pm$ 1.51 <sup>a</sup>	2.68 $\pm$ 0.09 <sup>a</sup>	11.43 $\pm$ 0.54 <sup>a</sup>
DC <sub>2%</sub>	1.42 $\pm$ 0.01 <sup>b</sup>	7.89 $\pm$ 0.03 <sup>bc</sup>	23.88 $\pm$ 0.18 <sup>a</sup>	2.62 $\pm$ 0.01 <sup>a</sup>	11.57 $\pm$ 0.11 <sup>a</sup>
DC <sub>3%</sub>	1.60 $\pm$ 0.10 <sup>a</sup>	9.77 $\pm$ 0.11	41.00 $\pm$ 1.02	3.15 $\pm$ 0.03	16.61 $\pm$ 0.22
<i>Compound Chocolate</i>					
CC <sub>0%</sub>	1.19 $\pm$ 0.02 <sup>c</sup>	5.51 $\pm$ 0.11	13.54 $\pm$ 0.31	2.09 $\pm$ 0.04 <sup>b</sup>	6.54 $\pm$ 0.13
CC <sub>1%</sub>	1.14 $\pm$ 0.02 <sup>c</sup>	6.33 $\pm$ 0.07	17.23 $\pm$ 0.17	2.12 $\pm$ 0.03 <sup>bc</sup>	8.23 $\pm$ 0.12
CC <sub>2%</sub>	1.18 $\pm$ 0.06 <sup>c</sup>	7.47 $\pm$ 0.20 <sup>bd</sup>	23.71 $\pm$ 0.82 <sup>a</sup>	2.26 $\pm$ 0.10 <sup>cd</sup>	11.83 $\pm$ 0.39 <sup>a</sup>
CC <sub>3%</sub>	1.23 $\pm$ 0.07 <sup>c</sup>	7.90 $\pm$ 0.29 <sup>acd</sup>	21.88 $\pm$ 0.82	2.34 $\pm$ 0.13 <sup>d</sup>	10.56 $\pm$ 0.20

Values superscripted sharing the same alphabet are not significantly ( $p > 0.05$ ) different. One-way ANOVA was conducted within each column.

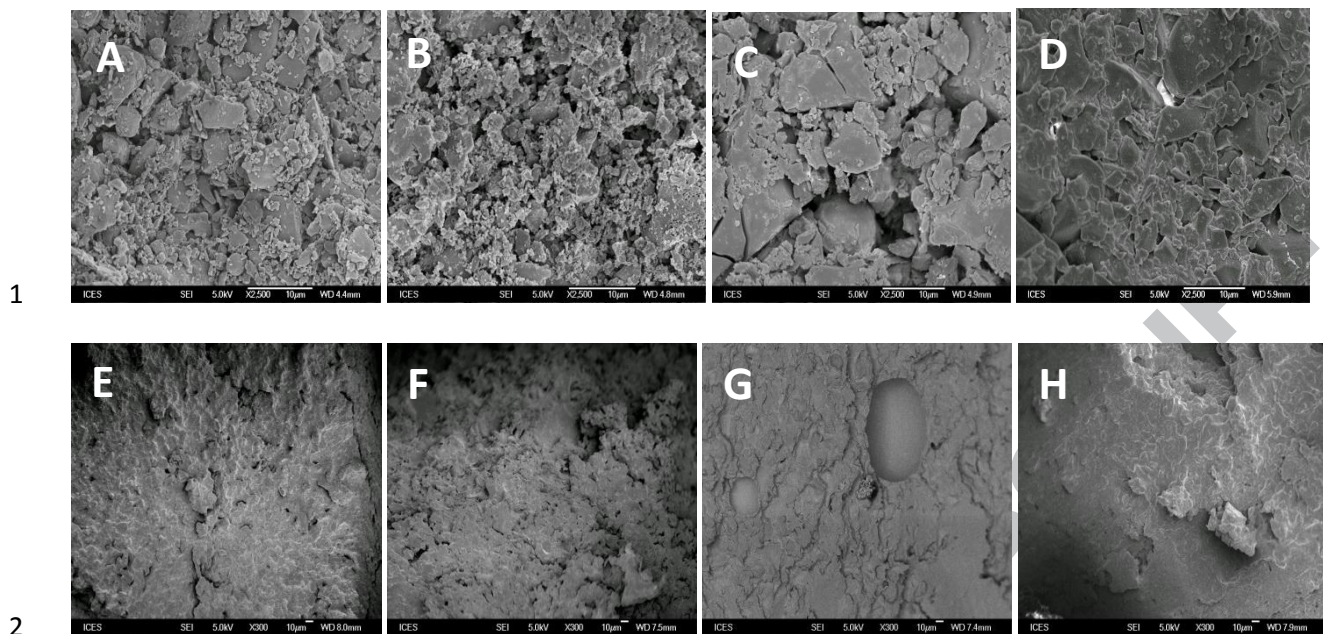
**Table 2**

Total phenolic content of mangosteen pericarp powder, dark and compound chocolates.

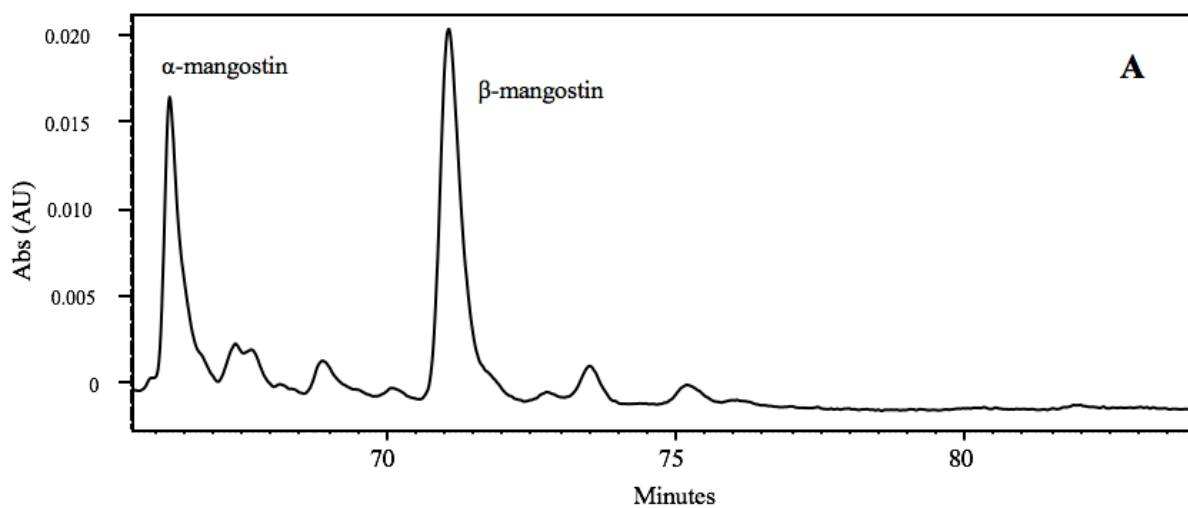
Total phenolic content mg GAE / g chocolate	Dark Chocolates			Compound Chocolates		
	DC <sub>0%</sub>	DC <sub>1%</sub>	DC <sub>3%</sub>	CC <sub>0%</sub>	CC <sub>1%</sub>	CC <sub>3%</sub>
	23.88 $\pm$ 0.74 <sup>a</sup>	24.08 $\pm$ 0.59 <sup>a</sup>	27.10 $\pm$ 0.53	7.71 $\pm$ 0.18	8.89 $\pm$ 0.25	11.60 $\pm$ 0.25
<i>Actual Difference vs 0%</i>		0.20 $\pm$ 1.33	3.22 $\pm$ 1.27		1.18 $\pm$ 0.43	3.89 $\pm$ 0.43
<i>Expected Difference vs 0%</i>		1.43 $\pm$ 0.07	4.29 $\pm$ 0.20		1.43 $\pm$ 0.07	4.29 $\pm$ 0.20
TPC of mangosteen pericarp powder: 143.23 $\pm$ 6.75 mg GAE / g of pericarp powder						

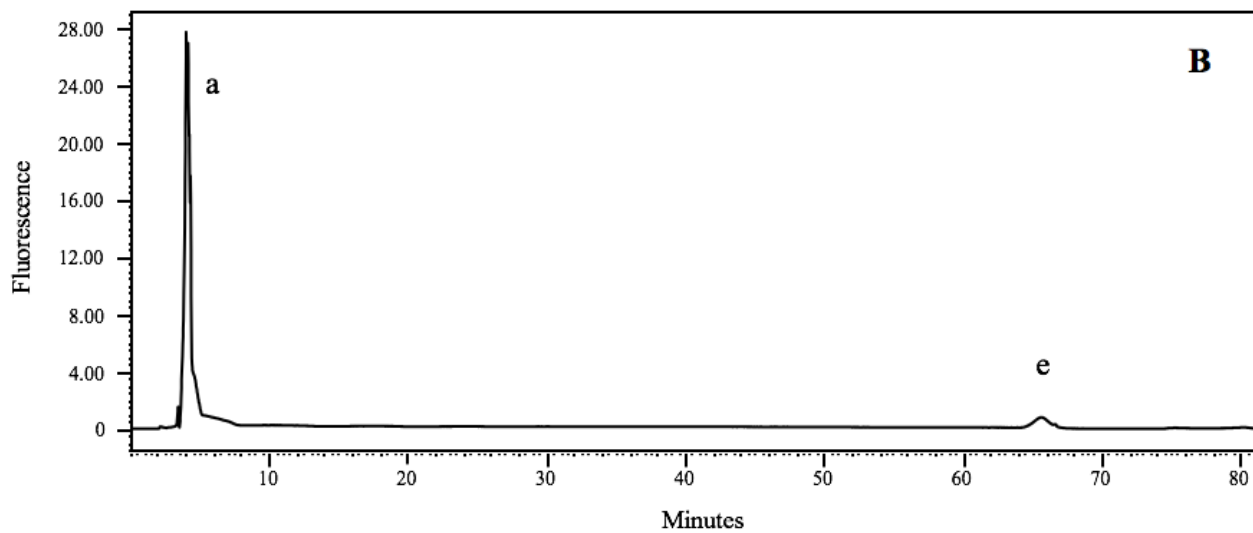
Values superscripted with the same alphabet are not significantly ( $p > 0.05$ ) different.



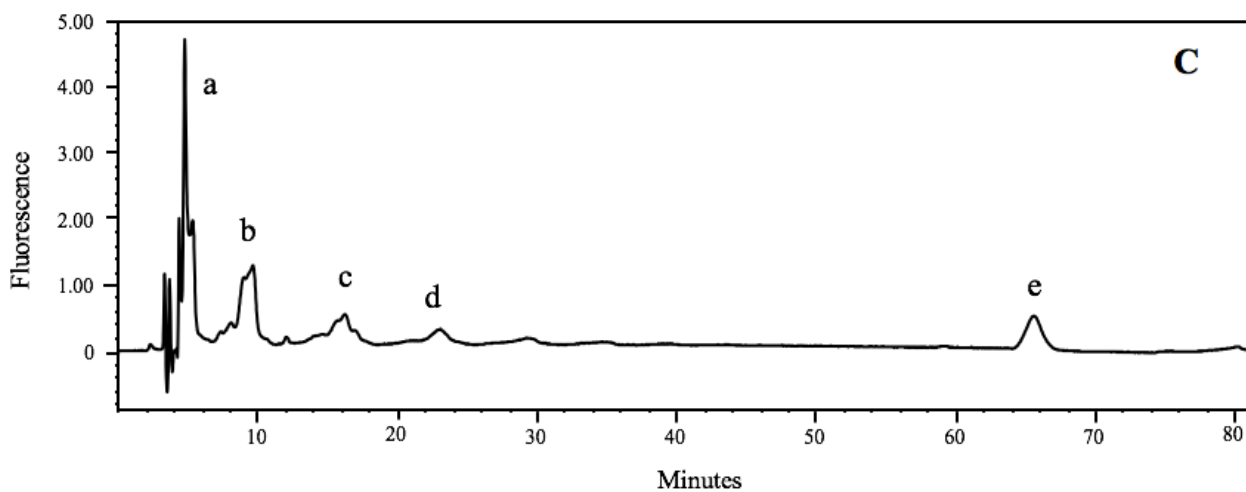


3 **Fig. 1.** Cryo-SEM micrographs showing morphology of plain and pericarp powder enriched chocolates. (A) defatted CC<sub>0%</sub>. (B)  
4 defatted CC<sub>3%</sub>. (C) defatted DC<sub>0%</sub>. (D) defatted DC<sub>3%</sub>. (E) CC<sub>0%</sub>. (F) CC<sub>3%</sub>. (G) DC<sub>0%</sub>. (H) DC<sub>3%</sub>.



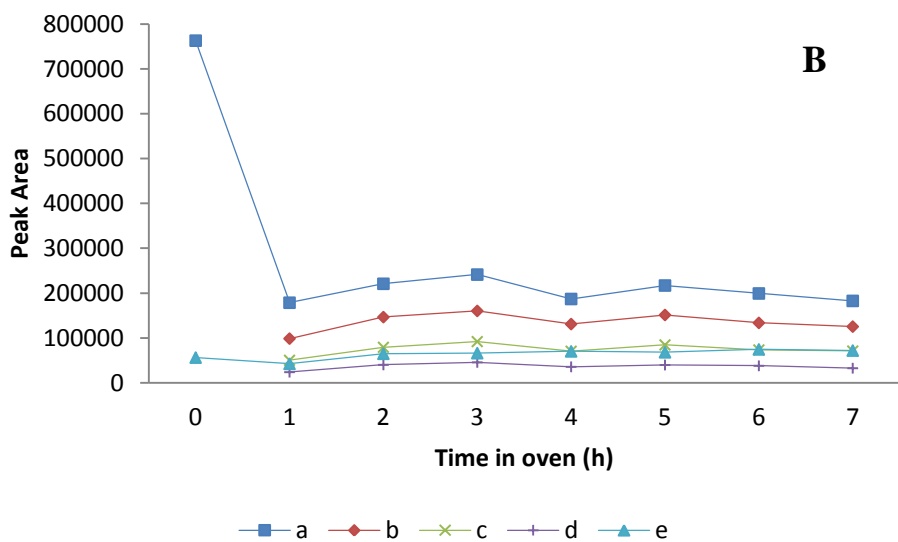
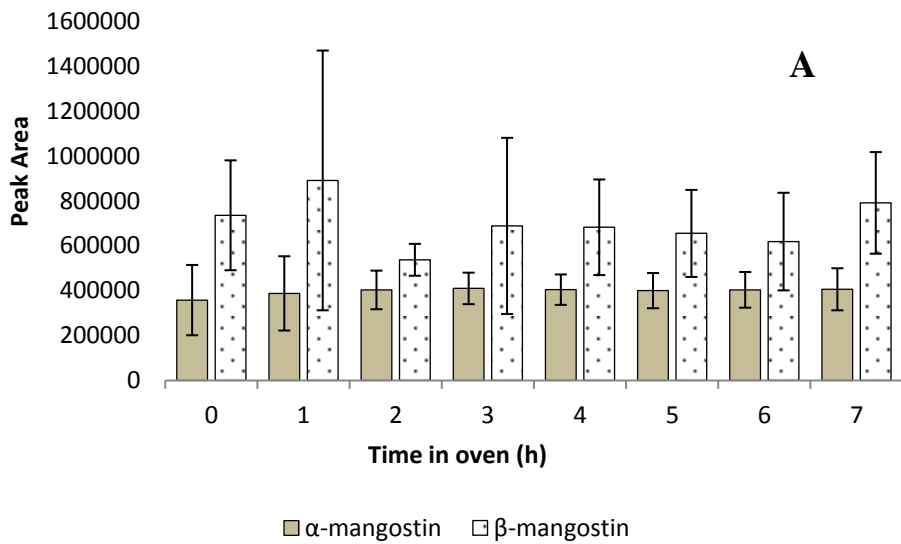


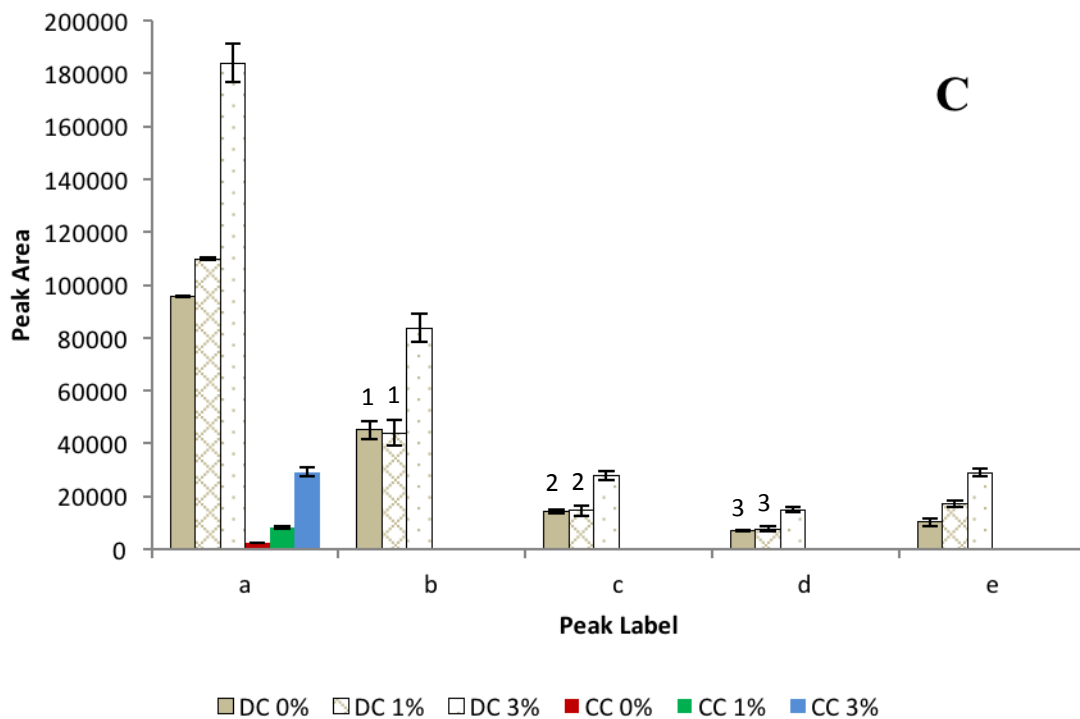
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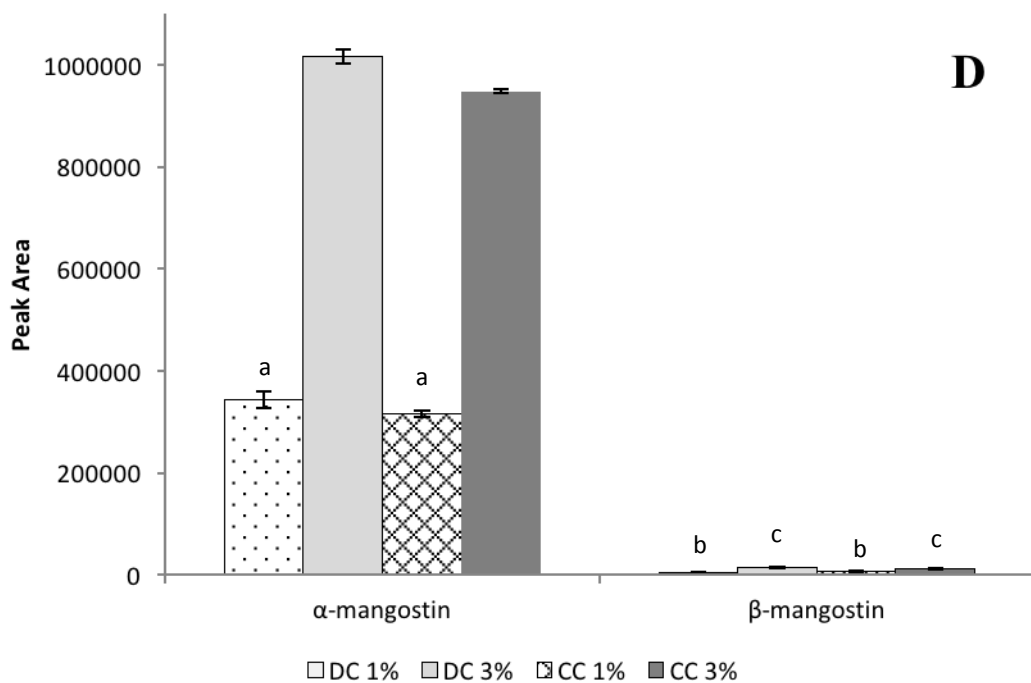
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8 **Fig. 2.** HPLC chromatogram of polyphenols in mangosteen pericarp powder. (A) xanthones, (B) procyanidins, (C) procyanidins  
9 after 1 h of heating at 80 °C. Peaks a to e correspond to the monomer, dimer and higher oligomers of procyanidins.





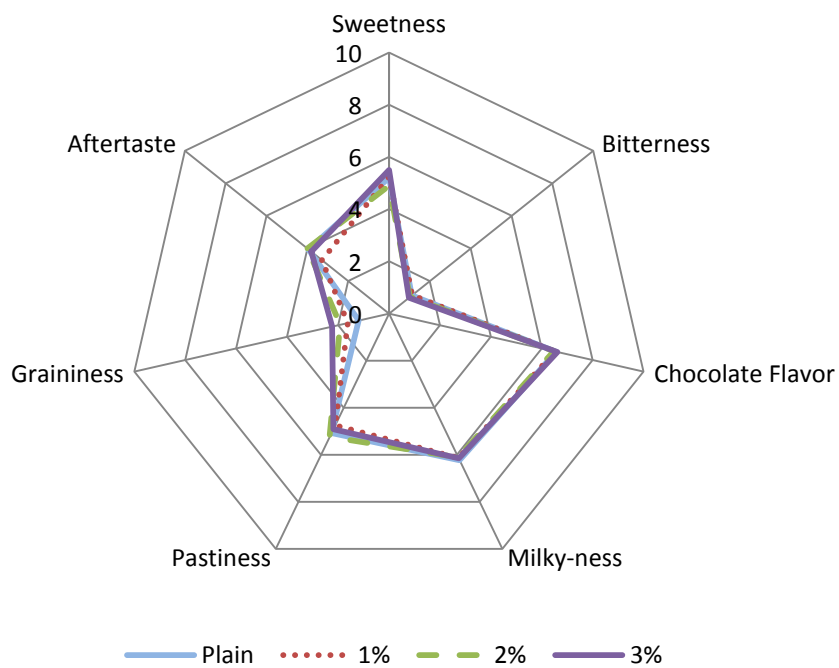
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14 **Fig. 3.** Polyphenol peak areas of (A)  $\alpha$ -mangostin and  $\beta$ -mangostin, (B) labelled procyanidin peaks, in mangosteen pericarp  
 15 powder over 7 h of heating at 80 °C. Polyphenol peak areas of (C) labelled procyanidins peaks, (D)  $\alpha$ -mangostin and  $\beta$ -mangostin  
 16 in plain, 1% and 3% pericarp powder-enriched dark and compound chocolates. Procyanidin peaks a to e correspond to the

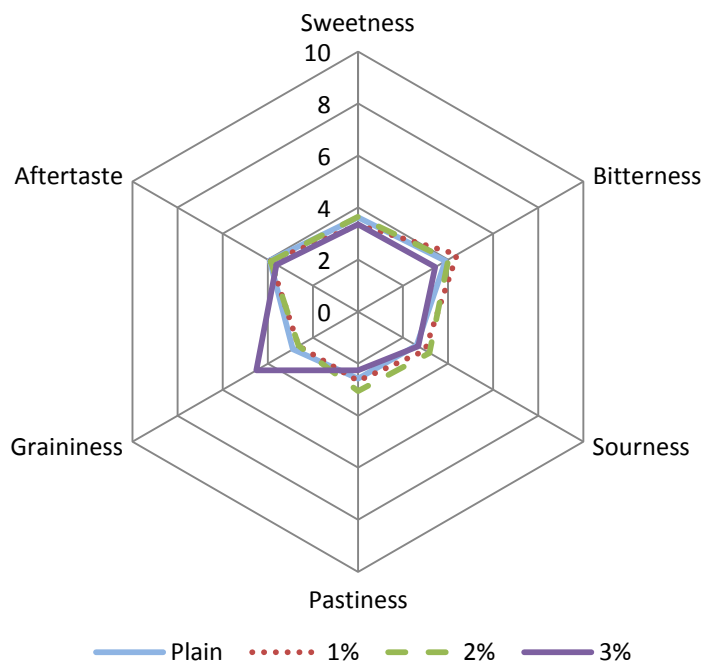
17 monomer, dimer and higher oligomers of procyanidins. Peak labels with the same number or alphabet are not significantly  
18 ( $p>0.05$ ) different. Statistical analysis was not done for (A) and (B) as they were not carried out in triplicate.

**A**

19

ACCEPTED

B



20

21 **Fig. 4.** Spider chart representing mean scores of the evaluated sensory attributes for (A) compound chocolates and (B) dark  
 22 chocolates with different concentrations of mangosteen pericarp powder. Only the 3% pericarp powder samples were significantly  
 23 ( $p < 0.05$ ) different in graininess to the other samples, for both dark and compound chocolates. The other attributes were not  
 24 significantly different between samples of different pericarp powder concentrations, for both dark and compound chocolates.

1. Mangosteen pericarp improved phenolic content without affecting sensory quality.
2. Plant polyphenols could boost flavor and bioactive profile of compound chocolates.
3. Plant polyphenols could be added during the mixing stage.

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