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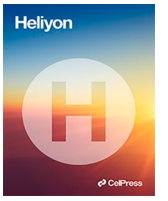

Research article

Characterization of microencapsulated powders rich in saponins from cocoa pod husk (*Theobroma cacao* L.) and medicinal plant an xoa (*Helicteres hirsuta* Lour.)

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A B S T R A C T

Cocoa pod husk (CPH) is a major residue of cocoa processing industry, while medicinal plant *H. hirsuta* is used for treatment of malaria and diabetes mellitus in folk medicine. This study aimed to produce microencapsulated powders from saponin-enriched CPH and *H. hirsuta* extracts and assess their physicochemical, phytochemical, antioxidant, and α-glucosidase inhibition properties. The findings show that the microencapsulated powders were achieved diserable physicochemical properties (moisture of 3.22–4.76 %, water activity of 0.43–0.46, water solubility index of 74.18–88.77 %, particle size of 254.2–719.7 nm, and zeta potential from −6.97 to −15.1 mV). The phytochemical content of microencapsulated CPH powders gained at high levels (total saponin content of 151.87–193.46 mg EE/g DS, total flavonoid content of 33.80–46.05 mg CE/g DS), total alkaloid content of 15.20–24.23 mg AA/g DS, and total phenolic content of 5.41–6.49 mg GAE/g DS). The antioxidant potential of microencapsulated CPH powders using ARSC and FRAP assays was 15.51–18.20 and 9.61–11.89 mg TE/g DS, respectively, while their α-glucosidase inhibition capacity at 100 μg/mL was found at 51.74–52.16 %. The phytochemical content (except total alkaloid content), antioxidant, and α-glucosidase inhibitory potential of microencapsulated CPH powders were smaller than those of microencapsulated *H. hirsuta* and combined powders. This study reveals that the microencapsulated CPH and *H. hirsuta* powders were prospective in reducing hyperglycemia activity. Therefore, this study provided an evidence for further application of CPH and *H. hirsuta* plant for functional food development.

1. Introduction

Diabetes Mellitus (DM) is a metabolic disease involving problems relating to insulin insufficiency and/or insulin resistance of target tissue. The World Health Organization classified DM into 2 major groups: Type I DM and Type II DM (T2DM), where 90–95 % of cases were diagnosed suffer from T2DM [1]. The hallmark of T2DM is hyperglycemia, which promotes mitochondrial dysfunction and the generation of reactive oxygen species (ROS), resulting in the development of numerous diabetic complications [2]. The status of high blood glucose levels, particularly postprandial rise in blood glucose results from an increasing rate of glucose absorption when intestinal α-glucosidase catalyzes the hydrolysis of carbohydrate [3]. Thus, there has been a remarkable trend that the secondary metabolites extracted from plants have been used as one of the most effective strategies to control T2DM by targeting α-glucosidase and ROS.

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Theobroma cacao Lour. originates from the rainforest regions of the Central and South America. The tree is classified as family *Malvaceae*, genus *Theobroma*, and species *Cocoa*. Cocoa cultivars can be classified into four types: Forastero, Criollo, Trinitario, and Nacional. Criollo was the dominant cultivar until the mid-eighteenth century when Forastero took over due to its stronger and more resilient traits, resulting in higher yields (representing 95 % of global cocoa production). However, Criollo possesses a more refined taste. Trinitario, originating from the lower part of the Amazon, combines the robustness of Forastero with the fine flavor of Criollo. Nacional is believed to have originated in Ecuador and, despite being less widely cultivated, it offers superior flavor and aroma [4]. In recent years, Vietnam has emerged as a larger cocoa producer, primarily cultivating a hybrid of Criollo and Trinitario (62.5 %), followed by Lower Amazon (26.25 %) and Upper Amazon Forastero cultivars (11.25 %) [5]. The cocoa industry is a billion-dollar industry, the combined value of exported cocoa beans witnessed 8.6 billion USD in 2017 and the annual growth rate for the global cocoa market is estimated to be 7.3 % from 2019 to 2025 [6]. Cocoa pod husk (CPH), which is assumed as a wasted residue that constitutes 67 % of the cocoa fresh weight, has been utilized as fertilizer, feed for livestock, and an alkaline source for soap manufacturing [7]. However, these applications could not exploit the potential value of CPH although several previous studies have indicated the presence of bioactive compounds in this material. The identification of gallic acid, quercetin, catechin, (−)-epicatechin, coumaric acid, and protocatechuic acid in both fresh and dried CPH has been done [8]. The retention of phenolic acids, flavonols, flavones, terpenoids, and fatty acids was reported in cocoa pod extract as well as its better antioxidant activity, anti-wrinkle, and inhibiting the tyrosinase enzyme [9]. The saponin-enriched CPH extract prepared by microwave assisted-extraction revealed high contents of total saponins, phenolics, and flavonoids as well as a strong antioxidant capacity [10]. The microencapsulated phenolic-enriched CPH powder was shown to have a high retention of total phenolic, flavonoid, and alkaloid contents, the strong ABTS radical scavenging ability and cupric ion reducing antioxidant power [11]. However, very few studies have been carried out to microencapsulate saponin-enriched CPH extract and evaluate its antidiabetic activity in the prospect of innovating a new functional food and reducing the impact on the environment.

Helicteres hirsuta Lour. is a traditional Southeast Asian medicine used for the treatment of malaria and diabetes mellitus [12]. The high contents of total phenolics, flavonoids, and saponins as well as the strong antioxidant capacity of *H. hirsuta* have been reported [13]. Due to its rich source of bioactive compounds, *H. hirsuta* has demonstrated its numerous health benefits including antioxidant, antimicrobial, anti-inflammatory, anticancer activity, and hepatoprotective effects. Six lignans from the *H. hirsuta* were isolated and identified, of which (±)-pinoresinol performed potent cytotoxic effects against a small panel of cancer cell line [14]. The high results of the activity against microorganisms including *E. coli* and *S. lugdunensis* and cancer cell lines (MIA PaCa-2, A2780, H460, A431) were revealed for the saponin-enriched fractions from the *H. hirsuta* [15]. Hepatoprotective effects of the *H. hirsuta* extract with methanol and ethanol were determined to protect the liver against fibrosis caused by CCl_4 in rats [16]. The aerial part of the *H. hirsuta* extracted with several solvents exhibited COX-1/COX-2 enzyme which is involved in prostaglandin biosynthesis, thus showed the potent anti-inflammatory activity of the bioactive compounds isolated from the *H. hirsuta* [17]. Although there are numerous studies demonstrating the activity of the *H. hirsuta* against oxidative stress, cancer, inflammation, and liver fibrosis. However, there is limited investigation related to the antidiabetic activity of the *H. hirsuta* plant as the evidence agents of traditional treatment.

Microencapsulation is a technique, in which the functional constituents are entrapped by the polymeric or nonpolymeric materials and released under particular conditions. Thus, these active components could be protected from extrinsic factors such as UV light, oxygen, temperature, and pH, as well as exhibit better physicochemical and sensory qualities and prolong their shelf life. With this in mind, this study aimed to prepare the saponin-enriched CPH extract using the previously optimized conditions for drying and extraction methods. Then, the saponin-enriched CPH extract was microencapsulated and assessed its physicochemical, phytochemical, and biological properties including antioxidant and α-glucosidase inhibitory capacity. In addition, the microencapsulated saponin-enriched *H. hirsuta* extract was used as a comparative sample and a combination of two these extracts was also prepared and evaluated accordingly.

2. Materials and methods

2.1. Analytical reagents and chemicals

All the chemicals used in this research were of analytical grade. Absolute chloroform, methanol, sodium nitrite, aluminum chloride, sodium hydroxide, Bromocresol green, citric acid, disodium phosphate, ABTS, potassium persulfate, sodium acetate, glacial acetic acid, monosodium phosphate and assay standard including escin, gallic acid, catechin, atropine, trolox were purchased from Sigma Aldrich (Missouri, United States) and Tokyo Chemical Industry Co. Ltd., (Tokyo, Japan). Folin-Ciocalteu reagent and 2, 4, 6-tripyridyl-*s*-triazine (TPTZ) were achieved from Shanghai Lanji Technology Development Co., Ltd. (Shanghai, China). Sodium carbonate anhydrous and iron (III) chloride were bought from Guangdong Guanghua Sci-Tech Com., Ltd. (Guangdong, China) Vanillin, sulfuric acid, and hydrochloric acid were purchased from Xilong scientific (Shantou, China). α-glucosidase, p-nitro-phenyl-α-D-glucopyranoside, and acarbose were purchased from Nanjing Duly Biotech Co. Ltd., (Nanjing, China). Encapsulated materials applied in this study were of food grade. Maltodextrin and gum Arabic were purchased from HiMedia Laboratories (Maharashtra, India) and Merck KGaA (Darmstadt, Germany).

2.2. Sample preparation

2.2.1. Saponin-enriched CPH extract

The ripe fresh cocoa fruits (*Theobroma cacao Trinitario*) were bought from the Thanh Trieu commune belonging to Chau Thanh

district of Ben Tre province in Vietnam (latitude 10.1732°N, longitude 106.1550°E) on April 24th, 2023. Once cocoa beans were removed from CPH, the CPH was sliced into thin pieces which were approximately 3 cm in thickness. The fresh CPH slices were packed in PA zip bags, stored in a freezer at −20 °C for the following stages, and would be thawed at room temperature (RT) if required. The CPH was dried using the microwave drying method, of which the parameter was formerly optimized at a power of 720 W and radiation time of 15 s/min [18]. Then, the dried CPH sample was ground into powder (particle size ≤1 mm) and stored in a freezer until used. The herbarium specimen of 240423 has been placed at the Research, Development and Teaching Group on Functional Foods (RDTGFF) Lab, Nha Trang University, Vietnam.

The extract of the CPH was accomplished by microwave-assissted extraction (MAE) method according to the previous research [10]. Initially, 30 g dried CPH powder was macerated in 1000 mL methanol 85 % for 30 min of pre-leaching. After that, the mixture was heated under 20 s at 600 W in the microwave three times, then applied MAE at the following conditions: power (600 W), radiation time (6 s/min), and extraction time (40 min). Then, the extract was rapidly cooled to RT in an ice water bath and the solid particles were entirely removed by vacuum filtration (Rocker 300, Taiwan). The condensed CPH extract was prepared using a vacuum rotary evaporator at 65 °C temperature, 120 mbar pressure, and 60 rpm velocity.

2.2.2. Saponin-enriched H.hirsuta and combined extracts

A similar protocol of sampling *H. hirsuta* plant as the protocol of sampling CPH, while the protocol of preparation the saponin-enriched extracts was also applied to the *H. hirsuta* and combined extracts with some modifications. Briefly, the *H. hirsuta* plant was collected from Vinh Tho commune, Nha Trang city, Khanh Hoa province, Vietnam (latitude of 12.27°N, longitude of 109.20°E) on April 25th, 2023 and being identified by the National Medicinal Materials Institute belonging to Health Ministry in Vietnam according to Circular 38/2021/TT-BYT, then cut into small chunks (about 3 cm length) and stored in a freezer for subsequent processing. The dried *H.hirsuta* was obtained by hot-air drying at 90 °C and air-flow of 30 % until dry [19], then ground into the powder (particle size ≤1 mm). The herbarium specimen of 250423 has been placed at the Group of Research, Development and Teaching on Functional Foods (RDTGFF) Lab, Nha Trang University, Vietnam.

The powder was soaked in methanol 100 % for 30 min before the *H. hirsuta* extract was carried out by utrasound-assisted extraction (UAE) method using an ultrasonic bath (Elmasonic, Germany). The extraction conditions applied for the *H. hirsuta* followed the former research and were described as temperature (60 °C), sample to solvent ratio (30 g/L), and extraction time (25 min) [20]. Afterward, the extract was filtered by vacuum filtration method and condensed using a vacuum rotary evaporator at the same parameter settings as the CPH extract. The combined extract was prepared by mixing the CPH and *H. hirsuta* condensed extract at a 1:1 (w/w) ratio.

2.2.3. Microencapsulated saponin-enriched CPH, H. hirsuta and combined powders

In this study, microencapsulation was used to protect bioactives and extend the shelf life of the extracts and powders for further application using the polymeric materials [21]. The mixture of maltodextrin combined with gum Arabic at a 8:2 (w/w) ratio was preferred according to previous research [11], which was indicated to have the best protection compared to chitosan, carrageenan, gelatin, and maltodextrin only. After hydration in the fridge at 4 °C overnight, the encapsulating mixture was homogenized with the CPH, *H. hirsuta*, and combined condensed extracts in the room condition (temperature of 25–26 °C) using a blender (Koninklijke Philips N.V., Netherlands) at the default velocity for 20 s. The ratio of extract to coating material mixture and the ratio of two components in the coating material mixture was briefly described in Table 1. The homogenous mixture then was subjected to freeze-drying at −40 °C, 0.01 mbar for 48 h to obtain the dried powders. The microencapsulated saponin-enriched CPH, *H. hirsuta*, and combined powders were then packed in zipped PA bags and kept at −20 °C in a freezer for further analysis.

The non-microencapsulated samples (control) were also used for evaluation and comparison of phytochemical, antioxidant, and α-glucosidase inhibition properties. The control samples were prepared by mixing maltodextrin combined with gum Arabic (8:2 w/w) with the CPH, *H. hirsuta,* and combined extracts at the same ratio as the microencapsulated samples.

To prepare the solutions for analysis, the microencapsulated and non-microencapsulated saponin-enriched CPH, *H. hirsuta,* and combined powders were soaked in 20 mL methanol 100 % at 50 °C for 10 min in a water bath before being dissolved using a vortex mixer for 5–10 min to fasten the solubility. The insoluble particles were removed using Newstar quantitative filter paper. The liquid

Table 1
The ratio of extract to coating material mixture and the ratio of 2 components in coating material mixture.

Sample	Extract (%)	Total coating materials (%)	Components of coating materials	
			Maltodextrin (%)	Gum Arabic (%)
C0	45	55	80	20
H0	50	50		
CH0	50	50		
C1	50	50	80	20
C2	40	60		
H1	50	50		
CH1	50	50		

C1: microencapsulated saponin-enriched CPH extract at 1:1 (w/w) ratio of extract and coating material mixture, C2: microencapsulated saponin-enriched CPH extract at 1:1.5 (w/w) ratio of extract and coating material mixture, H1: microencapsulated saponin-enriched *H. hirsuta* extract, CH1: microencapsulated saponin-enriched combined extract. C0: non-microencapsulated saponin-enriched CPH extract, H0: non-microencapsulated saponin-enriched *H. hirsuta* extract, CH0: non-microencapsulated saponin-enriched combined extract.

part containing bioactive components was stored at −20 °C in a freezer until tested.

2.3. Physicochemical properties analysis

2.3.1. Moisture analysis

The moisture content of microencapsulated powders was determined based on the method published by AOAC International [22], where 0.5 g of each powder was dried by a hot-air oven from Memmert at Schwabach in Germany at 105 °C until it achieved a balanced mass.

2.3.2. Water activity measurement

The water activity (a_w) of microencapsulated powders was measured by a water activity meter named Rotronic Hygrolab C1 from Rotronic Instruments Pte Ltd at Bukit Merah Central in Singapore at 25 ± 0.5 °C [23].

2.3.3. Water-soluble index (WSI) determination

Briefly, 0.5 g of microencapsulated powders was dissolved in 10 ml of deionized water. The mixture then was fastened to solubility using a vortex mixer, and filtered through dried, pre-weighted Newstar quantitative filter paper. The filter paper containing supernatant was then dried by a hot-air oven from Memmert at Schwabach in Germany at 105 °C until it achieved a balanced mass. The WSI was calculated based on the following equation (1) [24].

$$WSI = \frac{TDS}{TDP} \times 100 \quad (1)$$

Where: WSI assigns for water soluble index (%). TDS and TDP assign for total weight in g of dried supernatant and dried powder, respectively.

2.3.4. pH measurement

The solution collected after the determination of WSI was reused to measure its pH using a pH meter from Mettler Toledo in Switzerland calibrated with standard solutions at RT [23].

2.3.5. Microstructure analysis

The morphology of microencapsulated powders was visualized using a scanning electron microscope (SEM) JSM IT200 from JEOL Ltd. at Tokyo in Japan. The sample was mounted onto a sample holder with double-adhesive conductive tape that was coated by gold particles in a vacuum chamber. The surface structure was performed by SEM at various magnifications which operates at an accelerating voltage of 10 kV [24].

2.3.6. Particle size distribution and zeta potential

The median diameter (d.nm) and zeta potential (mV) of microencapsulated powders were evaluated by a particle size distribution and zeta potential analyser (Malvern, United Kingdom).

2.4. Phytochemical compounds analysis

2.4.1. Total saponin content (TSC)

TSC of saponin-enriched samples with and withoutmicroencapsulation was analyzed according to the described method with some modifications [25]. Escin was used as a standard. Initially, 0.25 mL sample or escin at various concentrations from 300 to 2500 μg/mL was mixed with 0.25 mL solution of vanillin 8 % (w/v) prepared in methanol 100 % and 2.5 mL H_2SO_4 72 % (v/v) solution. The mixture was hold at 70 °C for 15 min before rapidly cooling to RT in an ice water bath. The mixture absorbance was measured at a wavelength of 560 nm using a UV-VIS spectrophotometer (Biochrom Ltd., UK). Methanol 100 % was used as a control sample. The TSC was calculated as mg escin equivalents (EE)/g dried sample (DS).

2.4.2. Total phenolic content (TPC)

TPC of saponin-enriched samples with and withoutmicroencapsulation was estimated according to the described method with some modifications [26]. Gallic acid was used as a standard sample. Briefly, 0.5 mL sample or gallic acid at various concentrations from 20 to 100 μg/mL were mixed with 2.5 mL solution of Folin-Ciocalteu reagent 10 % (v/v) dilluted in deionized water and stabilized for 6 min. Then, the mixture was added to 2 mL solution of Na_2CO_3 7.5 % (w/v) prepared in deionized water and kept for 1 h in the dark at RT. The mixture absorbance was measured at a wavelength of 765 nm. Methanol 100 % was used as a control sample. The TPC was calculated as mg gallic acid equivalents (GAE)/g dried sample (DS).

2.4.3. Total flavonoid content (TFC)

TFC of saponin-enriched samples with and withoutmicroencapsulation was quantified based on the reported work with some changes [25]. Catechin was used as a standard sample. Firstly, the mixture of 0.5 mL sample or catechin at various concentrations from 2.5 to 100 μg/mL, 2 mL deionized water, and 0.15 mL solution of $NaNO_2$ 5 % (w/v) prepared in deionized water was hold for 6 min in

the dark at RT. Then, 0.15 mL $AlCl_3$ 10 % (w/v) solution was added to the mixture and kept for a further 6 min. Finally, 2 mL solution of NaOH 4 % (w/v) prepared in deionized water and 0.7 mL deionized water were poured into the mixture and kept for 15 min. The mixture absorbance was measured at a wavelength of 510 nm. Methanol 100 % was used as a control sample. The TPC was calculated as mg catechin equivalents (CE)/g dried sample (DS).

2.4.4. Total alkaloid content (TAC)

TAC of saponin-enriched samples with and withoutmicroencapsulation was estimated based on the prior work with some changes [27]. Atropine was used as a standard sample. Briefly, the Bromocresol Green (BCG) solution was made by heating 69.8 mg BCG in 3 mL NaOH solution 2 N and 5 mL deionized water until completely dissolved. Then, this solution was diluted to 1000 mL by deionized water. The phosphate buffer solution pH 4.7 was made by adjusting the pH of sodium phosphate sodium 0.2 M by citric acid solution 0.2 M. 1 mg pure atropine was prepared in 10 mL deionized water to make a standard solution.

Next, the solvent from 2 mL of each sample was completely removed by hot air-drying method at 50 °C and replaced by 2 mL HCl 2 N solution. After that, 1 mL solution or atropine at various concentrations from 50 to 200 μg/mL was transferred to the separatory funnel and washed three times by 10 mL chloroform. The pH of the solution was neutralized by adding NaOH solution 0.1 N. The mixture was then added by 5 mL BCG and 5 mL phosphate buffer solutions. The mixture was homogeneously shaken and completely separated by 1, 2, 3 and 4 mL chloroform. The chloroform extract was then removed and the remaining was measured the absorbance at the wavelength of 470 nm. Control and blank samples were 1 mL HCl 2 N solution replaced for the sample and deionized water, respectively. The TAC was calculated as mg atropine equivalents (AE)/g dried sample (DS).

2.5. Assessment of antioxidant activity

2.5.1. ABTS radical scavenging capacity (ARSC)

ARSC of saponin-enriched samples with and withoutmicroencapsulation was analyzed based on the previous publication with some changes [28]. Trolox was used as a standard sample. Firstly, $ABTS^+$ solution 7.4 mM was mixed with $K_2S_2O_8$ solution 2.6 mM prepared in deionized water at a 1:1 (v/v) ratio and hold in the dark at RT to prepare a stock solution. After 12 h, this stock solution was stored at −20 °C in a freezer for future requirements. To prepare a working solution, 1 mL stock solution was mixed with 60 mL methanol 100 % to achieve an absorbance of 1.1 ± 0.02 at 734 nm.

Next, the mixture of 0,15 mL sample or trolox at various concentrations ranging from 5 to 100 μg/mL and 2.85 mL working solution was kept for 3 h in the dark at RT. The mixture absorbance was then measured at the wavelength of 734 nm. The control and blank sample were methanol 100 %. The ARSC was presented as mg trolox equivalents (TE)/g dried sample (DS).

2.5.2. Ferric reducing antioxidant power (FRAP)

FRAP of saponin-enriched samples with and withoutmicroencapsulation was analyzed using the modified method according to the previous study [29]. Trolox was used as a standard sample. Reagent A (acetate phosphate solution 300 mM, pH 3.6), reagent B (TPTZ solution 10 mM dissolved in 40 mM HCl), and reagent C ($FeCl_3.6H_2O$ solution 20 mM prepared in deionized water) were mixed at a 10:1:1 (v/v) ratio to prepare the fresh FRAP reagent. The mixture of 0.15 mL sample or trolox at various concentrations from 5 to 60 μg/mL, and 2.85 mL of the fresh FRAP reagent was hold for 30 min in the dark at RT. The mixture absorbance was measured at the wavelength of 593 nm. Control and blank samples were methanol 100 %. The FRAP was calculated as mg trolox equivalents (TE)/g dried sample (DS).

2.6. Assessment of α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of saponin-enriched samples with and withoutmicroencapsulation was assessed using the established method with several adjustments [30]. The non-microencapsulated and microencapsulated saponin-enriched powders was dissolved in phosphate buffer solution 0.1 M, pH 6.8. The concentration of CPH, *H. hirsuta*, and combined samples with and without microencapsulation were prepared at 50, 100, and 100 μg/mL, respectively. For the α-glucosidase inhibitory activity assessment, the mixture of 1.5 mL phosphate buffer solution 0.1 M, pH 6.8, 0.3 mL enzyme α-glucosidase 1 U/mL, and 0.6 mL sample was hold at 37 °C for 15 min. Then, 0.6 mL pNPG solution 5 mM was added and kept at 37 °C for a further 20 min before being terminated by the addition of 1.5 mL Na_2CO_3 solution 0.1 M. The absorbance of *p*-nitrophenol released by the hydrolysis of pNPG catalyzed by enzyme α-glucosidase was measured at the wavelength of 405 nm. Acarbose at a concentration of 50 μg/mL was used as a positive control. The negative control sample was the reaction mixture without the presence of sample. The negative control blank sample was the buffer only. The test sample blank was the reaction mixture without the presence of enzyme and substrate. The percentage of inhibition (%) was calculated according to equation (2).

$$I(\%) = \frac{Abs(B) - Abs(S)}{Abs(B)} \quad (2)$$

Where: Abs (B) is the absorbance of the negative control sample - the absorbance of the negative control blank sample; Abs (S) is the absorbance of sample - the absorbance of a test sample blank.

2.7. Statistical analysis

All the experiments were conducted in triplicate. The results were presented as means ± standard deviations. The data was statistically analyzed using the SPSS software 22. One-way ANOVA coupled with Tukey's HSD post hoc test was used to analyze and compare the experiments at a significant level 5 % ($p < 0.05$).

3. Results and discussion

3.1. Physicochemical properties of microencapsulated saponin-enriched CPH, H. hirsuta, and combined powders

The secondary metabolites are environmentally sensitive and could suffer from serious degradation resulting from oxidation, pH, temperature, and so forth. The purpose of microencapsulation is to inhibit partly the negative influences of environmental conditions, therefore protecting the phytochemical components, prolonging the shelf life as well as promoting the powder quality. The moisture content (MC) and water activity (a_w) measure the availability of water for microbial, enzymatic, or chemical activity that has a critical role in the prediction of the shelf life of a product. While the water-soluble index (WSI) regards the solubility of powder in water. The physicochemical properties of microencapsulated saponin-enriched powder including MC, a_w, WSI, pH, microstructure, particle size, and zeta potential were described in Table 2, Fig. 1, Fig. 2, and Fig. 3.

The highest MC was observed for the C2 sample (4.76 %), while the lowest value was found in the H1 sample (3.22 %). MC of C1 and CH1 samples at 3.96 % and 4.23 % witnessed no significant difference according to a one-way ANOVA ($p < 0.05$). There was also a significant difference between MC of two CPH saponin-enriched microencapsulated powders with different proportions of extract to wall material, whereas the higher wall material concentration probably increased MC. In the studies about the effect of wall material types and concentrations on freeze-drying catechin nanoemulsion, the higher content of coating materials slightly gave rise to the MC [31]. In contrast, the reduction of MC associated with the increasing of carrier concentration [32]. An escalation of MC is probably affected by various external factors such as storage conditions, proper capping of storage containers as well and the different mechanisms of drying methods [33]. However, MC of all microencapsulated powders was lower than 6 %, the level at which the deterioration of product resulting from biochemical and microbiological reactions is inhibited [34], hence it is meaningful to preserve the powder quality. The a_w of 0.6 is the lower limitation of almost all microorganisms, while the acceleration in oxidation is minimized at the a_w of 0.4 [35]. The a_w of microencapsulated saponin-enriched powders ranged from 0.43 to 0.46, which was within the expected range of a powdered product for the purpose of microbial inhibition. The a_w of four microencapsulated powders was slightly higher than the freeze-drying CPH powder encapsulated with maltodextrin and gum Arabic with the ratio of 8:2 (0.41 ± 0.01) [11]. The WSI of three microencapsulated powders (C1, C2, and CH1 at 88.77, 87.14, and 83.12 %, respectively) was significantly greater ($p < 0.05$) than that of microencapsulated powder H1 at 74.18 %. The *H. hirsuta* extraction using methanol 100 % as a solvent may result in water insoluble components, which has influenced the solubility of microencapsulated powders. The solubility of black carrot juice extract power with the microencapsulation of maltodextrin and gum Arabic was 96.13 % and 73.47 % [36]. Microencapsulated bitter melon aqueous powder using maltodextrin and gum Arabic combination as carrier material displayed the WSI ranged from 89.9 % under optimized spray-drying conditions [34], which was higher than the WSI of all powder in the present study.

The pH of microencapsulated powder varied from 3.95 to 4.02, where the lowest value was observed in the CH1 sample and the H1 sample had the highest pH. The microencapsulated CPH aqueous powder ranged from 5.28 to 5.35, which did not alter significantly, indicating that the encapsulated agent had no influences on the pH of the powders [11]. The pH of CPH powders demonstrated was 4.82 [10], which was moderately higher than the pH of samples in this present study. The acidic environment might result from the amount of organic acid extracted from the plant materials. Moreover, various external factors such as the quality of water, the extraction conditions, environmental conditions, and so forth could have serious influences on the pH level of an extract. The pH of four these powders were around 4.00 indicating a strong ability in microorganism limitation because of their acidic environment. Fig. 1 depicts the microstructure of all microencapsulated powders. The C1 and H1 samples had irregular forms and hard structures, but the C2 and CH1 samples had a smooth surface and uniform appearance. This is perhaps owing to the sublimation of ice crystals during freeze-drying that results in rough and glass-broken structures. The similar morphology of freeze-dried microencapsulated powders also can be observed in the previous reports [37,38]. The C2 sample had higher coating material concentration than its counterparts and thus could perform better protection of bioactive compounds from extrinsic factors. Another reason for the

Table 2
Physicochemical properties of microencapsulated saponin-enriched CPH, *H. Hirsuta* and combined powders.

Physicochemical properties						
Sample	Moisture content (%)	Water activity	Water soluble index (%)	pH	Particle size (nm)	Zeta potential (mV)
C1	3.96 ± 0.08^{b}	0.43 ± 0.02^{b}	88.77 ± 0.44^{a}	4.00 ± 0.01^{ab}	719.7	−6.97
C2	4.76 ± 0.24^{a}	0.46 ± 0.01^{a}	87.14 ± 0.92^{a}	3.98 ± 0.02^{b}	666.1	−15.1
H1	3.22 ± 0.20^{c}	0.43 ± 0.01^{b}	74.18 ± 5.13^{b}	4.02 ± 0.02^{a}	369.6	−14.4
CH1	4.23 ± 0.25^{b}	0.46 ± 0.01^{a}	83.12 ± 2.36^{a}	3.95 ± 0.01^{c}	245.2	−14.0

All values are presented as mean ± standard deviation. Values denoted with different letters indicate significant differences between samples ($p < 0.05$). C1: microencapsulated CPH powder (CM: CPH = 1:1, w/w), C2: microencapsulated CPH powder (CM: CPH = 1.5:1, w/w), H1: microencapsulated *H. hirsuta* powder, CH1: microencapsulated combined powder, CPH: cocoa pod husk, *H. hirsuta*: *Helicteres hirsuta* Lour, CM: coating material.

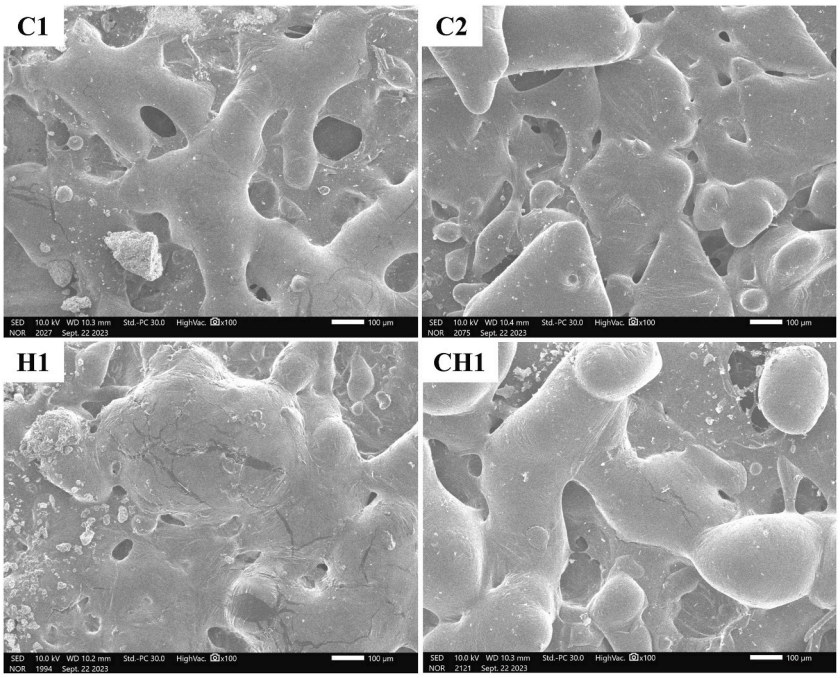

Fig. 1. Scanning electron micrographs of microencapsulated saponin-enriched CPH, *H. Hirsuta* and combined powders. C1: microencapsulated CPH powder (CM:CPH = 1:1, w/w), C2: microencapsulated CPH powder (CM:CPH = 1.5:1, w/w), H1: microencapsulated *H. hirsuta* powder, CH1: microencapsulated combined powder, CPH: cocoa pod husk, *H. hirsuta*: *Helicteres hirsuta* Lour., CM: coating material.

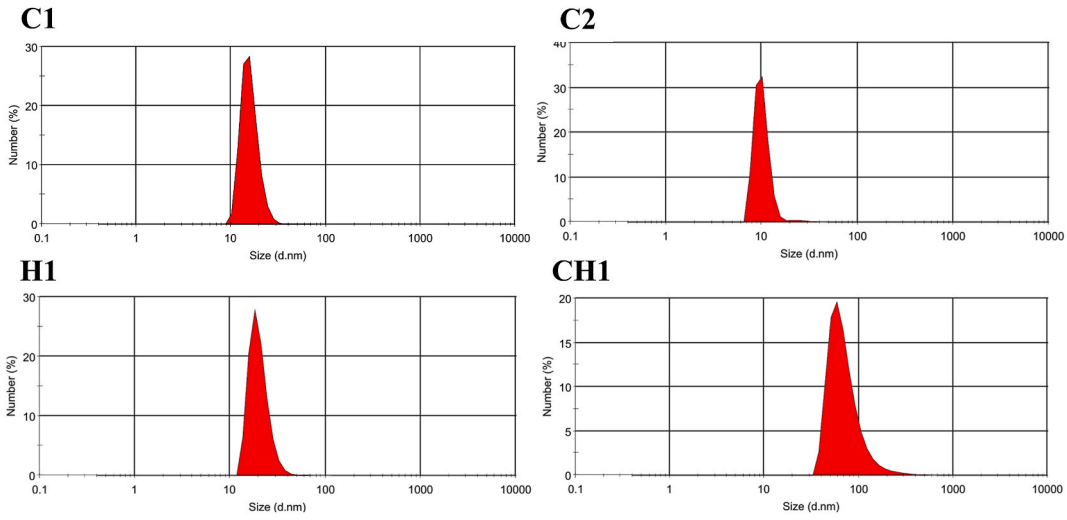

Fig. 2. Particle size distribution of microencapsulated saponin-enriched CPH, *H. Hirsuta* and combined powders. C1: microencapsulated CPH powder (CM:CPH = 1:1, w/w), C2: microencapsulated CPH powder (CM:CPH = 1.5:1, w/w), H1: microencapsulated *H. hirsuta* powder, CH1: microencapsulated combined powder, CPH: cocoa pod husk, *H. hirsuta: Helicteres hirsuta* Lour., CM: coating material.

differences in the morphology of these samples is homogenization, of which time and velocity have a critical role in forming homogeneous matrices around the core material. Accelerated time and velocity at the homogenization stage may enhance the structure of the freeze-dried powders [11].

The zeta potential determination allows to assess the stability of colloidal suspension. The colloidal system is stable, one a dominant role is played by the forces causing the mutual repulsion of the particles. The greater is an absolute value of the zeta potential, the better the probability that the suspension will be stable. A small value of the zeta potential (from +5 to −5 mV) indicates a tendency for the suspension destabilization. The colloidal suspensions exhibit the least stability at the isoelectric point, at which the total charge of the diffusion layer around the particles is equal to zero. The particle size and zeta potential of microencapsulated powders ranged from

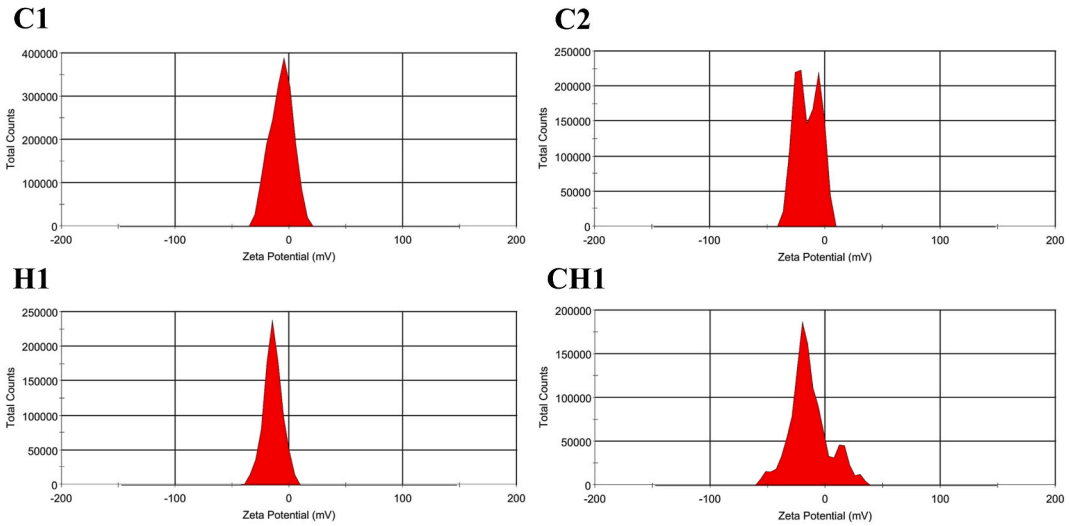

Fig. 3. Zeta potential of microencapsulated saponin-enriched CPH, *H. Hirsuta* and combined powders. C1: microencapsulated CPH powder (CM: CPH = 1:1, w/w), C2: microencapsulated CPH powder (CM:CPH = 1.5:1, w/w), H1: microencapsulated *H. hirsuta* powder, CH1: microencapsulated combined powder, CPH: cocoa pod husk, *H. hirsuta: Helicteres hirsuta* Lour., CM: coating material.

254.2 to 719.7 nm and from −6.97 to −15.1 mV, respectively (Table 2, Figs. 2, and Fig. 3). Of which, the smallest particle size was observed for microencapsulated *H. hirsuta* and combined powders (H1 and CH1) that is respossible for their lowest solubility, while the highest zeta potential was recorded for microencapsulated CPH powder (C2), which might display the best solubility and suspension stability. This outcome reveals that the particle size and zeta potential significantly affect the solubility of the microencapsulated powders, in which higher the zeta potential, the lower solubility and the smaler particle size, the lower solubility. In contrast, the particle size and zeta potential slightly affect the pH value of the microencapsulated powders.

3.2. Phytochemical content of non-microencapsulated and microencapsulated saponin-enriched CPH, H. Hirsuta, and combined powders

The phytochemical compounds involving total saponin content (TSC), total phenolic content (TPC), total flavonoid content (TFC), total alkaloid content (TAC) of microencapsulated saponin-enriched powder from the CPH, *H. hirsuta*, and combined extracts were described in Table 3. Of these, the bioactive compounds in the microencapsulated powders, irrespective TSC in the CPH and *H. hirsuta* powders, were not significantly lower ($p < 0.05$) than those of the non-microencapsulated powders. This phenomena may be the consequence of the encapsulated stage, where the wall materials entrap the active constituents, hence, when reconstituting the microencapsulated powder in a solvent, in which the wall materials may be not completely insoluble, the bioactive compounds might partly remain. While regarding TSC, TPC, and TFC, the value for the *H. hirsuta* powder was considerably higher than the CPH and combined powders, the opposite was true for TAC. The mixture addition of *H. hirsuta* extract at the concentration of 50 % to CPH extract also increased the TSC, TPC, and TFC compared to other powders.

TSC of the microencapsulated powder samples was in the range of 151.87–294.51 mg EE/g DS, where the H1 sample had the

Table 3
Phytochemical properties of saponin-enriched CPH, *H. hirsuta*, and combined powders with and without microencapsulation.

	Phytochemical components			
Sample	TSC (mg EE/g DS)	TPC (mg GAE/g DS)	TFC (mg CE/g DS)	TAC (mg AE/g DS)
C0	256.38 ± 7.82^{b}	6.88 ± 0.34^{d}	51.36 ± 1.97^{d}	27.14 ± 15.07^{a}
C1	193.46 ± 12.94^{c}	6.49 ± 0.41^{d}	46.05 ± 1.11^{d}	24.23 ± 13.35^{a}
C2	151.87 ± 2.70^{d}	5.41 ± 0.19^{e}	33.80 ± 1.12^{d}	15.20 ± 8.27^{a}
H0	294.51 ± 11.07^{a}	12.95 ± 0.46^{a}	316.25 ± 14.67^{a}	11.88 ± 7.66^{a}
H1	235.82 ± 9.35^{b}	12.12 ± 0.29^{a}	244.58 ± 14.82^{b}	10.49 ± 7.05^{a}
CH0	176.98 ± 5.99^{c}	10.74 ± 0.24^{b}	126.85 ± 2.90^{c}	15.42 ± 9.93^{a}
CH1	174.91 ± 5.48^{cd}	9.33 ± 0.35^{c}	121.43 ± 7.89^{c}	14.36 ± 11.04^{a}

All values are presented as mean ± standard deviation. Values denoted with different letters indicate significant differences between samples ($p < 0.05$). C0: non-microencapsulated CPH extract with placebo, C1: microencapsulated CPH powder (CM: CPH = 1:1, w/w), C2: microencapsulated CPH powder (CM: CPH = 1.5:1, w/w), H0: non-microencapsulated *H. hirsuta* extract with placebo, H1: microencapsulated *H. hirsuta* powder, CH0: non-microencapsulated combination of CPH and *H. hirsuta* extract with placebo, CH1: microencapsulated combined powder, CPH: cocoa pod husk, *H. hirsuta*: *Helicteres hirsuta* Lour, CM: coating material, TSC: total saponin content, TPC: total phenolic content, TFC: total flavonoid content, TAC: total alkaloid content, GAE: gallic acid equivalents, CE: catechin equivalents, EE: escin equivalents, AE: atropine equivalents, DS: dried sample.

highest value and the C2 sample was observed in the lowest retention. The TSC of the C2 sample was much lower than the C1 sample and C0 sample due to a higher proportion of coating material. In comparison with the TSC of the *H. hirsuta* sample, the CPH sample was also lower. The TSC of microencapsulated CPH powder in this study showed a slightly lower value than the that indicated in the former research, in which the microencapsulated CPH powder was 282.23–307.13 mg EE/g DS [11]. The samples with and withoutmicroencapsulation, in which the core component concentration in the powder was 50 %, resulted in much higher TSC value than 100 % bitter melon aqueous powder, in which the content was 77.1–113.6 mg EE/g DS [39].

TFC also witnessed a higher retention in all samples. Regarding CPH samples, there were no significant differences among three samples (256.38, 193.46, and 151.87 mg CE/g DS for C0, C1, and C2, respectively) based on one-way ANOVA analysis *($p < 0.05$)*. The TFC of *H. hirsuta* samples also considerably 6-folded about the CPH sample (316.25 mg CE/g DS for non-microencapsulated and 244.58 mg CE/g DS for microencapsulated sample). The TFC of microencapsulated saponin-enriched CPH powder extracted in methanol in this study was much higher than microencapsulated phenolic-enriched CPH powder extracted in water using various encapsulated material types, which varied from 13.10 to 24.96 mg CE/g DS [11]. This may be due to the difference in solvent chosen for extraction. In MAE, while absolute methanol is used as the solvent, its high dielectric constant and dielectric loss characteristic result in a rapid rate of heating solvent, and therefore, increase the removal of flavonoid from the plant matrices [40]. According to the previous report, the nonmicroencapsulated CPH powder rich in saponins was approximately one-half of CPH powder (115.50 mg CE/g DS) [10]. TFC value of freeze-dried *Moringa stenopetala* leaves extract encapsulated with maltodextrin and the mixture of maltodextrin/high methoxyl pectin at 9:1 ratio (w/w), and the core to coat ratio of 1:6 (w/w) was 45.42 and 46.13 mg CE/g DS [41], which was comparable to the TFC analyzed for microencapsulated CPH powder with the core to coat ratio of 1:1 (w/w) in this present study.

The retention of TPC in the CPH powder with and withoutmicroencapsulated agent was varied from 5.41 to 6.88 mg GAE/g DS. The TPC value for the C0 and C1 samples observed no significant difference, while the C2 sample was significantly lower than its counterparts. On the subject of TPC, the nonmicroencapsulated CPH powder was 15.69 mg GAE/g DS [10], which was approximately 2.5 times the TPC of microencapsulated CPH powder in this study. The TPC of the *H. hirsuta* sample also doubled the CPH sample. The comparative sample also yielded a considerably higher TPC than the CPH sample. The optimal encapsulation for red grape pomace with 10.10 % maltodextrin and 0.21 % carboxymethyl cellulose yielded the maximum TPC of 31.56 mg GAE/g DS [42], which was approximately 6 times of microencapsulated CPH powder sample and doubled the microencapsulated *H. hirsuta* powder sample in this study.

TAC of the microencapsulated powder from the CPH (15.20–27.14 mg AE/g DS) was observed higher than the microencapsulated *H. hirsuta* and the combined powders. The retention of alkaloid in the *H. hirsuta* sample witnessed the lowest value (11.88 mg AE/g DS and 10.49 mg AE/g DS for the sample with and withoutmicroencapsulation), which was only half of the CPH sample. The TAC of all samples with and without microencapsulated displayed no significant difference *($p < 0.05$)*.

3.3. Antioxidant capacity of non-microencapsulated and microencapsulated saponin-enriched CPH, H. Hirsuta, and combined powders

Oxidation stress could be defined as an imbalance between the free radicals and the antioxidants in the human body, hence resulting in a disruption of redox signaling and control and/or damage of cells and tissues [43]. Thus, the consumption of foods, functional foods, or medicines rich in antioxidants is critical for the prevention and treatment of numerous disorders. In the evaluation of the antioxidant properties of plant extract, ARSC and FRAP are the two common assays due to certain advantages. In the ARSC assay, the antioxidants stabilize the ABTS radicals by donating electrons, while FRAP assay determines the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) [44]. Table 4 illustrates the antioxidant capacity of microencapsulated saponin-enriched powders from the CPH, *H. hirsuta*, and their combination.

Table 4
Antioxidant capacity of saponin-enriched CPH, *H. hirsuta*, and combined powders with and without microencapsulation.

Antioxidant capacity		
Sample	FRAP (mg TE/g DS)	ARSC (mg TE/g DS)
C0	11.94 ± 0.41^{c}	18.77 ± 0.65^{bc}
C1	11.89 ± 0.71^{cd}	18.20 ± 0.57^{bc}
C2	9.61 ± 0.05^{d}	15.51 ± 0.81^{c}
H0	23.77 ± 0.85^{a}	31.76 ± 3.95^{a}
H1	21.94 ± 1.71^{a}	30.84 ± 8.31^{ab}
CH0	18.20 ± 0.26^{b}	31.75 ± 7.35^{a}
CH1	15.96 ± 0.58^{b}	23.00 ± 2.17^{abc}

All values are presented as mean ± standard deviation. Values denoted with different letters indicate significant differences between samples ($p < 0.05$). C0: non-microencapsulated CPH extract with placebo, C1: microencapsulated CPH powder (CM: CPH = 1:1, w/w), C2: microencapsulated CPH powder (CM: CPH = 1.5:1, w/w), H0: non-microencapsulated *H. hirsuta* extract with placebo, H1: microencapsulated *H. hirsuta* powder, CH0: non-microencapsulated combination of CPH and *H. hirsuta* extract with placebo, CH1: microencapsulated combined powder, CPH: cocoa pod husk, *H. hirsuta*: *Helicteres hirsuta* Lour, CM: coating material, FRAP: Ferric reducing antioxidant power, ARSC: ABTS radical scavenging capacity, TE: Trolox equivalent, DS: dried sample.

The antioxidant activity of microencapsulated powder by FRAP assay varied in the range of 9.61–21.94 mg TE/g DS, in which the highest value was sample H1 (21.94 mg TE/g DS), trailed behind samples CH1 and C1 (15.96 mg TE/g DS and 11.89 mg TE/DS, respectively) and the least activity was sample C2 (9.61 mg TE/g DS). There is no significant difference *($p < 0.05$)* in FRAP between nonmicroencapsulated and microencapsulated CPH, *H. hirsuta*, and combined powders. The FRAP of chitosan-encapsulated *Cannabis sativa* L., *Cannabis indica* L., and *Mitragyna speiosa* K. powder (wall material to extract at 1:1 ratio) were 17.2, 30.9, and 34.4 mg TE/g DS [45]. The flavonoids-enriched *Moringa oleifera* leaves extract using deep eutectic solvent, which was then encapsulated by the modified soybean protein isolate recording FRAP value of 15.6 mg TE/g, which was comparable with the microencapsulated combined powder in this study [46].

Regarding the ARSC assay, the microencapsulated *H. hirsuta* sample showed outstanding antioxidant activity in comparison to the CPH sample, which was about 1.7 times higher the sample C1. No significant difference was observed in the retention of *H. hirsuta* and combined samples with and without microencapsulation *($p < 0.05$)*. The addition of *H. hirsuta* extract also supported the antioxidant activity of the CPH extract, which demonstrated a higher ARSC and FRAP value than the only CPH extract. These results are relevant to the total bioactive compounds determined. The ARSC and FRAP values of microencapsulated phenolic-enriched powder from the CPH were 49.65 and 39.55 mg TE/g DS, respectively [11], which had the same core to coat ratio and maltodextrin to gum Arabic ratio to sample C1.

In advance T2DM, hyperglycemia enhances the generation of reactive oxygen species, therefore inducing oxidative stress. Moreover, the production of free radicals is promoted by the presence of advanced glycation end products formed by the non-enzymatic covalent attachment of glucose and its toxic derivatives to biological macromolecules [2]. Hence, the antioxidant activity investigation in this present study could express a notable meaning to further research to support T2DM management.

3.4. α-glucosidase inhibitory activity of non-microencapsulated and microencapsulated saponin-enriched CPH, H. Hirsuta, and combined powders

The α-glucosidase inhibition capacity of microencapsulated saponin-enriched powders was reported in Table 5. Of those, all the microencapsulated powders at 50–100 μg/mL expressed an inhibition percentage more than 50 % and much lower than acarbose at 50 μg/mL (96.36 %). At a concentration of 100 μg/mL, the α-glucosidase inhibition capacity of the microencapsulated CPH powders was not significantly unlike ($p < 0.05$) from nonmicroencapsulated powder (52.16 %, 51.74 %, and 52.85 % for C1, C2 and C0 samples, respectively). Both *H. hirsuta* powders with and without microencapsulation (76.81 % and 81.73 % for H1 and H0 samples, respectively) indicated a more effective α-glucosidase inhibition activity than the CPH powders. The combined powder at 50 μg/mL (65.45–66.39 %) also exhibited better inhibition capacity than only CPH samples at 100 μg/mL. The α-glucosidase inhibitory capacity of these samples also corresponded to the phytochemical contents as mentioned. Furthermore, the high water solubility of maltodextrin and gum Arabic promoted the solubility of microencapsulated powders in phosphate buffer, which is the solvent of solutions used in this assay. As a consequence, the release of bioactive components was better and demonstrated more precise results.

Control of hyperglycemia has been one of the effective clinical treatments of T2DM, in which the inhibition of α-glucosidase by plant-derived bioactive compounds has surged as a trend due to the hypoglycemia activity. The rate of glucose absorption could be delayed by competitive and reversible inhibition on α-glucosidase, thus the postprandial rise of blood glucose is decelerated [3]. Saponins, flavonoids, alkaloids, phenolic compounds, and so forth are the secondary metabolites that have been proven to have remarkable α-glucosidase inhibitory activity. The saponin compound namely daucosterol, which was isolated from *Eleocharis dulcis* peel presented the strongest α-glucosidase inhibitory activity in comparison to the stigmasterol glucoside and campesterol glucoside isolated from the same material. This mechanism of action was clarified as the formation of a ducosterol-α-glucosidase complex, where this saponin forms 7 hydrogen bonds with 4 residues of the enzyme's active site [47]. A corroboration of the better α-glucosidase inhibition of biflavonoids including amentoflavone and hinokiflavone than acarbose and monoflavonoids through a non-competitive mechanism has been found [48]. Anthocyanin and a phenylpropanoid glycoside extracted from black rice bran called cyanidin-3-glucoside and 6′-*O*-feruloylsucrose had high binding energies and good binding interactions with the active site residues of the receptor protein [49]. The high content of phytochemical components in saponin-enriched CPH extract, of which the hallmarks were total saponin and total flavonoid contents, was particularly potent in inhibition activity on α-glucosidase, thus could be expected as an ingredient for T2DM functional food development.

4. Conclusion and perspectives

The microencapsulated saponin-enriched CPH powder prepared by using previously optimized drying, extraction, and appropriate microencapsulated conditions presented desirable physicochemical properties including water soluble index, moisture content, water activity, pH, particle size, and zeta potential. The phytochemical contents were also evaluated with the amount of total saponins and flavonoids being the highest, followed by total alkaloids and phenolics. Antioxidant capacity assessed by ARSC and FRAP assays corresponded to the total bioactive components content. Associated with good antioxidant activity, the inhibition of α-glucosidase at certain concentrations also indicated the potential of CPH as a means to develop a functional food for supporting T2DM disorder as well as exploit the enormous quantities of this by-product. The combination of CPH extract with the other plant extracts was a novel investigation that the suggested combination findings may have a synergistic effect on hyperglycemia. Further research is necessary to reveal the α-amylase inhibition activity, and the mechanism of enzyme inhibition *in vitro*, as well as investigate the antidiabetic activity of saponin-enriched CPH extract *in vivo* by using a rat model.

Table 5
α-glucosidase inhibitory activity of saponin-enriched CPH, *H. hirsuta* and combined powders with and without microencapsulation.

α-glucosidase inhibitory activity		
Sample	Concentration (μg/mL)	Inhibition (%)
C0	100	52.85 ± 2.24^{d}
C1	100	52.16 ± 2.16^{d}
C2	100	51.74 ± 7.15^{d}
H0	50	81.73 ± 1.63^{b}
H1	50	76.81 ± 3.11^{bc}
CH0	50	66.39 ± 4.58^{c}
CH1	50	65.45 ± 6.02^{c}
[a]**Acarbose**	50	96.36 ± 2.93^{a}

[a] Positive control. All values are presented as mean ± standard deviation. Values denoted with different letters indicate significant differences between samples ($p < 0.05$). C0: non-microencapsulated CPH extract with placebo, C1: microencapsulated CPH powder (CM: CPH = 1:1, w/w), C2: microencapsulated CPH powder (CM: CPH = 1.5:1, w/w), H0: non-microencapsulated *H. hirsuta* extract with placebo, H1: microencapsulated *H. hirsuta* powder, CH0: non-microencapsulated combination of CPH and *H. hirsuta* extract with placebo, CH1: micro-encapsulated combined powder, CPH: cocoa pod husk, *H. hirsuta*: *Helicteres hirsuta* Lour, CM: coating material.

Data availability

All the data generated during the review is already reported within the manuscript and are obtained from the publicly available full-texts. No new data has been generated in our study.

Ethical statement

Not applicable.

CRediT authorship contribution statement

Van Tang Nguyen: Writing – review & editing, Validation, Software, Project administration, Funding acquisition. **Phuong Trang Thi Tran:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

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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