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Microencapsulated Asiatic Pennywort (*Centella asiatica*) fortified chocolate oat milk beverage: Formulation, polyphenols content, and consumer acceptability

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Abstract: This study investigated the use of microencapsulated Asiatic pennywort (*Centella asiatica)* (CA) as a functional ingredient to formulate a novel chocolate oat milk beverage. The main objectives of the study were to characterize and encapsulate bioactive components from CA and to determine the polyphenol content and sensory properties of the beverage. CA extract was microencapsulated using maltodextrin and gum Arabic as carriers and subsequently freeze-dried to produce microcapsules. Microencapsulated CA was incorporated into chocolate oat milk at varying concentrations. Polyphenol content of the beverages was quantified using liquid chromatography–mass spectrometry. Consumer acceptability and sensory perception of the beverages were evaluated through an acceptance test and a check-all-that-apply test, respectively, to assess the sensory characteristics of the chocolate oat milk beverage. CA fortified chocolate oat milk contained fourteen polyphenols. Increasing the concentration of microencapsulated CA led to an increase in the polyphenol content of the beverage. Among the identified polyphenols, asiatic acid and asiaticoside stood out as the unique and most abundant compounds in CA ($p < 0.05$). Additionally, the incorporation of cocoa powder into the beverage further contributed to the polyphenol content, introducing bioactive compounds such as benzoic acid, caffeic acid, catechin, chlorogenic acid, kaempferol, luteolin, madecassic acid, *p*-coumaric acid, and quercetin. Evaluation of consumer acceptability revealed that chocolate oat milk beverages containing 2% and 4% microencapsulated CA were liked by consumers. However, beverages with higher concentrations of CA were perceived as less acceptable, characterized by grassy, bitter, and earthy attributes. In conclusion, this study demonstrates the potential of microencapsulated CA as a functional ingredient in chocolate oat milk beverages.

KEYWORDS
Centella asiatica, consumer acceptability, microencapsulation, oat beverage, polyphenols

Practical Application: This study reveals new insights on the microencapsulation of bioactive compounds in CA, proposing its potential as a novel functional ingredient in food and beverage applications in Western markets. The study revealed microencapsulated CA retained polyphenols in CA including asiatic acid and asiaticoside responsible for its bioactive properties. Consumer perception of CA added to oat milk revealed that it can be added at an acceptable level of 4%; however, higher amounts can decrease consumer acceptability. As practitioners explore the incorporation of CA as a functional component in food products, it is crucial to explore preservation techniques for the sensitive bioactive components while balancing the optimal amount of CA to enhance overall consumer liking.

1 | INTRODUCTION

Centella asiatica (L.) Urb. (CA; Family: Apiaceae) has long been an important herb in traditional medicines practices in various Asian and African countries. CA optimally grows in swampy tropical climates; however, reports have mentioned its ability to tolerate heavy shade as well as dry land, which may explain why New Zealand's subtropical climate can support the growth of CA (Wulandari et al., 2020). According to the NZ plant Association Network, it could have been introduced by the Asian community as a salad vegetable and medicinal plant, which has now grown in the wild creeping its way into many New Zealanders gardens as a weed (New Zealand Plant Conservation Network, 2024). CA is not available in general supermarkets although Ministry for Primary Industries has included CA in their list of regulated plants for import for human consumption (Ministry for Primary Industries, 2019).

Although the focus of research on CA has primarily been its efficacy in wound healing and managing other chronic conditions, recent investigations are expanding into areas such as cosmetics, phytoremediation, and food packaging (Oyenihi et al., 2023). Studies have linked CA in improving neurological and many other bodily functions. It is natural antioxidant due to its notable ability to prevent oxidative damage as studies have proven its neuroprotection activity and antiaging effects (Gofir et al., 2021). This highlights the remarkable potential of CA utilization in functional foods and beverages, due to its many nutritional, functional and physiological benefits. CA is also abundant in vitamins A, B, B2, C, niacin, and carotene (Chandrika & Prasad Kumara, 2015).

CA exhibits significant antioxidant capacity comparable to other herbs such as rosemary and sage. Wong et al. (2006) studied the antioxidant capacity using DPPH and FRAP assays, demonstrating its ability to scavenge DPPH free radical and reducing ferric ions. Mohapatra et al. (2021) explored the efficiency of various extraction methods for phenolic compounds from CA. Microwave-assisted extraction coupled with methanol achieved the highest extractive yields for bioactive compounds. However, when considering its advantages in food applications, water can be considered a superior solvent for extracting CA due to its cost-effectiveness and avoidance of potential residue from organic solvents (Plaskova & Mlcek, 2023).

Interestingly, there are little studies on the incorporation of CA in food despite its health potential benefits. Only one study investigated the development of herbal noodles formulated using CA powder (Nongrum et al., 2020). It was found that 5% CA produced the best noodle in terms of sensory acceptability.

There have been no studies investigating the microencapsulation of bioactive components in CA to date. In the food industry, microencapsulation techniques are used to preserve important bioactive compounds found in food ingredients against any degradation due to heat, light, air, and moisture. It can also assist in masking unpleasant flavors or odors (Eghbal & Choudhary, 2018). This study will look at the microencapsulation of bioactives in CA to ensure these components are preserved and can enhance the therapeutic potential of CA when fortifying the oat beverage.

A food product that is fortified with a microencapsulated extract may be more acceptable to consumers than a product containing the free extract as the carrier can mask some attributes of the extract, such as aroma or taste of the plant material, which may come across as unpleasant. Moghadam et al. (2021) studied the effect of microen-

capsulated Pennyroyal (*Mentha pulegium* L.) extract on physiochemical and sensory properties in yoghurt. They conducted sensory analysis and had panellists compare yoghurt samples with the fresh extract added to the samples with maltodextrin-encapsulated extract added. The results revealed that yoghurt with the encapsulated extract scored higher in odor, taste, and general acceptance.

Nonetheless, previous studies have looked at the application of microencapsulation in leafy materials, including green tea leaves (James et al., 2016), maca leaf (Lee & Chang, 2020), and arjuna herb (Sawale et al., 2017). These studies reported that maltodextrin and gum Arabic had the highest encapsulation efficiency, therefore making them promising ingredients for microencapsulation of bioactive compounds. Maltodextrin resulted in better retention of total phenolic components and better encapsulation efficiency values. In addition, a combination of both maltodextrin and gum Arabic demonstrated better stability over time in terms of color changes, total phenolic content and phenolic retention, lipid oxidation, pH, sedimentation, and viscosity (James et al., 2016; Lee & Chang, 2020; Sawale et al., 2017).

In addition to an increasing interest in functional foods, consumers are also shifting toward sustainable and plant-based alternatives. Health concerns related to dairy or animal-based products are becoming more prevalent, such as allergies, lactose intolerance, and heart disease caused by high cholesterol and saturated fats (Voskuil et al., 2005). The combination of sustainability issues and health concerns linked to animal-based products has led to plant-based alternatives gaining more market share, especially in Western European countries, according to Transparency Market Research (2019). The majority of alternatives to animal milk are derived from plant sources such as seeds, legumes, nuts, cereal, or pseudo-cereal plants (Cardello et al., 2022).

For this project, oats will serve as the primary plant-based source for developing an oat milk beverage. Oats are rich in protein, vitamins, calcium, polyphenols, and dietary fiber (Shah et al., 2016). Oats are also a rich source of β-glucan, which has been found to have a cholesterol-lowering effect (Lyly et al., 2003). Research indicates that the bioavailability of polyphenolic compounds can be affected by the food matrix and the compounds present in the food matrix (Jakobek & Matić, 2019). Oat milk, being high in dietary fiber, is an ideal base for this nutritional beverage containing CA due to the interaction between dietary fiber and polyphenol compounds, making them more readily accessible during digestion (Edwards et al., 2017).

There is significant potential for using CA in functional foods and beverages due to its health benefits, antioxidant activity, and nutritional content. Further research and product development in this area is warranted to fully explore the possibilities of utilizing CA in various food and beverage applications. The aim of this study is to develop a novel oat-based beverage fortified with CA, taking advantage of its nutritional benefits, while ensuring sensory qualities and consumer acceptability. Specifically, the main objectives of this study were to: (1) characterize and encapsulate the bioactive components present in freeze dried CA powder, (2) determine polyphenol content in oat milk beverages containing CA, and (3) assess the sensory properties of the CA-fortified beverage.

2 | MATERIALS

2.1 | Centella asiatica

Fresh leaves of CA were collected directly from an experimental plot in Auckland, New Zealand. The collection took place in June 2021. Fresh leaves and stems of CA were first washed and stored in airtight bags and refrigerated at 4°C until further use.

2.2 | Oat flour

Oat flour (20 kg) was purchased in bulk from Harraway & Sons Limited, New Zealand. The oat flour is a finely ground powder produced by additional milling from kilned oats.

2.3 | Chocolate oat milk ingredients

Cadbury Bournville Cocoa Powder (premium dark), a fine powder made from 100% cocoa and Airborne Bush honey, was purchased from a supermarket in Auckland, New Zealand.

2.4 | Enzymes

AMG 300 L and BAN 480 L were supplied by Novozymes Australia Pty Ltd. BAN 480 L is an alpha-amylase that rapidly hydrolyses gelatinized starch, reducing its viscosity. AMG 300 L is a high-quality exo-glucoamylase. It hydrolyses maltose into glucose, which makes it suitable for producing sweet oat-based beverages.

2.5 | Coating material for encapsulation

The maltodextrin DE17-19 (DEXMM) was purchased in a bulk 25 kg bag from the Three Mac Company Limited.

Gum Arabic was sourced in a bulk 5 kg bag from Ingredient Stop. Both emulsifiers were in the form of a refined powder.

2.6 | Chemicals and reagents

Hexane with a purity of 95%–100%, methanol, formic acid, nitrogen gas, and acetonitrile were provided by AUT. Asiatic acid, asiaticoside, madecassic acid, madecassoside, chlorogenic acid, and rutin were obtained in powdered form from Extrasynthese.

3 | METHODS

3.1 | Freeze drying and extraction *Centella asiatica*

The fresh leaves were distributed evenly across three sterile ceramic plates. These plates were carefully placed on the shelves of the freeze dryer (VirTis Advantage Pro Freeze Dryer/Lyophilizer). The freeze dryer was set to treat the CA samples at −45°C for a duration of 72 h (3 days). After the freeze-drying process was completed, the dried CA was removed and ground into a fine powder using a Nutribullet blender. Drying was carried out according to Mohd Zainol et al. (2009). The powder was then stored in an air-tight zip lock bag and stored in a freezer set at −20°C until further use. Approximately 165 g of fresh CA leaves produced 25 g of freeze-dried ground CA.

The CA extract was prepared from dried CA powder rather than fresh leaves to remove moisture, reduce water activity, and inhibit microbial growth, as well as inhibit any enzymatic or biochemical reactions from occurring (Buchaillot et al., 2009). Freeze drying also helps standardize the sample, reduces weight for efficient storage, and increases shelf life (Saifullah et al., 2016). In addition, freeze drying prevents degradation to heat sensitive compounds such as polyphenols, making it a suitable drying method for CA. The grinding of the CA leaves allows for a larger surface area for extraction (Oikonomopoulou et al., 2011).

A water extract of CA was prepared based on the method described by Mohapatra et al. (2021). Water was used as the extraction solvent in this experiment to maintain a food-grade product. Twelve grams of freeze-dried CA powder were dissolved in 480 mL of distilled water at ambient temperature (25 ± 1°C) in sterile 800 mL Schott bottles. The bottle was then placed in a sonicator, which had a cold-water bath. The sonication process was carried out using a 90% pulse rate to prevent any temperature increase during the extraction while ensuring enhanced ultrasonic exposure. The sample was sonicated for 20 min at a frequency of 20 kHz (Sabaragamuwa et al., 2022).

After sonication, the bottle containing the extract was stored at room temperature in a dark cupboard for 48 h to allow complete hydration. After 48 h, the water extract was passed through filter paper under vacuum to remove any unwanted plant materials. The filtered extract was transferred to centrifuge tubes and then centrifuged for 15 min at 3000 rpm at 4°C to further separate any plant material that may sediment at the bottom of the tubes. The CA water extract was then immediately used for microencapsulation.

3.2 | Microencapsulation of *Centella asiatica* extract

To prepare the emulsion for freeze drying, a 4.33% by weight of carrier consisting of MD-DE 17–19, and GA was prepared. A preliminary test on the ratio of MD to GA was carried out to determine the most effective combination of carriers for achieving the highest yield of microencapsulated CA. The combination of 20% maltodextrin and 15% gum Arabic resulted in the highest encapsulation efficiency of the bioactive compounds. A total of 250 mL of CA water extract was used for the microencapsulation process. In small batches of 25 mL CA water extract carefully poured into a 50 mL beaker, the appropriate amounts of MD and GA were weighed and added into the respective beakers. The mixture was thoroughly stirred using a magnetic stirrer to form an emulsion. This was followed by homogenization using the Silverson L4RT homogeniser at 7000 rpm for 5 min. The emulsions were then transferred into plastic 50 mL tubes with caps that were immediately stored in a freezer at −20°C until fully frozen. Once frozen, the tubes were removed from the freezer and exposed in liquid nitrogen for 5 min to further freeze the samples. To facilitate the subsequent freeze-drying, holes were drilled on the caps of the tubes. Once the samples were fully freeze dried, the tubes were retrieved. The conditions for freeze drying are shown in Table 1. The samples were then ground into a fine powder using a mortar and pestle. The microencapsulated powder obtained was stored in a plastic airtight bag until further use.

3.3 | Oat milk formulation

To prepare the oat milk, oat flour and water were weighed into beakers. The ratio of oat flour to water was 1:4. Therefore, to produce 1 L of oat beverage, 200 g of flour and 800 mL of water are mixed. Two enzyme combinations at different concentrations were trialed for the oat milk formulation. The oat flour and water mixture were stirred for 15 min. Novozymes enzymes, BAN (0.30%) + AMG (0.10%), were added to the oat flour and water mixture using a pipette, followed by mixing for another 10 min.

TABLE 1 Conditions of freeze-drying steps for microencapsulated *Centella asiatica*.

Step	Shelf temp (°C)	Ramp (min)	Hold (min)	Vacuum (μbar)
1	−20	60	30	950
2	−20	60	30	900
3	−20	120	30	700
4	−20	120	999	400
5	−20	120	999	350
6	−20	120	999	300
7	−20	120	999	300
8	−20	120	999	300

The beaker was then placed in a temperature-controlled incubator at 65°C for 45 min. Enzyme deactivation was then initiated by increasing the temperature to 120°C for 80 s. The beaker was then removed from the incubator and placed into an ice water bath to cool the mixture to 70°C. The mixture was stirred every 10 min and the internal temperature monitored using a digital thermometer until the mixture reached 70°C. The mixture was poured and squeezed through a straining filter bag to discard the unwanted oat fiber. The strained oat milk was then refrigerated at 4°C. Once chilled, cocoa, honey, and CA were added to the oat milk. Cocoa was added at 15% w/v and bush honey was added at 5% w/v. The oat milk samples were refrigerated at 4°C for sensory testing for no longer than 4 days.

Six liters of oat milk were prepared for sensory testing. The typical shelf life of fresh oat milk without a preservation method is 4–5 days refrigerated (Sante, 2023). To ensure consumers had a fresh sample, the oat milk was prepared in two batches; the first 3 L batch was prepared for the first week of sensory testing, and the second 3 L batch was prepared for the following week. An additional 0.5 L was prepared for LC–MS analysis.

A small scale preliminary sensory test was conducted to determine the maximum amount of CA that could be added to the drink before negatively impacting the sensory profile. It was found that concentrations beyond 10% would be perceived as unacceptable. As a result, varying concentrations of CA from 2% to 10% were chosen for this study.

3.4 | Liquid chromatography–mass spectrometry (LC–MS) analysis of polyphenols

Standard solutions: Pure standards of asiatic acid, asiaticoside, madecassic acid, madecassoside, chlorogenic acid, rutin, benzoic acid, kaempferol, catechin, gallic acid, quercetin, *p*-coumaric acid, caffeic acid, and luteolin were purchased from Extrasynthese. A stock standard solution for each of the polyphenols was prepared at 1 mg/mL by dissolving 1 mg of the solid analyte into 1 mL of pure methanol. The solids were weighed at approximately 1 mg and methanol was added accordingly. The standard solutions were stored in darkness at −20°C.

To develop the LC–MS parameters for polyphenol analysis, individual 20 mg/mL solutions were prepared by diluting 20 mL of the stock solutions into 980 mL pure methanol.

Calibration standards: Series of dilutions of the polyphenol stock solutions in pure methanol were prepared.

3.5 | Sample preparation and extraction of bioactive compounds

The following samples were prepared for analysis: oat milk without cocoa and microencapsulated CA powder at concentrations of 2%, 4%, 6%, 8%, and 10%, and a control. Oat milk and cocoa powder and microencapsulated CA powder 2%, 4%, 6%, 8%, and 10%, and a control. The control samples contained no microencapsulated CA powder. Experimental replication of the 12 treatments was performed, as each treatment was prepared and analyzed in triplicates for accuracy.

Defatting: Each oat milk beverage sample (10 mL) was pipetted into 80 mL falcon tubes. Then, 10 mL of pure hexane was added into each of the falcon tubes containing the samples, followed by mixing by vortexing for 15 s or until the samples were visibly homogenized. The tubes were centrifuged at 4000 rpm for 20 min at 4°C. The supernatant was then removed using a pipette and was yellow in color indicating that fat was removed. This procedure was repeated three times, and each time the supernatant was discarded.

Protein precipitation: The defatted sample (3 mL) was taken using a pipette and placed into a new falcon tube. Methanol (12 mL) was added to each falcon tube contain-

ing the sample. The tubes were then vortexed for 15 s and stored at −20°C for 1 h to allow for protein precipitation to occur. Then, the samples were centrifuged at 3950 rpm for 5 min at 4°C. After centrifugation, the supernatant layer was transferred into a new falcon tube. A methanol/formic acid mixture (9:1) was prepared by mixing 36 mL of 100% methanol with 4 mL of 100% formic acid in a beaker. The methanol/formic acid mixture (12 mL) was pipetted into each of the falcon tubes containing the remaining precipitate. The tubes were vortexed for 15 s and stored at −20°C for 1 h to allow further protein precipitation. Afterward, the tubes were centrifuged at 3950 rpm for 5 min at 4°C. The acid supernatant layer was removed and combined with the previously removed supernatant. The tubes containing the combined supernatants were centrifuged again at 4000 rpm for 30 min at 20°C. The samples were evaporated under a stream of nitrogen flow to remove the methanol and formic acid. The resulting residue was then reconstituted to a final volume of 3 mL using methanol. The reconstituted samples were centrifuged again at 3225 rpm for 5 min at 4°C and then stored in the freezer.

Preparation for LC–MS injection: The supernatant layer (1 mL) obtained from the previous protein precipitations steps was transferred using a pipette into a 2 mL plastic micro centrifuge tube vial. The vials were then centrifuged in a micro centrifuge for 5 min at 1000 rpm. Using a glass pipette, the supernatant of each sample was collected and passed through a syringe-driven filter with a 13 mm nylon membrane, 0.22 mm pore size. The filtered samples were collected in glass vials, which were then placed into the LC–MS system for analysis.

Chromatography: The chromatographic conditions of the standard solutions, including the analytical column, mobile phase composition, flow rate, column temperature, and detector wavelength, were optimized to obtain optimal resolution, peak shape, and retention time.

LC–MS analyses were conducted using an Agilent 1260 Infinity Quaternary LC System. The system consisted of the following components: 1260 quaternary pump (model number: G1311B), 1260 infinity ALS sampler (model number: G1329B), 1260 infinity TCC column component (model number: G1316A), and 1260 infinity diode array detector (model number: G4212B), connected to a 6420 triple quadrupole LC/MS system with electrospray ionization source (model number: G1948B).

The MS ionization source conditions were set as follows: capillary voltage of 4 kV, drying gas temperature of 300°C, drying gas flow of 10 L/min, and nebulizer pressure of 40 psi. The negative ionization mode was performed with multiple reaction monitoring for quantitative analysis.

The Agilent Poroshell EC-C18 (2.1 × 150 mm, 2.7 μm) column was used for this analysis. The mobile phases consisted of water containing 0.1% (v/v) formic acid (A) and acetonitrile containing 0.1% (v/v) formic acid (B). The flow rate was set to 0.30 mL/min and the column temperature was maintained at 40°C. The initial gradient condition was 95:5 (A:B) and held for 1 min. From 1 to 15 min, the proportion of B was increased to 90% and held for 3 min. From 18 to 20 min, the proportion of B was decreased to 5%. The total run time was 29 min and the injection volume was 3 μL.

3.6 | Sensory evaluation

Consumer testing was carried out on chocolate oat milk by a total panel of 53 participants over a period of 2 weeks. Due to restrictions imposed by the COVID-19 pandemic, the number of participants that could attend the sensory sessions at any given time was limited. As a result, multiple small sessions were conducted until the total of 53 participants was achieved. The participants were students and staff of the Auckland University of Technology, as well as members of the public.

Data collection took place in standard sensory booths under controlled conditions, including white lighting and a temperature between 22 and 23°C, with controlled air flow. All samples were labeled with 3-digit random codes and were served chilled after being refrigerated at 4°C. Products were presented in a balanced order to mitigate presentation order and carry-over effects using the Williams design. FIZZ Nomad (Biosystemes) was used for the consumer testing, and participants were provided with a QR code to access an online sensory questionnaire for acceptability and check-all-that-apply (CATA) tests.

Each participant evaluated a total of six samples, including chocolate oat milk with 2%, 4%, 6%, 8%, and 10% encapsulated CA powder, as well as a control sample with 0% encapsulated CA. The samples were served in three-digit coded plastic cups containing about 15 mL of the sample, accompanied by a glass of water. This sample size was chosen to allow for more than one sip, while also minimizing the risk of a carry-over effect from intense stimuli, such as chocolate flavor, CA flavor, or sweetness. The samples were presented in a random order before the participants started sensory testing. The sensory testing involved participants individually tasting and smelling the samples according to the order in the sensory questionnaire, followed by rinsing their palettes with water during the 1-min interstimulus intervals. After tasting each sample, the participants rated the samples on an unstructured line scale for overall liking, liking of flavor, liking of aroma, and liking of mouthfeel. The unstructured line scales were anchored with "dislike very much" and "like very much" at each end.

Participants were also asked to select the CATA attributes that best described the flavor of each sample. CATA questions are versatile multiple-choice questions that have been increasingly used for product sensory characterization with consumers (Xu et al., 2019). This method is a simple and reliable approach for sensory product characterization across a wide range of products, providing similar results to descriptive analysis with trained assessors (Alexi et al., 2018).

3.7 | Statistical analysis

The LC–MS data were analyzed using mixed-model analysis of variance (ANOVA), multivariate ANOVA (MANOVA), and principal components analysis (PCA). Mixed-model ANOVA was employed to understand the main effects of CA (e.g., 0.02 and 0.04) and matrix (chocolate oat milk and oat milk), as well as their interaction. Fisher's LSD test was applied for attributes that showed statistical significant differences. MANOVA was used to generalize the results and understand the impact of cocoa addition in the oat milk base. PCA was used to generalize the results and identify correlations between samples and polyphenols.

The sensory data were analyzed using generalized ANOVA model to determine if significant differences existed between the chocolate oat milk samples. Tukey's post hoc test was applied for attributes that reached statistical significance. The CATA data were analyzed using the Cochran *Q*-test with the Sheskin grouping procedure, if the attribute reached statistical significance. Cochran's *Q*-test was carried out separately for each experimental treatment to identify significant differences among samples for each sensory term (Limbad et al., 2020). Correspondence analysis (CA) was performed on the frequency table for each experimental treatment. CA was carried out to visualize the relationship between products and attributes. CA was performed considering Hellinger's distances, similarly to Lin et al. (2022). All univariate and multivariate analysis were conducted using XLSTAT 2022 (Addinsoft).

4 | RESULTS AND DISCUSSION

4.1 | Development of oat milk

To develop a novel oat milk fortified with CA, enzymes were used. The addition of enzymes can reduce viscosity during starch gelatinization, enhance the nutritional value of the grain, release more flavor components, increase desired sweetness without the added sugar (natural), mitigate protein precipitation, and increase the stability of the beverage over time and during storage (Rosa-Sibakov et al., 2022). For this study, a custom enzyme blend of BAN and AMG was incorporated in the development o the oat milk base through preliminary tests.

Enzymes were used to treat the oat milk by starch hydrolysis through liquefaction and saccharification processes. Liquefaction reduces viscosity of the blend, enhancing its mouthfeel and overall appeal. Saccharification, on the other hand, generates a natural sweetness without the requirement of additional sweeteners. The use of enzymes is key in obtaining a consumer-acceptable mouthfeel, viscosity, and sweetness of the oat milk.

4.2 | Polyphenol composition of oat milk samples varying in CA concentration

The potential effect of adding cocoa powder was analyzed by comparing two treatments: one with cocoa and one without. Additionally, LC–MS analysis was carried to determine the polyphenols present in cocoa that was included in the beverages (Figure S1–S12). Resultsin Table 3 show the effects of cocoa addition on polyphenols in oat milk at varying concentrations of CA. All polyphenols showed a significant impact of cocoa addition, with the exceptions of madecassic acid ($p = 0.506$) and *p*-coumaric acid ($p = 0.225$). Varying amounts of CA significantly impacted all polyphenol concentrations except for benzoic acid, kaempferol, and *p*-coumaric acid.

Oat milk samples containing cocoa had significantly higher content of asiatic acid, catechin, kaempferol, luteolin, quercetin, and rutin when compared to oat milk without cocoa, with increasing concentration of encapsulated CA. Hence, the addition of cocoa powder made a significant contribution to the polyphenol content in the oat milk beverage containing the encapsulated CA. The antioxidant capacity and polyphenol composition of cocoa powder and cocoa bean have been reported to contain primarily flavonoids (Mohapatra et al., 2021). Cocoa beans are a major source of catechins, with an average of 260 μg of catechin per gram of cocoa powder, as reported by Ackar et al. (2013). On the other hand, oats naturally do not contain catechins (Soycan et al., 2019). Only gallic acid was significantly higher in oat milk without cocoa than in chocolate oat milk.

According to Han et al. (2020), CA has been found to contain chlorogenic acid, rutin, asiaticoside, asiatic acid, madecassoside, madecassic acid, and other components. The authors added that asiatic acid and asiaticoside are unique to CA, and it is Asiatic acid and its derivatives that possess a wide range of physicochemical properties. Interestingly, the amount of asiatic acid was significantly higher in chocolate oat milk than oat milk samples with varying concentrations of CA. It is possible that the increase in

TABLE 2 Calibration standard preparation and concentrations.

Calibration standard	Concentration (mg/mL)	Volume of solution	Methanol (μL)
Std A	20	20 μL of each stock standard	900
Std B	10	500 μL std A	500
Std C	5	500 μL std B	500
Std D	2.5	500 μL std C	500
Std E	1.25	500 μL std D	500
Std F	0.62	500 μL std E	500
Std G	0.31	500 μL std F	500
Std H	0.15	500 μL std G	500

asiatic acid content could be attributed to potential interactions between the polyphenols present in cocoa and the polyphenolic compounds in CA.

Existing literature does not provide an explanation for this cocoa-induced effect on the bioavailability of asiatic acid in the chocolate oat milk beverage. Only one study has investigated the interaction between cinnamon and cocoa extract in terms of antioxidant activity (Muhammad et al., 2017). In this study, representative compounds of chocolate (epicatechin and catechin) were combined with various polyphenolic compounds in cinnamon (gallic acid, tannic acid, quercetin, sinapic acid, cinnamic acid, eugenol, and cinnamaldehyde) in different ratios. The authors found that the addition of cinnamon extract significantly increased the antioxidant capacity of the cocoa extract. The total antioxidant activity of the mixture containing these polyphenolic compounds was influenced by the interactions among the phenolics, which can be either synergistic or antagonistic. In the current study, the cocoa powder might have a synergistic interaction with the CA extract, resulting in a higher amount of CA polyphenols in the chocolate oat milk. As a result, there is an opportunity for further research to investigate these aspects and gain a deeper understanding of the relationship between cocoa, CA extract, and their combined effects on polyphenol content and antioxidant activity.

Asiaticoside levels were found to be significantly higher in oat milk samples without cocoa addition containing 2%, 4%, and 6% encapsulated CA compared to cocoa-containing oat milk samples. However, the highest levels of asiaticoside were observed with the addition of 8% and 10% encapsulated CA, with no significant differences between chocolate oat milk and oat milk-only samples. Regarding chlorogenic acid and madecassic acid, the highest amounts were found in oat milk samples (both with and without cocoa addition) containing 10% and 8% encapsulated CA, respectively. These findings suggest that the concentration of encapsulated CA in oat milk, regardless of the presence of cocoa, has an impact on the levels of asiaticoside, chlorogenic acid, and madecassic acid.

Based on these observations regarding the polyphenol content and the effects of the addition of cocoa, as well as the increasing concentration of CA, it can be concluded that oat milks containing cocoa and CA have a significant amount of polyphenols, including asiatic acid and asiaticoside, which are unique to CA. Additionally, the addition of cocoa has a positive influence on the polyphenol content of oat milk, particularly in terms of catechin.

However, it is important to note that further research is needed to investigate the specific mechanisms underlying these observations and to understand the potential synergistic effects between cocoa and encapsulated CA on the bioavailability and content of these compounds in oat milk. Additional studies would contribute to a better understanding of the interactions between cocoa and encapsulated CA and their combined impacts on the polyphenol profile of oat milk.

4.3 | Principal components analysis of the polyphenol composition in oat milk beverages

There is clear separation between oat milk samples containing cocoa and those without along Factor 1 (*F*1) that explained 48.16% of the variance (Figure 1). Along *F*1, oat milk samples containing cocoa and CA at 2%, 4%, 6%, 8%, and 10% all had positive scores, which corresponded to higher loadings of caffeic acid, rutin, kaempferol, catechin, asiatic acid, luteolin, and quercetin. This was supported by ANOVA results shown in Table 3, where the concentrations of these polyphenols were significantly higher than the oat milk samples without cocoa powder. The oat beverages without cocoa powder and with CA (2%, 4%, 6%, 8%, and 10%) had high negative scores along *F*1. Oat milk with 6%, 8%, and 10% negative scores were correlated to gallic acid. This is consistent with the ANOVA results (Table 2) that showed significantly higher concentrations of these polyphenols in cocoa-containing samples as compared to those without cocoa. Oat milk samples (with and

TABLE 3 Polyphenol composition of oat milk beverages with and without cocoa powder and with varying concentrations of encapsulated *Centella asiatica*.

		Control	0.02	0.04	0.06	0.08	0.1	F and *p*-value (matrix)	F and *p*-value (concentration)	F and *p*-value (matrix × concentration)
Asiatic acid	Cocoa	57.649 c	77.582 bc,x	86.284 ab,x	92.81 ab,x	58.173 c,x	105.436 a,x	513.96	6.106	511.84
	Oat	N/A	4.003 d,y	7.529 c,y	11.58 b,y	14.907 a,y	12.852 b,y	<0.0001	0.002	0.002
Asiaticoside	Cocoa	0 d	0 d,y	75.554 c,y	133.167 b,y	250.998 a,x	249.318 a,x	26.41	41.12	2.95
	Oat	N/A	86.678 c,x	172.177 b,x	251.835 a,x	263.448 a,x	263.616 a,x	<0.0001	<0.0001	0.045
Benzoic acid	Cocoa	278.316 ab	375.88 ab,x	279.007 ab,x	430.836 a,x	230.741 b,x	421.924 a,x	5.432	1.70	3.75
	Oat	N/A	235.01 bc,x	344.79 a,x	309.748 ab,x	291.781 ab,x	240.001 b,y	0.030	0.189	0.020
Caffeic acid	Cocoa	117.804 b	116.273 b,x	127.641 ab,x	132.505 ab,x	137.008 a,x	125.377 ab,x	24.84	8.29	2.26
	Oat	N/A	78.532 c,x	96.046 bc,y	117.148 ab,x	124.448 a,x	120.61 a,x	<0.0001	0.000	0.099
Catechin	Cocoa	1049.5 b	3204 a,x	3200.586 a,x	3332.426 a,x	1939.767 b,x	3137.597 a,x	2063.52	8.72	45.72
	Oat	N/A	10.58 d,y	26.309 cd,y	89.645 c,y	768.039 a,y	635.28 b,y	<0.0001	0.000	<0.0001
Chlorogenic acid	Cocoa	17.778 f	162.355 e,x	260.329 d,y	409.264 c,y	645.989 a,x	573.586 b,x	11.91	151.46	2.46
	Oat	N/A	163.291 d,x	335.06 c,x	523.799 b,x	639.432 a,x	646.839 a,x	0.003	<0.0001	0.078
Gallic acid	Cocoa	0 a	0 a,x	0 a,x	0 a,y	0 a,y	0 a,y	125.66	22.90	22.90
	Oat	N/A	0 d,x	0 d,x	110.557 c,x	290.118 b,x	381.299 a,x	<0.0001	<0.0001	<0.0001
Kaempferol	Cocoa	16.466 a	13.603 b,x	14.242 ab,x	14.988 ab,x	12.613 b,x	16.466 a,x	229.46	1.71	1.46
	Oat	N/A	6.341 a,y	6.06 a,y	6.485 a,y	6.632 a,y	6.69 a,y	<0.0001	0.187	0.250
Luteolin	Cocoa	128.499 b	154.551 a,x	161.245 a,x	165.356 a,x	101.332 c,x	161.207 a,x	1085.11	14.56	11.35
	Oat	N/A	33.745 ab,y	38.194 ab,y	45.761 a,y	33.681 ab,y	28.145 b,y	<0.0001	<0.0001	<0.0001
Madecassic	Cocoa	0 f	5.794 e,x	11.645 d,y	19.645 c,x	30.619 b,x	35.458 a,x	0.46	229.05	7.37
	Oat	N/A	5.12 e,x	15.185 d,x	23.217 c,x	32.85 a,x	29.119 b,y	0.506	<0.0001	0.001
p-Coumaric acid	Cocoa	57.889 a	61.546 a,x	65.857 a,x	66.824 a,x	59.513 a,x	64.898 a,x	1.37	2.10	0.86
	Oat	N/A	62.671 ab,x	75.203 ab,x	77.587 a,x	63.355 ab,x	58.898 b,x	0.255	0.119	0.504
Quercetin	Cocoa	198.181 b	278.922 a,x	297.212 a,x	288.193 a,x	138.001 c,x	277.551 a,x	1010.51	14.14	13.54
	Oat	N/A	0 a,y	0 a,y	0 a,y	0 a,y	0 a,y	<0.0001	<0.0001	<0.0001
Rutin	Cocoa	0 c	0 c,x	0 c,x	0 c,x	0.999 b,x	1.907 a,x	33.69	14.69	14.69
	Oat	N/A	0 a,x	0 a,x	0 a,x	0 a,y	0 a,y	<0.0001	<0.0001	<0.0001

Note: Means with different letters (a–e) within column show significant effect of concentration of encapsulated *Centella asiatica* in each carrier beverage; means with different letters (x and y) within row show significant effect of carrier beverage in each set concentration. Hotelling–Lawley's MANOVA also revealed that there were significantly more polyphenol content in the cocoa base compared to the oat base ($F_{(13,16)} = 116.476$ and $p < 0.001$).

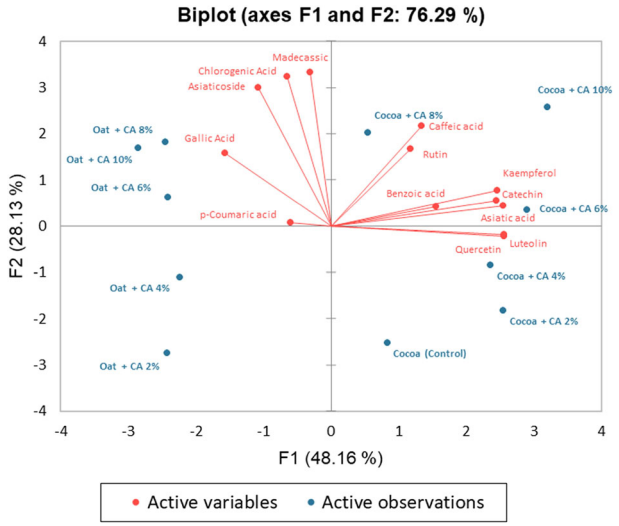

FIGURE 1 A principal components analysis biplot based on the polyphenol composition in oat milk beverages, both with and without cocoa addition, and with varying amount of *Centella asiatica* indicated as a percentage. The active vector variables are the polyphenols compounds, and the observation loadings are the oat milk samples.

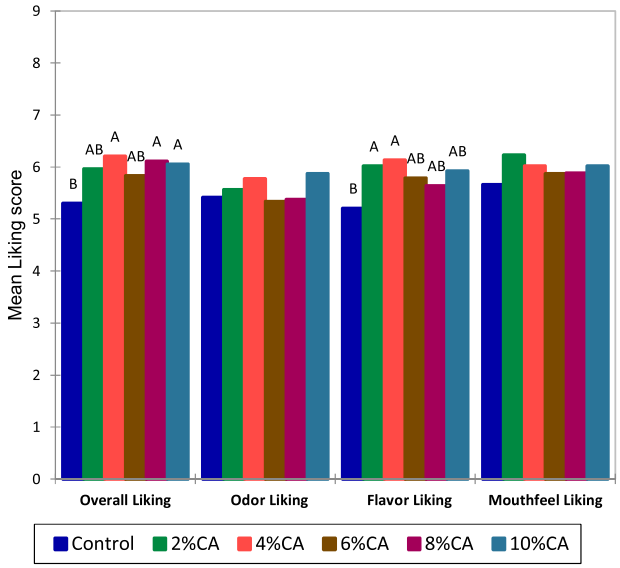

FIGURE 2 Summary of means of overall liking, liking of odor, liking of flavor, and liking of mouthfeel of the different chocolate oat milk beverages fortified with encapsulated *Centella asiatica* powder. Different letters indicate significant differences in chocolate oat milk beverages for each hedonic characteristic. No significant differences were observed for odor and mouthfeel liking values.

without cocoa), with CA concentrations of 8% and 10%, had high positive scores along *F*2 that explained 28.13% of the variance in the data. These samples were associated with madecassic acid, chlorogenic acid, and asiaticoside, which was consistent with the ANOVA results (Table 3) that showed significantly higher concentrations of these polyphenols in these samples as compared to those with 2%, 4%, and 6% CA (Figure 2).

4.4 | Consumer testing results

Chocolate oat milk beverages containing encapsulated CA at varying concentrations did not show significant differences between them in terms of overall liking and liking of flavor. However, the chocolate oat milk samples containing 4%, 8%, and 10% CA were significantly higher in terms of overall liking compared to the control chocolate oat milk beverage without CA. Additionally, the chocolate oat milk beverages containing 2% and 4% CA were significantly more liked in terms of flavor compared to the control sample. This was an interesting observation, considering CA being known to have a "green" or "leafy" taste profile. Based on the findings, it can be inferred that the addition of encapsulated CA to chocolate oat milk has the potential to enhance the liking of the beverage, specifically in terms of flavor, when used in the appropriate concentrations. Therefore, it is important to carefully consider the concentration of CA to achieve the desired flavor profile and overall consumer liking for chocolate oat milk.

Further studies and experimentation may be necessary to determine the optimal concentration of encapsulated CA for achieving the desired flavor in chocolate oat milk. Additional hedonic analysis could be conducted to evaluate the specific sensory attributes of the chocolate oat milk samples to further guide product optimization. For example, the use of just about right scales could be used to understand how the flavor of the oil milk samples can be improved to increase consumer liking.

To analyze the CATA flavor characteristics, multiple pairwise comparisons were conducted using the critical difference Sheskin procedure. The results presented in Table 4 indicate the percentage of participants who selected each attribute for each sample. These results provide valuable insights into the impact of CA concentration on specific liking attributes of the chocolate oat milk beverages. Analysis revealed significant differences in the grassy, earthy, and oaty attributes among the samples at a 10% significance level, as indicated in Table 3. Interestingly, the grassy attribute was found to be significantly higher in the samples with 6% CA and 10% CA compared to the Control (0% CA), 2% CA, and 8% CA samples. Moreover, the earthy attribute was significantly higher in the 10% CA sample when compared to the 8% CA sample. Lastly, it is worth noting that the oaty attribute was found to be signif-

TABLE 4 Summary of percentage ratio for check-all-that-apply (CATA) flavor characteristics of the different chocolate oat milk beverages fortified with encapsulated *Centella asiatica* powder.

Attributes	Control	2% CA	4% CA	6% CA	8% CA	10% CA	*p*-value
Cocoa	0.906 a	0.811 a	0.830 a	0.868 a	0.943 a	0.811 a	0.080
Sweet	0.736 a	0.755 a	0.811 a	0.774 a	0.717 a	0.698 a	0.618
Grassy	0.151 a	0.151 a	0.208 ab	0.358 b	0.151 a	0.358 b	0.000
Malty	0.340 a	0.226 a	0.226 a	0.283 a	0.283 a	0.321 a	0.309
Caramel	0.170 a	0.208 a	0.283 a	0.245 a	0.226 a	0.245 a	0.333
Creamy	0.208 a	0.283 a	0.245 a	0.302 a	0.245 a	0.283 a	0.852
Nutty	0.208 a	0.226 a	0.208 a	0.170 a	0.170 a	0.208 a	0.872
Bitter	0.208 a	0.170 a	0.245 a	0.283 a	0.208 a	0.264 a	0.470
Earthy	0.396 ab	0.434 ab	0.453 ab	0.396 ab	0.340 a	0.528 b	0.093
Oaty	0.396 ab	0.358 ab	0.472 b	0.321 ab	0.283 a	0.340 ab	0.092

Note: Different letters indicate significant difference within row (between samples) for each sensory attribute.

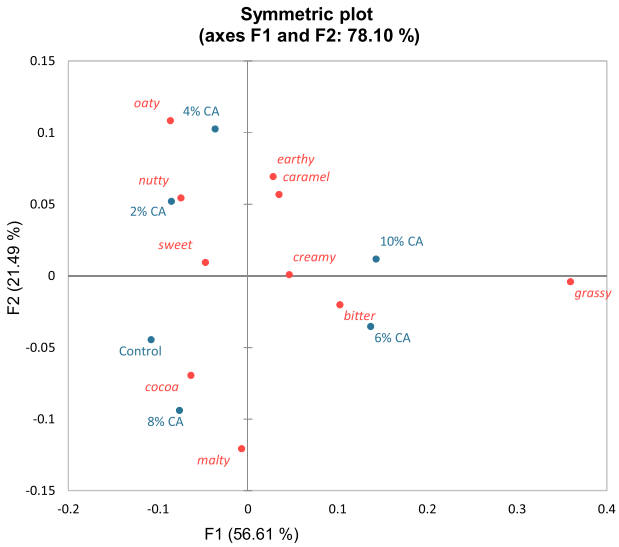

FIGURE 3 Biplot generated from correspondence analysis on the check-all-that-apply (CATA) sensory attributes rated for the chocolate oat milk beverages.

icantly higher in the sample with 4% CA in comparison to the sample containing 8% CA.

Figure 3 depicts a biplot of the CATA sensory attributes identified as being present in each of the chocolate oat milk samples, with a total of 78.10% of the variance explained along *F*1 and *F*2. Clear separation can be observed among the samples, where 10% CA and 6% CA exhibited positive scores, whereas samples 4% CA, 2% CA, and the control showing negative scores along *F*1. Samples with 10% CA and 6% CA were correlated with sensory attributes of bitter, grassy, and earthy, whereas samples with 4% CA, 2% CA, and control were closely correlated with sweet, oaty, and nutty attributes. In a study conducted by Akter et al. (2022), the use of CA extract in meatballs was investigated through sensory analysis, where participants rated meatballs containing 0%, 1%, 2%, and 3% CA extract based on color, flavor, tenderness, and overall acceptability. The results indicated that meatballs with 3% CA extract received the most favorable ratings, suggesting an improvement in consumer acceptability with the addition of CA. Similarly, a study exploring the incorporation of CA powder in noodle formulations found no significant difference in the overall liking ratings of flavor between the sample with no CA and the sample with 5% CA, both of which received higher ratings compared to samples containing 10% and 15% CA, which likely contained less favorable attributes (Nongrum et al., 2020).

Based on the results obtained, it is evident that incorporating cocoa and honey, along with lower to mid concentrations of CA, effectively masks the bitter, earthy, and grassy sensory attributes typically associated with CA. This masking effect allows other desirable attributes such as sweetness, oaty notes, and caramel flavors to dominate, resulting in a more pleasant and palatable chocolate oat milk beverage. Previous research has identified sesquiterpene compounds, such as α-humulene, β-caryophyllene, bicyclogermacrene, germacrene B, and myrcene, present in CA and other plant materials, which contribute to the bitter and earthy aromatics, similar to hops used in beer. The presence of these compounds in CA further supports the observed sensory perceptions of bitterness and earthiness when higher concentrations of CA are present in the beverage (Wongfhun et al., 2010).

Taking into consideration both consumer testing and CATA results, it can be concluded that chocolate oat milk beverages containing 2% and 4% encapsulated CA were found to be acceptable by consumers without exhibiting the undesirable grassy, bitter, and earthy attributes associated with higher amounts of CA. This highlights the

potential for developing a healthy chocolate oat milk beverage incorporating CA, which can offer both nutritional benefits and consumer appeal.

As there is limited research examining the use of CA as a functional ingredient in the food industry, the results from this study provide new insights into the potential incorporation of CA in food and beverage applications. This study also demonstrates the effectiveness of microencapsulation in preserving and delivering bioactive components from plant materials. The sensory evaluation conducted in this study showed that CA can be added to beverages at a level that is acceptable to consumers. Therefore, these findings may serve as catalyst for future product development in the functional food and beverage sector, opening up the potential for incorporating ingredients that offer both nutritional and physiological benefits.

5 | CONCLUSION

This study demonstrates that CA can be microencapsulated, preserving its bioactive components for in food and beverage application. This study also reveals the potential to develop a novel oat milk fortified with CA. The incorporation of CA into the beverages showed promising results in terms of both polyphenol content and sensory evaluation. The polyphenol analysis highlights the potential nutritive benefits associated with incorporating CA into the beverages. Higher sensory ratings for samples containing CA suggest that CA not only enhances the nutritional profile but also contributes to their sensory acceptably. CATA results reveal that varying concentrations of CA significantly impact attributes such as bitterness, grassiness, earthiness, sweetness, oatiness, and nuttiness. Higher concentrations of CA positively correlated with bitter, grassy, and earthy attributes, whereas lower concentrations and the control were associated with sweet, oaty, and nutty characteristics. These findings provide insights of the relationship between CA concentration and specific attributes, contributing to a better understanding of the sensory characteristics of the beverages. Future research can explore optimization strategies byevaluating the impact of CA addition on overall sensory experience and shelf stability of the beverages.

AUTHOR CONTRIBUTIONS

Roselle Samaratunga: Conceptualization; investigation; writing—original draft; methodology; visualization; writing—review and editing; formal analysis; project administration. **Kevin Kantono**: Investigation; methodology; data curation; supervision; resources; formal analysis; software. **Rothman Kam**: Conceptualization; investigation; supervision; formal analysis; project administration; writing—review and editing; writing—original draft; methodology. **Swapna Gannabathula**: Conceptualization; investigation; methodology; data curation; resources; supervision; writing—original draft; writing—review and editing; project administration. **Nazimah Hamid**: Conceptualization; funding acquisition; methodology; writing—review and editing; writing—original draft; software; project administration; supervision; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

This study was approved by the Auckland University of Technology Ethics Committee (AUTEC 19/381). Participants were provided written and informed consent prior to commencement of the study.

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SUPPORTING INFORMATION

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