ORIGINAL ARTICLE

Effect of Various Diluents on the Result of Particle Size Analyzing Process of *Theobroma cacao* Pod Husk Extract

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ABSTRACT

Introduction: Extract of *Theobroma cacao* pod husk has been inspected for its application in dental therapy. Conventional maceration and ultrasonic-assisted extraction technique are several ways to produce the extract. In the process, particle size reduction could happen, which has a beneficial effect. Various tools could be used to examine the particle size of materials. Particle Size Analyzer is the most accurate tool to meet the requisite. A diluent is needed to perform the test using a particle size analyzer. Water is a suggestive diluent, but other diluents could be used, such as ethanol. **Methods:** In this study, extract of *Theobroma cacao* pod husk was prepared using conventional maceration and ultrasonic-assisted extraction. Those extracts were examined using a particle size analyzer with different diluents. **Results:** The study showed that ethanol as a diluent produced smaller particles than water. On conventional maceration extract, when ethanol was used as the diluent, the average particle size was 221.9 ± 49.7 nm; when water was used as the diluent, the average particle was 1,689 ± 359 nm. On ultrasonic-assisted extraction extract, the average particle size was 107.8 ± 27 nm when ethanol was used as the diluent. When water was used as the diluent, the average particle was 320.8 ± 81.6 nm. **Conclusion:** The diluent used in the particle size analysis process will determine the results. Therefore, it is necessary to consider the diluent used in the particle size analysis process to have reliable results.

Keywords: particle size analyzer, nanoparticle, *Theobroma cacao* pod husk extract, good health and well-being

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INTRODUCTION

As an exile product, *Theobroma cacao* pod husk or cocoa pod husk (CPH) has been inspected for its bioactive components. Phenolic compounds extracted from CPH have beneficial effects on dental health for their antioxidant, anti-inflammatory, anti-cariogenic and antimicrobial activities. (1,2) However, the large particle size of bioactive compounds affected their ability to cross cell membranes. Therefore, influence enhancement of the extract could be held by reducing the particle size of those compounds. (3) The extraction process could obtain size reduction of particle size. In a previous study, it was reported that ultrasonic-assisted extraction (UAE) on spinach leaf resulted in a smaller size of particles than conventional maceration (CM). (4) The particle size of materials can be examined using a scanning electron microscope (SEM) and particle size analyzer (PSA). Particle size analyzer has eminence compared to SEM, as it minimizes the sampling errors, allows fast particulate systems characterization, and has reproducible results. The particle size analyzer has several methods: laser diffraction and scattering, photo sedimentation, dynamic light scattering, and others. (5,6)

This study used PSA based on dynamic light scattering methods. Light scattering methods have become the standard method to measure particle size, and it works by quantifying the diffusion coefficients of the particles undergoing Brownian motion. However, a diluent is required to perform the test using this method. (7) According to the system, several common diluents could be chosen. Another study on casein micelles showed that diluent used on PSA process affected the result of particle measurement. (8) Based on that study, water and ethanol were used as diluents to compare the measurement results in the particle size analysis process in this research.

MATERIALS AND METHODS

CPH extract was prepared using CM and UAE techniques.

The pod husk was dried for seven days, mashed into powder using a blender, and sieved using an 80 mesh sieve. The CM technique was conducted by soaking 20 grams of dried samples in 200 mL of 80% ethanol for 48 hours at room temperature. Whatman paper number four was used as a paper filter and continued with the solvent evaporation process at 40°C and 100 rpm. (2) The extract was labeled as CM extract.

UAE technique was conducted in the same ratio as the CM technique. The suspension was put into a sonication bath machine, and the process was established in the 40 kHz frequency, 296 W power, and 55°C for 45 minutes. (3) Finally, the filtrate was filtered and evaporated in the same manner as the CM technique. The extract was labeled as UAE extract.

The particle measurement was carried out using a particle size analyzer (Delsa Nano C, Beckman Coulter). First, the CM and UAE extract samples were prepared in a water bath at 20°C, one hour before the measurement. After this, the samples were diluted using water (for group water) and ethanol (for group ethanol), each in 150 µL of extract/1 L of diluent in a disposable cuvette. (8) Then, the test was performed at 25°C. Data were collected using Delsa Nano C software, resulting in particle size and distribution.

RESULTS

CPH extract obtained from the UAE technique showed a smaller average particle size than the CPH extract obtained from the CM technique on both water and ethanol utilized as the diluent in particle size measurement (Fig. 1). The average particle size of CPH extract using water as a diluent was 1,689 ± 359 nm on the CM technique and 320.8 ± 81.6 nm on the UAE technique. When ethanol was used as the diluent, the average particle of CM extract was 221.9 ± 49.7 nm, and UAE extract was 107.8 ± 27 nm.

All measurements of CPH extract showed a polydispersity index (PDI) under 0.7 (Fig. 2), classified as uniformity in the distribution of size population. This also indicates that the particle size examination result was accurate.

DISCUSSION

The comparison of particle size results on UAE and CM technique was in line with the previous study on spinach leaf extract. Cavitation bubbles formed in the UAE process create several physical effects, including shockwave, microjet, and turbulence. Particle damage, following the effect, results in releasing active substances and reducing particle size. (4,9)

Figure 1 pointed out that ethanol utilization as a diluent on CPH extract measurement resulted in smaller particles than water. This result is affected by the characterization of the extract. Due to ethanol utilization as a solvent in CPH extract preparation, the extract has a hygroscopic character. (10) Nature leads to water absorption and particle swelling when water is used as a diluent. Then it produces greater measurement in the particle size analyzing process.

It was noted that there was a more significant gap between the utilization of water and ethanol in the CM extract's particle measurement results compared to the UAE extract. The recent research mentioned things that might affect the extracted feature, such as drying methods, conditions, and procedures. (11) Nor of these things is relevant in this research. It was also known that the nano-scale particles have a high surface area and tend to agglomerate. (12) In this case, UAE should have the more significant gap, as it has the smaller size particles. Nevertheless, the research found the opposite result. The feasibility reason for the phenomenon was because the ultrasonic cavitation influences the rheological properties of the extract, as seen in the other study on chitosan emulsion preparation using ultrasonic. (13)

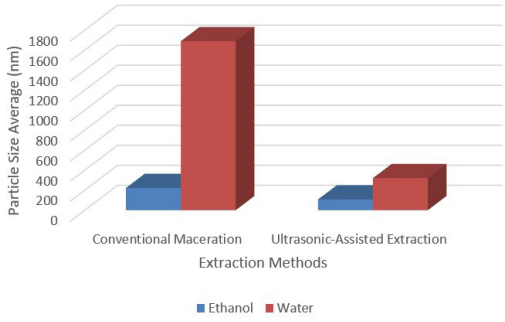

Fig. 1: Diagram of particle size average of CPH extract using water and ethanol as the diluents. CPH extract was prepared using conventional maceration and ultrasonic-assisted technique, followed by particle size measurement using the dynamic light scattering method.

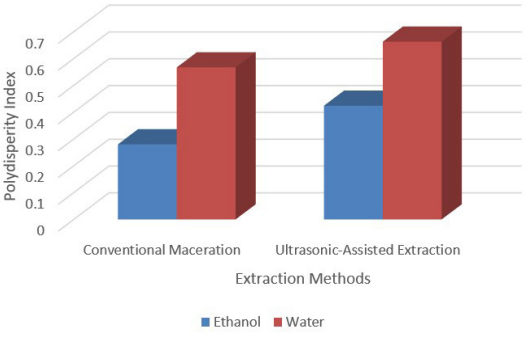

Fig. 2: Diagram of polydispersity index of CPH extract using water and ethanol as diluents. All of the measurements below the value of 0.7 concluded that all extracts have homogeneity in particle size distribution

The PDI ranges from 0.0 to 1.0. While the value under 0.05 indicates the high homogeneity size standard of particles, values 0.05-0.7 showed uniformity, and it is suitable to evaluate using the dynamic light scattering

method. A value more prominent than 0.7 indicates the particle size is broadly distributed or needs to be evaluated using other methods of particle size analyzer. (14)

CONCLUSION

The diluent used in the particle size analysis process affected the measurement. CPH extract using CM and UAE techniques showed a smaller particle size when ethanol was used as the diluent. CPH extract has a hygroscopic character that may be involved in the result.

CONFLICT OF INTEREST

Author declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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