Pertanika Proceedings 1 (7): 151 - 154 (2025)

PERTANIKA JOURNALS

PERTANIKA PROCEEDINGS

Journal homepage: http://www.pertanika.upm.edu.my/

The Characterisation of Colloidal Gas Aphrons Generated with Whey Protein Isolate Solution

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ABSTRACT

Colloidal gas aphrons (CGAs) represent a low-cost separation approach utilizing microbubbles covered by surfactant multilayers. This study aimed to determine the characteristics of CGA generated using 1, 3, and 5% whey protein isolate (WPI) solutions including gas hold-up, half-life, and aphron size. CGAs were generated by intensely stirring WPI solutions using a high-speed homogenizer at 14,000 RPM for 5 min. The results revealed that CGA generated with 5% WPI exhibited significantly higher gas hold-up (51.17%) and stability (half-life = 2192 s) compared to those generated with 3% WPI and 1% WPI, which achieved gas hold-up values of 48.75% and 39.31%, and half-lives of 1454 s and 1063 s, respectively. These findings highlight the superior stability of CGA produced with 5% WPI, attributed to its ability to maintain gas within the bubbles over time, preventing rapid coalescence or collapse. Additionally, CGA generated with 5% WPI also had the smallest aphron size (12.56 μm), compared to CGA generated with 3% WPI (15.49 μm), and 1% WPI (19.09 μm). Considering the stability characteristics observed for CGA generated with 5% WPI, this approach offers a promising natural alternative to reduce reliance on synthetic surfactants in CGA production.

Keywords: Colloidal gas aphrons, gas hold-up, half-life, stability, whey protein isolate

INTRODUCTION

Colloidal gas aphrons (CGAs) are microbubbles with increased interface area that can adsorb charged and/or hydrophobic molecules. The efficiency of target compounds separation or recovery in CGA systems significantly depends on the type of surfactant used. Whey protein isolate (WPI) is a natural surfactant that

ARTICLE INFO

Article history:
Received: 27 October 2025
Published: 10 December 2025

DOI: https://doi.org/10.47836/pp.1.7.027

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e-ISSN: 3083-9475

can be used to encapsulate perishable compounds. According to Cao and Xiong (2017), WPI is widely employed in protein-polyphenol conjugate formation due to its solubility, functionality, and diverse applications in the food industry.

MATERIALS AND METHODS

CGA Generation

Whey protein isolate (WPI) was purchased from LushProtein Ltd (Selangor, Malaysia). Whey protein isolate (WPI) solutions (1%, 3%, and 5%) were prepared by dispersing the powder in distilled water at 25°C for 2 h, then stored overnight at 4°C. The next day, 500 mL of each solution was stirred at 14,000 rpm for 5 min using a high-speed homogenizer before being pumped into the flotation column via a peristaltic pump.

Determination of Gas Hold-up

Colloidal gas aphrons (CGAs) were poured into a 1000 mL measuring cylinder. After one min, the volume of clear surfactant below the CGAs dispersion was recorded. Once the CGAs were fully collapsed, the liquid volume was measured as the initial surfactant volume ($V_{surfactant}$). The method for determining gas hold-up was adapted from Maidin et al. (2018) and calculated using Equation 1, where V_{CGA} is the CGA volume after 5 mins of intense stirring, and $V_{surfactant}$ is the surfactant volume before CGA generation.

$$\text{Gas hold-up, } \varepsilon = \frac{V_{CGA} - V_{Surfactant}}{V_{CGA}} \times 100\ (\%) \qquad [1]$$

Determination of CGAs Stability

Colloidal gas aphron (CGA) stability was assessed by measuring its half-life (τ), defined as the time required for half of the initial surfactant volume to drain. CGA was first generated by stirring a known surfactant volume at 14,000 rpm for 5 min. Then, the time was recorded immediately after the CGA was transferred into a measuring cylinder (Fuda et al., 2005).

Determination of Aphron Size (in diameter)

The aphron of CGA was analyzed using a light microscope (Jauregi & Varley, 1999).

Statistical Analysis

All analyses were conducted in triplicate (n = 3). Data were analysed using one-way ANOVA in Minitab Version 20, and results are presented as mean ± standard deviation. Different letters indicate statistically significant differences between values ($P < 0.05$).

RESULTS AND DISCUSSION

CGA Characterisation

Overall, it was observed that CGAs generated with 5% WPI solution exhibited higher gas hold-up and smaller aphron size (in diameter), which could maximize the interfacial area, and consequently, enhance their efficiency in the recovery process of target compounds. Moreover, their half-life was longer than that of CGAs generated with 1% and 3% WPI solutions (Table 1), as well as those produced using Tween 20 (10 mM), which had a half-life of 628.07 secs, as reported by Aimara et al. (2025). These results demonstrate that surfactant concentration appeared to significantly influence the characteristics and stability of the generated CGAs.

Table 1
The characteristics of colloidal gas aphrons generated using different concentration of whey protein isolates solution

WPI concentration	Gas hold-up (%)	Half-life (s)	Aphron size (μm)
WPI 1%	39.31 ± 2.85^{a}	1062.67 ± 96.69^{a}	19.09 ± 1.42^{a}
WPI 3%	48.75 ± 2.97^{a}	1453.67 ± 163.57^{b}	15.49 ± 0.37^{b}
WPI 5%	51.19 ± 2.52^{b}	2192.00 ± 49.51^{c}	12.56 ± 0.55^{c}

*WPI: Whey protein isolate. Values are expressed as mean ± SD (n = 3). Different letters in the same column denote significant difference $P < 0.05$.

CONCLUSION

This study confirmed that high concentration of 5% WPI improved gas retention, enhanced overall stability, and led to small aphron sizes, preventing rapid bubble collapse. These results suggest that WPI-based CGAs could serve as an effective and sustainable alternative to conventional synthetic surfactants in separation processes.

ACKNOWLEDGEMENT

This research was supported by the Ministry of Higher Education (MOHE) Malaysia through the Fundamental Research Grant Scheme (FRGS/1/2024/STG04/UMT/02/2).

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