

A novel water-in-water microfluidic droplet system enhances cyanidin-3-O-glucoside content in red pigments from defective mulberry fruits

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Defective mulberry fruits, which are considered to be rubbish, are abandoned a large amount every year due to the insufficient preservation technology. The random disposal of defective mulberry fruits causes not only environmental pollution but also resource waste. However, the red pigments in defective mulberries, are safe and nontoxic, and are recognized as a suitable source of anthocyanins in nature. Consequently, reusing defective mulberry fruits can be helpful. Cyanidin-3-O-rutinoside (C₃R) accounts for approximately 50% of the total mulberry red pigments, while cyanidin-3-O-glucoside (C₃G) accounts for approximately 40% of the total (Natić et al., 2015). In recent years, a large number of studies indicated that C₃G has many beneficial effects on the human body. C₃G, as a natural antioxidant, could prevent and improve cardiovascular complications in diabetic rats (Li et al., 2018). C₃G could also protect the biosynthesis of progesterone in rat leydig cells by regulating mitochondrial dysfunction (Wen et al., 2018). Due to these functions of C₃G, it is essential to further enhance the content of C₃G in mulberry red pigments. C₃G and C₃R only differ by the presence of a rhamnosidic bond in the latter. Using α -L-rhamnosidase, C₃R can be specifically transformed into C₃G, and this can improve the content of C₃G in mulberry red pigments and yield a new high-quality mulberry red pigment.

Microfluidic droplet technology is a new microfluidic flow mode. In the microfluidic device, the dispersed phase is dispersed in the continuous phase in a minute volume to form micro-droplets. According to the difference between the dispersed phase and continuous phase, microfluidic droplets can be divided into two types, oil-in-water droplets (W/O) and water-in-oil droplets (O/W). In addition, special water-in-water (W/W) and multiple droplets (W/O/W) can be formed (Ziemecka et al., 2011). Microfluidic droplet technology has been widely studied in chemistry, biology and other fields because of its small volume, large specific surface area, high throughput, precise controllability and other advantages (Liu et al., 2019). Besides, Kim et al. used a micro-droplet system to evaluate drug efficacy by encapsulating pathogenic bacteria to test strains in an antibiotic gradient (Kim et al., 2019). In addition, Liu et al. prepared water-in-oil microfluidic droplets to hydrolyze lipase, the hydrolysis reaction efficiency was increased by 40% to 100%, compared with conventional reactors (Liu et al., 2016). In this paper, the microfluidic droplet technology is used to catalyze mulberry red pigment and further enriches the types of microfluidic devices in the yield of mulberry pigments industrial production.

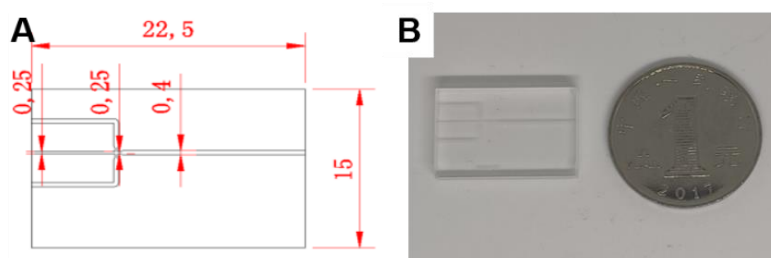


Fig. 1 The flow-focused chip. (A) The internal diameter diagram of flow-focused chip, (B) The diagram of flow-focused chip.

However, the common W/O and O/W droplets are limited by the addition of the oil phase and/or surfactants in bioavailability applications, which requires a large amount of post-treatment to remove the interference of the oil phase. Meanwhile, the waste organic reagents and/or surfactants would also pollute the environment (Kaminski and Garstecki, 2017). The W/W droplets could overcome the above disadvantages. The W/W droplets are generally formed by two incompatible polymer solutions, or a polymer solution and certain salts solution (Hardt and Hahn, 2012). First of all, W/W droplets have good biocompatibility. Liu et al. used PEG/Dex to prepare W/W droplets to encapsulate rat islet cells and the encapsulated islet cells retain high viability and the function of insulin secretion after cultivation for 7 days (Liu et al., 2018). Afterwards, W/W droplets are easy to recycle without causing environmental pollution. Zhang et al. used W/W droplets as microreactors, monodispersed hydrogel particles are synthesized by either UV light or calcium ions, and recovered conveniently without cumbersome post-processing (Zhang et al., 2019). The W/W droplets provide a full water, microscale, and reaction-separation coupling platform for intensification of the enzymatic catalytic process, conducive to environmental protection and resource recycling.

In the present study, PEG was used as the continuous phase and Dex was the dispersed phase to form water-in-water droplets. In order to obtain droplets of appropriate size and stability, by adjusting the two-phase flow rate and observing the formation process of the droplets, it was found that the smaller the inner diameter of the fusion place, the easier it was to form droplets. Therefore, the flow-focusing microchip was selected for subsequent experiments. Fig. 1A shows the inner diameter diagram of the flow-focusing chip and Fig. 1B shows the diagram of the flow-focusing chip. At the intersection of the two phases, the inner diameter of the two phases is 250 μm , and the inner diameter of the part after the fusion is 400 μm . Fig. 2 shows the diagram of the W/W microfluidic droplet reactor. Inlet A was injected with the Dex phase, while inlet B was injected with the PEG-rich phase and rhodamine B. By adjusting the two-phase flow rate, Fig. 2B shows the formation of the W/W droplets in the channel of the flow-focused chip through microscope. When the flow rate of Dex and PEG were respectively fixed at 0.05 and 0.2 $\mu\text{L}/\text{min}$, uniform and stable W/W droplets were formed. In conclusion, the developed W/W microfluidic droplet reactor could be used for conversion of mulberry red pigment in further research.

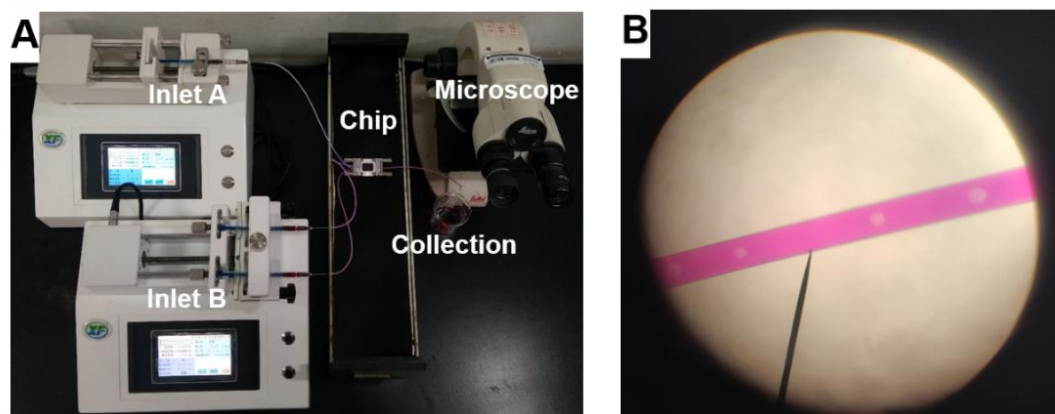


Fig.2 The diagram of the W/W microfluidic droplet reactor. (A) The W/W microfluidic droplet reactor, (B) The photomicrograph of the W/W droplet in the flow-focused chip.

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References:

- [1] Hardt, S. and Hahn, T. Microfluidics with aqueous two-phase systems[J]. *Lab Chip*, **2012**, 12(3), 434-442.
- [2] Kaminski, T.S. and Garstecki, P. Controlled droplet microfluidic systems for multistep chemical and biological assays[J]. *Chemical Society Reviews*, **2017**, 46(20), 6210-6226.
- [3] Kim, S., Masum, F., Jeon, J.S. Recent developments of chip-based phenotypic antibiotic susceptibility testing[J]. *BioChip Journal*, **2019**, 13(1), 43-52.
- [4] Li, W., Chen, S., Zhou, G., Li, H., Zhong, L., Liu, S. Potential role of cyanidin 3-glucoside (C3G) in diabetic cardiomyopathy in diabetic rats: An in vivo approach[J]. *Saudi Journal of Biological Science*, **2018**, 25(3), 500-506.
- [5] Liu, H.T, Wang H., Wei W.B., Liu H., Jiang L., Qin J.H. A microfluidic strategy for controllable generation of water-in-water droplets as biocompatible microcarriers[J]. *Small*, **2018**, 1801095.
- [6] Liu, L., Jiang, L., Xie, X., Yang, S. Amphiphilic carbonaceous microsphere-stabilized oil-in-water pickering emulsions and their applications in enzyme catalysis[J]. *ChemPlusChem*, **2016**, 81(7), 629-636.
- [7] Liu, L., Xiang, N., Ni, Z. Droplet-based microreactor for the production of micro/nano-materials[J]. *Electrophoresis*, **2019**, 0, 1-19.
- [8] Natić, M.M., Dabić, D.Č., Papetti, A., Akšić, M.M.F., Ognjanov, V., Ljubojević, M., Tešić, Ž.L. Analysis and characterisation of phytochemicals in mulberry (*Morus alba* L.) fruits grown in Vojvodina, North Serbia[J]. *Food Chemistry*, **2015**, 171, 128-136.
- [9] Wen, L., Jiang, X., Sun, J., Li, X., Li, X., Tian, L., Li, Y., Bai, W. Cyanidin-3-O-glucoside promotes the biosynthesis of progesterone through the protection of mitochondrial function in Pb-exposed rat leydig cells[J]. *Food & Chemical Toxicology*, **2018**, 112, 427-434.
- [10] Zhang, Q., Chen, J., Gai, H. High-throughput-generating water-in-water droplet for monodisperse biocompatible particle synthesis[J]. *Journal of Materials Science*, **2019**, 54(24), 14905-14913.
- [11] Ziemecka, I., van Steijn, V., Koper, G.J.M., Kreutzer, M.T., van Esch, J.H. All-aqueous core-shell droplets produced in a microfluidic device[J]. *Soft Matter*, **2011**, 7(21), 9878-9880.