



Comparing the formation of lutein nanodispersion prepared by using solvent displacement method and high-pressure valve homogenization: Effects of formulation parameters



Tai Boon Tan ^a, Nor Shariffa Yussof ^a, Faridah Abas ^b, Hamed Mirhosseini ^a,
Imededdine Arbi Nehdi ^c, Chin Ping Tan ^{a,*}

^a Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

^b Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

^c King Saud University, College of Science, Chemistry Department, Riyadh, Saudi Arabia

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ABSTRACT

In this study, we compared the effect of formulation parameters on the physicochemical properties of lutein nanodispersions prepared using a low- and high-energy approach, i.e., solvent displacement (SD) and high-pressure valve homogenization (HPH), respectively. The lutein concentration had a significant effect on the particle size (PS) and particle size distribution (PSD) of nanodispersions that were prepared by using both methods. However, Tween 80 concentration and organic-phase-to-aqueous-phase volume ratio (OAR) only had a significant effect on the PS of nanodispersions prepared by SD. Under all the variations in the formulation parameters, the PSs and PSDs of nanodispersions prepared by SD and HPH were not significantly different. At 0.1% lutein concentration, 0.1% Tween 80 concentration and OAR of 1:9, the nanodispersions prepared by using both methods displayed minimum PS and excellent lutein retentions (>90%). This study showed that SD is a suitable alternative to HPH for preparing lutein nanodispersions.

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1. Introduction

Lutein is a non-provitamin A carotenoid that is usually found in the human eye, primarily in the macula region. As is the case with many other carotenoids, the human body is unable to synthesize lutein; thus, it must be obtained through dietary means. In recent times, there has been a growing interest in lutein because of its biological properties, which may be important in terms of its relevance to human health (Roberts et al., 2009). However, lutein is generally insoluble in water, which hinders its uptake by the human body. Numerous approaches have been taken to increase the bioavailability of lutein (and carotenoids as a whole), with nanotechnology being touted as one of the more promising approaches for creating delivery systems, such as nanoemulsions or nanodispersions, which are able to overcome this problem (Chaudhry and Castle, 2011).

In the field of nanotechnology, the various techniques used to

fabricate nanodispersions can be broadly categorized as either high-energy or low-energy approaches. The high-energy approach usually involves the use of specific equipment, such as high-pressure valve homogenizer, Microfluidizer and ultrasound probe, which are capable of producing highly disruptive forces. The low-energy approach involves methods such as solvent displacement (SD), spontaneous emulsification and phase inversion methods (McClements and Rao, 2011). In the past, the focus among manufacturers and researchers has been on the high-energy approach because it provides ease in terms of industrial scale ups, and the mechanism of droplet breakup via this approach is straightforward and easily understood. However, in recent times, there has been more of a focus on using a low-energy approach in preparing nanodispersions. This change has occurred because the low-energy approach involves only basic and low-cost equipment such as magnetic stirrer, which is relatively cheaper and easier to handle compared with equipment such as high-pressure valve homogenizers that are usually used in the high-energy approach (Saberi et al., 2013). More importantly, the high-energy approach requires high energy input for the fabrication of nanodispersions.

* Corresponding author.

E-mail address: tancp@upm.edu.my (C.P. Tan).

This energy input will consequently generate a huge amount of heat, which is undesirable because most bioactive compounds and food samples are heat-sensitive. Thus, more and more studies are currently being pursued on the low-energy approach because it is more sample-friendly.

Numerous studies have been made to compare the methods used *within* the high-energy and low-energy approaches. For example, Lee and Norton (2013) compared the methods used within the high-energy approach by evaluating the performances of a high-pressure valve homogenizer and a Microfluidizer in terms of droplet breakup. In contrast, Mora-Huertas et al. (2011) compared the low-energy methods of SD and emulsification–diffusion for producing nanoemulsions. However, only a few comparative studies, such as the one by Yang et al. (2012), have been performed *between* these approaches. In their study, high-pressure homogenization (using a Microfluidizer) and spontaneous emulsification methods were compared. They found that both methods were capable of producing ultrafine emulsions. However, the spontaneous emulsification method required the usage of higher concentration of surfactants when compared with the high-pressure homogenization method.

Although there has been a shift in focus towards the use of low-energy approach to prepare nanodispersions, with SD being one of the methods thought to have the potential to replace the high-energy, high-pressure homogenization method, no direct comparison has been made between these two methods. The SD method is a simple, one-step method that relies on the complete water-miscibility of certain solvents, such as acetone and tetrahydrofuran, to form nanodispersions (Beck-Broichsitter et al., 2010). In this method, the compound of interest is dissolved in a water-miscible solvent to form the organic phase. This organic phase is then added to an aqueous phase (usually consisting of surfactant dissolved in deionized water), under continuous magnetic stirring. As the solvent diffuses rapidly into the aqueous phase, the hydrophobic solute becomes more and more supersaturated. This supersaturation of solute leads to spontaneous nucleation and growth of small particles. Once this nucleation and growth process is complete, a stable nanodispersion will be formed. Meanwhile, the formation of nanodispersions by high-pressure valve homogenization (HPH) is related to droplet breakups that are caused by cavitation and/or turbulence in the homogenization chamber (Håkansson et al., 2011).

Therefore, in this study, a direct comparison of lutein nanodispersions prepared with SD and HPH methods was performed. The nanodispersions consisted of lutein (as stabilized by Tween 80 emulsifier) as the dispersed phase and water as the continuous phase. Since both techniques involved distinctly different processing parameters and to provide a fair comparison, only the effects of the formulation parameters, such as the lutein and Tween 80 concentrations and the organic-phase-to-aqueous-phase volume ratio (OAR), on the particle size and particle size distribution were investigated. We were interested in finding whether the nanodispersions prepared via the SD method will differ from those prepared using HPH, in terms of their particle sizes, particle size distributions and lutein content.

2. Materials and methods

2.1. Materials

Lutein was purchased from Rui Heng Industry Co. Limited (Hefei City, China). Polyoxyethylene sorbitan monooleate (Tween 80) and HPLC grade acetone were purchased from Merck (Darmstadt, Germany). Ultrapure water was produced by using a Sartorius Stedim Biotech ARIUM 611DI system (Goettingen, Germany).

2.2. Preparation of lutein nanodispersions

For nanodispersions prepared via SD method, the organic phase was prepared by dissolving lutein in acetone. The organic phase was then added (in one step) to the aqueous phase containing Tween 80; the mixture was stirred for 15 min by using a magnetic stirrer at 500 rpm (MR Hei-Tec; Heidolph, Schwabach, Germany). Subsequently, the acetone was removed from the nanodispersion by subjecting the sample to rotary evaporation (Eyela NE-1101; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) under reduced pressure (0.25 bar at 40 °C). As summarized by Mora-Huertas et al. (2011), most researchers who have employed the SD method used vague terms to describe the stirring speed they applied during their studies, such as gentle, moderate and vigorous stirring; there were only a few who reported their stirring speed in exact rpm (ranging from 250 to 3000 rpm). In this study, a constant stirring speed of 500 rpm was used to ensure consistency. All samples were subjected to a stirring time of 15 min to allow for the complete mixing of the two phases.

For nanodispersions prepared with HPH, the organic phase (consisting of lutein dissolved in acetone) was added (in one step) to the aqueous phase containing Tween 80. The mixture was then homogenized by using a rotor-stator homogenizer (Silverson L4RT; Silverson Machines, East Longmeadow, MA, USA) for 5 min at 5000 rpm. The resulting coarse emulsion was then passed through a high-pressure valve homogenizer (Panda Plus 2000; GEA Niro Soavi, Parma, Italy) for one homogenizing cycle at 80 MPa. Acetone was then evaporated from the nanodispersions under the same conditions as described above. For the HPH method, the samples were passed only once through the high-pressure valve homogenizer at 80 MPa. We chose these parameters based on a study by Cheong et al. (2008), who employed a similar valve-type high-pressure homogenizer and found that a one-cycle homogenization at 80 MPa was sufficient for producing nanodispersions with particle sizes less than 100 nm. The study also showed that increasing the number of homogenizing cycles would lead to bigger particle sizes (from over-processing) and a higher content loss of the compound of interest; thus, only one homogenization cycle was applied in this study.

For both methods, several batches of nanodispersions were prepared by varying the lutein concentrations (0.1–0.5 wt. %) in acetone, the Tween 80 concentrations (0.1–0.5 wt. %) in water, and the OAR (1:9, 2:8 and 3:7).

2.3. Characterization of nanodispersions

2.3.1. Particle size and particle size distribution measurements

The particle size (z-average) and particle size distribution (polydispersity index, or PDI) of the nanodispersions were measured with a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). All measurements were performed in duplicates with five readings for each replicate.

2.3.2. Sample preparation for lutein determination

A reversed phase solid-phase extraction (SPE) C18 cartridge (Phenomenex, Torrance, CA, USA) was conditioned with 6 ml of methanol, followed by 6 ml of deionized water. Then, 1 ml of sample was applied to the conditioned cartridge and washed with 3 ml of deionized water, followed by elution with 10 ml of acetone. The final elution (10 ml) was collected and used for lutein determination. The aforementioned steps were carried out to remove the surfactants from the sample so that only lutein was retained in the final elution. To ensure that only lutein was retained in the final elution, a 10 µl aliquot of the elution was assayed by using Waters e2695 HPLC separation modules equipped with a Waters 2489

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