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Olive oil emulsions formed by catastrophic phase inversion using bacterial cellulose and whey protein isolate

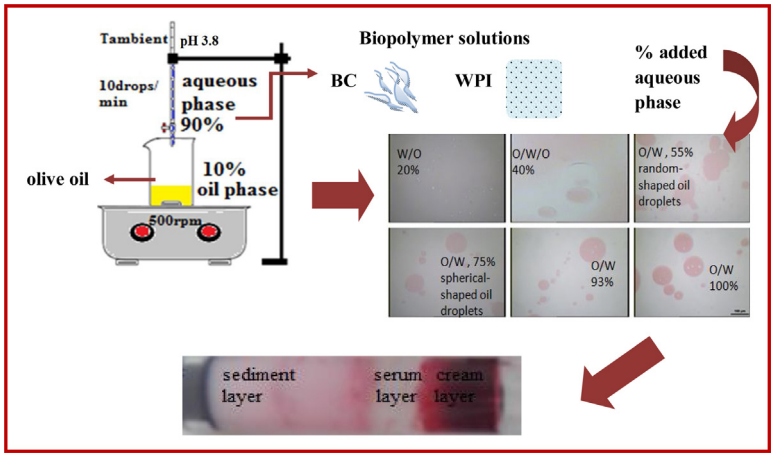
Elli Panagopoulou, Erminda Tsouko, Nikolaos Kopsahelis, Apostolis Koutinas, Ioanna Mandala, Vasiliki Evageliou *

Department of Food Science and Human Nutrition, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece

HIGHLIGHTS

- Olive oil emulsions formed by the catastrophic phase inversion (CPI) technique.
- Bacterial cellulose and whey protein isolate were used as stabilizers.
- Their phase behavior, emulsifying capacity and viscosity were evaluated.
- Phase inversion occurred within the area of 55–70% of the added water phase.
- Produced emulsions were not stable.

GRAPHICAL ABSTRACT



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ABSTRACT

Low energy emulsification methods have gained ground the recent years because of their simplicity in use and low cost. Our work was focused on the use of catastrophic phase inversion (CPI) technique for the formation of olive oil emulsions in the presence of bacterial cellulose (BC) and whey protein isolate (WPI). Firstly, phase behavior of the biopolymers was established. Then, surface and interfacial tension, as well as viscosity measurements of the WPI and BC solutions and their mixtures were carried out. Based on these findings, emulsions were prepared at pH 3.8 with an oil to water ratio of 10:90. The aqueous phase, containing BC and WPI at various concentrations or a mixture of both, was titrated into the oil phase, gradually forming an oil-in-water emulsion. According to our findings, phase inversion happened at the 55–70% of the added water phase. Stabiliser type and concentration had great influence on the oil droplet size. However, all the emulsions produced presented instability phenomena. Thus, indicating that the energy offered to the system by mixing was not sufficient.

1. Introduction

Emulsions find great applications in the Food Industry. They consist of two immiscible liquids, typically the oil phase and the

* Corresponding author. Fax: +30 210 5294691.
E-mail address: evageliou@aua.gr (V. Evageliou).

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aqueous phase. One of the immiscible liquids is dispersed as small spheres in the other. Due to the interfacial tension between the oil and the water phase, emulsions are thermo dynamically unstable and they tend to break down over time. In order to increase stability, emulsifiers are added. Emulsifiers adsorb on the interface between the oil and the water, creating a barrier between the oil droplets and thus, preventing coalescence [1]. Further emulsion stabilization is achieved by the presence of thickening agents. These are usually polysaccharides and their role is to increase the viscosity of the continuous phase and, as a result, retard the droplet movement [2].

Both high and low energy methods are used for emulsion preparation. High energy homogenization is achieved by mechanical devices (e.g., homogenisers, sonicators, etc.) but it is often quite expensive [3]. On the other hand, low energy methods take advantage of the chemical energy stored in the system and they do not use any special equipment. Thus, their cost is rather low. There are several low energy methods among them phase inversion methods. Phase inversion is the conversion of an oil-in-water emulsion (o/w) to water-in-oil (w/o) or vice versa. There are two basic types of phase inversion: transitional and catastrophic [4].

In the present work, emulsions were formed using the catastrophic phase inversion (CPI) which is caused by an increase in the volume fraction of the dispersed phase in the continuous phase. CPI is usually achieved by the titration of the dispersed phase into the continuous phase (in the present study the aqueous phase into the oil phase) under stirring [5]. As the amount of the aqueous phase added is increasing, the system passes through various structures, i.e., from an initial w/o emulsion, to a double o/w/o emulsion and finally an o/w emulsion [6,7].

Additionally, we focused on the use of Whey protein isolate (WPI) and bacterial cellulose as stabilizers. WPI is a mixture of globular proteins, exhibiting great surface activity. Thus, it is widely used as an emulsifier in the Food Industry [8]. BC is produced by strains of *Acetobacter* as an extracellular polysaccharide. It is produced as pellicles consisting of randomly assembled fibrils with width less than 100 nm [9]. Due to its physical properties (e.g., low density, high purity, high water holding capacity), BC has promising applications in the Food Industry in various areas [10], one of them being its use as a stabilizer in food emulsions [11,12]. BC, as well as cellulose from other sources, is used as particles in order to stabilize the so called Pickering emulsions.

Overall, the main goal of our study was to determine the efficiency of the CPI method to form stable olive oil-in-water emulsions containing WPI and BC. The use of environmental friendly stabilizers, such as WPI and BC, is crucial in order to reduce the use of synthetic ingredients in the Food Industry [13]. A primary step of our work was to evaluate the phase behavior, the rheological properties and the emulsifying capacity of the biopolymer solutions used.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) Lacprodan DI-9224 was kindly donated by Arla Foods Ingredients (Amba-Denmark). Bacterial cellulose (BC) was produced by the method of Tsouko et al. [14]. Extra virgin olive oil Altis Klasiko (Elais, Unilever S.A., Athens, Greece) was purchased from a local supermarket and used without further purification. Oil Red O was obtained from Sigma Aldrich (Steinheim, Germany). Citric acid monohydrate and tri-Natrium citrate—Dehydrat were from Merck KGaA (Darmstadt, Germany). Sodium azide was obtained by Fisher Scientific (U.K.). Deionized water was used to prepare all the solutions needed.

2.2. Methods

2.2.1. Preparation of biopolymer stock solutions

The citrate buffer solution (pH 3.8) was prepared by dissolving the required amounts of citric acid monohydrate and tri-Natrium citrate—Dihydrat in deionized water.

A WPI stock solution (10% wt) was prepared by dissolving the appropriate amount in citric acid 0.1 M by magnetic stirring for 90 min at room temperature. Then, sodium azide was added at a final concentration of 0.01% wt as an antimicrobial agent. The solution was stored overnight at 4 °C to ensure complete hydration. Solutions of lower WPI concentration (down to 1% wt) were prepared by dilution with the citrate buffer solution. When not in used, all solutions remained at 4 °C.

Prior to use, the water content of the purified BC pellicles was determined by cutting the pellicles in small pieces and oven drying them at 40 °C. According to our calculations, the water content was 99% wt. With that taken into account, a stock BC solution (0.5% wt) was prepared. BC pellicle pieces were diluted in the citrate buffer solution, treated with a high shear blender (2 runs of 10 min each, 15,000 rpm, Ultra Turrax T25, IKA, Germany), homogenized using ultrasounds (1 min, 20 kHz, 20% amplitude, Sonopuls 3200 with a 13 mm diameter MS 73-492 probe, Bandelin Gmbh & Co., Berlin, Germany). Sodium azide solution at a concentration of 0.01% wt was added to prevent microbial growth. Solutions of lower BC concentration (down to 0.0625% wt) were prepared by dilution with the citrate buffer solution. When not in used, all solutions remained at 4 °C.

2.2.2. Phase behavior of the biopolymer mixed solutions

Appropriate amounts of the WPI and BC stock solutions were mixed, stirred for 1 min and placed in 10 mL test tubes. The concentration of the two biopolymers in the mixed solutions varied from 1–10% wt and 0.0625–0.5% wt for WPI and BC, respectively. Their compatibility was evaluated by visual observation at ambient temperature.

2.2.3. Apparent viscosity

Steady flow curves were obtained using a Discovery HR3Hybrid Rheometer (TA Instruments, New Castle, DE, USA) equipped with concentric cylinder geometry (30 mm cup diameter, 28 mm bob diameter) at shear rates from 1 to 100 s^{-1}. Temperature was kept constant at 25 °C via a Peltier system.

2.2.4. Surface and interfacial tension

Air–water surface tension and olive oil–water interfacial tension were determined at 25 °C by the Du Nouy ring method using a KSV Sigma 701 tensiometer (KSV Instruments Ltd., Finland). Approximately 40 g of the solutions to be tested were transferred into the measurement vessel and in the case of the interfacial tension, 40 g of olive oil were poured on top afterwards. Prior to measurement, the samples were left to equilibrate for 10 min for the surface and 1 h for the interfacial tension measurements, respectively. All measurements were performed in triplicate.

2.2.5. Emulsion preparation

Emulsions were prepared at pH 3.8 with an oil to water ratio of 10:90. Olive oil was used as the oil phase. The aqueous phase, containing BC and WPI at various concentrations or a mixture of both, was titrated via a burette into the oil phase, which was previously dyed with Oil Red O, under constant stirring (500 rpm) at ambient temperature (25 °C) with a flow rate of 10 drops/min.

2.2.6. Microstructure and phase inversion point

The oil phase was dyed with Oil Red O in order to distinguish the oil droplets from the aqueous phase. An optical trinocular