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# The influence of polysaccharide on the stability of protein stabilized oil-in-water emulsion prepared by microchannel emulsification technique

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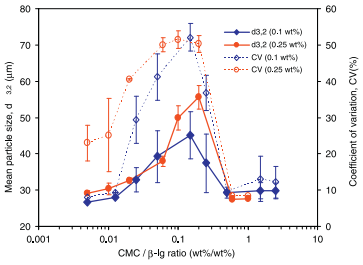
## HIGHLIGHTS

- Monodisperse beta-lactoglobulin (β-lg) stabilized O/W emulsion was produced using MC emulsification.
- The influence of a carboxymethylcellulose (CMC) on the stability of monodisperse β-lg stabilized O/W emulsion was studied.
- The presence of high concentration ratios of CMC/β-lg in the emulsion system enhanced the stability of emulsion.
- The enhanced emulsion stability was observed even at pH near the isoelectric point of β-lg upon heating.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The influence of carboxymethylcellulose (CMC) on the stability of monodisperse beta-lactoglobulin (β-lg) stabilized oil-in-water (O/W) emulsion was investigated. Monodisperse emulsion droplets with particle size ($d_{3,2}$) of 29.6 μm and coefficient of variation (CV) of 9% were generated by microchannel (MC) emulsification using a hydrophilic asymmetric straight-through MC silicon 24 mm × 24 mm microchip consisting of 23,348 microchannels. This study demonstrates that the stability of the emulsions produced was mainly governed by the CMC/β-lg concentration ratios, pH and heating conditions. Emulsions were highly susceptible to aggregation at intermediate CMC/β-lg concentration ratios (0.02–0.25) but were relatively stable at high ratios (higher than 0.5). At the pH near the isoelectric point of β-lg, β-lg stabilized emulsions were more prone to destabilization after heating and prolong storage in contrast to those emulsions with CMC present in their system.



## 1. Introduction

In many food formulations, proteins are often used simultaneously with polysaccharides. Proteins are commonly used as emulsifiers to aid in the formation and stability of emulsions by lowering the interfacial tension between the oil–water interfaces and conferring a protective membrane around the droplets to prevent them from coalescence. Polysaccharides on the other hand, are known for their water-holding and thickening properties and function as stabilizers to provide long-term stability to emulsions against gravitational separation [1]. The nature and strength of the protein–polysaccharide interactions are critical determining factors on the overall stability and structural properties of the food [2]. Knowledge of their interactions is of great importance in the control and manipulation of processed foods, particularly since many of these foods are often exposed to changes in pH, ionic strength,

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temperature, freeze–thawing and competitive interactions with other system components (sugars, lipids, surfactants, etc.) [1].

Whey protein is one of the most commonly used proteins in the food industry. β-lactoglobulin (β-lg) at ca. 55% is the major component of whey protein [3]. β-lg is a globular protein with a molecular weight of 18.3 kDa and isoelectric point (pI) of 5.2. Native β-lg possesses a tertiary structure stabilized by intramolecular disulfide bonds in addition to a free sulfhydryl group which is buried in the interior of the protein structure. The sulfhydryl groups only become available for interaction once the protein unfolds due to changes in pH, ionic strength, temperature or addition of certain chemicals with implications on emulsion stability. Previous studies have shown that globular proteins are susceptible to aggregation when heated above a certain temperature [4]. The extent to which the protein denatures will affect the solubility and other functional properties of the protein, and hence might limit its use in various food applications. Interactions of proteins with polysaccharides have been proposed as an alternative method to increase its utility. In a mixed protein–polysaccharide system, associative electrostatic interactions can lead to coacervation or soluble complex while thermodynamic incompatibility can cause separation into two phases, one rich in protein and the other rich in polysaccharide. Their interactions have been shown to be capable of enhancing or destabilizing the emulsion systems, depending on factors such as the nature of the biopolymers involved, the solution composition and the prevailing environmental conditions [5].

Carboxymethylcellulose (CMC), an anionic water soluble polysaccharide is commonly used in the food industry due to its exceptional water binding capacity, thickening properties, mild taste and odor and its ability to impart desirable textural attributes and mouthfeel to a wide range of food products [6]. Various works have studied the interactions between whey protein and CMC in O/W emulsions [7,8]. Many of these works though, were based on very fine and polydisperse emulsions produced through high pressure homogenization method, making it rather difficult to discern changes to their droplet–droplet interactions.

Here, we make use of a novel emulsification technique called microchannel (MC) emulsification to produce monodisperse emulsion droplets with very narrow particle size distributions. Emulsions produced via MC technique confer many advantages over conventional emulsions in terms of their stability against coalescence due to Ostwald ripening since all the droplets are uniformly sized. In addition, the unique features of the emulsions offer a better understanding of phenomena such as the short-term destabilization mechanisms involving bridging/depletion flocculations and coalescence at the individual microscopic droplet level in a way that is impossible with normal fine polydisperse emulsion droplets made by high pressure homogenization method.

The aim of this study is to investigate the stability of β-lg stabilized monodisperse oil-in-water emulsion prepared by MC emulsification technique and to compare their properties in the presence of CMC.

## 2. Materials and methods

### 2.1. Materials

Powdered β-lg (Biopure) was a gift from Davisco Foods International, Inc. (Lot #JE003-3-922, USA). Refined soybean oil (SBO), sodium acetate, hydrochloric acid (HCl), sodium azide ($NaN_3$) and carboxymethyl cellulose sodium salt (CMC) were obtained from Wako Pure Chemical Industries Ltd. (Japan). Milli-Q water was used for the preparation of all solutions.

### 2.2. Preparation of solutions

Buffer solution was prepared by dispersing sodium acetate into Milli-Q water containing 0.02 wt% $NaN_3$ as an antimicrobial agent to form 10 mM acetate buffer solution. Beta-lg and CMC solutions were prepared by dispersing 0.5 wt% of β-lg and CMC powder individually in buffer solution. Both the solutions were stirred for at least 2 h to ensure complete dissolution, after which they were left to stand overnight at refrigeration temperature to ensure full hydration. CMC solution was adjusted to pH 4 with 1 M HCl while no pH adjustment done for β-lg solution. The solutions were filtered with 0.45 μm cellulose acetate hydrophilic filter (Advantec Toyo Kaisha, Ltd., Japan) before used.

### 2.3. Preparation of monodisperse O/W emulsion by MC emulsification

Monodisperse O/W emulsion was obtained by microchannel (MC) emulsification based on the method of Kobayashi et al. [9]. The experimental setup was as shown in Fig. 1a. Briefly, a 24 × 24 mm asymmetric straight-through MC silicon plate (EP Tech. Co. Ltd., Japan, model WMS1-3) after 20-min of ultrasonic degassing in the continuous phase was assembled into the module (Fig. 1b). Both the dispersed and continuous phases were supplied into the module via two syringe pumps (Model 11, Harvard Apparatus Inc., USA). Soybean oil at a flow rate of 0.2 ml/h and β-lg solution at a flow rate of 9.8 ml/h were used as the dispersed and the continuous phase respectively. Droplet generations occurred when the pressure of the dispersed phase reached the back of the silicon plate in the module in which the dispersed phase was then pushed out into the continuous phase via through-holes to form emulsion droplets. The emulsion droplets formed were swept away from the plate by the continuous phase flow. The formed emulsion was then adjusted to pH 4 with 1 M HCl. CMC of varying concentrations were mixed with the emulsions to give a final emulsion compositions of 1 wt% SBO, 0.25 wt% or 0.1 wt% β-lg and CMC/β-lg ratios (wt%/wt%) of 0.001 to 2.5 at pH 4.

### 2.4. Zeta (ζ)-potential analysis

The ζ-potential of the emulsion droplets was determined using a ζ-potential analyzer (ZEECOM; Microtec Co. Ltd., Japan). This instrument has the capability to measure the ζ-potential of droplets within the particle size range of 0.02–100 μm. Emulsions were first diluted to a droplet concentration of approximately 0.5 wt% oil by using buffer solution prior to analysis. This instrument initially determines the electrophoretic mobility of the droplets by measuring the direction and velocity of the droplet movement when electric field was applied (20 mV). The software then converts these electrophoretic mobility data to ζ-potential values based on the Smoluchowsky mathematical model. ζ-potential measurements are reported as the average ζ-potential and standard deviation of at least two separate experiment runs with at least 20 measurements made per sample.

### 2.5. Particle size analysis

The diameters of the emulsion droplets ($d_{3,2}$) and its particle size distribution (PSD) were determined using a laser diffraction particle size analyzer (Coulter LS 13 320; Beckman-Coulter Ltd., USA). The instrument can measure particle sizes in the range of 0.04–2000 μm. A small amount of emulsion droplets was added to the measuring cell containing Milli-Q water and measurement was started when the droplets were fully dispersed and reached an obscuration of at least 8%. This instrument measures the PSD according to the principles of light scattering whereby the forward