Optimisation of an emulsification process for wheat bran oil encapsulation with proteins

Sandra Fraile, María Olga Ruiz, María Teresa Sanz, José Manuel Benito, and Sagrario Beltrán (beltran@ubu.es)
Department of Biotechnology and Food Science, Chemical Engineering Division, University of Burgos, Burgos, Spain

INTRODUCTION

Wheat bran oil (WBO, Table 1) is an interesting industrial by-product of the flour milling industry due to present large proportions of polyunsaturated fatty acids (PUFA) and natural antioxidants. Nowadays, food grade oil-in-water nanoemulsions from the PUFA rich oils (or PUFA-oil capsules after drying of such emulsions) are commercially attractive for the formulation of new healthier foods with low fat content and new functional foods.

Food proteins can be employed as emulsifying agents due to their ability to quick self-assembly as aggregates at the O/W interface, allowing the formation of a continuous film around the oil droplets. The protein molecule is an adequate carrier material due to its influence on electrostatic and steric repulsions between droplets that help avoiding coalescence and flocculation effects, thus ensuring a long term physical stability for the stored emulsion [1-3].

The main aim of this study was to analyse the use of bovine serum albumin (BSA), sodium caseinate (SC) and whey protein concentrates (WPC) for the preparation of WBO nanoemulsions. Different process parameters, including formulation variables and operating conditions, were studied to determine their effects on the oil encapsulation efficiency, the emulsion droplet size distribution and its colloidal stability for 2-120 h.

Table 1. Profile of fatty acids, alkylresorcinols (AR) and tocopherols (T) for WBO used in this work.

Fatty acids	
C16:0 fatty acid (%)	17.30 ± 0.10
C16:1n-7 fatty acid (%)	0.20 ± 0.01
C18:0 fatty acid (%)	1.10 ± 0.01
C18:1 n-9 fatty acid (%)	16.10 ± 0.02
C18:1 n-7 fatty acid (%)	1.05 ± 0.01
C18:2 n-6 fatty acid (%)	57.70 ± 0.07
C18:3 n-3 fatty acid (%)	5.20 ± 0.04
C20:0 fatty acid (%)	0.19 ± 0.01
C20:1 n-9 fatty acid (%)	0.70 ± 0.02
C20:2 n-6 fatty acid (%)	0.10 ± 0.01
C22:0 fatty acid (%)	0.18 ± 0.01
C24:0 fatty acid (%)	0.18 ± 0.01
Alkylresorcinols	
C15-AR (mg/g oil)	0.5 ± 0.1
C17-AR(mg/g oil)	2.0 ± 0.1
C19-AR (mg/g oil)	6.9 ± 0.1
C21-AR (mg/g oil)	10.5 ± 0.3
C23-AR (mg/g oil)	3.1± 0.3
C25-AR (mg/g oil)	1.4 ± 0.2
Tocopherols	
α-T (mg/g oil)	1.68 ± 0.04
β-T (mg/g oil)	0.15 ± 0.01
γ-T (mg/g oil)	0.89 ± 0.04
δ-T (mg/g oil)	-

MATERIALS AND METHODS

Raw whey with 25 g/L of lactose was supplied by a local cheese factory. Removal of lactose and subsequent concentration of whey protein were performed by ultrafiltration using a 100 kDa tubular multichannel Filtanium ceramic membrane (7-channel configuration, Tami Industries) with an effective membrane area of 132 cm^2. A final WPC at pH = 7 with 10 g/L of total proteins and 0.017 g/L of lactose was obtained.

BSA and SC dissolved in water (pH = 7) or in several buffers (pH = 2, 7, and 10) and WPC were used as the continuous phase. The oil content in the formulation was 1.0 wt% for all samples. Quantities of each sample component were measured using an analytical balance (± 0.001 g) to obtain a final weight of 10.00 ± 0.01 g. The mixture was manually shaken for 5 min before the emulsification process.

O/W emulsions with identical formulations were prepared (Fig. 1) using a high speed blender (Miccra D-9, ART Labortechnik, Mülheim, Germany), an ultrasound homogeniser (Vibra-Cell VCX500, Sonics & Materials Inc., USA) and the combination of both mechanical devices under several operating conditions.

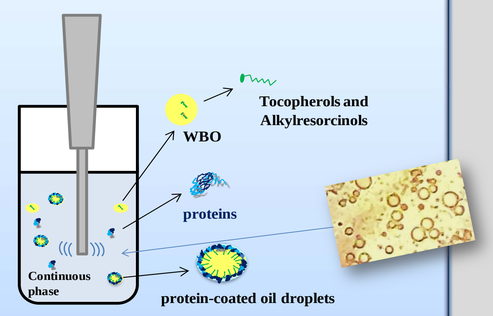

Figure 1. Schematic diagram of the emulsification process.

All samples were immersed in an ice bath to avoid overheating of the sample during process. Then, all formed emulsions were stored in darkness at 20.0 ± 0.5 ºC and analysed experimentally after 2-120 h to determine the physical stability of the emulsions

RESULTS AND DISCUSSION

A. Effect of the Continuous Phase Viscosity for SC/WBO Samples

All droplet size distributions for these initial emulsions of Fig. 2 were multimodal (at least two or three peaks), without providing uniform sized oil droplets.

A high reduction of D_{43} (Fig. 2) from 1988 ± 18 nm to 181 ± 27 nm was reached when SC amount decreased from 8 to 1 wt% in the continuous phase. This behaviour can be justified in terms of the continuous phase viscosity (η) due to the modification of Reynolds number (Re=9,061-2,100 for samples with 1-8% of SC, respectively). Thus, η has been found to be a limiting factor to achieve a fully turbulent flow for the entire mixer, which is required to enhance the efficiency of WBO droplet rupture during the emulsification process.

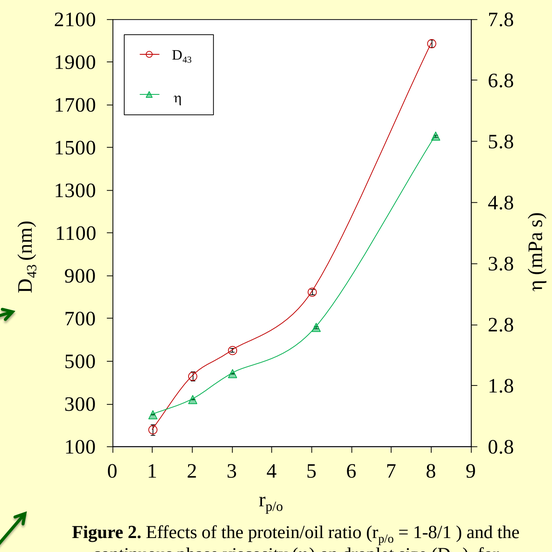

Figure 2. Effects of the protein/oil ratio ($r_{p/o}$ = 1-8/1) and the continuous phase viscosity (η) on droplet size (D_{43}) for SC/WBO samples formulated in water at pH=7. **Operating conditions for shear emulsification with a high speed blender: 29,000 rpm for 300 s.**

A partial destabilisation (creaming layers and/or a visible phase separation) was observed after 72-120 h for all samples of Fig. 2.

B. Results for Ultrasonic Emulsification

Comparison of BSA and SC as Encapsulating Agents

Droplet size distributions with narrower peaks and lower D_{43} were reached when using SC as encapsulating agent, as can be seen in Fig 3. In addition, higher SC concentrations (η = 1.33-2.00 mPa s for samples with 1-3 wt% SC, respectively) have been found to increase shear effect of cavitation on droplet surface during the emulsification process.
Stable protein film around oil droplet, with no sample modification after 120 h was obtained for all SC/WBO samples of Fig. 3.

Effect of ultrasound time

D_{43} was not significant affected by the modification of ultrasound time from 50 to 100 s for SC/WBO formulations with a $r_{p/o}$=1/1.

A pre-emulsification stage with a high speed blender at 29,000 rpm for 300 s

In SC/WBO samples, the best result providing uniform sized droplets (D_{43}= 214 nm) was achieved using a shear pre-emulsification stage before ultrasonication. Furthermore, D_{43} remained constant and independent of the protein concentration (1-3 wt% of SC). This result indicates that the use of a premixing stage removed the effect of η on cavitation for SC/WBO samples with $r_{p/o}$ from 1/1 to 3/1.

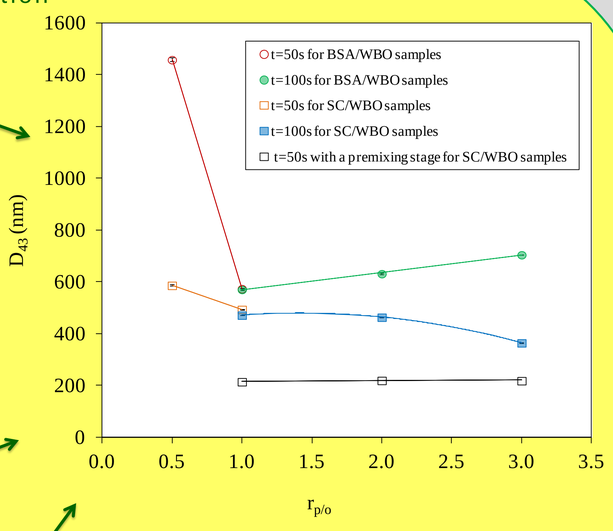

Figure 3. Effects of protein/oil ratio ($r_{p/o}$ = 0.5-3/1), ultrasound time and the use of a premixing stage on droplet size (D_{43}) for BSA/WBO and SC/WBO samples formulated in water at pH=7. **Operating condition for ultrasonic emulsification: effective time of 50 or 100 s, with pulses every 5 s (5 s on and 5 s off, 20% amplitude, 500 W, 20 kHz frequency).**

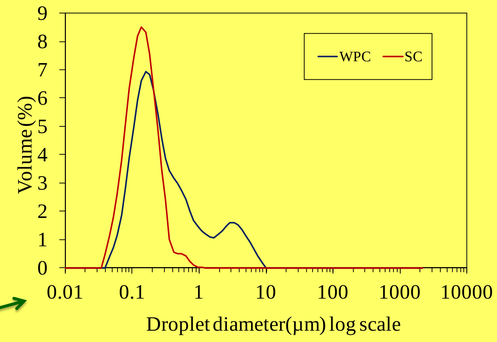

Figure 4. Comparison of WBO droplet size distributions for SC/WBO and WPC/WBO emulsions formulated with a $r_{p/o}$ = 1/1 at pH 7. **Operating conditions for combined emulsification process: 29,000 rpm and 300 s for pre-emulsification stage; 100 s for ultrasonication stage.**

Whey Protein Concentrates as Encapsulating Agent

The use of WPC promotes the formation of wider peaks, a decrease of the small size structures and the formation of larger size structures with values from 1 to 7 µm.

C. Effect of the Ionic Strength and pH of the Continuous Phase

A ultrasound time of 300 s was required in order to decrease the value of D_{43} in these emulsions formulated in buffers.

Best emulsions, with uniform and small oil droplet size, were obtained at pH about 7 (in buffers and water), slightly above the isoelectric point of SC.
The comparison of emulsions at pH 7 indicates that the presence of Na^+ and K^+ increase the oil droplet interfacial tension and continuous phases with salts reduce the bubble coalescence rate [4,5], thereby both combined effects reducing shear forces upon the droplet surface during its deformation and rupture

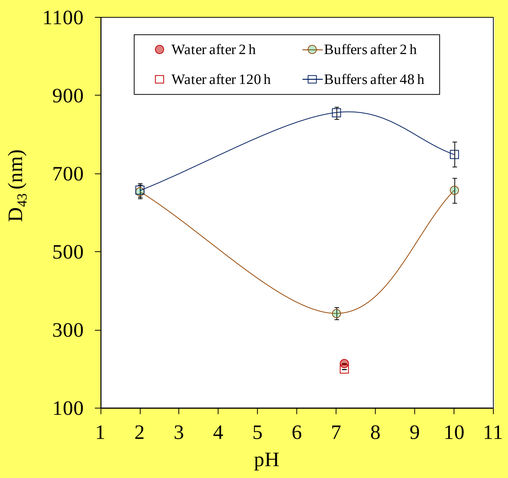

Figure 5. Results of D_{43} for SC/WBO emulsions formulated in water (pH =7) or in different buffers (pH = 2, 7, and 10) with a $r_{p/o}$=1/1. **Operating conditions for combined emulsification process: 29,000 rpm and 300 s for pre-emulsification stage; 50 s or 300 s for ultrasonication stage in water formulations or in buffer formulations, respectively.**

After 48 h, a partial destabilisation was observed for all emulsions formulated in buffers.

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