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Original article

Optimization of dynamic loop mixer operating conditions for production of o/w emulsion for cell microencapsulation

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Abstract – Emulsification and spray-drying were selected to develop a low-cost cell microencapsulation method adaptable to large-scale production to improve the stability of sensitive probiotic bacteria. The aim of the present study was to determine the optimum operating conditions to produce anhydrous milk fat/whey protein emulsion using a dynamic loop mixer as a first step for the microencapsulation process. The effect of various parameters of the two-phase dispersion process on fat globule size and their distribution measured at steady state was examined: the rotation speed of the helical impeller in the mixer (2000, 2500 and 3000 rpm), hydrophilic/hydrophobic phase ratio (95/5, 92.5/7.5 and 90/10 w/w), and percentage of dry material to encapsulate in the hydrophobic phase (5, 10 and 15% w/w). Fat globule size distribution was found to be only dependent on the internal mixing speed in the dynamic loop mixer ($P < 0.05$). A rotation speed of 2500 rpm, corresponding to a fat globule population with $D[3, 2]$ of 23 μm, $D(v, 0.1)$ of 15 μm, $D(v, 0.5)$ of 25 μm, and $D(v, 0.9)$ of 49 μm was selected for the production of multiphase spray-dried microcapsules with a diameter smaller than 100 μm, containing micronized powder particles of freeze-dried bacteria. This research showed that anhydrous milk fat/whey protein emulsions with controlled fat-globule size distribution and small size dispersion can be prepared with a continuous mode and high productivity with a dynamic loop mixer.

Emulsification / dynamic loop mixer / whey protein / anhydrous milk fat / microencapsulation

Résumé – Optimisation des conditions d'opération d'un mélangeur à boucles pour la production d'une émulsion de type huile-dans-eau pour l'encapsulation cellulaire. L'émulsification et le séchage par atomisation ont été sélectionnés pour développer une méthode de microencapsulation cellulaire à faible coût adaptable à une production à grande échelle pour améliorer la stabilité des bactéries probiotiques sensibles. Le but de la présente étude était de déterminer les conditions d'opération optimales permettant de produire une émulsion huile de beurre/protéines sériques en utilisant un mélangeur à boucles comme première étape du procédé de microencapsulation. L'effet de différents paramètres du procédé de dispersion de phase sur la taille des globules gras et leur distribution, mesuré à l'état stationnaire, a été par la suite étudié : vitesse de rotation de la turbine hélicoïdale dans le mélangeur (2000, 2500 et 3000 rpm), rapport phase hydrophile/phase hydrophobe (95/5, 92,5/7,5 et 90/10 p/p), pourcentage de matériel sec à encapsuler dans la phase hydrophobe (5, 10 et 15 % p/p). Il a été montré que la distribution de taille des globules

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gras était uniquement dépendante de la vitesse de recirculation dans le mélangeur à boucles ($P < 0,05$). Une vitesse de rotation de 2500 rpm, correspondant à une population de globules gras avec un $D[3, 2]$ de 23 µm, un $D(v,0,1)$ de 15 µm, un $D(v,0,5)$ de 25 µm, et un $D(v,0,9)$ de 49 µm a été sélectionnée pour la production par séchage-atomisation de microcapsules multiphasées possédant un diamètre inférieur à 100 µm, contenant des particules de poudre micronisée de bactéries lyophilisées. Cette recherche a montré que le mélangeur à boucle permet de produire, en continu et avec une productivité élevée, une émulsion huile de beurre/protéines sériques avec un contrôle élevé de la taille des globules de gras et une faible dispersion.

Émulsification / mélangeur à boucles / protéine de lactosérum / huile de beurre / microencapsulation

1. INTRODUCTION

Microencapsulation has recently been reported to provide protection to sensitive probiotic lactic cultures from high oxygen levels [34], freezing [30] and acidic environments during the manufacture and storage of yoghurt [1] and during transit through the gastro-intestinal tract of consumers [17]. However, although promising on a laboratory scale, the technical difficulty and high cost of the process have made it unattractive to manufacturers for industrial-scale applications.

Microencapsulation is defined as a technology for packaging solids, liquids or gaseous materials in miniature sealed capsules that can release their contents at controlled rates under specific conditions [31]. Water-insoluble dry microcapsule preparations with low (< 100 µm) and controlled particle size are desirable for incorporation of immobilized probiotic bacteria in food products for various reasons, including higher stability, easier handling and storage of the cultures, and limited effects on the sensorial properties of the products, especially texture. Among the numerous methods proposed for the manufacture of microcapsules, spray-drying is certainly the most appropriate technique for developing such a system [31]. Spray-dry encapsulation is a well-established process, involves readily available equipment, and is able to produce large amounts of capsules. However, this economical and effective technology for protecting active materials is rarely considered for cell immobilization because of the high mortality resulting from simultaneous dehydration and thermal inactivation of the microorganisms [13]. The use of bacterial cultures in dried form for capsule formation before drying has not been investigated yet. Suspending fine powder particles of freeze-dried cells in a hydrophobic material and dispersing the resulting suspension in a solution of wall material prior to atomization might be an interesting alternative for limiting the extent of cellular injury and increasing the ability of microorganisms to withstand the high temperatures employed in the process. However, there are obvious challenges associated with the production of multiphase spray-dried microcapsules with diameters lower than 100 µm for cell immobilization, particularly concerning the control of the size of the different components entering into the composition of the capsules (powder particles of freeze-dried culture, oil/fat globule size).

Industrial-scale emulsification is routinely conducted in the food industry and may be easily applied to the production of macroemulsions with a droplet size suitable for efficient coating of fine powder particles of freeze-dried culture (i.e., ranging from 10 to 50 µm). The reactors typically used to prepare these types of emulsion are cylindrical vessels agitated by means of various impellers (a turbine with blades of various designs, marine-style impeller or grid device). In such devices, shear, energy dissipation and dynamic pressure are not

homogeneously distributed. Dead volumes or stagnant zones may be present as the vessel volume increases or when mixing viscous fluids, leading to large size dispersions. One alternative for minimizing these problems may be provided by a dynamic loop mixer. The operating principle of this new type of mixer is based on a succession of loops through a rotor/stator mixing unit inside a small, closed, specifically designed vessel, which ensures the homogeneous mixing of the substances even if their viscosity and composition are different. However, despite its promising potential the use of a dynamic loop mixer for emulsification and the effects of homogenization conditions on the properties of o/w emulsion have not yet been reported in the scientific literature.

Whey proteins are extensively used as functional ingredients in food systems [24]. The physico-chemical and functional properties of whey proteins have been extensively reviewed [19, 24] and would appear to satisfy the requirements of an encapsulating agent. Microencapsulating properties of whey proteins have been investigated and reported in recent years [18, 29]. Particularly, it has been shown that whey protein isolate (WPI) might be an effective wall system for anhydrous milk fat-containing microcapsules prepared by spray-drying, providing an effective barrier against oxidation [23]. Milk fat and other hydrophobic substances have previously been reported as potential cell immobilization matrices [2, 7, 21]. Diffusion of H^+, organic acids, water and oxygen across membranes of lipid capsules has been shown to be very limited. This property can be particularly useful for protecting viable probiotic bacteria during storage in fermented dairy products. Another important functional characteristic of whey proteins is their ability to form salt-induced gels at room temperature. Cold-set gelation is generally achieved by adding Ca^{2+} ions to a WPI suspension preheated under specific conditions [3, 15]. The production of water-insoluble whey-protein beads using an emulsification/cold gelation process has recently been proposed for protection and subsequent release in the intestine of bioactive molecules sensitive to the gastric environment [4]. The gastroresistance properties of cold-set gel of whey proteins along with their aforementioned microencapsulating properties may offer unique opportunities for developing capsules with controlled release in the intestine of active food or nutraceutical ingredients, such as probiotic organisms.

Emulsification and spray-drying were selected to encapsulate milk fat droplets containing freeze-dried probiotic bacteria in water-insoluble whey protein-based microcapsules. Within this context, the main objective of the present study was to determine the optimum operating conditions to produce an anhydrous milk fat/whey protein emulsion with a fat globule size distribution suitable for large-scale production of multiphase spray-dried microcapsules smaller than 100 μm. Another aspect of this work was to examine the effects of various emulsion parameters (hydrophilic/hydrophobic phase ratio, internal mixing speed in the mixer and percentage of non-fat solids in the hydrophobic phase) on fat globule size and their distribution using a dynamic loop mixer for emulsification.

2. MATERIALS AND METHODS

2.1. Ingredients in the emulsion

Anhydrous milk fat (AMF) containing 99.9% (w/w) lipids was purchased from Ault Foods (Mitchell, ON, Canada), and whey protein isolate (WPI) containing 97.5% (w/w) proteins from Davisco International (Le Sueur, MN, USA).

Low heat skim milk powder was obtained from Agropur (Granby, QC, Canada) and used as a model powder to replace the expensive powder of freeze-dried

bacteria during development of the microencapsulation technology. The milk powder particles were micronized before use with a spiral jet mill 50 AS (Hosokawa Alpine AG, Augsburg, Germany) in order to get a final population with a $D(v, 0.9)$ (i.e., the size at which the cumulative volume reaches 90% of the total volume) lower than 25 μm. The particle size distribution of the treated powder was measured with a laser diffractometer (Mastersizer S, Malvern Instruments, Southborough, MA, USA).

2.2. Dynamic loop mixer

A 1 L IMT continuous dynamic loop mixer (International Mixing Technology, Dunkerque, France) was used for phase dispersion. The operating principle of this new type of dynamic mixer is illustrated in Figure 1 and briefly described below. The different products to be mixed are introduced through the lower inlet port of the mixer and directed into a central tuyere equipped with a worm screw that is used to guide the product flow upwards and control the internal mixing speed and number of loops. The feed components are forced through the mixing elements; the resulting dispersion is first returned downwards along the walls of the outer mixing chamber, and then redirected towards the inner tube where it is mixed with a new incoming vein of products. This loop process is repeated until the final product is directed to the outlet of the mixer via the exit ports of the outer mixing chamber. Different rotor/stator equipment and internal mixing speeds can be used to produce homogeneous final products with different characteristics. For our study, the rotor/stator mixing unit with fine transverse grooves designed for producing fine emulsions was used.

2.3. Preparation of the emulsion

Whey protein solution containing 10% (w/w) solids was prepared in distilled water and kept overnight at 4 °C. The solution was adjusted to pH 7.0 ± 0.1, heat-treated at 80 °C for 30 min under agitation in a Groen steam-jacketed cooker/mixer (Model DTA/3, Groen, Jackson, MS, USA) to denature proteins, and cooled to 45 °C. Milk fat was preheated at 60 °C in a water bath to dissolve all crystals and cooled to 45 °C. Skim milk powder was then dispersed into the hydrophobic phase using a Caframo stirrer (Model RZR 50, Caframo Ltd., Wiarton, Ontario, Canada) at the lowest speed for 20 min. The resulting suspension was kept at 45 °C while stirring. The hydrophobic phase containing non-fat solids (skim milk powder particles) and the whey protein solution were simultaneously pumped in the dynamic loop mixer at various ratios with calibrated progressive cavity pumps (Seepex MD 006, Seepex Inc., Enon, OH, USA), for a total flow rate of 30 $kg \cdot h^{-1}$ entering the vessel. The effects of the percentage (w/w) of hydrophobic phase in the emulsion, ε, internal mixing speed (i.e., the rotation speed of the helical impeller in the mixer), Ω, and percentage (w/w) of non-fat solids (NFS) in the hydrophobic phase, Ψ_{NFS}, on the fat globule size distribution were investigated using a Box-Benhken design (Tab. I).

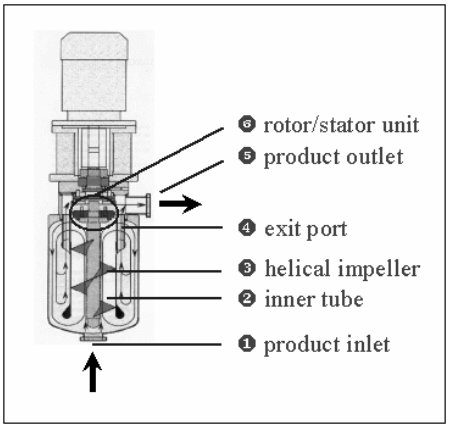

Figure 1. Schematic representation of the 1 L IMT continuous dynamic loop mixer.

Table I. Process variables, coded variables and responses of the Box-Behnken design (BBD).

	Process variables			**Corresponding coded variables**			**Responses**				
Run	$\varepsilon^{(a)}$ (%)	$\Psi^{(b)}_{NFS}$ (%)	$\Omega^{(c)}$ (rpm)	**x1**$^{(d)}$	**x2**$^{(e)}$	**x3**$^{(f)}$	Φ_{10-50} (%)	***D*[3,2]** (μm)	***D*(v, 0.1)** (μm)	***D*(v, 0.5)** (μm)	***D*(v, 0.9)** (μm)
1	5	5	2500	–1	–1	0	92.0	21.1	13.8	22.2	42.6
2	5	10	2000	–1	0	–1	64.6	34.7	20.6	38.9	75.5
3	5	10	3000	–1	0	1	92.4	16.8	10.8	18.3	32.9
4	5	15	2500	–1	1	0	90.5	21.5	13.2	23.7	43.0
5	7.5	5	2000	0	–1	–1	69.7	32.3	18.8	34.6	79.4
6	7.5	5	3000	0	–1	1	94.2	17.1	11.4	18.0	33.9
7	7.5	15	3000	0	1	1	92.6	17.6	11.8	18.9	32.9
8	7.5	15	2000	0	1	–1	64.5	35.6	21.7	39.4	79.1
9	10	5	2500	1	–1	0	89.8	21.5	13.7	22.9	46.0
10	10	10	3000	1	0	1	92.4	16.5	10.6	17.6	33.7
11	10	15	2500	1	1	0	91.0	21.0	13.0	22.7	44.4
12	10	10	2000	1	0	–1	68.4	33.4	19.7	36.5	76.4
13	7.5	10	2500	0	0	0	87.3	23.8	14.6	25.8	50.0
14	7.5	10	2500	0	0	0	86.9	23.3	14.6	25.2	49.9
15	7.5	10	2500	0	0	0	88.8	23.3	14.8	25.3	48.4

$^{(a)}$ ε = % (w/w) of hydrophobic phase in the emulsion; $^{(b)}$ Ψ_{NFS} = % (w/w) of NFS in the hydrophobic phase; $^{(c)}$ Ω = internal mixing speed; $^{(d)}$ xl = coded variable for ε; $^{(e)}$ x2 = coded variable for Ψ_{NFS}; $^{(f)}$ x3 = coded variable for Ω.

Once the system was stabilized and the steady state was reached, samples were collected at the exit of the mixer and immediately observed by phase-contrast microscopy for fat globule size distribution analysis. To determine the stabilization time of the system, the residence time distribution in the mixer was studied.

2.4. Determination of the residence time distribution

The residence time distribution (RTD) of material in the dynamic loop mixer was determined using a step input tracer test, according to the procedure reported by Levenspiel [20]. The method consisted of feeding the vessel with water, switching from water to a concentrated tracer solution, and measuring the tracer concentration of the fluid leaving the vessel until it reaches the concentration at the entry. The mixer was initially filled with deionized water and the flow was changed at time $t = 0$ from water to a 5 g·L^{-1} NaCl solution. Samples of 25 mL were collected at the mixer's outlet every 15 s during the first 2 min and every 30 s during the remainder of the experiment, then kept at 20 °C overnight before measuring conductivity. The distribution of the exit tracer concentration C_e as a function of time (C-curve) was

determined from conductivity data. To compare the *C*-curves and in order to make them independent of the process conditions, the tracer output concentration must be normalized. The *C*-curve obtained experimentally was directly transformed to a normalized step function, called the *F*-curve, by changing the concentration scale so that the values lie between 0 and 1 [20]. From this dimensionless step response curve, the mean residence time in the vessel, t_m, is given by:

$$t_m = \frac{\int_0^{C_{max}} t\,dC}{\int_0^{C_{max}} dC} = \frac{1}{C_{max}} \int_0^{C_{max}} t\,dC$$

$$= \int_0^1 t\,dF_t$$

where C_{max} corresponds to C_i, the inlet tracer concentration. The $F(t)$ function can also be expressed by using a dimensionless time variable (θ), which measures time directly in terms of units of t_m, i.e.,

$$\theta = \frac{t}{t_m} \; ; \; F(\theta) = F(t).$$

The time required to achieve equilibrium in the system, λ, was defined as the time necessary to obtain $C_e/C_i = 0.99$ and was expressed in units of normalized time (t_m). Two sets of experiments were carried out to measure t_m and λ for different operating conditions. The first one was performed at three different flow rates (25, 30 and 40 kg·h^{-1}) using a constant rotation speed of the helical impeller (2500 rpm). In the second set, three different internal mixing speeds, 2000, 2500 and 3000 rpm, were used with a constant flow rate of 30 kg·h^{-1}. All the step experiments were repeated three times.

2.5. Conductivity measurement

Electrical conductivity was measured at 20 °C using a portable conductivity meter (Horiba ES-14, Horiba Ltd., Kyoto, Japan). The values obtained were converted to salt concentrations with a standard curve developed from NaCl solutions in a concentration range from 0.05 to 5 g·L^{-1}.

2.6. Fat globule size measurements

The emulsion particle size distribution was determined from microscopic observations coupled with image analysis. Three microscopic slides were prepared for each experiment by placing one drop of the emulsion on a slide immediately after production. The drop was carefully covered with a cover slide whose edges were subsequently varnished in order to limit the diffusion phenomena. A phase-contrast microscope (Leitz laborluxS, Leica, Wetzlar, Germany) was used to examine between 8 and 10 areas of each emulsion sample preparation at magnification X100. Pictures were taken with a coupled video camera device (High resolution CCD-Iris:RGB color video camera with camera adaptator CMA-D2, Sony Co. Ltd., Tokyo, Japan) interfaced with image analysis software (Matrox Inspector 1.71, Matrox Electronic System Ltd., Dorval, QC, Canada). Images in a black and white format were calibrated by converting pixels to microns and processed by blob analysis. The mean Feret diameter, calculated as the mean value of twelve Feret diameters measured between 0° and 360° with intercalated angles of 30°, was determined for 250–1000 fat globules on each microscopic slide. Particle sizes were converted from individual diameter to the volume of the diameter equivalent sphere and used to calculate the volume size distribution of fat globules. The Sauter mean diameter or volume-surface mean diameter $D[3, 2]$, defined as the diameter of the particle with volume to surface area ratio equal to the arithmetic mean of the volume to surface area ratio calculated for all particles in the sample, the 10% volume fractile of the particle size distribution $D(v, 0.1)$, the volume median

diameter $D(v, 0.5)$, the 90% volume fractile of the particle size distribution $D(v, 0.9)$, and the percentage (% v/v) of fat globules with sizes ranging from 10 to 50 μm, Φ_{10-50}, were determined.

2.7. Experimental design and statistical analysis

A Box-Benhken design (BBD) with three independent variables was selected for the emulsion experiments. This type of design is typically used to obtain full second-order polynomial models, with information on the linear and quadratic effects as well as two-factor interactions. The process variable levels studied and coded variables are presented in Table I. The BBD was used in the present study to determine the relationships between the fat globule size distribution parameters (D[3, 2], $D(v, 0.1)$, $D(v, 0.5)$, $D(v, 0.9)$ and Φ_{10-50}) and the process parameters studied (hydrophilic/hydrophobic phase ratio, internal mixing speed and percentage of NFS in the hydrophobic phase). These relationships were subsequently used to select the optimum values of processing parameters in order to produce an emulsion with specific characteristics. Analysis of variance and regression calculations were all carried out using Statgraphics Plus 4.0 (Statistical Graphics Co., Rockville, MD, USA).

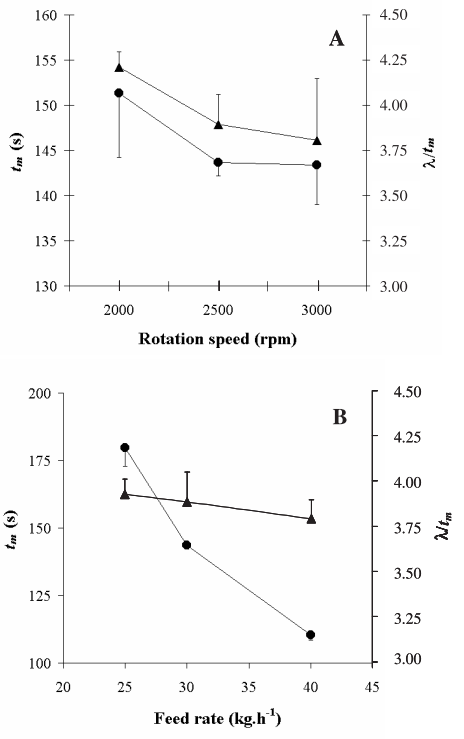

Figure 2. Mean residence time, t_m (●), and number of t_m necessary to obtain system equilibrium, λ/t_m (▲), as a function of (A) internal mixing speed for a feed rate of 30 kg·h^{-1}, and (B) feed rate at internal mixing speed of 2500 rpm. Error bars represent standard deviation.

3. RESULTS AND DISCUSSION

3.1. Residence time distribution in the dynamic loop mixer

The objective of this preliminary part of the study was to investigate the effects of feed rate and internal mixing speed on the RTD of material in the dynamic loop mixer using water as a model fluid. Increasing the rotation speed of the helical impeller from 2000 to 3000 rpm at a feed rate of 30 kg·h^{-1} slightly reduced both t_m, the mean residence time, and λ, the time necessary to obtain $C_e/C_i = 0.99$ (Fig. 2a); however, this effect was not significant ($P > 0.05$). On the other hand, the increase in feed rate from 25 to 40 kg·h^{-1} at an internal mixing speed of 2500 rpm resulted in a significant decrease of t_m ($P < 0.05$), but did not change λ (Fig. 2b).

The RTD of material is generally measured to characterize and predict the exact flow behavior in a vessel such as a chemical reactor or a bioreactor. In our particular application, the RTD was used to estimate when equilibrium of the dispersion was reached once the emulsion was started. This information also gave the time necessary for the system to return to a stationary state after modifying the process conditions.

Because of the difficulties relating to the use and the quantification of a tracer in a complex emulsion produced at 30 kg·h^{-1}, we used water as a model fluid. Our results indicated that reaching equilibrium in the mixer required between 3.8 and 4.2 t_m, depending on the feed rate and the internal mixing speed. Low hydrophobic/hydrophilic phase ratios (up to 10%) and whey protein concentrations (up to 10%) were used to prepare the anhydrous milk fat/whey protein emulsions, which had low viscosity. Although o/w dispersions are non-Newtonian fluids, data obtained with water can be reasonably extrapolated to emulsions produced in this study. Consequently, a minimum stabilization period of 4.2 t_m was used before sampling the emulsion after starting the mixer or changing the operation conditions.

3.2. Effects of emulsification conditions on fat globule size distribution

Core-in-wall emulsion characteristics have been reported to be of critical importance to the quality and functionality of microencapsulated systems [28, 32]. In particular, retention of core during the microencapsulation process and core release from microcapsules are strongly affected by core particle size distribution, especially for microencapsulation of apolar core materials [18]. A main objective of the present study was therefore to examine the effects of key parameters of the emulsion on fat globule size distribution in order to select the optimum operating conditions for production of multiphase spray-dried microcapsules with diameters lower than 100 μm. The effects of the internal mixing speed, hydrophilic/hydrophobic phase ratio and percentage of NFS in the hydrophobic phase on the fat globule size distribution parameters for each experimental unit are shown in Table I.

When preparing oil-in-water dispersion for spray-drying, a mean particle size smaller than 2 μm and preferably from 0.3 to 0.7 μm is usually recommended to increase emulsion stability and prevent droplet coalescence during the drying process [29, 33]. In our particular application, a fat globule population diameter ranging from 10 to 50 μm was required to allow an efficient coating of particles of freeze-dried cells with $D(v, 0.9) < 25$ μm prior to atomization. Table II presents the analysis of variance of Φ_{10-50}, the percentage (% v/v) of fat globules with sizes ranging from 10 to 50 μm. The linear and quadratic effects of the internal mixing speed (Ω and Ω^2) were both highly significant ($P \leq 0.001$), explaining 77.7% and 18.6% of the total variation, respectively. None of the other effects was significant ($P > 0.05$). Analysis of variance of the volume-surface mean particle diameter $D[3, 2]$ is shown in Table III. In the same way as for Φ_{10-50}, the linear and quadratic effects of the internal mixing speed (Ω and Ω^2) were the only significant effects ($P \leq 0.001$), accounting for 91.2% and 5.7% of the total variation, respectively. The analysis of variance of the 10% volume fractile of the particle size

Table II. Analysis of variance of Φ_{10-50}, the percentage (% v/v) of fat globules with diameters ranging from 10 to 50 μm.

Source	d.f.[(a)]	S.S.[(b)]	F[(c)]
ε	1	0.546012	0.25
Ψ_{NFS}	1	6.24811	2.81
Ω	1	1364.51	613.37***
ε^2	1	5.13028	2.31
$\varepsilon\Psi_{NFS}$	1	1.75562	0.79
$\varepsilon\Omega$	1	3.5344	1.59
Ψ^2_{NFS}	1	14.6035	6.56
$\Psi_{NFS}\Omega$	1	3.4225	1.54
Ω^2	1	326.166	146.62***
Total error	1	11.123	
Total (corr.)	14	1755.51	

[(a)] d.f. = degree of freedom; [(b)] S.S. = sum of squares; [(c)] F = Fisher ratio. Significance levels: *: $P_\alpha \leq 0.05$; **: $P_\alpha \leq 0.01$; ***: $P_\alpha \leq 0.001$.

Table III. Analysis of variance of $D[3, 2]$, the volume-surface mean particle diameter.

Source	d.f.[(a)]	S.S.[(b)]	F[(c)]
ε	1	0.39605	0.72
Ψ_{NFS}	1	1.6562	3.00
Ω	1	578.34	1048.95***
ε^2	1	2.69256	4.63
$\varepsilon\Psi_{NFS}$	1	0.1681	0.30
$\varepsilon\Omega$	1	0.3025	0.55
Ψ^2_{NFS}	1	3.20493	5.81
$\Psi_{NFS}\Omega$	1	1.7956	3.26
Ω^2	1	36.2503	65.75***
Total error	1	2.75677	
Total (corr.)	14	634.08	

[(a)] d.f. = degree of freedom; [(b)] S.S. = sum of squares; [(c)] F = Fisher ratio. Significance levels: *: $P_\alpha \leq 0.05$; **: $P_\alpha \leq 0.01$; ***: $P_\alpha \leq 0.001$.

distribution $D(v, 0.1)$, the volume median diameter $D(v, 0.5)$, and the 90% volume fractile of the particle size distribution $D(v, 0.9)$ provided results very similar to those obtained with $D[3, 2]$, with linear and quadratic effects of the internal mixing speed as the only significant effects (data not shown).

The polynomial regression equation and plot obtained from the significant effects of the analysis of variance for each response parameter are shown in Table IV and Figure 3, respectively. In all cases, the fitted model explained a large part of the total variation ($R^2 > 0.95$). The effects of internal mixing speed on the percentage (% v/v) of fat globules with diameters ranging from 10 to 50 μm are shown in Figure 3a. A maximum value of 92.2% was found for a rotation speed of the helical impeller of 2850 rpm. For higher internal mixing speeds, the volume fraction of fat globules with diameters less than 10 μm increased, and consequently, Φ_{10-50} decreased. Figure 3b illustrates the effects of internal mixing speed on fat globule size distribution parameters. As expected, $D[3, 2]$, $D(v, 0.1)$, $D(v, 0.5)$ and $D(v, 0.9)$ decreased on increasing the rotation speed of the helical impeller from 2000 to 3000 rpm. The fat globule size is directly related to the mixing speed since the mixing device provides mechanical energy needed to split the droplets. When the mixing speed is increased, the hydrophobic phase is finely dispersed, forming small droplets suspended in the aqueous phase, and resulting in a macroemulsion. The dynamic loop mixer is activated by the rotation at variable speed of a helical impeller which creates a circulating energy and allows the control of the internal mixing speed.

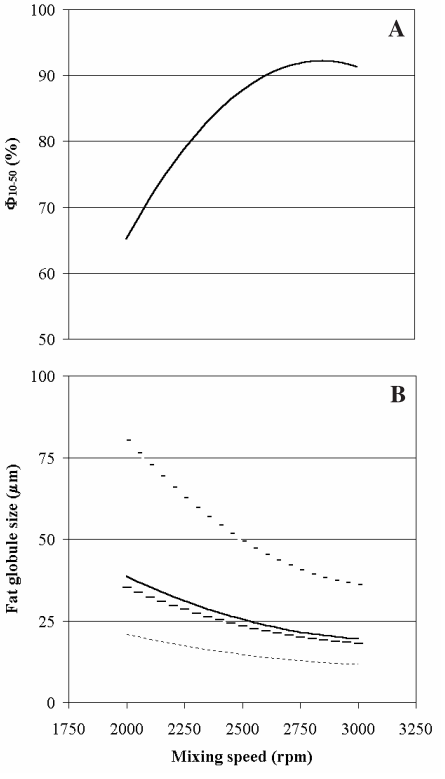

Figure 3. Modeled effect of internal mixing speed on (A) Φ_{10-50}, the percentage (% v/v) of fat globules with diameters ranging from 10 to 50 μm, and (B) fat globule size distribution parameters: $D[3, 2]$ (– –), $D(v, 0.1)$ (------), $D(v, 0.5)$ (——), $D(v, 0.9)$ (— —).

Table IV. Second-order models of Φ_{10-50}, $D[3, 2]$, $D(v, 0.1)$, $D(v, 0.5)$, $D(v, 0.9)$.

Size distribution parameters	Second-order model [(a)]	R^2
Φ_{10-50} (%)	87.67 + 13.06 Ω – 9.39875 Ω^2	0.9823
$D(3, 2]$ (μm)	23.45 – 8.50 Ω + 3.13333 Ω^2	0.9878
$D(v, 0.1)$ (μm)	14.66 – 4.55 Ω + 1.63375 Ω^2	0.9557
$D(v, 0.5)$ (μm)	25.44 – 9.57 Ω + 3.61833 Ω^2	0.9853
$D(v, 0.9)$ (μm)	49.43 – 22.12 Ω + 8.77292 Ω^2	0.9948

[(a)] Regression equation calculated from the significant effects of the analysis of variance. Values of the variables are specified in their coded units.

Increasing the rotation speed of the helical impeller, and as a consequence the number of loops that the whole liquid or suspension made through the rotor/stator mixing unit before its exit, reduced the fat globule diameter.

Surprisingly, the percentage (w/w) of hydrophobic phase in the emulsion was not retained in second-order models. As shown in Table II for Φ_{10-50} and in Table III for $D[3, 2]$, the main and interaction effects of ε never reached the 95% level of significance. Increasing the hydrophobic/aqueous phase ratio did not significantly affect the fat globule size, even at high internal mixing speeds. Hogan et al. [14] recently reported that particle size of soya oil/whey protein emulsions decreased with increasing homogenization pressure but was not affected by oil/protein ratios ranging from 0.25 to 3.0. The highest oil/protein ratio (w/w) tested in our study was 1.11, which was much less than the values normally used (up to 12.5) to examine emulsifying properties of whey proteins [6, 9, 10]. However, the experimental studies conducted on oil-in-water droplets emulsified with commercial milk protein ingredients (i.e., sodium caseinate, whey protein concentrate and isolate) are largely based on the production of fine dispersions by high pressure homogenization to ensure good physical stability of the emulsions [11]. A very small average particle size (< 1 μm) is generally obtained, which contrasts with the relatively high $D[3, 2]$ values measured in the present work, particularly for a rotation speed of the helical impeller of 2000 rpm. Such large particle diameters may favour the instability and propensity of the oil droplets for rapid creaming and coalescence, resulting in an increase in emulsion particle size.

Whey proteins are largely used as emulsifiers [8, 16, 26]. Due to their tensioactive properties, they form a protective membrane at the surface of oil droplets that prevents the droplets from coalescing. Several approaches have been proposed in order to alter the whey-protein structure and improve their functional properties. Among these approaches, heat treatments have received considerable attention. Under controlled conditions (pH and mineral environment), heat increases the emulsifying properties of whey proteins and the stability of emulsions [5, 6]. The WPI solution used in the present study was heat-treated at 80 °C, pH 7.0 for 30 min in order to induce the formation of water-soluble polymers for subsequent drying of the emulsion and cold-set gelation of the microcapsule wall when dried microcapsules are added to mineral-rich dairy products. As indicated by the absence of instantaneous creaming and coalescence observed in all the emulsions produced, the emulsifying capacities of whey protein polymers were suitable for our application (spray-drying of o/w emulsion in a continuous two-step process), with emulsion particle size distributions unchanged after 150 min of storage at 42 °C

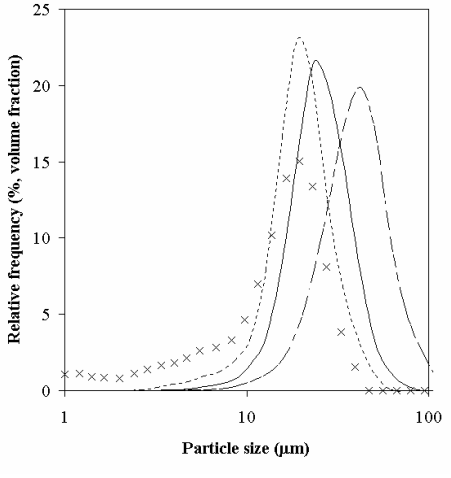

Figure 4. Particle size distribution of micronized milk skim powder (×) and milk fat globules obtained at 2000 rpm (— — ; run 5), 2500 rpm (——— ; run 15), and 3000 rpm (------ ; run 10).

under low speed agitation (data not shown). Therefore, since the amount of WPI available to stabilize newly exposed droplet interfaces was not limiting at the fat/protein ratios used and the emulsion stability was satisfactory for our microencapsulation application, emulsion oil droplet size was determined primarily by the homogenizing conditions, i.e., the internal mixing speed.

The percentage (w/w) of non-fat solids in the hydrophobic phase Ψ_{NFS} was also not retained in the second-order models. In designing multiphase microcapsules for cell immobilization, effort must be made to attain the desired distribution of the different elements constituting the capsules. In our particular application, the size of the powder particles of NFS must be larger than the size of individual bacterial cells (2–5 μm) or chains and smaller than the fat globule size. Since $D(v, 0.9)$ of the commercial skim milk powder employed was as high as 77 μm, a drastic particle size reduction (i.e., micronization) was necessary. The largest particles of the resulting micronized powder were markedly larger than the $D(v, 0.9)$ of the fat-globule populations produced experimentally at a rotation speed of the helical impeller of 3000 rpm (43 vs. 33 μm) (Fig. 4). The particle size analysis of a three-dimensional dry particle is conventionally carried out using the equivalent sphere theory, i.e., the volume of a particle is converted into that of a sphere. Because micronized skim milk particles are far from spherical and their stability inside the oil droplets was presumably relatively poor in the aforementioned operating conditions, a transfer of NFS from the hydrophobic to the aqueous phase was expected, particularly at high Ψ_{NFS} values. Depending on its importance, this phenomenon can have a significant effect on emulsion droplet size distribution and may eventually lead, in extreme cases, to destabilization of the emulsion. The rehydration of calcium and salts present in micronized skim milk powder in the suspension of soluble whey protein polymers may, in addition, result in a dramatic reduction of the electrostatic repulsion and cause a premature gelation of the proteins before spray-drying through polymer-polymer associations. However, according to our results, the transfer of NFS from the hydrophobic to the aqueous phase did not appear to have a significant influence on the milk fat globule size and the equilibrium of the oil-in-water dispersion.

Industrial production of microcapsules not only implies scale-up, but also the need to control the mean size and size dispersion of the core droplets. Both gelation or membrane formation and final properties of the microcapsules are correlated by the initial dispersion quality [27]. The selection of mean size (large or small) is a function of the applications. In all cases, size dispersion will constitute a hindrance to the process control. Core-in-wall emulsions to be spray-dried are commonly prepared using a high-pressure homogenizer or microfluidizer [12, 22, 25], leading to a very small mean particle size which is unsuitable for bacteria microencapsulation. The emulsifying

equipment used to produce o/w emulsions with fat globule diameters ranging from 10 to 50 μm, a dynamic loop mixer, permitted us to overcome the potential limitations existing with batch emulsion processes for large-scale production of such macroemulsions: non-homogeneous distribution of the mixing energy in the reactor, resulting in large particle size dispersion, lack of control of particle diameter and particle size distribution, batch-to-batch variability, and the technical difficulties of integrating to a continuous multiple-stage process and industrial scale-up. The principal characteristic of this new type of mixer is based on a mixing of the products by recirculation in a loop inside a small, closed, specifically designed vessel. The flow velocity and flow direction of the veins of products to be mixed are controlled and ducted, so that no part of the products can be deviated from the trajectory imposed by the internal forms of the mixer. As homogeneous shear is applied to the whole liquid, a narrower size distribution is obtained, compared with classically-used systems for phase dispersion. Unimodal particle size distributions showing normal distribution were observed for all the emulsions produced in the present work, regardless of operating conditions. Figure 4 illustrates typical particle size distributions of the fat globule population obtained during the emulsification process at 2000, 2500 and 3000 rpm. Increasing the rotation speed of the helical impeller resulted in a reduction in the size dispersion. Using second-order models reported in Table IV, the fat globule size distribution parameters of the two-phase dispersion process studied can be determined when the internal mixing speed is selected. By comparing the predicted values of $D[3, 2]$, $D(v, 0.1)$, $D(v, 0.5)$, $D(v, 0.9)$ and Φ_{10-50} at 2850 and 3000 rpm (Fig. 3), one can reasonably hypothesize that particle size distributions obtained with these two operating conditions would be very similar. The particle size distribution of skim milk powder with specific $D(v, 0.9)$ prepared for our study is represented in Figure 4 with the emulsion particle size spectra. As discussed earlier, the largest particles of the micronized powder could not be properly coated and remain stable inside the fat globules produced at 3000 rpm (similar size, non-spherical shape). Rehydration of NFS particles during emulsification did not have any significant effects on fat globule size but may have seriously affected both the encapsulation efficiency and stability of the NFS during spray-drying. Consequently, a rotation speed of the helical impeller of 2850 rpm giving the maximum Φ_{10-50} value cannot be considered as satisfactory within the context of our application. According to Figure 4 and to the predicted values of fat globule size distribution parameters in Figure 3, particle size distribution of milk fat/whey protein emulsions obtained at 2500 rpm is best suited to allowing an efficient coating of NFS particles with $D(v, 0.9) < 25$ μm. As a result, a helical impeller rotation speed of 2500 rpm, corresponding to a fat globule population with a Φ_{10-50} value of 87.7%, a $D[3, 2]$ of 23.5 μm, a $D(v, 0.1)$ of 14.7 μm, a $D(v, 0.5)$ of 25.4 μm, and a $D(v, 0.9)$ of 49.4 μm was selected for the production of multiphase spray-dried microcapsules with diameters less than 100 μm and containing fine powder particles of freeze-dried bacteria.

4. CONCLUSION

This study provided information on the effects of emulsion composition and homogenization conditions using a dynamic loop mixer on the properties of o/w emulsions for spray-dry encapsulation of bacterial cells. Our results indicated that the percentage of hydrophobic phase in the emulsion and the percentage of material to encapsulate in the hydrophobic phase both had non-significant effects on emulsion particle size in the studied intervals. The fat globule size distribution was only found to be dependent on the internal mixing speed. Considering the fat globule size

required for the cell microencapsulation process in the range from 10 to 50 μm, a helical impeller rotation speed of 2500 rpm was selected from the second-order polynomial models of fat globule size distribution parameters.

This research also demonstrated that anhydrous milk fat/whey proteins emulsions with controlled fat globule size distribution and small size dispersion can be prepared on a continuous mode with a dynamic loop mixer. Due to the special concept of the mixer, data obtained with the laboratory system used (up to 100 $L \cdot h^{-1}$) can be directly extrapolated to the industrial model (up to 10000 $L \cdot h^{-1}$). The use of this original mixing system might prove to be very useful for large-scale production of multiphase spray-dried microcapsules in a two-step continuous process. The properties of powders produced by spray-drying of anhydrous milk fat/whey protein emulsions with conditions selected in this study are reported in another paper.

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