Physicochemical properties and encapsulation of silicon in double emulsions for healthier food applications

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Abstract This article analyses the potential use of double emulsions as silicon delivery systems with reference to the influence of the composition of the inner aqueous phase (W_1 , containing NaCl and sodium caseinate or gelatin) on silicon encapsulation and physicochemical properties of food-grade $W_1/O/W_2$. Irrespective of W_1 , DEs initially showed a well-defined monomodal distribution, with the widest range registering in the sample with gelatin. All samples developed a bimodal distribution during storage (3 ± 2 °C). Heating increased the range of droplet size distribution. DEs exhibited high physical stability (creaming), decreasing over storage; this behaviour was generally unaffected by W_1 composition, which maintained similar stability (95–96%) at the end of storage. Viscosity was generally unaffected by formulation, storage time or heating treatment. Si encapsulation efficiency (72.4 and 78.3%) was not affected by W_1 composition, while Si encapsulation stability was generally unaffected by either storage or heating. These DEs can be used as potential ingredient (with lower fat content, better fatty acid profile and with the potential Si health benefits) for the development of healthier foods including meat products.

Keywords Silicon · Double emulsion · Encapsulation · HR-CS FAAS silicon determination · Creaming · Droplet size

Introduction

Silicon is not generally recognized as an essential nutrient in humans because it has not been shown to have a defined biochemical function, but emerging research suggests that consumption of water-soluble forms may be beneficial. This has prompted renewed and growing interest in the potential beneficial effects of silicon on human health (Keith 2013). Recent findings provide additional evidence that silicon in nutritional and supra nutritional amounts promotes bone and connective tissue health, may have a modulating effect on the immune or inflammatory response, and has been associated with mental health (Nielsen 2015). In addition, silicon has been shown to reduce aluminium (Al) levels with possible preventive effects in Alzheimer's disease (González-Muñoz et al. 2008) and to exert hypocholesterolemic effects (Garcimartín et al. 2014).

Silicon exists in the food chain, with concentrations tending to be much higher in plant-based (mainly unrefined grains, cereal products and root vegetables) than in animal foods. Beverages, however, are the major contributors to dietary silica, or silicon, and include water, coffee, and beer (from barley, hops, etc.) (Keith 2013). The availabilities of silicon from meat, milk and beers are high, but low in the case of seafood and cereal products (Robberecht et al. 2008). The intake of Si in humans is therefore greatly affected by consumption habits, i.e. dependent on the amount and proportion of Si-poor (e.g. meat) or Si-rich foods,

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on the extent to which they are processed, and on the use of silicon additives (as anti-foams or texture modifiers). The estimated overall dietary intake from all sources is approximately 20–50 mg silicon/day for Western populations (Keith 2013), although much of the silicon in silicate form is not absorbed, which indicates that absorption of dietary silicon is inefficient (Nielsen 2003). Then again, factors such as ageing or diminishing oestrogen levels appear to reduce absorptive capacity (González-Muñoz et al. 2008). Dietary supplements containing orthosilicic acid or other forms that are presumably modified to a form that is water-soluble absorbed and bioavailable, are alternative silicon sources (Keith 2013). Silicon-rich plant extracts offer some of the more important possibilities for Si dietary supplementation; however, their use as food ingredients can present some limitations due to their colour and/or flavour characteristics. In this regard, one procedure that has been identified as suitable for promoting intake is to diversify Si sources and to enrich foods where Si levels are naturally low, such as meat products.

There are now reports of various delivery systems with different functional characteristics (McClements et al. 2009; Jiménez-Martín et al. 2015). However, double emulsions (DEs) offer specific possibilities as Si delivery systems since they could be used as food ingredients, providing a promising technological strategy to optimize dietary active components for the development of healthier foods, including functional foods. DEs are multi-compartmentalized systems in which oil-in-water (O/W) and water-in-oil (W/O) interfaces co-exist and where the globules of the dispersed phase themselves contain even smaller dispersed droplets (Garti 1997). The most common forms are water-in-oil-in-water emulsions ($W_1/O/W_2$), which offer numerous advantages for food applications, since this has been found to be a potentially useful strategy, including for encapsulation (masking flavours and colours), protection and release of hydrophilic components (Jimenez-Colmenero 2013). DEs can be used to get around the limitations associated with the use of silicon-rich plant extracts as food ingredients. When certain structural properties are needed, DEs can be designed for the intended food application. In this regard, aspects relating to the characteristics of inner water phases of $W_1/O/W_2$ emulsions have been considered as a composition factor for the purpose of stabilizing and structuring. In stabilizing emulsions, the combination of different emulsifiers can produce synergistic effects (Benichou et al. 2004). In this connection, Su et al. (2006) reported improved stability of the primary emulsion when concentrations of caseinate in

the internal phase were increased (up to 1%). An alternative approach is to increase the viscosity of these structures by incorporating macromolecular substances such as sugars, proteins and polysaccharides into the inner aqueous phase (Dickinson 2011; Su et al. 2006). When biopolymers that are readily converted to the gel state (e.g. by thermal processing) are present, the inner emulsion droplets are converted into soft solid-like particles (Dickinson 2011). For instance, thickening or gelling biopolymers have been added to the dispersed aqueous phase of W/O emulsions such as gelatin (Sapei et al. 2012; Khalid et al. 2013; Oppermann et al. 2015; Tepsongkroh et al. 2015), locust bean gum and l-carrageenan (Patel et al. 2015), alginate (Tepsongkroh et al. 2015) etc., to improve their stability, and hence their encapsulation efficiency (EE).

The objective of this study was to assess the potential of double emulsions as silicon delivery systems. To that end we examined the influence of the inner aqueous phase on silicon encapsulation and physicochemical properties of food-grade $W_1/O/W_2$ emulsions and assessed the potential for increasing the stability of multiple emulsions by adding caseinate as a synergistic emulsifier or by gelling the inner aqueous phase with gelatin. As far as the authors are aware, no studies have been published on the use of double emulsions for silicon encapsulation. Also, despite studies performed on biopolymers involving the incorporation of proteins as potential active ingredients in $W_1/O/W_2$ emulsions, there is a lack of knowledge on the effect of the inner aqueous phase for successful encapsulation of Si. In order to study the potential benefit of internal phase engineering on encapsulation efficiency and stability, samples were subjected to a relatively unbalanced osmotic pressure by means of an aqueous plant extract incorporated into the inner water phase. This extract was a natural Si source, and the lipid phase was olive oil. In addition, to the best of authors' knowledge the direct determination of silicon in a complex matrix such as $W_1/O/W_2$ has not been reported to date. Also, since DEs are highly susceptible to breakdown when exposed to different conditions commonly occurring in the food industry, the effect of chilling storage and thermal processing on DEs has also been considered. Thus, it has been necessary the optimization of a new methodology in order to carry out this work. Given these conditions, the preparation of these DEs was especially important as one more element in the development of healthier foods. The emulsions can be used in different food applications, but they were specifically designed for the replacement of animal fat by an ingredient with lower fat content, a better fatty acid profile and potential Si health benefits in meat product development.

Materials and methods

Materials

The lipid phase used for the DE formulation was olive oil (Carbonell Virgen Extra, SOS Cuétara S.A. Madrid, Spain). The lipophilic surfactant, polyglycerol polyricinoleate (PGPR), was purchased from Bavaro Chemicals S.L. (Sant Cebrià de Vallalta, Spain). Sodium caseinate (SC) was from Friesland Campina DMV (Excellion EM 7, Veghel, The Netherlands). Sodium chloride was from Panreac (Panreac Química, S.A.; Barcelona, Spain). Sodium azide was from Sigma-Aldrich (Madrid, Spain). A natural soluble silicon source (from plant extract) containing 369.0 ± 4.7 mg Si/kg was used (Ergysil, Laboratorios Nutergia, San Sebastián, España). Cold-soluble gelatin (G), 220 bloom (Tradissimo, Trades, S.A., Barcelona, España) was also used. All other reagents and solvents used were of ACS grade unless specified.

Design and preparation of double emulsions (DE)

Three different DEs were designed and formulated (with three different inner aqueous phases) containing a final Si concentration of 30 mg/kg. These emulsions must be considered in the context of their practical application. They are designed for use as food ingredients, for example in meat product reformulation processes. Since the addition of these types of DEs to meat systems at levels ranging from 10 to 15 g/100 g is realistic (Cofrades et al. 2013), this means a content of 3–4.5 mg of Si/100 g of food (meat product), which covers at least 15% of the suggested adequate intake (between 10 and 25 mg/d) to achieve the beneficial effects of silicon (Nielsen 2015). As formulated, these DEs were expected to have similar olive oil contents (32 g/100 g).

A two-stage procedure was used to prepare DEs (Bou et al. 2014a). Briefly, the Si-rich plant extract was used to prepare the three inner aqueous phases (W_1). One contained NaCl (0.58%) and the other two contained the same amount of NaCl plus SC (0.5%) or gelatin (3%). In all cases sodium azide (0.02% to prevent microbial growth) was incorporated. A similar outer (W_2) phase (0.58% NaCl + 0.5% SC) was used in all samples. The lipid phase (O), consisting of olive oil (94 g/100 g) plus PGPR as a lipophilic surfactant (6 g/100 g), was mixed for 15 min at 60 °C in a Thermomix TM-31 food processor (Vorwerk, Germany) at setting 5 (2400 rpm). Then, three primary coarse emulsions (W_1/O) were prepared by dropwise addition of the corresponding inner (W_1) aqueous phase (20 g/100 g) to the lipid phase (80 g/100 g) in the

Thermomix food processor at 60 °C, 15 min, setting 5. Each primary coarse emulsion was passed twice through a two-stage high pressure homogenizer at 55/7 MPa (Panda Plus 2000, GEA NiroSoavi, Parma, Italy). The resulting primary fine emulsions (W_1/O) were then allowed to cool at room temperature. DEs were prepared by gradually adding each W_1/O fine emulsion (40 g/100 g) to the outer (W_2) aqueous phase (60 g/100 g) in the Thermomix food processor at 37 °C, setting 3. The resulting DEs were passed twice through a two-stage high pressure homogenizer (Panda Plus 1000) at 15/3 MPa to obtain the final $W_1/O/W_2$. Thus, the three final samples were prepared with different inner aqueous phases: a control DE (C/DE) with NaCl only, a DE with NaCl plus sodium caseinate (SC/DE) and a DE with gelatin (G/DE). These DEs were aliquoted in flat-bottomed screw-capped glass tubes and Petri dishes and stored (3 ± 2 °C) for 28 days. All samples were analysed at 1, 7, 14 and 28 days of storage unless otherwise specified. At each storage time, the influence of the thermal treatment (70 °C for 30 min) on each type of DE was also evaluated.

Microscopy

Optical microscopy was used to examine the $W_1/O/W_2$ emulsion morphology. Samples were placed on microscope slides and gently covered with a coverslip. The microstructure of double emulsions was observed at 100 and 160 magnifications using an inverted microscope (Leica AF6000 LX; Wetzlar, Germany).

Particle size characteristics

The particle size and distribution of oil droplets in the DEs was determined with a Malvern Mastersizer S laser diffraction particle size analyser (Malvern Instrument Ltd, Worcestershire, UK) equipped with a He–Ne laser ($\lambda = 633$ nm). The measurement range was 0.05–900 μm . Obscuration was in the range of 8–15%. Particle size calculations were based on the Mie Scattering theory. Surface average diameter (d_{32}), as the most sensitive parameter for measurement of the presence of small droplet size (Tepsonkroh et al. 2015), was measured immediately after addition to the dispersion unit. This parameter was determined in triplicate.

Physical stability

Gravitational separation (creaming) of DEs was recorded in triplicate over storage in terms of phase separation and expressed as percentage of initial sample height. This parameter was determined in triplicate.

Viscosity

The viscosity at each storage time and after thermal treatment of the different DEs was determined at 25 °C, in a CP1/60 cone/plate cell (1° angle and 60 mm diameter) with a 30 µm gap on a Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK) at a constant shear stress of 0.5 s⁻¹. Results, expressed as Pa.s, were averages of three determinations.

Colour

Colour, CIE-LAB tristimulus values, lightness (L*), redness (a*) and yellowness (b*) of DEs were evaluated on a CR-400 Chroma Meter (Konica Minolta Business Technologies, Inc., Tokyo, Japan). The measurements were carried out on sample aliquots placed on glass Petri dishes. Five determinations were performed from each case.

Determination of silicon and the encapsulation efficiency and stability

Silicon determination procedure

Silicon determination was carried out using a ContrAA 700 high-resolution continuum source atomic absorption spectrometer (HR-CS AAS) equipped with both flame and graphite furnace atomizers in two separate sample compartments, and coupled to the autosampler module AS 52S for flame mode (Analytik Jena AG, Jena, Germany). The optical system comprises a xenon short-arc lamp operating in “hot-spot” mode as the continuous radiation source, a compact high-resolution double monochromator consisting of a prism pre-monochromator and an echelle grating monochromator, and a linear charge-coupled device (CCD) array detector.

The main line for silicon at 251.611 nm was employed for all the analyses. Three pixels (the central peak plus the adjacent ones (CP ± 1)) were selected for all measurements. Wavelength selected absorbance (WSA), obtained from the sum of the individual mean absorbance values of the selected pixels, was chosen as the analytical signal for quantification purposes. A nitrous oxide-acetylene flame was used for the atomization of Si. High-purity acetylene (C₂H₂) 99.6% and high-purity nitrous oxide (N₂O) 99.998% (Carburros Metálicos, Madrid, Spain) were employed as fuel gas and oxidant gas respectively. Flame stoichiometry and burner height were optimized. A model SFS 6 injection module (Analytik Jena AG, Jena, Germany) was used for aspiration of blank, standard and sample solutions. The aspiration rate was fixed at 10 mL min⁻¹, and all measurements were carried out in triplicate.

Silicon standard solutions were prepared daily by appropriate dilution of a 1.000 g L⁻¹ silicon commercial stock solution for atomic absorption spectrometry (Scharlau, Barcelona, Spain) in ultrapure water with resistivity not less than 18.2 MΩ cm at 25 °C (Ultra Clear™ TWF UV EDI, Siemens). Analysis of the commercial soluble silicon material was carried out after dilution of 1.0 mL of sample with ultrapure water up to 25.0 mL. The content of silicon in the outer aqueous phase W₂ was directly analysed without the need of a dilution step.

Silicon encapsulation efficiency (EE) and stability (ES)

Silicon encapsulation efficiency (after preparation) and stability (during storage) were determined by measuring Si release to the outer phase. This was done by diluting the DE with 4 volumes of the W₂ phase. Samples were then centrifuged at 1300 g for 15 min (Heraeus multifuge 3L-R DJB Labcare Ltd.; Buckinghamshire, UK) to separate the fat globules from the outer aqueous phase and frozen at -80 °C. The crystallized aqueous phase was then separated from the lipid fraction and stored frozen at -20 °C pending determination of silicon content as reported above. Encapsulation efficiency and stability were calculated with respect to the amount of silicon added to the inner aqueous phase using the following equation (Sapei et al. 2012): Encapsulation (%) = 100 - (Cw₂/Cw₁) × 100, where Cw₁ is the concentration of silicon in the emulsion considering the amount of the Si initially added to the inner aqueous phase, and Cw₂ is the concentration of silicon determined in the outer aqueous phase considering the volume fraction. All samples were analysed in duplicate at each storage time and after thermal treatment.

Statistical analysis

The experiment was carried out in duplicate. Series of one-way ANOVAs were performed for each factor (type of emulsion, storage time and thermal treatment) by fixing the other factors at each specific level in order to determine the existence of significant differences in the measured parameters. Means were separated by Tukey's HSD test (α = 0.05). *P* ≤ 0.05 was deemed significant in all calculations. The statistical analyses were carried out using IBM SPSS Statistics 21 software.

Results and discussion

Various food biopolymers have been investigated with respect to their influence on W₁/O/W₂ properties and stability. These include gelatin and SC, which have been used to condition the nature of the aqueous inner phase and

could hence potentially affect the silica encapsulation and physicochemical properties of food-grade $W_1/O/W_2$ emulsions.

Droplet size characteristics

Figure 1 shows the droplet size distribution in the samples prepared with different inner aqueous phases at the initial stage and at the end of storage time, as well as after thermal treatment (at each storage time). Irrespective of inner aqueous phase, DEs initially showed a well-defined monomodal distribution, the widest range occurring in the G/DE sample, although the three samples had similar ($P > 0.05$) initial mean droplet size (d_{32}) ranging between

Fig. 1 Particle size distribution of double emulsions with different inner aqueous phases as affected by storage and heating. For sample denomination see footnote of Table 1

2.30 and 2.46 μm (Table 1). The droplet sizes are in agreement with those of similar double emulsions prepared with PGPR and SC as lipophilic and hydrophilic emulsifiers respectively (Cofrades et al. 2013; Bou et al. 2014a, b). However, unlike in this experiment, the inclusion of gelatin in the inner phase has been reported to induce some modifications in droplet size. For example, Khalid et al. (2013) observed that the average droplet diameter of double emulsions in the presence of gelatin in the inner aqueous phase was greater than that of similar emulsions without gelatin. Tepsongkroh et al. (2015) reported that d_{32} values of $W_1/O/W_2$ emulsions containing PGPR and biopolymers (gelatin or sodium alginate) ranged from 3.18 to 3.94 μm . Monomodal, bimodal or even trimodal particle size distributions have been reported in double emulsions (Cofrades et al. 2013). The initial monomodal distribution of DEs (Fig. 1) is consistent with

Table 1 Effect of the inner aqueous phase composition, storage time and heating on emulsions' droplet surface-weighted mean diameter and emulsions' viscosity of DEs

Days at 3 °C	C/DE	SC/DE	G/DE
<i>Surface average diameter (d_{32}; μm)</i>			
1	2.37 $\pm$ 0.12 ^x (3.62 $\pm$ 0.25) ^y	2.30 $\pm$ 0.07 ^x (3.62 $\pm$ 0.25) ^y	2.46 $\pm$ 0.04 ² (2.47 $\pm$ 0.07)
7	2.37 $\pm$ 0.10 ^x (2.87 $\pm$ 0.01) ^y	2.40 $\pm$ 0.18 (2.89 $\pm$ 0.50)	2.40 $\pm$ 0.03 ¹² (2.42 $\pm$ 0.02)
14	2.48 $\pm$ 0.25 –	2.31 $\pm$ 0.23 ^x (3.05 $\pm$ 0.16) ^y	2.35 $\pm$ 0.06 ¹² (2.37 $\pm$ 0.02)
21	2.40 $\pm$ 0.17 –	2.30 $\pm$ 0.24 (3.44 $\pm$ 0.97)	2.44 $\pm$ 0.06 ² (2.40 $\pm$ 0.02)
28	2.44 $\pm$ 0.18 –	2.50 $\pm$ 0.47 ^x (3.87 $\pm$ 0.38) ^y	2.30 $\pm$ 0.09 ¹ (2.38 $\pm$ 0.01)
<i>Emulsions' viscosity (Pa.s)</i>			
1	5.18 $\pm$ 0.22 (9.34 $\pm$ 1.79)	5.66 $\pm$ 0.05 (7.39 $\pm$ 1.32)	5.24 $\pm$ 0.02 ¹ (5.99 $\pm$ 0.85)
7	5.61 $\pm$ 0.52 (6.67 $\pm$ 0.77)	5.51 $\pm$ 0.97 (7.13 $\pm$ 1.85)	5.10 $\pm$ 0.35 ¹ (4.92 $\pm$ 0.06)
14	4.37 $\pm$ 0.51 –	4.33 $\pm$ 0.37 (7.51 $\pm$ 2.23)	4.39 $\pm$ 0.02 ^{1x} (5.13 $\pm$ 0.01) ^y
21	4.50 $\pm$ 0.10 –	4.41 $\pm$ 1.15 (6.94 $\pm$ 1.32)	5.66 $\pm$ 1.21 ¹² (4.45 $\pm$ 0.11)
28	4.93 $\pm$ 0.10 ^a	4.96 $\pm$ 1.15 ^a (5.76 $\pm$ 0.35)	8.12 $\pm$ 0.14 ^{2,b,x} (5.40 $\pm$ 0.32) ^y

Sample denomination: C/DE, SC/DE and G/DE are double emulsions containing NaCl, NaCl + sodium caseinate and NaCl + gelatin in the inner aqueous phase, respectively. Mean $\pm$ standard deviation, in parenthesis for the same sample after heating (when—the determination was not possible). Unheated samples with different letters (a–b) within the same row or numbers in the same column indicate significant differences ($P < 0.05$). Different letters (x–y) indicates significant differences ($P < 0.05$) between unheated and heated samples at each storage time

Fig. 2 Optical microscopy images of SC/DE as affected by storage and heating: **a** and **b** stored at 3 °C for 1 and 28 day, respectively; **c** and **d** heated at 70 °C for 30 min after 1 and 28 days, respectively. For sample denomination see footnote of Table 1

reports by other authors in DEs using PGPR as a lipophilic emulsifier (Carrillo-Navas et al. 2012; Frasc-Melnik et al. 2010; Weiss et al. 2005), and additionally with those in which SC was used as a hydrophilic emulsifier in the outer aqueous phase (Cofrades et al. 2013; Bou et al. 2014a, b). However, bimodal distributions have also been reported in DEs when proteins are included in the inner phase (O'Reagan and Mulvihill 2009; Sapei et al. 2012; Su et al. 2006). For instance, Su et al. (2006) reported that DEs prepared with SC (0.5% w/v) and without it in the inner aqueous phase (and with 0.5 and 2% w/v PGPR) exhibited similar bimodal distributions, without any change in droplet size of DEs. DEs containing 2% (w/w) NaCl stabilized with varying amounts of gelatin (0, 3 and 10%) presented bimodal oil globule size distributions, with a dominant 10–100 μm distribution and a small distribution in the 2–10 μm range (Sapei et al. 2012). However, Weiss et al. (2005) reported that a double emulsion in which W_1 contained 5% of gelatin and the lipid phase consisted of various mixtures of vegetable oils (with different solid fat contents) or medium chain triglyceride oil plus 2 or 8% PGPR, the oil globules presented a monomodal distribution. Differences in droplet sizes and distributions may be

explained by differences in emulsion composition (inner and outer aqueous phases and lipid phase, emulsifiers, etc.) and processing conditions (pressure level, number of passes, etc.).

The effect of storage on the particle size distribution of unheated DEs was affected by the inner aqueous phase composition (Fig. 1). During storage a bimodal distribution developed in all samples, and while all showed similar ranges of droplet size distribution, the second population of droplets was lowest in G/DE and highest in the sample without protein in the inner phase C/DE (Fig. 1). Despite this, mean particle size (d_{32}) was not clearly affected by storage, even when significant changes were observed, as in the case of G/DE (Table 1); in this sample, d_{32} decreased slightly at the end of the storage period, as shown in the microscopic images (Fig. 2). There have been reports of mean particle diameters increasing or remaining unchanged in the course of storage (O'Reagan and Mulvihill 2009; Choi et al. 2009; Jiménez-Alvarado et al. 2009; Carrillo-Navas et al. 2012; Bonnet et al. 2009), or even of diameters diminishing (Fechner et al. 2007). As in this experiment, Sapei et al. (2012) reported that the oil globule size distribution of DE

containing NaCl and gelatin in the inner aqueous phase was unaffected after 1 month of storage (4 °C). To the best of the authors' knowledge no studies have been reported about the influence of SC in the inner aqueous phase on DE storage stability.

The effect of heating on particle size distribution was influenced by inner aqueous phase composition (Fig. 1). While DEs containing protein in the inner phase were thermally stable over storage, the control sample (C/DE) was thermally unstable after 7 days of storage, and so after that point this (and other) properties could not be determined. Heating increased the range of droplet size distribution, favouring the formation of the bimodal distribution (particularly in SC/DE), as observed in the fresh samples over storage (Fig. 1). In confirmation of this, Table 1 shows that the average lipid globule size (d_{32}) of C/DE, and especially in SC/DE, increased during storage owing to the thermal treatment, while in G/DE no effect of heating treatment was observed over storage and sizes remained practically unchanged after 28 days of storage (Table 1 and Fig. 1). Overall, the addition of biopolymers in the inner water phase improved stability to thermal treatments. It has been reported that after heating, different type of DEs retained their structure, and lipid droplet sizes were unaffected (Bou et al. 2014a; Bonnet et al. 2009; Oppermann et al. 2015). Unlike in this experiment, a recent report has found that the effect of gelatin on droplet size of multiple emulsions seems to be affected by their concentration (Oppermann et al. 2015). In this connection, these authors have reported that while there was no change in samples containing a gelled inner dispersed phase with 5% gelatin, as observed in Table 1, the incorporation of 10% gelatin increased droplet size after heating.

Microscopy

Microscopic images show the characteristic compartmentalized structure of the double emulsions (Fig. 2, sample SC/DE as representative), consisting of relatively large oil droplets (of different sizes) with some smaller water droplets inside. The oil droplets observed were very similar irrespective of formulation (data not shown).

As with the reported effects on droplet size (Table 1 and Fig. 1), storage was found to have little effect on oil droplet appearance (Fig. 2), whereas the effect of the thermal treatment applied to DEs at each storage period depended on the composition of the inner aqueous phase. As observed in particle size distribution (Fig. 1), heating increased the number of lipid droplets in SC/DE up to the end of the storage and in C/DE at the beginning of storage. After 7 days DE was unstable after heating, as noted earlier. However, thermal treatment seemed to have no effect on the microscopic characteristics of G/DE (data not shown). These results are consistent with a report by Sapei et al. (2012) indicating that there was no change in the morphology of a DE with NaCl and gelatin in the inner aqueous phase after 1 month of storage (4 °C).

Physical stability

The high physical stability of DEs (Fig. 3), reflected by the limited changes occurring in them as reported above (Table 1, Fig. 1), indicated that these multi-compartmentalized systems were in general stable to water diffusion/expulsion, droplet coalescence and Ostwald ripening throughout this period (Choi et al. 2009). Initially and irrespective of the inner aqueous phase used, DEs showed no signs of phase separation (Fig. 3). However, the

Fig. 3 Gravitational stability of DEs as affected by storage. Different letters (between samples for the same storage time) and numbers (between storage days for the same sample) are significantly different ($P < 0.05$). Error bars represent standard deviations. For sample denomination see footnote of Table 1

gravitational stability (creaming) of DEs decreased over storage and this behaviour was generally not affected by the inner aqueous phase, showing similar ($P > 0.05$) stability at the end of storage, around 95–96% (Fig. 3), and demonstrating that these DEs are quite stable to creaming. This can be related to the fact that the mean particle size of the oil droplets was relatively small and to the sizes observed in the DE, and to the limited changes that occurred during storage (Table 1; Figs. 1 and 2). In similar types of DE to the control sample (C/DE), gravitational stability has been reported to decrease to values ranging between 75 and 90% after period of 7–10 days storage at 4 °C (Cofrades et al. 2013; Bou et al. 2014a, b). DEs prepared with gelatin in the inner aqueous phase have been reported to be more stable to creaming than in absence of gelatin (Khalid et al. 2013). The DEs prepared with both NaCl and gelatin in the W_1 were stable to sedimentation for a month in storage at 4 °C (Sapei et al. 2012). Gelation of the inner aqueous phase with gelatin increased the stability and yield of multiple emulsions after preparation, storage, shearing and heat treatment (Oppermann et al. 2015). The improvement of emulsion stability in G/DE could be attributed to several factors including the gelatin thickening or gelling processes in the inner aqueous phase, the droplet size and the viscosity as discussed below.

Viscosity

Viscosity is an important characteristic of emulsions. It influences the rate of creaming, giving information about the type of emulsion instability occurring during storage. Generally, viscosity was not affected by formulation, storage time or heating treatment (Table 1). The only exception occurred in sample G/DE at the end of storage, where it registered the highest ($P < 0.05$) viscosity. Some differences in viscosity were to be expected since the composition of the inner aqueous phase was different in each DE, including a gelled W_1 phase in sample G/DE. Moreover, the viscosity of DEs has been associated with particle size and particle size distribution and swelling processes (Bou et al. 2014a; Iqbal et al. 2013). Sapei et al. (2012) reported that gelatin and electrolyte had a synergistic effect on emulsion stability and swelling in the inner water phase. Results are difficult to interpret, but in the case of G/DE, the existing relationship between viscosity and droplet size (Table 1) suggests that the viscosity increase may be related to the instability of the larger droplets during the course of storage.

Colour

The inner aqueous phase of DEs affected lightness (Table 2), with generally no noticeable effects on redness

and yellowness (data not shown). Initially the inner aqueous phase had no observable effect ($P < 0.05$) on L^* ; however, this parameter decreased ($P < 0.05$) over storage in all samples, but in different pathways. The decrease of lightness occurred earlier in G/DE than in C/DE and SC/DE, although in similar proportions, so that the final values were similar. Heating generally produced a slight (if significant) reduction of L^* . Bou et al. (2014b) observed that the lightness of a DE containing olive oil, as in this experiment, was not affected by thermal treatment but decreased during storage at 4 °C.

Si encapsulation efficiency and stability

Double emulsions have been used as a strategy to encapsulate bioactive compounds in the inner aqueous phase. Minerals such as Ca, Fe or Mg have been encapsulated, and in some cases have even been used as model species to test the release mechanism in these systems (Jimenez-Colmenero 2013). The initial silicon EE, ranging between 72.4 and 78.3%, was not affected ($P > 0.05$) by inner aqueous phase composition (Table 2). The authors know of no studies relating to Si encapsulation, although there have been some on other bioactive compounds, including minerals. For instance, Bonnet et al. (2009) reported initial magnesium EE higher than 99% irrespective of the oil type used in the formulation. Iron has been encapsulated within the internal aqueous phase of DEs with a high loading efficiency (>99%) (Choi et al. 2009). Also, mango seed kernel extract registered high (>90%) EE values (4–8% PGPR) in the presence of gelatin (1–5%), although even in the absence of gelatin EE values were in the range 85–88%, suggesting that internal water droplets in the DEs were stable (Tepsongkroh et al. 2015). Then again, encapsulation efficiency of 70–95% has been reported with hydrosoluble vitamins such as riboflavin or ascorbate incorporated in an inner aqueous phase (Bou et al. 2014a).

Encapsulation stability was determined by the amount of Si remaining entrapped in the inner aqueous phase as a result of storage or exposure to thermal treatment. Given that the release rates of hydrophilic compounds encapsulated in DEs have been associated with droplet sizes, the similarity of the droplet sizes in the W/O/W emulsions (Table 1) facilitates direct comparison of encapsulation efficiency and release rates (Bonnet et al. 2009). The fact that silicon ES was generally unaffected (with some exceptions) during storage and heating (Table 2) indicates high Si encapsulation stability in these systems. Studies on the effect of storage on encapsulation stability have produced varying results. In general, storage has been found to reduce encapsulation stability. Depending on lipid phase levels, it has been reported that up to 30% of the

Table 2 Effect of internal aqueous phase composition, storage time and heating on lightness (L^*) and Si encapsulation efficiency and stability of DEs

Days at 3 °C	C/DE	SC/DE	GDE
<i>Lightness (L^*)</i>			
1	81.22 $\pm$ 0.10 ^{3,x} (80.58 $\pm$ 0.01) ^y	80.90 $\pm$ 0.31 ² (80.94 $\pm$ 0.09)	81.20 $\pm$ 0.23 ² (80.93 $\pm$ 0.12)
7	81.25 $\pm$ 0.03 ^{3,b} (80.75 $\pm$ 0.02)	81.50 $\pm$ 0.38 ^{2,b} (80.37 $\pm$ 0.67)	76.85 $\pm$ 0.09 ^{1,a} (76.58 $\pm$ 0.09)
14	76.66 $\pm$ 0.10 ^{2,b} –	76.29 $\pm$ 0.09 ^{1,a,x} (75.50 $\pm$ 0.21) ^y	76.80 $\pm$ 0.02 ^{1,b,x} (75.92 $\pm$ 0.03) ^y
21	75.98 $\pm$ 0.19 ¹ –	76.17 $\pm$ 0.25 ¹ (75.65 $\pm$ 0.30)	76.60 $\pm$ 0.03 ^{1,x} (76.14 $\pm$ 0.13) ^y
28	75.67 $\pm$ 0.27 ¹ –	76.16 $\pm$ 0.45 ¹ (75.39 $\pm$ 0.36)	76.58 $\pm$ 0.07 ^{1,x} (75.76 $\pm$ 0.14) ^y
<i>Si encapsulation efficiency and stability (%)</i>			
1	76.9 $\pm$ 1.72 ¹² (77.8 $\pm$ 3.74)	72.4 $\pm$ 3.65 (74.4 $\pm$ 1.81)	78.3 $\pm$ 1.38 (79.9 $\pm$ 2.21)
7	79.5 $\pm$ 1.24 ¹ (75.6 $\pm$ 0.83)	76.0 $\pm$ 1.41 (76.5 $\pm$ 0.75)	78.4 $\pm$ 1.24 (78.9 $\pm$ 1.29)
21	71.9 $\pm$ 1.06 ^{2,a} –	76.9 $\pm$ 0.80 ^{ab} (77.1 $\pm$ 0.63)	78.4 $\pm$ 1.92 ^b (81.2 $\pm$ 1.84)
28	76.4 $\pm$ 2.01 ^{12,a} –	77.9 $\pm$ 0.29 ^{1,ab,x} (84.7 $\pm$ 1.38) ^y	81.9 $\pm$ 0.83 ^b (82.2 $\pm$ 0.92)

Sample denomination: C/DE, SC/DE and G/DE are double emulsions containing NaCl, NaCl + sodium caseinate and NaCl + gelatin in the inner aqueous phase, respectively. Mean $\pm$ standard deviation, in parenthesis those for the sample after heating (when—the determination was not possible). Unheated samples with different letters (a–b) within the same row or numbers (1–2) in the same column indicate significant differences ($P < 0.05$). Different letters (x–y) indicates significant differences ($P < 0.05$) between unheated and heated samples at each storage time

encapsulated magnesium was released after 1 month at 4 °C (Bonnet et al. 2009). Conversely, Sapei et al. (2012) reported that NaCl encapsulation efficiency in DEs remained at 94–95% during a month-long study irrespective of NaCl load and gelatin concentration in the inner aqueous phase. Similarly, only a small amount of iron leakage (from the iron encapsulated in the internal aqueous phase) was observed over 7 days storage (Choi et al. 2009).

When entrapped in the inner aqueous phase, water-soluble species such as Si tend to migrate from the internal to the external phase without film rupturing (Bonnet et al. 2009); however, no such effect was observed in our case. As far as the authors are aware, there have been no studies dealing with encapsulation of minerals with a gelled inner aqueous phase. Gelatin has been included to solidify the inner aqueous phase of DEs and so increase the encapsulation efficiency of the system (Fechner et al. 2007; Muscholik 2007); Si encapsulation was clearly affected in this way, and that effect presumably contributed to fact that stability increased more with longer storage times than with the other DEs (Table 2). This may involve processes associated with silico-protein interaction. Silicon apparently binds hydroxyl groups of proteins (Nielsen 2015);

also, it has been suggested that gelatin solution interacts with orthosilicic acid (Jaffery and Viswanathan 1987). There are not many available references to the effect of thermal treatment on encapsulation stability, but heating of DEs has been reported to promote magnesium release (Bonnet et al. 2009). In our case, the addition of caseinate or gelatin improved stability as compared to thermal treatments.

In conclusion, our results showed that DEs exhibited good stability as regards size characteristics. Although the gravitational stability of DEs decreased over storage, minimal changes were observed at the end of the storage period (28 days). Silicon encapsulation was unaffected by the composition of the inner aqueous phase used in the DE formulation, but the addition of gelatin may have contributed to greater encapsulation stability during storage. Overall, DEs with a gelled inner aqueous phase were more stable to processing in terms of storage and heat stability than the control sample.

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