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ORIGINAL PAPER

Microencapsulation of a Low-*trans* Fat in Trehalose as Affected by Emulsifier Type

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Abstract A low-*trans* fat blend formulated with high linoleic sunflower seed oil (SFO) and a high melting fraction (HMF) of milk fat was encapsulated by freeze-drying emulsions. The selected emulsifiers were a mixed of the palmitic sucrose esters (SE) P-170 and P-1670, sodium caseinate (NaCas) or a blend of SE and NaCas. The ability to retain the core material with time was studied by storing the powders at different water activities (a_w). Efficiency of encapsulation was strongly dependent on emulsifier type. NaCas formulation was more efficient retaining core material during storage. The formulation with a protein and a small surfactant had the lowest performance. The stabilizer also influenced droplet size distribution and matrix crystallinity. For NaCas-stabilized powder volume weighted mean diameter ($D_{4,3}$) remained small for up to 2 months of storage (0.56 ± 0.5 μm) and then grew notably in agreement with matrix collapse. There were no significant differences in $D_{4,3}$ with water content. For NaCas/SE-stabilized powder, however, $D_{4,3}$ was high at the beginning (100 ± 0.5 μm) and then decreased most likely due to particle break-up. Although particle size distribution showed the same behavior for all a_w, retention was strongly dependent on water content. Retention with time was determined by the counteracting effects of these factors.

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Introduction

It is well known that stability of lipids is improved by encapsulation since it is a physical means offering protection against oxidation without the need of antioxidants. Milk fat has been encapsulated to extend storage life at ambient temperature in order to use it as an ingredient for making dairy creamer and other related products [1–3]. It is also interesting to be able to encapsulate a low *trans* fat blend rich in PUFA as it is for a blend of high linoleic sunflower oil (SFO) in a high-melting fraction of milk fat (HMF).

There are several methods of encapsulating sensitive materials. Spray drying is the most common method as the cost of drying is 30–50 times less than freeze-drying. Spray-drying provides a very large surface area which enhances oxidation, if the wall material is not thick or dense enough to provide a good oxygen barrier. Freeze-drying occurs at a low temperature from the frozen state avoiding any water phase reactions and most oxidation because of the vacuum. Thus, it is the preferred method for the flavor industry. The efficiency of encapsulation in both, freeze and spray-drying is comparable [4].

The choice of encapsulant, core material and emulsifiers is critical as they will influence emulsion stability before drying. Superior emulsifying capacity and oil retention have been reported for some materials. Typical wall components for microencapsulation by spray-drying are low molecular weight carbohydrates like maltodextrins [5] since they have a high glass transition temperature and are resistant to

caking and structural collapse. In this context, the physicochemical properties of trehalose, a known cryoprotectant, appear to be very promising concerning its use in microencapsulation especially in freeze-drying where no hot air is used. Trehalose possesses a uniquely high glass transition temperature. The glass transition temperature ranges from 79 to 115 °C and this is attributed to polymorphism in the crystallization pattern [6]. If trehalose crystallizes, the predominant form is the trehalose dihydrate, thus immobilizing water and keeping the water activity at a low level. For these reasons, trehalose is particularly effective because of its ability to prevent lipids from oxidizing and its having the capability of stabilizing proteins or carbohydrates [7]. Sodium caseinate has been reported to be the most effective emulsion stabilizer for fats [8]. It is also of interest to study the effects of sucrose esters (SE) on oil retention since in addition to their major function of producing and stabilizing emulsions, SE contribute to numerous other functional roles such as texturizers and film formers [9].

The removal of preadsorbed proteins by surfactants is called "elutability". Surfactant elutability is influenced not only by protein properties but also by the type of surfactant. Factors relating to the structural stability of the protein are of major importance [10]. Nonionic surfactants are generally found to be ineffective in removing protein from hydrophilic surfaces. At hydrophobic surfaces the removal is generally high. Therefore, in a previous study we investigated how surfactants/protein interactions affect initial retention [11]. The aim of the present study was to compare the ability of three different formulations to encapsulate a low-*trans* fat blend formulated with a high-melting fraction of milk fat (HMF) and high-linoleic sunflower oil (SFO). The performance of a protein (sodium caseinate) or small surfactants (the palmitic sucrose esters P-170 and P-1670) or the blend of both, protein and small surfactants, was studied by following the temporal evolution of the retention of the fat phase in a trehalose matrix when the powders were stored at different water activities (a_w). The effects of factors such as droplet size, water content and physical properties of the matrix on retention with time were also investigated.

Materials and Methods

Starting Materials

α,α-Trehalose dihydrate (α-D-glucopyranosyl-(1-1)-α-D-glucopyranoside) from *Saccharomyces cerevisiae*, 99% obtained from Sigma (Sigma-Aldrich, St. Louis, Mo., USA) were used without any further purification. HPLC water was used for all experimental work. The fat phase was a blend of 40 wt% high linoleic sunflower seed oil (SFO) in a high-melting fraction of milk fat (HMF). The SFO composition was as follows: $C_{14:0}$ 0.1%, $C_{16:0}$ 6.7%, $C_{18:0}$ 3.6%, $C_{18:1t}$ 0.7%, $C_{18:1c}$ 21.9%, $C_{18:2c}$ 66.3%, $C_{20:0}$ 0.2%, and $C_{22:0}$ 0.5%. The HMF was obtained by fractionation of anhydrous milk fat (AMF) with ethyl acetate (3:1). After 2 h at 5 °C, the solids were separated by filtration and the solvent was evaporated. Dropping point (the temperature at which a solid fat just begins to flow under controlled conditions) of the HMF and the 40 wt% SFO-in-HMF blend was determined with the Mettler FP 80 dropping point apparatus (Mettler Instruments A.G., Greifensee-Zurich, Switzerland), using a heating rate of 1 °C/min. The Mettler dropping point of HMF and the 40 wt% SFO-in-HMF blend were 49.5 and 46.5 °C, respectively. The solid fat content (SFC) of the fat blend measured by nuclear magnetic resonance (NMR) at 22.5 °C was 45%. Palmitic sucrose ester (SE) (P-170) with hydrophilic/lipophilic balance (HLB) = 1 and Palmitic SE (P-1670) with HLB = 16 were supplied by Mitsubishi-Kasei Food Corp. (Tokyo, Japan). The SE had Mettler dropping points of 58.0 and 44.0 °C, respectively. The monoester content of P-170 were 1 wt%, with di-, tri-, and polyesters comprising 99 wt%. P-1670 had 80% monoester and 20% di-, tri-, and polyester. The average HLB for the emulsifier blend was nine.

Emulsion Preparation

Three emulsions were prepared by mixing 100 mL of aqueous phase and 4 g of fat phase. In all cases aqueous phase was a 20 wt/v% solution of trehalose. Three different stabilizers were formulated: SE, NaCas, and a 50 wt% blend of SE and NaCas. For SE emulsions, 0.250 g of P-1670 were dissolved in 100 mL aqueous phase while 0.250 g of P-170 were dissolved in 4.000 g of fat phase (a blend of 40 wt% SFO-in-HMF). For NaCas emulsions 0.500 g of NaCas was used while for SE/NaCas emulsions 0.125 and 0.250 g of P-1670 and NaCas, respectively, were dissolved in 100 mL aqueous phase, and 0.125 g of P-170 were added to the 4.000 g of fat phase. The SE concentration was within the those usually employed in foods for these esters. We selected SE with extremes HLB (1 and 15) because it is often the case in food products, and in confectionery too, that a combination of two emulsifiers in a recipe formula containing two distinct phases will result in the longer lasting and more uniform product. In these cases, combinations of low- and high-HLB emulsifiers give the best results [9]. The fat phase was blended with the trehalose solution using an Ultra-Turrax T25, S25N10G device, operated at 20,000 rpm for 1 min to give pre-emulsions. The resultant emulsions were further homogenized at 40 MPa with four recirculations using a high-pressure laboratory valve homogenizer Stansted Fluid Power model

n674004:050 (Stansted, Essex, UK) and subsequently analyzed for particle size distribution.

Powder Production

Emulsions were frozen with liquid nitrogen (−190 °C) and stored over night at −80 °C before freeze-drying to allow the highest amount of freezable water to crystallize. A Heto-Holten A/S, cooling trap model CT 110 freeze-dryer (Heto Lab Equipment, Allerød, Denmark) was operated at −110 °C and at a chamber pressure of 4.10^{-4} mbar. The dried emulsions were broken into powder using a mortar and pestle and subsequently washed with hexane (HPLC grade) to remove nonencapsulated fat. The washing process was repeated twice. Powders were separated from hexane by filtering. Hexane was then evaporated and the fat phase was weighed to determine the initial efficiency of encapsulation [12, 13]. The washed powder was further dehydrated under vacuum over $Mg(ClO_4)_2$ for 24 h. Then, it was analyzed for water content, thermal behavior and particle size distribution.

Storage Study

Aliquots of about 2 g of the dried samples were placed in glass vials (5 mL capacity) and exposed to atmospheres of saturated salt solutions of water activities 0.11 (LiCl), 0.33 ($MgCl_2$), 0.44 (K_2CO_3), 0.54 ($Mg(NO_3)_2$), and 0.76 (NaCl) into evacuated desiccators at ambient temperature (22.5 °C). At 2, 7, 14, 28, 42, 56, 70 and 84 days, samples were removed and analyzed for fat retention, thermal transitions, crystallinity and particle size distribution. Water content was analyzed at 2, 7, 14, 28 and 84 days. During storage, the initial losses were not considered and 100% retention corresponds to the resultant amount of HMF/40 wt% SFO blend in each powder after drying. All experiments were performed in duplicate and the average value was reported.

Particle Size Distribution

The droplet size distribution of emulsions was determined immediately after emulsion preparation by light scattering using a Beckman Coulter Particle Analyzer model LS™ 230 (Beckman Coulter, Fullerton, CA, USA). Calculations from 0.003 to 300 μm were expressed in differential volume. The particle size data were reported as the volume-weighted mean diameter ($D_{4,3}$), the 10 and 90% volume percentiles of the size distribution (d_{10} and d_{90}) and the volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$). The span (S) of the distribution was expressed as $S = (d_{90} - d_{10})/d_{50}$, where d_{50} is the 50% volume percentile, also known as the median of the distribution. S indicates the width of the distribution regardless of the median size. Determinations were conducted in duplicate. Droplet size of powder after freeze-drying and further dehydration with $Mg(ClO_4)_2$ was measured from the reconstituted emulsion using the same conditions described for emulsions. The powder was reconstituted to 25 g solids per 100 g reconstituted emulsion by dissolving 1 g of powder in 4 mL of distilled water; 5 min after reconstitution, the emulsion was analyzed for droplet size distribution. Powders stored at different water content levels were analyzed in the same way as dried powders.

Determination of Water Content

The water content of the equilibrated samples was determined by difference in weight before and after drying in vacuum ovens at 98 °C for 48 h. These conditions had proved themselves to be adequate for assessing constant weight after drying.

Extractable and Encapsulated Fat

Extractable fat was determined by dispersing 2 g powder in 15 mL hexane and shaking for 15 min. The soluble fraction was filtered and the solvent was evaporated, leaving the fat. The weight of the dried material representing the extractable fat was calculated as a percentage of the total fat in the dry powder. Then, the powder, free of extractable fat, was mixed with 15 mL water and 15 mL ethanol. The resulting solution was extracted with 40 mL sulfuric ether. The clear organic phase was collected and this extract containing the encapsulated fat was then dried and weighed. During storage, the initial losses were not considered and 100% retention corresponds to the resultant amount of fat in each powder after freeze-drying and further dehydration with $Mg(ClO_4)_2$.

Thermal Transitions

Differential scanning calorimetry (DSC) was used to determine glass transition temperatures (T_g), melting point of trehalose matrix (T_m), and heats of fusion (ΔH_m) for the dried systems and storage powders. A DDSC Mettler Toledo model 822^e (Mettler Toledo, Schwerzenbach, Switzerland) was used with a thermal analysis software Mettler Star^e. Calibration was carried out at a heating rate of 10 °C/min by using indium proanalysis (p.a.) as standard. From 5 to 9 mg of each sample in hermetically sealed aluminium pans was placed in the DSC and held at −70 °C for 5 min prior to melting at a heating rate of 10 °C/min from −70 to 120 °C. A single empty pan was employed as a reference. Two replicates were performed for each sample, and means and

standard deviation of peak temperatures and melting enthalpies are reported. The melting endothermic peak that appeared at a temperature almost identical to that corresponding to crystalline trehalose dihydrate [14] was used to indicate the amount of crystalline dihydrate present. The crystallization degree (DC) was calculated as:

$$\mathrm{DC} = \frac{\Delta H_{\mathrm{m}}}{\Delta H_T} \quad (1)$$

where ΔH_{m} is the melting endotherm area for a given sample and ΔH_T is the melting endotherm area corresponding to pure trehalose (149 J/g).

Crystallinity

Samples were analyzed for their crystallinity by X-ray diffraction (XRD). A Philips 1730 X-ray spectrometer fitted with a system for temperature control (Philips Argentina, S.A., Buenos Aires, Argentina). $K_{\alpha 1 \alpha 2}$ radiation from copper was used at 40 kV, 20 mA, and a scanning velocity of 1°/min from 5° to 50°. Experiments were performed at ambient temperature (22.5 °C).

Statistical Analysis

Significant differences between means were determined by the Student's t test. An α level of 0.05 was used for significance.

Results and Discussion

Powders Dehydrated Over $Mg(ClO_4)_2$

Initial Efficiency of Matrices

The efficiency of the three stabilizers for successfully encapsulating the HMF/SFO blend was determined measuring the amount of fat extracted in each powder after freeze-drying and further dehydration with $Mg(ClO_4)_2$. The retention values corresponding to the powders stabilized with NaCas, SE, and NaCas/SE were 89.7 ± 0.1, 64.9 ± 0.8, and 55.5 ± 1.0, respectively. Clearly, incorporation of SE decreased the efficiency of the NaCas stabilizer. In addition, the efficiency of the NaCas/SE mixture is even lower than that found for each stabilizer separately. These results indicate that the ability of the small surfactant molecules to remove protein from the interface had a great effect on retention [11]. Although stabilizers were minor components in emulsion formulation, their chemical structure and interactions played a key role in retention.

Oil Particle Size of Dried Powder Reconstituted Emulsions

Figure 1 shows the particle size distribution for the reconstituted emulsions of dried NaCas (a), SE (b), and NaCas/SE (c)-stabilized powders and Table 1 summarizes $D_{4,3}$ and S of distributions in Fig. 1. NaCas and SE-stabilized powders showed a bimodal distribution while NaCas/

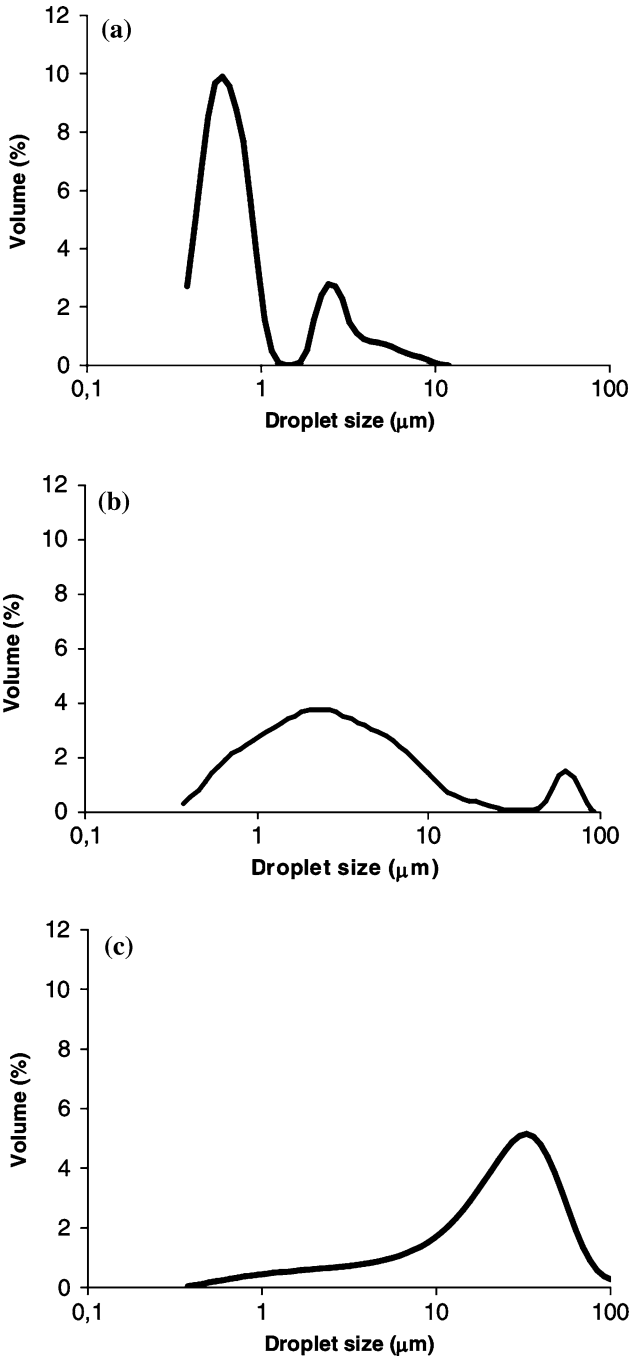

Fig. 1 Particle size distribution for emulsions reconstituted from powders dehydrated under vacuum over $Mg(ClO_4)_2$ for 24 h. Selected stabilizers were **a** sodium caseinate (NaCas), **b** sucrose esters (SE), and **c** a 50 wt% NaCas/SE blend

Table 1 Volume-weighted mean diameter ($D_{4,3}$) and Span (S) of the distribution for NaCas, SE or NaCas/SE-stabilized emulsions immediately after preparation and for the reconstituted powders after freeze drying

Samples	Emulsions immediately after preparation			Reconstituted powders after freeze-drying		
	$D_{4,3}$	S	$\%V_{d>1}$	$D_{4,3}$	S	$\%V_{d>1}$
NaCas	0.56 ± 0.22^{a}	2.53^{e}	27.81^{h}	0.56 ± 0.22^{a}	2.72^{e}	25.31^{h}
SE	1.60 ± 0.19^{b}	2.25^{e}	67.40^{i}	4.11 ± 0.19^{c}	7.86^{f}	83.74^{j}
NaCas/SE	2.00 ± 0.20^{b}	2.93^{e}	72.13^{k}	28.12 ± 0.33^{d}	52.23^{g}	97.52^{l}

Values without the same superscript letter in the same column are significantly different ($p < 0.05$). Units of $D_{4,3}$ and S are micrometers (μm)
NaCas sodium caseinate, *SE* sucrose esters

SE-stabilized powder had a unimodal distribution. However, in the last case, the mean diameter was the greatest of the three distributions. Values of S indicated that not only median particle size was greater but the span of the distribution as well. It might be expected that a unimodal droplet size distribution would lead to higher retention. Surprisingly, as was discussed in the previous paragraph, the opposite results were obtained. However, in both, NaCas or SE distributions, particles with smaller diameter represented a higher percentage of total particles indicating that a large proportion of small particles was a more important property of the distribution related to retention than the monodispersity of the system. Particle size distribution was in agreement with retention values. The lower the median particle size and span the higher the retention. All samples were prepared using the same processing conditions and therefore differences in droplet size were mostly due to the interfacial processes. The interaction among stabilizers and aqueous or oil phases is driven by electrostatic or hydrophobic forces or in many cases it is a combination of the two. NaCas interacted strongly with the lipid phase giving small particles. Addition of SE might have destabilized protein structure and therefore the NaCas/SE-stabilized powder had a greater droplet size.

Table 1 also summarized values of $D_{4,3}$ and S for emulsion immediately after preparation. NaCas-stabilized emulsion had a $D_{4,3}$ and S that showed no significant differences between emulsion immediately after preparation and the reconstituted powder after freeze-drying. This result suggests that the structure of the protein layer remained stable after those treatments. SE and NaCas/SE-stabilized emulsions, however, showed significant differences in $D_{4,3}$ and S after freeze drying ($p < 0.05$). $\%V_{d>1}$ dramatically increased for SE and NaCas/SE emulsions when both the oil and water phases crystallized ($p < 0.05$), indicating the great effect of interface composition on particle size.

Thermal Behavior of Dried Powders

NaCas, SE, and NaCas/SE-stabilized powders containing the encapsulated HMF/40 wt% SFO blend had an initial water content of 2.08 ± 0.21, 3.02 ± 0.25, and $3.67 \pm 0.43\%$ (dry basis), respectively. DSC diagrams after freeze drying showed that powder formulated with NaCas had a DC of $0.3 \pm 0.2\%$, and a T_g of 88 ± 3 °C after dehydration under vacuum for 24 h and a DC of $3.0 \pm 0.5\%$ after washing with hexane. SE-stabilized powder had a DC of $0.4 \pm 0.2\%$ immediately after freeze drying and 3.7 ± 0.2 after washing with hexane. The DSC diagram of powder after freeze drying showed a T_g of 86 ± 4 °C. When the stabilizer was the 50:50 wt% blend NaCas and SE, T_g was 84 ± 3 °C. A small peak at 99 ± 1 °C, which represents a DC of $0.4 \pm 0.2\%$, was present. A small amount of trehalose also crystallized when washing with hexane leading to a DC of $3.0 \pm 0.1\%$. These results indicated that the trehalose matrix was mostly amorphous in all cases.

Degree of Crystallinity of Powders

X-ray experiments were performed to confirm the DSC studies. As a representative example, Fig. 2 shows X-ray patterns for NaCas-stabilized powder dehydrated under

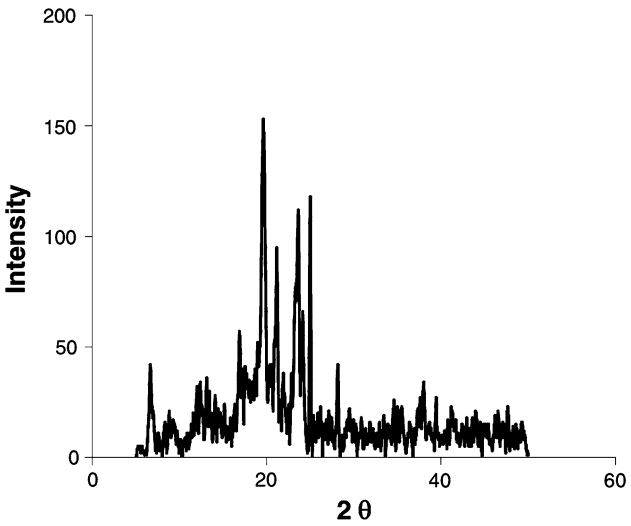

Fig. 2 X-ray diffraction pattern of NaCas-stabilized powder dehydrated under vacuum over $Mg(ClO_4)_2$ for 24 h. *NaCas* sodium caseinate

$Mg(ClO_4)_2$ for 24 h. Fats solids represented 8.75%. There were two very weak signals at 4.3 and 3.8 Å corresponding to the β'-form of the fat blend. According to the DSC diagrams, the DC was 0.3 ± 0.2%. In agreement with the calculated DC, very weak signals, the greatest of which appeared at 3.6 Å, corresponding to the sugar pattern were found. The pattern showed that trehalose was mostly amorphous. SE and NaCas/SE-stabilized powders showed very similar patterns to NaCas powder (data not shown). Immediately after preparation, the low degree of crystallinity of the powders was similar in all cases. Mazzobre and Buera [15] obtained a similar pattern for amorphous trehalose.

Storage Study

Water Content

Figure 3 shows water content versus time for NaCas (a), SE (b) and NaCas/SE (c)-stabilized powders at a_w 0.11, 0.33, 0.44, 0.54, and 0.76. For NaCas-stabilized powders, water content varied in greater extent until 7 or 14 days of storage. After 28 days of storage water content decreased until the end of storage (84 days). These changes might be related to equilibration process and sugar crystallization. Labrousse et al. [13] reported decreased in water content when lactose crystallized. Amorphous sugars are highly hygroscopic and they may absorb large amounts of water from the surroundings. Once crystallization is initiated, sugar molecules become tightly packed, and the amount of water that can be held therefore decreases [7]. Water content for trehalose, however, changed to a lesser extent than values reported for lactose which is not surprising with regard to its unique properties. SE showed similar behavior to NaCas-stabilized powders. Although values for SE were higher in several cases than the ones for NaCas, differences were only slightly significant ($p < 0.05$). NaCas/SE powders, however, showed significant differences in water content with time when compared with the other two cases. Firstly, values of the water content were lower for all a_w studied. Secondly, there were almost no changes in water content after 28 days of storage for a_w 0.33, 0.44, 0.54, and 0.76. These results are intimately related with the crystallinity of the systems and are discussed later on.

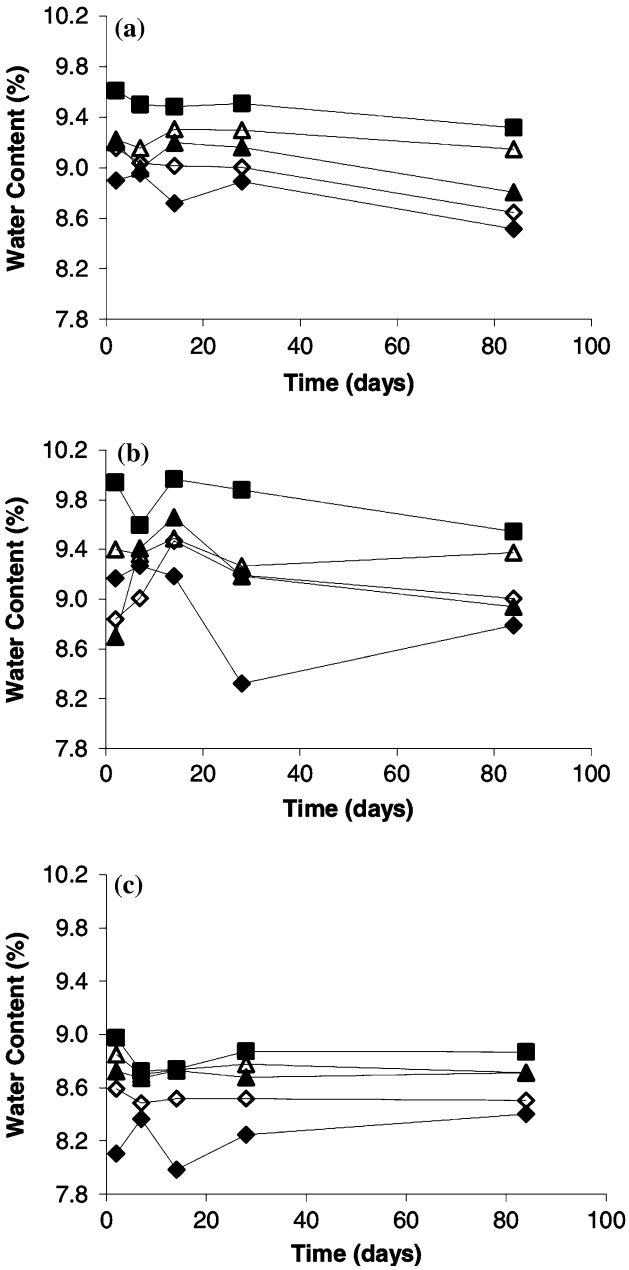

Fig. 3 Water content with time for **a** NaCas, **b** SE, and **c** NaCas/SE-stabilized powders stored at a_w 0.11 (*filled diamond*), 0.33 (*open diamond*), 0.44 (*filled triangle*), 0.54 (*open triangle*), and 0.76 (*filled square*). Data points are the average of two replicates. Standard deviations were within symbols size. Abbreviations as in Fig. 1

Fat Retention During Storage

Figure 4 shows retention versus time for the NaCas, SE, and NaCas/SE-stabilized powders stored at a_w 0.11, 0.33, 0.44, 0.54, and 0.76. The ability of trehalose matrix to encapsulate fat depended on the stabilizer selected. The trehalose-NaCas matrix was the most effective for encapsulating the 40% SFO in HMF blend. Retention values with time, at all a_w were higher than values for the other two stabilizers ($p < 0.05$). Labrousse et al. [12] reported the release of an encapsulated material upon exceeding T_g owing to the collapse and crystallization of the matrix. When methyl linoleate was encapsulated in lactose-gelatin, collapse occurred above T_g, resulting in re-encapsulation of the oil. NaCas-stabilized powder suffered collapse after 2 months of storage at all a_w and in agreement with their

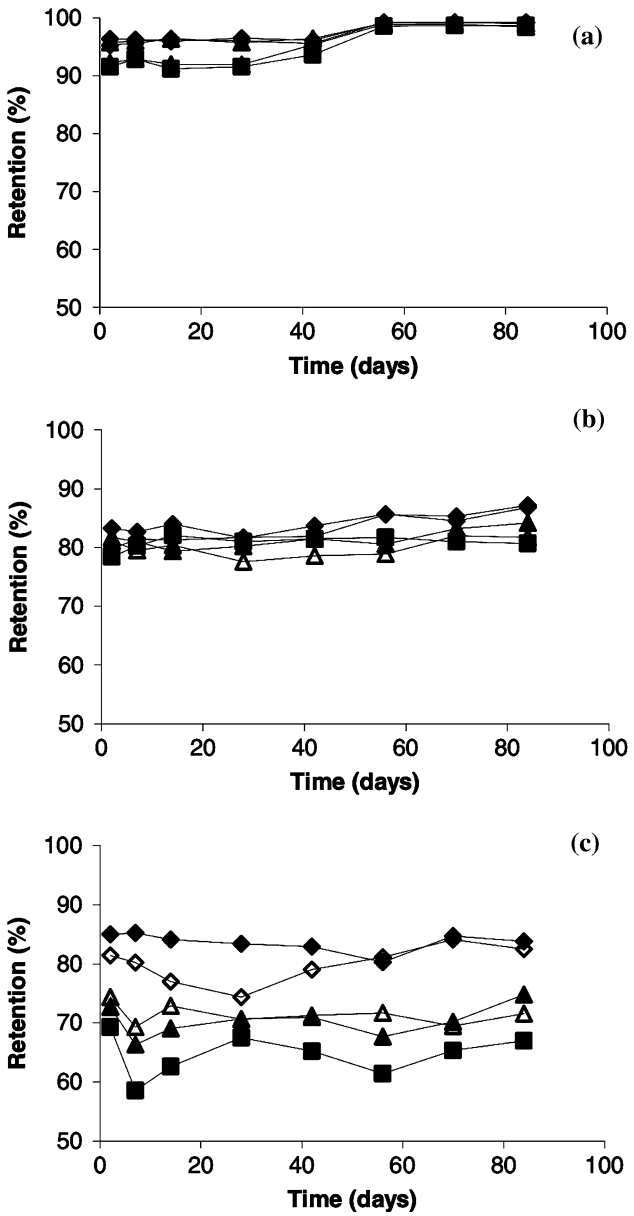

Fig. 4 Retention with time for **a** NaCas, **b** SE, and **c** NaCas/SE-stabilized powders stored at a_w 0.11 (*filled diamonds*), 0.33 (*open diamonds*), 0.44 (*filled triangles*), 0.54 (*open triangles*), and 0.76 (*filled squares*).Data points are the average of two replicates. Standard deviations were within the symbol size. Abbreviations as in Fig. 1

results the system showed even higher retention values from that time. Most likely, the protein changed its conformation in a time-dependent way at the interface which was observed from the decreasing volume of the powder at that time (collapse). The new matrix with a different structure was able to re-encapsulated release material. The trehalose NaCas and SE matrices were efficient encapsulants for HMF/SFO blend, and the ability to retain fat was affected by the water content to a low extent. NaCas/SE-stabilized powders had similar retention values at a_w 0.11 and 0.33. Retention values with time for a_w 0.44, 0.54, and 0.76, however, diminished significantly ($p < 0.05$). Although the water content in these systems was lower than for NaCas and SE stabilizers, it affected the retention to a greater extent. The formulation with a protein and a small surfactant had the lowest performance. It might be expected that molecular mobility in these systems was low. Despite the small motions of molecules, in the NaCas/SE-stabilized powder, NaCas elutability at the hydrophobic surface of the fat droplet was high as evidenced by lower retention values. Water content of the samples stored at a_w 0.44, 0.54, and 0.76 were high enough to allow elutability. Competition between both stabilizers had a great effect on retention of the core material with time.

Particle Size Distribution

Figure 5 shows, as a representative example, the particle size distribution of NaCas-stabilized powder stored at a_w 0.11 and 0.76 for 1, 8 and 12 weeks. Volume weighted mean diameter ($D_{4,3}$), the 10 and 90% volume percentiles of the size distribution (d_{10} and d_{90}), and volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$) of all samples are summarized in Table 2. No significant differences in particle size distribution among the powder dehydrated over $Mg(ClO_4)_2$ (Fig. 1a) and powders stored at a_w 0.11 and 0.76 for 1 week (Fig. 5a, d) were found ($p < 0.05$). After 8 weeks of storage, the powders showed a monomodal distribution and a notable growth in the median particle size (Table 2, Fig. 5b, e) most likely due to coalescence or partial coalescence since the fat phase is partially crystallized at the storage temperature. Particle growth was in agreement with changes in the matrix structure, that is, occurrence of matrix collapse and re-encapsulation. After 12 weeks, the mean size diminished as a result of droplet break-up which led to the formation of smaller particles of various sizes that follow the distributions shown in Fig. 5c, f. Perhaps protruding crystals were involved in the mean droplet size decrease. As can be seen in Fig. 5, there were no significant differences in $D_{4,3}$ with water content. SE-stabilized powder showed a different behavior (Table 2). At a_w 0.11 and 0.33, $D_{4,3}$ significantly increased with storage time. When the powder was stored at a_w 0.44, 0.54, and 0.76, $D_{4,3}$ decreased after 8 weeks most likely due to particle break-up. However, surprisingly, retention values were close for both a_w showing no correlation with particle size. For the SE-stabilized powder, the matrix structure was stable since no collapse was observed. Structural stability seemed to be a determining factor for retention. NaCas/SE-stabilized powder stored at all a_w (Table 2) showed a similar behavior to SE-stabilized powder stored at a_w 0.44, 0.54, and 0.76 but $D_{4,3}$ decreases occurred to a lesser extent. Although particle size

Table 2 Volume-weighted mean diameter ($D_{4,3}$), volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$), and 10 and 90% volume percentiles of the size distribution (d_{10} and d_{90}) of powders stored at a_w 0.11, 0.33, 0.44, 0.54, and 0.76 for 1, 8 and 12 weeks

Sample	1 week				8 weeks				12 weeks			
	$D_{4,3}$	$\%V_{d>1}$	d_{10}	d_{90}	$D_{4,3}$	$V_{d>1}$	$\%d_{10}$	d_{90}	$D_{4,3}$	$V_{d>1}$	$\%d_{10}$	d_{90}
NaCas												
a_w 0.11	0.57	15.20	0.43	1.99	87.14	100.00	55.08	132.62	0.77	30.90	0.49	3.57
a_w 0.33	0.58	18.20	0.41	2.03	87.32	97.23	47.08	129.21	0.74	27.23	0.48	3.79
a_w 0.44	0.55	17.70	0.43	2.08	87.34	100.00	53.11	111.00	0.71	24.20	0.47	2.65
a_w 0.54	0.58	18.10	0.42	2.04	87.29	99.05	53.87	117.42	0.76	31.80	0.48	4.03
a_w 0.76	0.56	19.90	0.43	2.10	87.49	95.48	44.28	124.70	0.79	34.60	0.49	4.55
SE												
a_w 0.11	15.38	89.50	0.97	103.70	21.19	93.76	1.48	123.10	32.79	98.87	1.13	136.10
a_w 0.33	16.02	90.05	1.03	102.31	25.44	94.15	1.34	121.78	37.09	99.03	1.08	133.15
a_w 0.44	69.82	93.97	1.50	154.20	23.20	91.06	1.08	142.70	16.23	87.10	0.86	77.96
a_w 0.54	69.57	93.99	1.48	153.29	18.16	90.83	1.05	139.89	15.34	88.25	0.91	100.04
a_w 0.76	69.82	94.00	1.50	154.21	17.78	90.36	1.03	123.50	14.67	89.60	0.97	141.01
NaCas/SE												
a_w 0.11	100.25	98.50	49.05	165.50	77.52	95.86	2.46	117.60	60.42	94.03	1.25	99.87
a_w 0.33	100.00	98.21	47.22	167.11	77.92	95.77	2.88	117.50	61.07	94.01	1.30	101.80
a_w 0.44	100.20	98.50	45.31	168.37	78.13	95.69	4.14	118.90	62.38	93.52	1.47	110.90
a_w 0.54	99.80	98.43	44.56	168.89	79.03	96.09	4.57	123.90	64.28	93.71	1.42	103.27
a_w 0.76	99.50	98.00	43.67	169.05	80.27	96.23	4.79	138.90	69.95	93.98	1.49	102.09

$D_{4,3}$ values that differed by more than 0.5 are significantly different ($p < 0.05$)

NaCas sodium caseinate, *SE* sucrose esters, a_w water content

distribution showed the same behavior for all a_w, retention was strongly dependent on water content. It is likely that the water content increased molecular mobility and enhanced elutability. These findings might also indicate that SE induced unfolding of the protein. As a result, more core material was released at higher a_w.

Trehalose Crystallinity

Figure 6 shows DC versus time for NaCas, SE, and NaCas/SE-stabilized powder calculated from the DSC diagrams. For NaCas stabilizer (Fig. 6a), at all a_w, powders showed no noticeable T_g after 2 days (data not shown). DC was higher than for the dehydrated powder. The higher the water content, the greater the DC. Powders stored at a_w 0.11, 0.33, and 0.44 had similar behavior. There was a period of rapid crystallization up till 28 days, and then DC remained almost constant. At a_w 0.54 and 0.76 DC grew rapidly the first week and then more slowly up till 28 days when values reached a plateau. SE-stabilized powder displayed the same behavior as NaCas-stabilized powder (Fig. 6b). NaCas/SE-stabilized powder however, had DC closer to the plateau values after 2 days at all a_w (Fig. 6c). A higher DC was in agreement with a lower water content measured for NaCas/SE-stabilized powders compared to the other two stabilizers which means that in these powders trehalose crystallization was faster.

Figure 7 shows X-ray patterns of NaCas-stabilized powders stored for 1 week at (a) a_w 0.11, (b) a_w 0.76, and for 3 months at (c) a_w 0.11. The sharp peaks that appeared after a week corresponded to trehalose crystallization. Fat signals are so weak compared to trehalose ones that they are not noticeable on this scale (25 times greater than in Fig. 2). For NaCas-stabilized powder stored at a_w 0.11 for 1 week, DC was 16.4 (Fig. 6a) while for 3 months DC was 75.8. In agreement with calculated DC intensity of the line that appeared at 3.6 Å was higher in c than in a. However, in both cases, Fig. 7a and c, patterns corresponded to a crystalline material. As shown in these figures, trehalose matrix crystallization continued during storage. When the powder was stored at a_w 0.76 for 1 week DC was 58.7 and intensity of the line that appeared at 3.6 Å (Fig. 7b) was higher than when it was stored at a_w 0.11 (Fig. 7a). All patterns (a, b, c) differed from the one of the amorphous trehalose shown in Fig. 2.

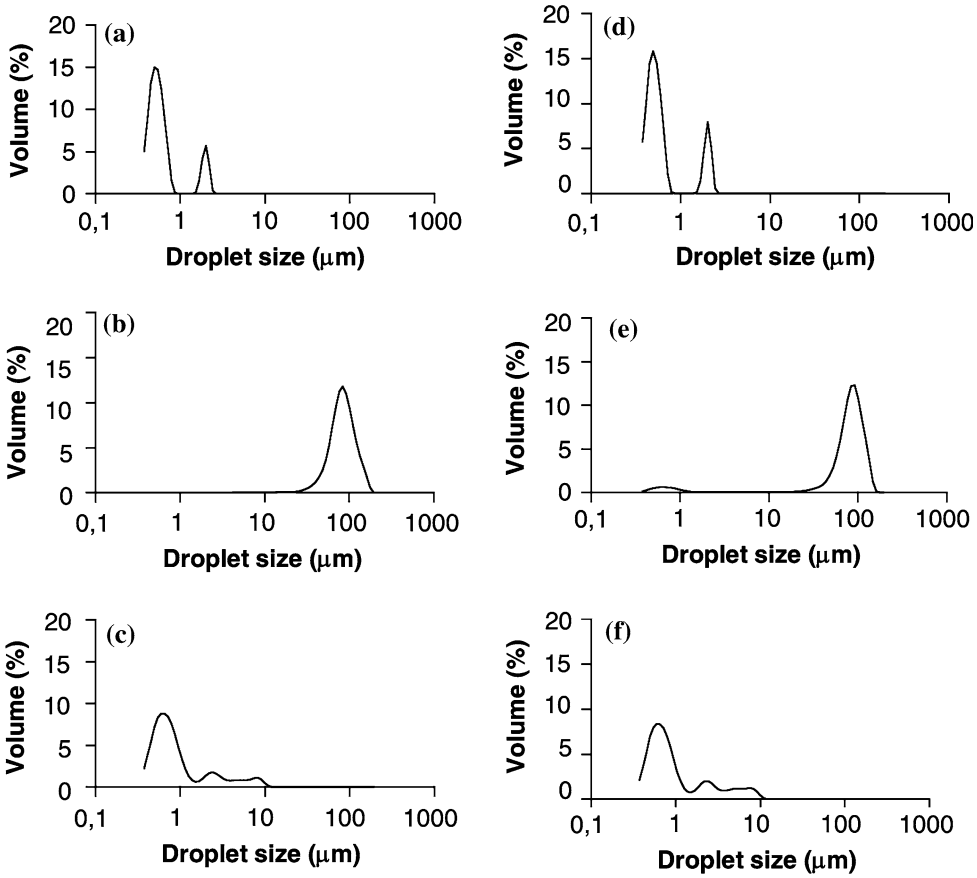

Fig. 5 Particle size distribution of reconstituted emulsions of NaCas-stabilized powder stored at a_w 0.11 for **a** 1 week, **b** 8 weeks, and **c** 12 weeks, and at a_w 0.76 for **d** 1 week, **e** 8 weeks, and **f** 12 weeks. Abbreviations as in Fig. 1

It was reported that both, caking or collapse and crystallization may lead to a release of the encapsulated substance from the matrix. Several authors claimed that the protective action of the solid matrix is lost when crystallization occurs [7]. Other authors observed a compromise between structural collapse (favorable for retention of encapsulated compounds) and matrix crystallinity (promoting the release of encapsulated compounds) [13, 16]. However, in the systems selected in this study, retention values did not correlate to DC showing the complex interactions that took place among matrix, stabilizer and lipid phase. Elizalde et al. [17] found that despite a matrix DC value of 83%, retention of β-carotene in trehalose stored for 156 days at a_w 0.44 was 75%. The kinetics of β-carotene loss did not correlate with matrix crystallinity and was strongly accelerated in samples with an excess of water above that necessary for crystallization. These results are in contrast to the ones reported by Senoussi et al. who showed that diacetyl retention decreased sharply in a lactose matrix, when about 60% of lactose crystallized [16]. Most of the systems reported in the literature showed a similar behavior to the diacetyl encapsulated-in-lactose system [7]. In those studies matrices were lactose or sucrose. Sussich et al. [18] reported that the anhydrous crystalline form of α,α-trehalose is almost isomorphous with the dihydrate crystal structure and possesses the unique feature of reversibly absorbing water to produce the dehydrate without changing the main structural features. The reversible process could play an important role in retention of encapsulated HMF/SFO blend with time since it is likely that during storage, structural changes of the trehalose matrix were small and therefore did not cause a large release of the core material. The effect of trehalose crystallization on the release of lipids is related to crystal size, shape and spatial distribution. Sugar crystals can exclude the solute and it is released, or, depending on crystallization conditions, the crystals can encapsulate the solutes [7]. This behavior differed from that reported for other sugars such as lactose or sucrose which maintained their protective action or encapsulation properties exclusively when vitrified [7, 13].

It has been reported that encapsulation properties are determined by different factors that have been postulated as isolated reasons to explain retention values: particle size and distribution, water content and T_g, crystallization and collapse. However, none of them was the sole factor responsible for HMF/SFO blend loss. The counteracting effects of all these factors and the interactions among

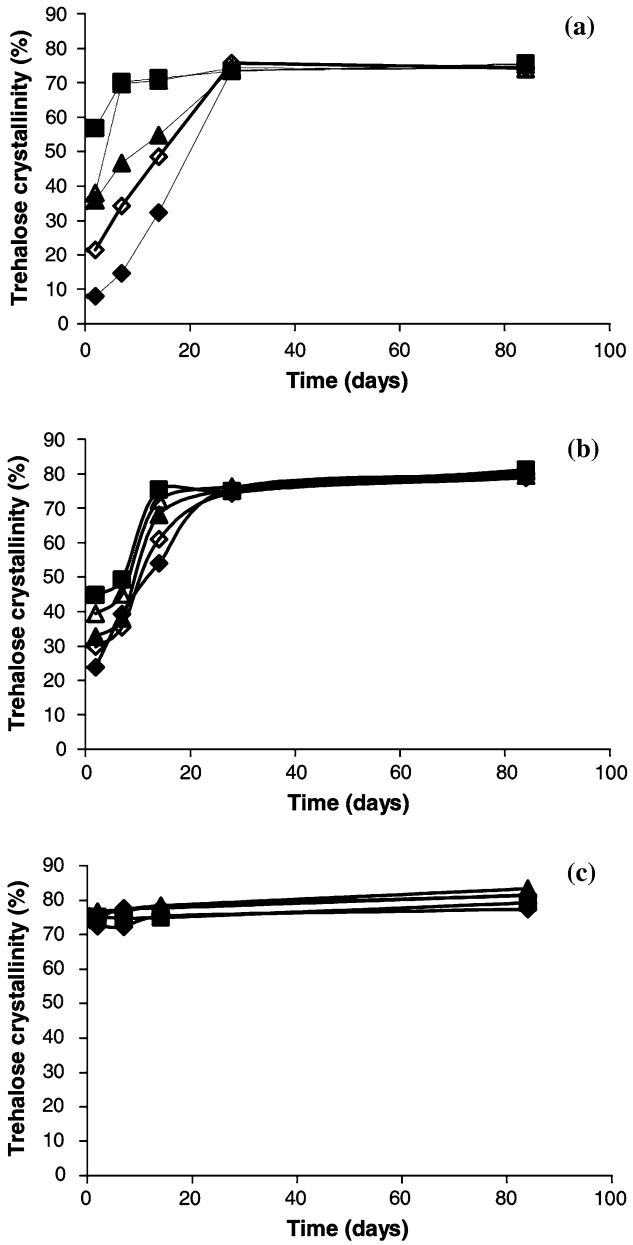

Fig. 6 Degree of crystallization (DC) versus time of **a** NaCas, **b** SE, and **c** NaCas/SE-stabilized powder stored at a_w 0.11 (*filled triangles*), 0.33 (*open diamonds*), 0.44 (*filled triangles*), 0.54 (*open triangles*), and 0.76 (*filled squares*). Data points are the average of two runs. Standard deviations were within symbol sizes. *NaCas* sodium caseinate

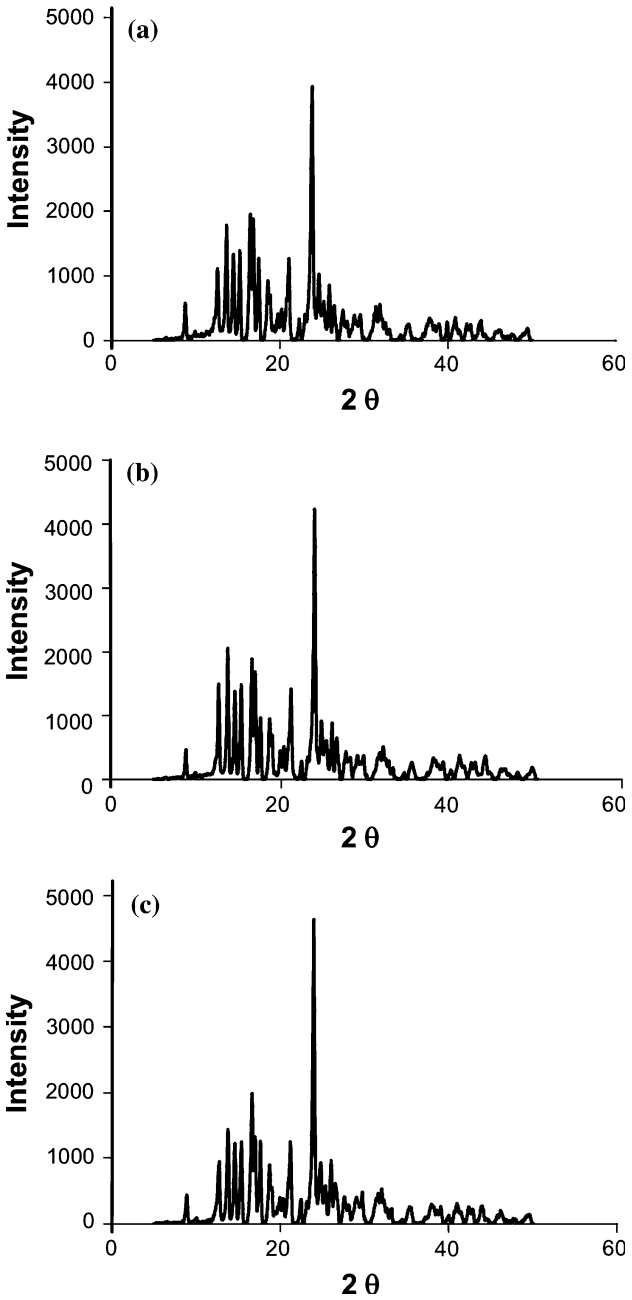

Fig. 7 X-ray diffraction patterns of NaCas-stabilized powders **a** stored at a_w 0.11 for 1 week, **b** stored at a_w 0.76 for 1 week, **c** stored at a_w 0.11 for 3 months

stabilizer, matrix and fat phase determined retention. In addition, retention was closely related to structural changes with time of the encapsulating matrix. NaCas was the most effective of the three stabilizers selected in this study. The powder had small droplet size as a consequence of the strong interactions among protein and aqueous and fat phases which remained unchanged up to 2 months of storage for all a_w. In addition, sugar crystallization did not destabilize the protein layer since structural changes of trehalose from amorphous to crystalline did not modify its main structural features.

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