Resveratrol entrapped niosomes as yogurt additive

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Abstract

Nanodesign of niosomes containing resveratrol (RSV) was carried out using food-grade surfactants with dodecanol to stabilize the membrane. Niosomes were prepared using a modified thin film hydration method.

A factorial design analysis was carried out to reduce the number of experiments. The response factors were: mean size, polydispersity index (PDI) and entrapment efficiency (EE). Agitation speed and surfactant to dodecanol weight ratio were selected as key parameters for niosomes preparation. Parameter contribution was determined using a statistical analysis of variance (ANOVA).

Niosomes formulated with Span 60 or Maisine 35-1 as surfactants and dodecanol as stabilizer were able to incorporate RSV. These niosomes exhibited a small mean size, narrow size distribution, high RSV entrapment efficiency and good stability. RSV addition did not involve changes in the textural properties of regular yogurt demonstrating that RSV entrapped niosomes are suitable additives in these dairy products.

Keywords: niosome, resveratrol, functional food, yogurt, factorial design, ANOVA

1. Introduction

Natural products have been widely used to prevent or mitigate various diseases and there has been lately a growing interest in research, development and commercialization of functional foods, nutraceuticals and dietary supplements (Shahidi, 2009; Ortuño *et al.*, 2010; Dewapriya & Kim, 2014). The term "*nutraceutical*" is defined as any food-based substance that provides health benefits, including prevention and treatment of diseases (Defelice, 1995). This term is commonly used in marketing although there is no regulatory definition, and in certain countries the terms functional food and nutraceutical are used interchangeably. In all cases, the main focus is to improve health and reduce the risk of diseases mainly through prevention (Shahidi, 2009).

Moreover, pharmaceutical and biotechnology companies have made significant investments in the discovery and production of nutraceuticals / functional foods (Nelson, 1999; Kalra, 2003).

Resveratrol (RSV) is a natural polyphenol that may be considered as a *nutraceutica*l because of benefits that include anticancer activity (Jang & Surh, 1997; Surh, Hurth, Kang, Lee, Kong & Lee, 1999), lifespan extension (Howitz *et al.*, 2003), cardioprotection (Hung, Chen, Huang, Lee & Su, 2000), antioxidant activity (Frankel*,* Waterhouse & Kinsella, 1993; Fremont, Belguendouz & Delpal, 1999), inhibition of platelet aggregation (Chung, Teng, Cheng, Ko & Lin, 1992; Bertelli *et al.*, 1995) and antiinflammatory activity (Pace-Asciak, Hahn, Diamandis, Soleas & Goldberg*,* 1995). Consequently, RSV may prove to be a useful ingredient for functional foods.

RSV is a photosensitive molecule that exists in *cis* and *trans* structural isomers, but only *trans*-RSV demonstrates health benefits. The change from the active *trans* isomer to the inactive *cis* isomer is mainly caused by exposure to light. Thus, RSV should be encapsulated. Encapsulation also facilitates control of the rate of RSV release and protects the molecule during digestion because of its degradation under pancreatic conditions (Tagliazucchi, Verzelloni, Bertolini & Conte, 2010), and could also help to mask undesired flavours.

RSV encapsulation studies have been done in pharmaceutical and cosmetic industries with the aims of preventing degradation, increasing its solubility in water, and targeting to specific locations in the body via use of multiparticulate forms and colloidal carriers

(Lucas-Abellán, Fortea, López-Nicolás & Núñez-Delicado, 2007; Caddeo, Teskač, Sinico & Kristl, 2008; Kristl, Teskač, Caddeo, Abramovic & Sentjurc, 2009; Peng *et al.*, 2010; Teskač & Kristl, 2010; Donsi, Sessa, Mediouni, Mgaidi & Ferrari, 2011; Wang *et al.*, 2011; Amri, Chaumeil, Sfar & Charrueau, 2012; Pando, Caddeo, Manconi, Fadda & Pazos, 2013a; Pando, Gutiérrez, Coca & Pazos, 2013b; Matos, Gutiérrez, Coca & Pazos, 2014; Sessa *et al.*, 2014).

Niosomes are vesicles formed by the self-assembly of non-ionic surfactants in aqueous media resulting in closed bilayer structures (Uchegbu & Vyas, 1998). These vesicles are commonly used to encapsulate both hydrophilic and lipophilic compounds, for either food, pharmaceutical or cosmetic applications. Hydrophilic compounds are entrapped in the aqueous compartments between the bilayers while the lipophilic components are preferentially located within the surfactant bilayer (Devaraj, Parakh, Devraj, Apte, Ramesh Rao & Rambhau, 2002).

Surfactants are versatile products of the chemical industry and the large number of available non-ionic surfactants enables the design of niosomes for specific applications (Manosroi *et al.*, 2003). The main advantage of niosomes, with respect to other encapsulation technologies, such as liposomes (Fang & Bhandari, 2010; Gibis, Zeeb & Weiss, 2014), is their low cost, high stability, and biocompatibility (Kopermsub, Mayen & Warin, 2011).

Size distribution, stability and entrapment efficiency are the key parameters to obtain optimal niosomal system. Two interacting variables involved in niosomes preparation (*i.e.* agitation speed and surfactant to stabilizer weight ratio) have to be tested to properly analyse the behaviour of the system and to optimize system parameters. Changing one factor at a time is not an efficient and economic strategy because it does not provide information regarding the optimum location and it does not take into account interactions of parameters. Factorial design and analysis of variance (ANOVA) methodology are appropriate and efficient statistical tools, which permit to study the effects of several factors that influence responses by varying them simultaneously within a limited number of experiments (Martínez-Sancho, Herrero-Vanrell & Negro, 2004; Kincl, Turk & Vrečer, 2005)

The aim of this work is to formulate RSV entrapped niosomes for oral administration, using a modified thin film hydration method. Dodecanol was selected as a membrane stabilizer, because it has previously been reported that stable niosomes could be

prepared with fatty alcohols (Devaraj *et al.*, 2002), instead of cholesterol, which avoids the gel-liquid phase transition of niosomes. By contrast cholesterol may not be suitable for use in functional foods because of potential adverse health effects.

The key parameters involved in niosomes preparation (*i.e*. surfactant to dodecanol weight ratio and agitation speed) were optimised by a factorial design of experiments and statistical analysis of variance (ANOVA) to assess their contributions to mean size, polydispersity index (PDI), and entrapment efficiency (EE). Finally, the best formulation for each surfactant was selected for subsequent preparation of yogurts enriched with RSV. The textural properties of these yogurts were also analysed.

2. Material and methods

2.1. Materials

Trans-resveratrol (RSV), with a purity >99%, sorbitan monostearate (Span 60, S60), lauryl alcohol (dodecanol, Dod) and absolute ethanol were supplied by Sigma-Aldrich (USA). Labrasol (Lab) and Maisine 35-1 (Mai) were a gift from Gattefossé (France). Methanol, acetonitrile, 2-propanol and acetic acid of HPLC-grade were obtained from Sigma-Aldrich (USA). Deionized water was used in all experiments.

2.2. Preparation of niosomes

Niosomes were prepared by the thin film hydration method (Bangham, Standish & Watknis, 1965; Baillie, Florence, Hume, Muirhead & Rogerson, 1985) with minor modifications, followed by agitation-sonication (Pando *et al*., 2013b). Accurately weighed amounts of surfactant (S60, Lab or Mai) and dodecanol in different weight ratios, from 1:0.5 to 1:1.5, were dissolved in 20 mL of a solution of ethanol containing a known concentration of RSV and placed into a 100 mL round bottom flask. Then, ethanol was removed at 40 ºC under reduced pressure in a rotary evaporator (Buchi, Switzerland). The dried film was hydrated with 40 mL of deionized water at 60 ºC to achieve a RSV concentration of 150 mg/L. The resulting sample was subsequently homogenized (SilentCrusher M, rotor model 22G, Heidolph, Germany), at speeds, ranging from 5,000 to 15,000 rpm depending on the experiment, and further sonicated for 30 min (CY-500 sonicator, Optic Ivymen System, Spain), using 45% amplitude, 500 W power and 20 kHz frequency.

2.3. Characterization of RSV entrapped niosomes

125 2.3.1. Vesicle size and zeta potential measurements

126 Mean (Z-Average) sizes and PDI of niosomes were determined via Dynamic Light
127 Scattering (DSL) using a Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire,
128 UK). Three independent samples were taken from each formulation, and each was
129 measured three times at room temperature without dilution.

130 For determination of zeta potentials (ζ-potential), three independent samples were also
131 taken from each formulation and measure three times at room temperature. Each of
132 them were diluted (1:10 v/v) before measuring vesicle electrophoretic mobility using
133 M3-PALS (Phase Analysis Light Scattering) technique. High absolute values of ζ-
134 potential indicate electrostatic repulsion between vesicles. Such conditions are linked to
135 high stability.

136 2.3.2. Stability measurements

137 The stability of niosomes was determined by measuring backscattering (BS) profiles in
138 a Turbiscan Lab® Expert apparatus (Formulaction, France) provided with an Ageing
139 Station (Formulaction, France). Undiluted niosomes samples were placed in cylindrical
140 glass test cells and the backscattered light was monitored as a function of time and cell
141 height for 15 days, every 3 hours, at 30 °C. The optical reading head scans the sample
142 in the cell, providing BS data every 40 μm in % relative to standards (suspension of
143 monodisperse spheres and silicone oil) as a function of the sample height (in mm).
144 These profiles provide a macroscopic fingerprint of the niosomes at a given time,
145 providing useful information about changes in vesicle size distribution and/or
146 appearance of a creaming layer or a clarification front with time (Pando *et al*., 2013a;
147 Pando *et al*., 2013b).

148 *2.4. RSV entrapment efficiency (EE)*

149 Entrapped RSV was separated from free RSV by dialysis. Samples (2 mL) were placed
150 into a dialysis bag, which was immersed in 1000 mL of deionized water at room
151 temperature and stirred at 500 rpm for 2 hours. Dialyzed and non-dialyzed samples
152 were diluted 1:10 (v/v) with methanol to rupture the vesicle membranes enabling
153 extraction of RSV. Later, RSV was determined by chromatography (HP series 1100
154 chromatograph, Hewlett Packard, USA). The system was equipped with a UV/VIS
155 absorbance detector HP G1315A and a fluorescence detector 1260 Infinity A (Agilent
156 Technologies, USA). A wavelength of 305 nm was used for the UV/VIS detector while

157 fluorescence detector used 310/410 nm of $\lambda_{excitation}/\lambda_{emission}$. The analytical column was a
158 Zorbax Eclipse Plus C_{18} of 5 µm particle size, 4.6 mm × 150 mm (Agilent Technologies,
159 USA).

160 The mobile phase consisted of a mixture of (A) 100% milliQ-water and (B) 100%
161 methanol with gradient elution at a flow rate of 0.8 mL/min. The step gradient started
162 with a mobile phase of 80% (A) running 100% mobile phase (B) in min 5 for 10 min.
163 The mobile phase (B) was fed for 2 min after each injection to prepare the column for
164 the next sample. The separation was carried out at 30°C.

165 *2.5. Preparation of yogurt enriched with RSV*

166 To prepare yogurt enriched with RSV, 10.6 mL of the niosomal suspension containing
167 RSV was placed into a beaker and diluted to 200 mL with a mixture of fresh
168 pasteurized milk and natural yogurt (8:1 v/v).

169 The sample was incubated for 12 hours at 40 ºC in a yogurt maker, model OU-YG01
170 (Moulinex, France). Once the yogurt was formed, it was cooled and stored at 4 ºC in a
171 refrigerator for at least 2 hours.

172 *2.6. Textural analysis of yogurt enriched with RSV*

173 A textural analysis was carried out both for regular yogurt and yogurt containing RSV to
174 study possible changes resulting from the addition of RSV entrapped niosomes.

175 The firmness and adhesiveness of both types of yogurt were measured with a
176 TA.XTplus texture analyser (Stable Micro Systems, USA) using a single compression
177 cycle test. A SMSP/0.5S probe was employed using as operating parameters a
178 penetration speed of 2 mm/s and a penetration depth of 5 mm. The test was carried out
179 immediately after removing the sample from refrigeration at 4°C.

180 Firmness was defined as the force necessary to reach the maximum depth and
181 adhesiveness was calculated as the negative force area of the cycle, representing the
182 work necessary to pull the compressing plunger away from the sample (Bourne, 2002).

183 *2.7. Statistical analysis*

184 All data were expressed as the mean ± SD (standard deviation) of three independent
185 experiments and statistical analysis of variance (ANOVA) was applied. Fisher's test
186 ($p<0.05$) was used to calculate the least significance difference (LSD) using statistical
187 software (Microsoft Excel 2010).

3. Results and discussion

3.1. Experimental design

Niosomes were prepared at three different agitation speeds (5,000, 10,000 and 15,000 rpm) and surfactant:dodecanol weight ratio values (1:0.5, 1:1, 1:1.5). The objective was to study, using ANOVA statistical analysis, how these key factors affected on the response parameters: mean size, PDI and EE. Factors and levels were combined according to the orthogonal array L_9 indicated in Table 1.

Table 1

Dispersions were characterized to determine the best formulation and method of preparation for each surfactant. Then, these best formulations were selected for the subsequent preparation of yogurt enriched with RSV.

3.1.1. Mean size

Table 2 shows mean sizes, PDI and EE values of the niosomes obtained for the three surfactants operating at different agitation speeds and surfactant:dodecanol weight ratios.

Table 2

The results in Table 2 exhibit a clear relationship between the type of surfactant used and the resulting mean size of the niosome ($p < 0.05$), for all experimental conditions tested. The smallest mean size corresponded to S60 niosomes, while Lab niosomes showed the highest values for this response factor. The niosomes formulated with S60 had mean sizes between 139-227 nm with an average value of 183 nm, while Mai-niosomes mean size ranged from 164 to 368 nm, being its average value 242 nm. These two surfactants are similar molecular structures, with the same number of C (17) for the alkyl chain, but differing in the hydrophilic portion with sorbitan monosterate for S60, and glycerol monosterate for Mai. Consequently, their hydrophilic – lipophilic balance (HLB) is quite similar, being 4.7 and 4.0 for S60 and Mai, respectively. Thus, it was expected that niosomes formulated with these surfactants should be similar in size, as was observed experimentally.

Moreover, when Mai was used as surfactant, a close correlation between the agitation speed and niosome mean size ($p < 0.05$) was observed; smaller niosome mean sizes

were produced at higher agitation speeds as it was previously reported (Pando *et al*., 2013b). No such influence was observed when S60 or Lab was employed.

Changes in niosome mean size resulting from different surfactant:dodecanol weight ratios were also observed when Lab was used as surfactant ($p < 0.05$). This result may be attributed to its largest HLB value (14) which results in a greater affinity for the aqueous phase, thereby preventing vesicles formation in the absence of a stabilizer. This situation leads to a close link between surfactant:dodecanol weight ratio and mean size.

For niosomes formulated with Lab, the smallest mean size was obtained at a surfactant:dodecanol weight ratio of 1:0.5. However, dodecanol did not stabilize niosomal structure well. The Lab–dodecanol system began to coagulate, leading to larger vesicle sizes and phase separation.

3.1.2. Polydispersity index (PDI)

PDI values of niosome size distributions for the three surfactants are also shown in Table 2.

The PDI results exhibit a clear ($p < 0.05$) dependence on the type of surfactant. Niosomes with the smallest PDI were obtained with S60 surfactant, followed by those using Mai and Lab. Niosomes prepared with S60 led to PDIs in the range 0.204-0.352, being 0.279 the average PDI value. PDI of niosomes formulated using Mai ranged from 0.211 to 0.488, with an average value of 0.314. Narrower distributions were obtained with these two surfactants, since niosomes obtained from formulations containing Lab led to PDI values in the range 0.129-0.582 with an average value of 0.378, which was too close to the limit value of 0.4. Once this value is exceeded, the corresponding PDI values are no longer considered narrow.

All the surfactants resulted in a significant PDI dependence of the agitation speed ($p < 0.05$). For formulations containing S60 and Mai, lower PDI values were obtained at higher agitation speeds, while for niosomes prepared using Lab, lower agitation speeds were associated with lower PDI values.

As it was stated earlier, only the Lab surfactant showed a close relationship between surfactant:dodecanol weight ratio, the mean size and the PDI of the niosomal system. For the Lab-Dod niosomes, the lowest PDI value was obtained for a surfactant:dodecanol weight ratio of 1:0.5, according to the lowest niosome mean size.

3.1.3. RSV entrapment efficiency (EE)

The EE was strongly dependent on the surfactant and surfactant:dodecanol weight ratio ($p < 0.05$), while agitation speed had no effect ($p > 0.05$).

For niosomes prepared with S60, EE values ranged between 16.8-72.5%, with an average value of 42.0%, while for niosomes prepared with Lab, the range of EE was 16.3-63.9%, with an average value of 40.6%. The lowest EE values were obtained with Mai (25.1-58.6%).

With respect to RSV entrapment efficiency, each surfactant exhibited different behaviour at the various surfactant:dodecanol weight ratios tested. Niosomes prepared with S60 and Mai led to higher EE values at higher surfactant:dodecanol weight ratio (1:1.5), while for niosomes formulated with Lab, higher EE values were obtained at the lowest surfactant:dodecanol weight ratio (1:0.5). These findings may be explained in terms of the similar HLB value of two of the surfactants (HLB = 4.7 and 4.0 for S60 and Mai respectively). Lab is much more hydrophilic (HLB =14).

3.1.4. Optimal operating conditions

For S60-Dod niosomes, the best results were obtained when agitated at 15,000 rpm, corresponding to the lowest PDI value, for 1:1.5 surfactant:dodecanol weight ratio.

However, for the Lab-Dod niosomes the lowest PDI value was obtained at the lowest agitation speed (5,000 rpm) and lowest surfactant:dodecanol weight ratio (1:0.5). These conditions lead to smaller mean size and a higher EE value.

Finally, for Mai-Dod niosomes, lower PDI values were observed at higher agitation speeds (15,000 rpm). Higher surfactant:dodecanol weight ratios led to smaller mean sizes.

A summary of the optimal key parameters is displayed in Table 3.

Table 3

3.2. Niosomes stability

The stability of the niosomes was determined from the variation of the BS (ΔBS) with time. Changes in niosome size are directly related to the ΔBS values measured in the middle zone of the Turbiscan cell (ΔBS_M). An increase in niosome size indicates that

coagulation and/or flocculation have taken place, leading to system destabilization. In addition, BS variations at the bottom (BS_B) and top (BS_T) of the measuring cell are linked to sedimentation and creaming phenomena respectively. Turbiscan equipment has been widely used to select the best formulation for colloidal systems employed in food applications (Márquez, Palazolo & Wagner, 2007; Pando *et al.,* 2013a; Pando *et al.,* 2013b; Matos *et al.*, 2014).

The ζ-potential is strongly linked to stability of the niosomes. High absolute values of ζ-potential imply repulsive force between niosomes with a concomitant increase in stability. On the other hand, close to zero or even low absolute values lead to unstable systems with resultant coagulation or flocculation processes.

The stability of samples from the best formulations was monitored every 3 hours for 15 days.

S60-Dod niosomes prepared at 15,000 rpm with a surfactant:dodecanol weight ratio of 1:1.5 were highly stable with no changes in niosome size, although a slight variation in BS values was observed. Thus, the ΔBS_M values were quite low and destabilization processes, such as coagulation or flocculation, did not occur. However, a creaming phenomenon was observed by an increase in BS at the top of the cell (ΔBS_T), due to particle accumulation in this zone and clarification at the bottom of the cell. These phenomena were accompanied by a corresponding decrease in BS (ΔBS_B) (Figure 1A). Creaming can be attributed to the presence of free dodecanol molecules, which did not form niosomal bilayer but migrated towards the top of the cell because of their lower density (850 kg/m^3).

Moreover, this niosomal system was characterized by a ζ-potential value of -50.1 ± 0.8 mV. This result agreed well with the high stability observed via Turbiscan measurements, confirming the absence of coagulation/flocculation processes resulting from electrostatic repulsion effects among niosomes.

Figure 1

Lab-Dod niosomes prepared at 5,000 rpm with a surfactant:dodecanol weight ratio of 1:0.5 showed lower stability along time. A sharp variation in BS values was observed in the middle zone of the cell (Figure 1B), which indicated a significant change in niosome

size. A creaming layer also appeared at the top of the cell leading to an increase in ΔBS_T and a simultaneous increase of ΔBS_B caused by sedimentation phenomena.

This niosomal system had a ζ-potential of -18.7 ± 0.6 mV. Thus, electrostatic repulsion would be weaker, making the increase in niosome size more likely.

Niosomes prepared at 15,000 rpm using Mai-Dod with a surfactant:dodecanol weight ratio of 1:1.5 exhibited acceptable stability. An increase in mean sizes of the niosomes, resulting from the BS decrease in the middle zone of the cell (ΔBS_M) was observed. In addition, a creaming phenomenon appeared due to free dodecanol molecules with the corresponding decrease in ΔBS_B and ΔBS_T increase (Figure 1C).This system had a ζ-potential of -39.9 ± 0.3 m corresponding to the acceptable stability of these niosomes.

The best formulation of the RSV entrapped niosomes was obtained using S60-Dod.This niosomal system demonstrated much better stability than niosomes formulated with Lab-Dod.

3.3. Textural analysis of yogurt enriched with niosomes containing resveratrol

The textural analysis of regular yogurt and yogurt enriched with RSV, by adding niosomes prepared with S60, Lab and Mai, was carried out in order to ascertain if any textural difference caused by addition of niosomes was present.

Figure 2 shows the textural analysis for each sample obtained by measuring the force applied on yogurt surface as a function of time.

Figure 2

Values of the textural parameters studied (firmness and adhesiveness) are summarized in Table 4.

Table 4

Only the yogurt containing RSV Lab-Dod niosomes showed significant differences in firmness with respect to control regular yogurt.

The yogurt containing RSV Mai-Dod niosomes exhibited adhesiveness closer to that of the control, followed by yogurt enriched with RSV S60-Dod niosomes. By contrast, addition of RSV Lab-Dod niosomes to the control yogurt led to significant decrease in adhesiveness.

Thus, yogurts with RSV entrapped niosomes formulated with S60 or Mai were characterized by textural properties similar to those of the control, but the yogurt enriched with Lab niosomes containing RSV led to textural properties slightly different, which could be readily apparent in the final product.

4. Conclusions

Combination of factorial experimental design with statistical analysis of variance (ANOVA) is a good methodology to employ in ascertaining the best formulation for niosomal systems entrapping RSV within a moderate number of experiments.

Dodecanol could replace cholesterol as stabilizer in formulations of food-grade niosomes.

Niosomes formulated with Span 60 or Maisine 35-1 as surfactants and dodecanol as stabilizer are successful preparations for incorporation of RSV. These niosomes exhibit a small mean size, narrow size distribution, high RSV entrapment efficiency, and good stability.

Addition of RSV did not involve changes in the textural properties of regular yogurt demonstrating that RSV entrapped niosomes are suitable additives in these dairy products.

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Figure captions

Figure 1. Backscattering profiles of RSV entrapped niosomes made of: A) S60 and dodecanol (1:1.5 w/w) at 15,000 rpm; B) Lab and dodecanol (1:0.5 w/w) at 5,000 rpm; C) Mai and dodecanol (1:1.5 w/w) at 15,000 rpm

Figure 2. Textural properties of yogurt: A) Regular yogurt, no additives; B) Yogurt with RSV entrapped S60-Dod niosomes; C) Yogurt with RSV entrapped Lab-Dod niosomes; D) Yogurt with RSV entrapped Mai-Dod niosomes

Table 1

Table 1. Experimental design schedule for the L_9 array

Trial	**Agitation speed (rpm)**	**Surfactant:dodecanol ratio (w/w)**
1	5,000	1:0.5
2	5,000	1:1
3	5,000	1:1.5
4	10,000	1:0.5
5	10,000	1:1
6	10,000	1:1.5
7	15,000	1:0.5
8	15,000	1:1
9	15,000	1:1.5

Table 2

Table 2. Experimental design results for niosome mean size, PDI and EE as response parameters

Trial	Size (nm)			PDI			EE (%)		
	S60 Dod	*Lab Dod*	*Mai Dod*	*S60 Dod*	*Lab Dod*	*Mai Dod*	*S60 Dod*	*Lab Dod*	*Mai Dod*
	205	333	237	0.342	0.251	0.386	29.0	59.1	37.0
1	220	351	216	0.326	0.276	0.282	29.3	51.8	25.4
	222	401	221	0.333	0.240	0.302	28.3	55.5	27.9
	139	294	272	0.269	0.229	0.305	33.7	46.7	37.4
2	162	233	296	0.241	0.129	0.488	52.0	52.7	36.9
	163	329	368	0.236	0.217	0.426	41.1	52.6	41.5
	196	368	331	0.352	0.332	0.357	52.0	16.3	44.9
3	175	543	269	0.289	0.556	0.321	47.4	24.5	27.0
	183	412	302	0.331	0.452	0.392	43.2	19.7	31.1
	163	427	223	0.204	0.403	0.367	22.9	61.7	39.6
4	161	344	277	0.215	0.349	0.410	21.5	63.9	25.1
	171	429	254	0.233	0.365	0.395	23.0	60.2	30.1
	185	314	213	0.271	0.281	0.253	40.5	30.3	48.5
5	188	389	224	0.272	0.412	0.232	40.0	48.6	35.1
	196	285	230	0.259	0.344	0.271	36.7	33.6	39.6
	178	378	311	0.261	0.501	0.429	56.8	27.7	55.1
6	183	350	252	0.270	0.448	0.302	58.5	25.5	49.7
	191	454	295	0.251	0.403	0.365	61.1	32.4	56.0
	162	418	211	0.255	0.403	0.232	22.5	50.0	39.2
7	195	379	222	0.261	0.379	0.292	16.8	34.3	29.9
	218	302	216	0.219	0.421	0.257	26.1	41.9	32.6
	227	373	203	0.235	0.364	0.265	59.1	41.4	37.3
8	190	544	164	0.271	0.490	0.249	53.2	33.6	47.3
	178	465	205	0.346	0.431	0.218	49.3	42.8	42.1
	165	688	170	0.267	0.481	0.241	58.5	31.8	56.4
9	167	408	166	0.248	0.582	0.241	72.5	22.8	58.6
	173	586	191	0.225	0.472	0.211	60.3	35.9	43.4

Table 3

Table 3. Optimal key parameters for preparation of RSV entrapped niosomes

Niosomal system	**Agitation speed (rpm)**	**Surf:Dod ratio (w/w)**	**Size (nm)**	**PDI**	**EE (%)**
S60-Dod	15,000	1:1.5	168 ± 4	0.247 ± 0.018	63.8 ± 7.6
Lab-Dod	5,000	1:0.5	362 ± 35	0.255 ± 0.021	55.4 ± 3.7
Mai-Dod	15,000	1:1.5	175 ± 13	0.260 ± 0.025	52.8 ± 8.2

Table 4 revised

Table 4. Firmness and adhesiveness values of different yogurts

System	**Firmness (g_F)**	**Adhesiveness (g_F)**
Regular yogurt (control)	7.535 ± 0.321	-4.035 ± 0.518
RSV S60-Dod yogurt	7.642 ± 0.106	-3.538 ± 0.173
RSV Lab-Dod yogurt	5.005 ± 0.105	-2.615 ± 0.085
RSV Mai-Dod yogurt	7.186 ± 0.124	-4.166 ± 0.055

Figure 1 color

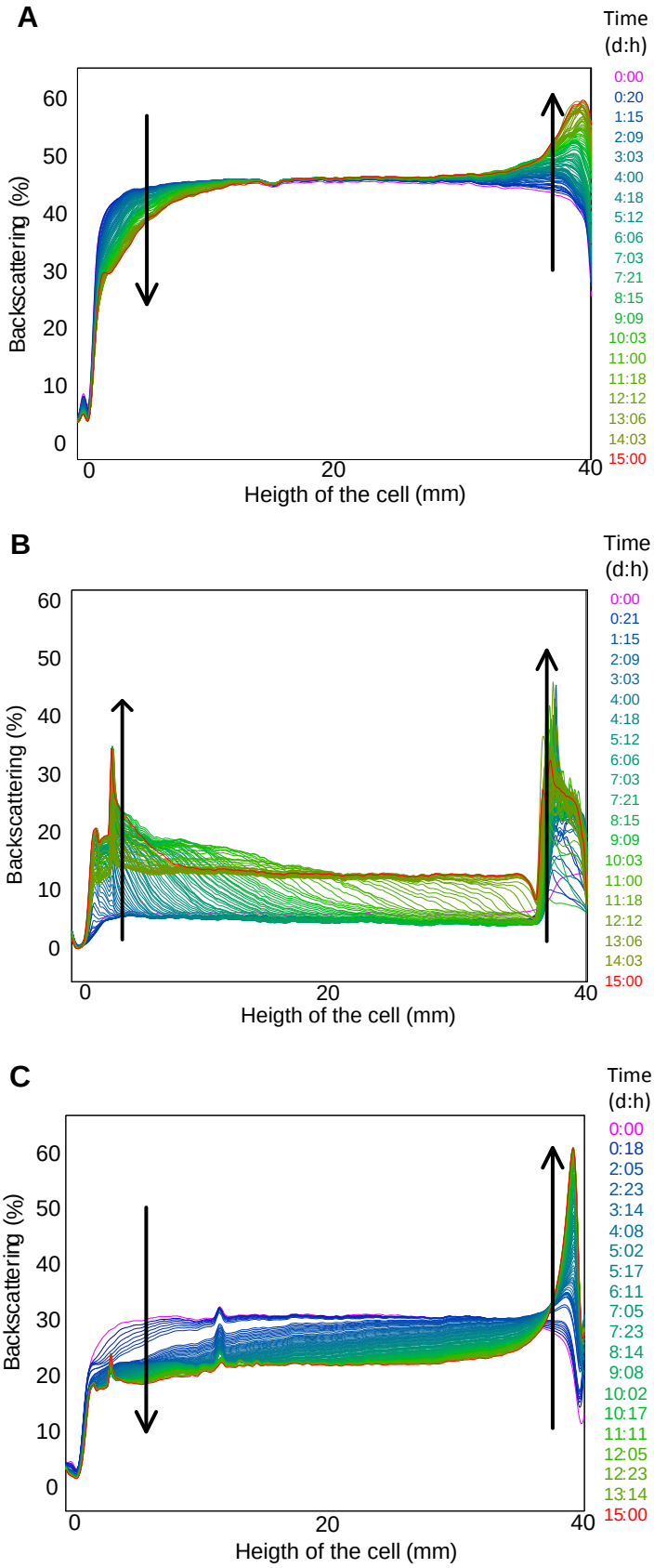

Figure 1

Figure 1 Black and White

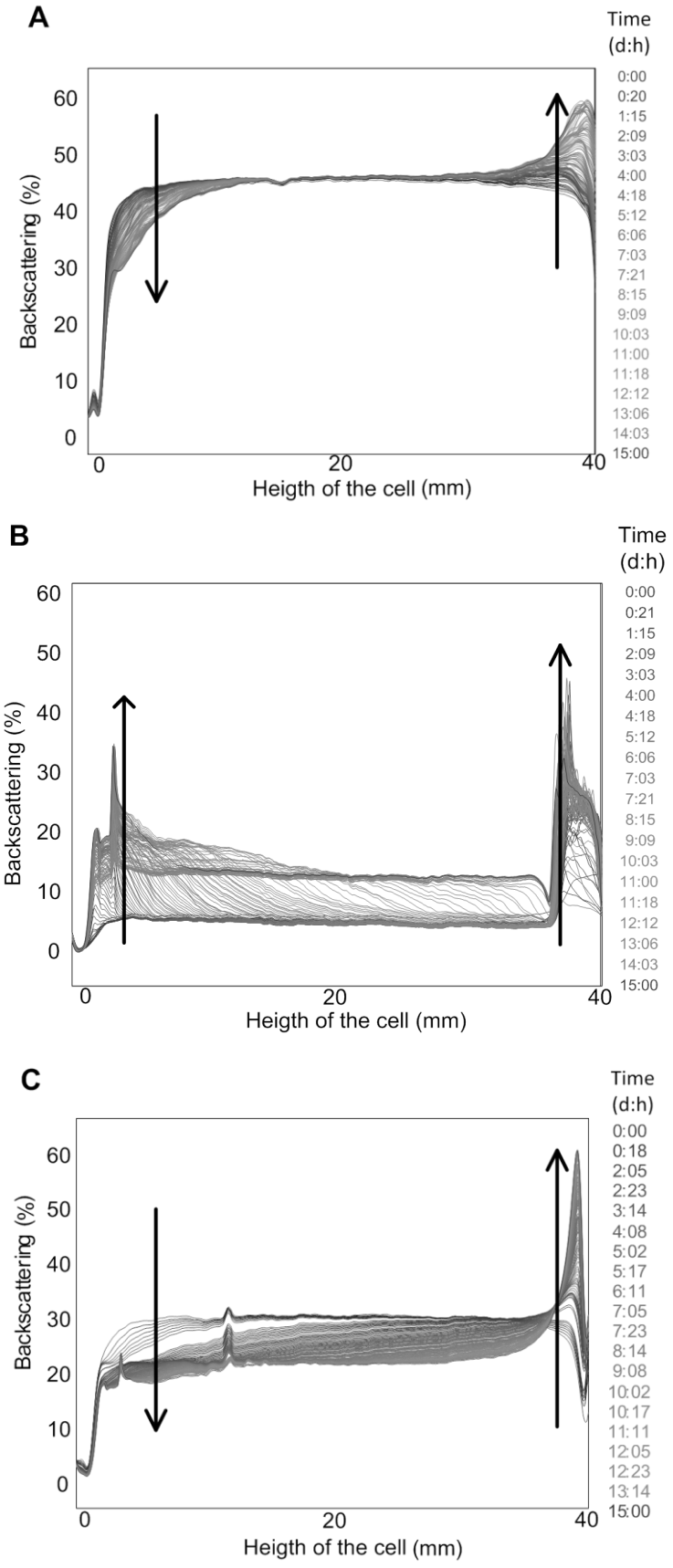

Figure 1

Figure 2 color

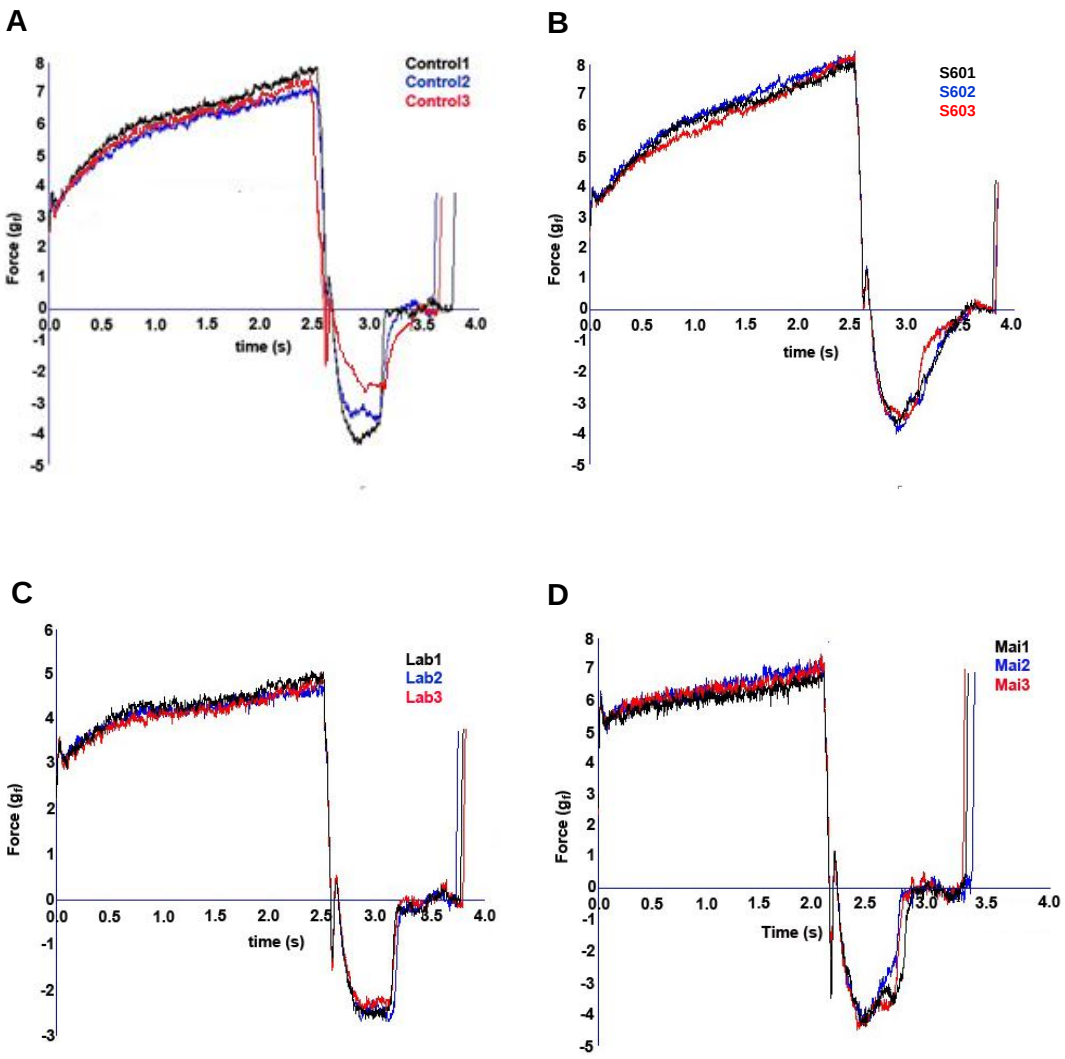

Figure 2

Figure 2 Black and White

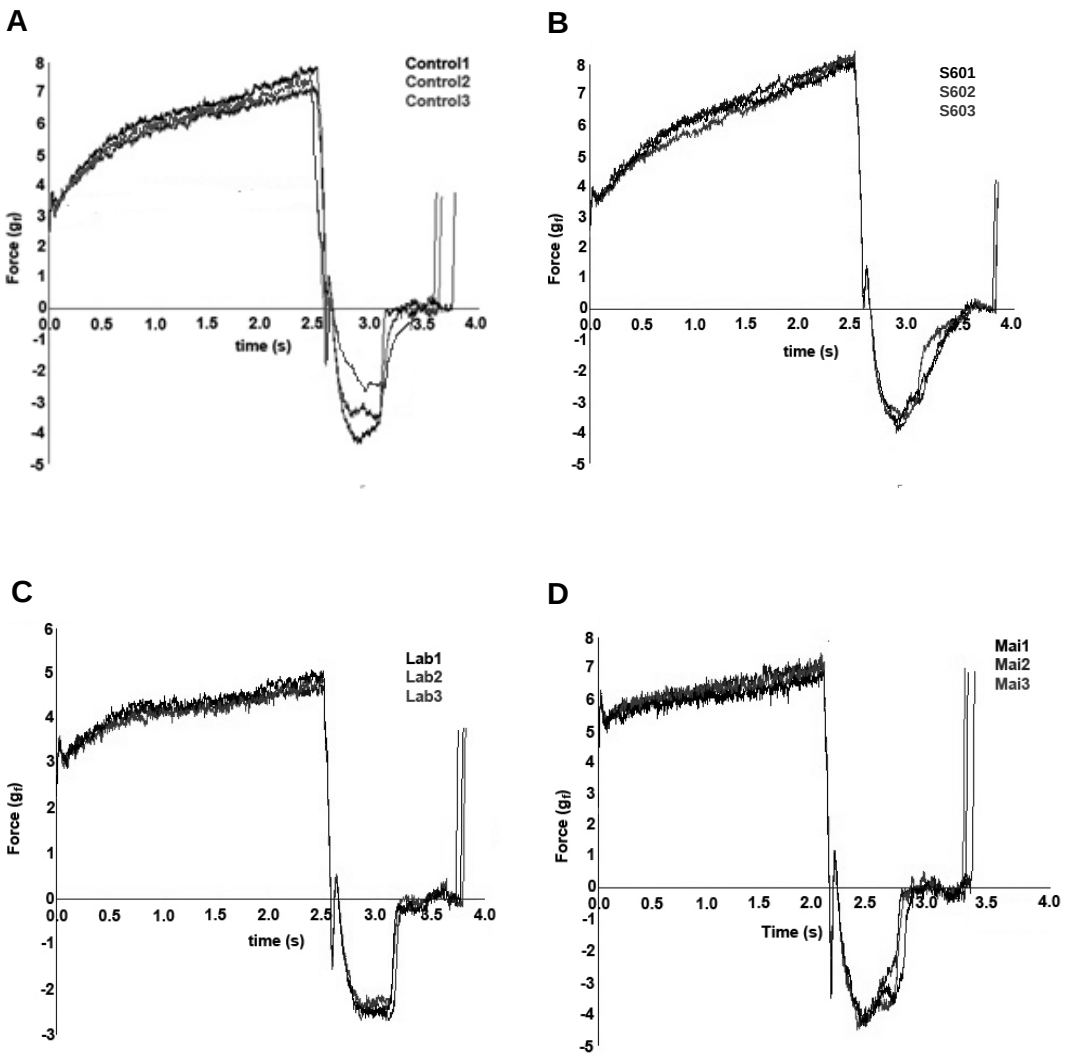

Figure 2