LWT - Food Science and Technology 65 (2016) 689–694

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Comparison between the spray drying and spray chilling microparticles contain ascorbic acid in a baked product application

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ARTICLE INFO

Article history:
Received 23 February 2015
Received in revised form
5 July 2015
Accepted 17 August 2015
Available online 19 August 2015

Keywords:
Microencapsulation
Spray drying
Spray chilling
Ascorbic acid
Biscuits

ABSTRACT

One of the functions of microencapsulation is the protection of sensitive substances. Many encapsulation methods can be employed for the same substance, and the degree of protection may vary according to the particle characteristics (i.e. dry or wet matrix, hydrophilic or hydrophobic surface, etc.). This study aimed to compare the characteristics of microparticles containing ascorbic acid (AA) by spray drying (SD; wall material: arabic gum) and spray chilling (SC; wall material: stearic acid + hydrogenated vegetable fat) to assess the degree of protection of the microstructures formed in their application in biscuits. The microparticles obtained by the two methods showed typical spherical morphologies and high polydispersity. The average diameters were 9.3 ± 0.2 and 31.2 ± 0.7 μm for SD and SC particles, respectively. The encapsulation efficiencies were high (>97%) for both samples. In the biscuits application, both structures suitably protected the AA (preservation of more than 85% of the added content of AA) compared with the addition of the free active substance (loss of 28% of AA). The SD microparticles providing a greater protective effect than the SC microparticles. Both microencapsulation methods can be used to obtain microparticles as potential protection vehicles in the application of sensitive substances in baked products.

1. Introduction

The food industry has been diversifying its product portfolio to meet the demands of a growing group of more critical and demanding consumers regarding the nutritional quality of processed products (MacAulay, Petersen, & Shank, 2006; Schrooyen, van der Meer, & De Kruif, 2001). The incorporation of functional substances in products has become a strategy of interest to the industry but has several limitations due to the sensitivity of these substances to conditions related to processing, storage and consumption of products (Champagne & Fustier, 2007; De Vos, Faas, Spasojevic, & Sikkema, 2010; Dubey, Shami, & Bhasker Rao, 2009; Wang & Bohn, 2012).

Among the technologies available to add special properties to foods, microencapsulation can be used. This technology, based on the creation of a barrier (matrix) between the substance of interest (active substance) and the environment (food), allows the addition of special characteristics to existing products or the development of new ones (Dziezak, 1988; Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Zuidam & Shimoni, 2010). Microencapsulation can protect, release in a controlled manner, manipulate the physical form and mask the flavor and odor of substances (bioactive nutrients, aromas, enzymes, yeasts, acids, salts, etc.). This technology allows the incorporation of sensitive substances into processed foods avoiding degradation and negative changes (Augustin & Hemar, 2009; Byun, Kim, Desai, & Park, 2010; Desai & Park, 2005; Gouin, 2004; Wang & Bohn, 2012).

Various methods of microencapsulation may be used for many substances, and the choice of the most appropriate method will depend on the type of substance, its special function and the desired application (Desai & Park, 2005).

The spray drying (SD) method is one of the oldest and most traditional in the encapsulation of aromas, and it is being increasingly applied for the protection of bioactive substances such as natural antioxidants, polyunsaturated fatty acids, probiotics, etc.

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http://dx.doi.org/10.1016/j.lwt.2015.08.049
0023-6438/

The technique is based on the atomization of a liquid mixture of a barrier material (carbohydrates, proteins or mixtures of both) and the active substance within a chamber containing a heated gas followed by evaporation of the solvent from the microdroplets formed to obtain dry microspheres containing the active substance (Reineccius, 1989; Dziezak, 1988; Gharsallaoui et al., 2007; Zuidam & Shimoni, 2010).

In contrast, the spray chilling (SC) method is based on the atomization of a mixture of the active substance and a melted lipid material in a cold chamber (temperature below the lipid's melting point) in which the droplets in contact with the cool air solidify to form solid lipid microparticles (SLMs) that retain and protect the active substance (Zuidam & Shimoni, 2010).

The two methods have the advantages of good protection of sensitive active substances, the possibility of industrial scale production and relatively low costs (Gouin, 2004; Zuidam & Shimoni, 2010).

This study aimed to compare the characteristics of microparticles obtained by SD and SC containing ascorbic acid (AA) as the active substance, using arabic gum and stearic acid + hydrogenated vegetable fat as wall material for SD and SC respectively, to assess the degree of protection of these microstructures with respect to the active substance when applying them to a baked product (biscuits).

2. Materials and methods

2.1. Materials

The following materials were used in the production of the microparticles: ascorbic acid (AA; 100% purity, Synth, Diadema, Brazil), arabic gum (AG; Instant Gum; Nexira, São Paulo, Brazil), stearic acid (SA; melting point of 55.0 °C, determined by open capillary slip point AOCS method Cc 3-25, AOCS, 1998, Synth, Diadema, Brazil) and hydrogenated vegetable fat (HVF; melting point of 41.0 °C, determined by open capillary slip point AOCS method Cc 3-25, AOCS, 1998, Iodine Index: 55, informed by producer. Bunge, Gaspar, Brazil). For the analysis, the following chemicals, all with analytical grade, were used: absolute ethyl alcohol (99.5%, Synth, Diadema, Brazil), oxalic acid monohydrate (Synth, Diadema, Brazil), 2-6-dichlorophenol-indophenol sodium salt (DCFI; Vetec, Duque de Caxias, Brazil).

2.2. Preparation of the mixtures of wall material and active substance and microparticles production

For the production of microparticles by SD (M-SD), a mixture of arabic gum (wall material) and AA (core) was solubilized in distilled water (mechanical stirrer, IKA-Werke, RW 11 Lab egg – stirrer, Staufen, Germany) at the total solid content of 25 g/100 g and the proportion of core: wall was 1:4, representing 20 g of core/100 g of total solid content. After stirring the active substance and wall material, the mixture was dried using the processing conditions described in Table 1.

For the production of microparticles by SC (M-SC), the wall material used consisted of a mixture of HVF and SA at a 1:1 ratio (melting point of 49.5 °C, determined by open capillary slip point AOCS method Cc 3-25, AOCS, 1998). The lipid materials were weighed in glass beaker and melted in a microwave oven in 30-s time intervals so that the final temperature of the mixture did not exceed 70 °C. The liquid lipid mixture was maintained at 70 °C on a hotplate until use. The AA was gently milled (mortar and pestle), sieved (mesh = 0.150 mm) and the fine portion (<0.150 mm) was added to the lipid mixture and homogenized by mechanical stirring (mechanical stirrer, IKA-Werke, RW 11 Lab egg – stirrer, Staufen, Germany) on a hotplate. The final mixture was subjected to an ultrasonic bath (70 °C for 1 min) to well disperse the AA crystals suspension and was spray chilled under the conditions described in Table 1.

Table 1
Processing conditions to obtain the SD and SC microparticles.

Processing conditions	Spray drying	Spray chilling
Sample Temperature (°C)	40 ± 2	70 ± 2
Inlet Temperature (T_{in}; °C)	150 ± 2	5 ± 3
Outlet Temperature (T_{out}; °C)	75 ± 3	15 ± 2
Feed sample rate (mL/min)[a]	8	12
Aspiration (m^3/h)	35[b]	24.5[c]
Nozzle diameter (mm)	0.7	
Atomization gas flow (L/min)	10	

[a] Spray Drier: peristaltic pump; Spray Chiller: gravity.
[b] Equivalent to 100% of aspirator rate.
[c] Equivalent to 70% of aspirator rate.

The same equipment (Mini Spry Dryer B290, Büchi, Flawil, Switzerland) was used for SD and SC processes (nozzle Ø = 0.7 mm). To work as a spray chiller, the Mini Spray Dryer B290 was adapted to produce cooled air using the Dehumidifier B296 (Fig. 1C) (Büchi, Flawil, Switzerland) and the mixture of wall lipid material and AA was kept warmed and in liquid state by the spray chilling accessory (Büchi, Flawil, Switzerland) (Fig. 1B, including a heated container for lipid + core material, heater, temperature probe and metering valve to feed the nozzle) before the atomization in the cooled chamber. The Fig. 1 shows the equipment configuration for spray drying process (Fig. 1A) and spray chilling process (Fig. 1A, B and C).

The samples produced in each process were collected and stored refrigerated for further characterization.

2.3. Characterization of microparticles

2.3.1. Total encapsulation efficiency (TEE)

The total microencapsulation efficiency was defined as the percentage of active substance (AA) measured in the microparticles after processing in relation to the amount of active substance initially used. Equation (1) was used to calculate the TEE (Alvim &

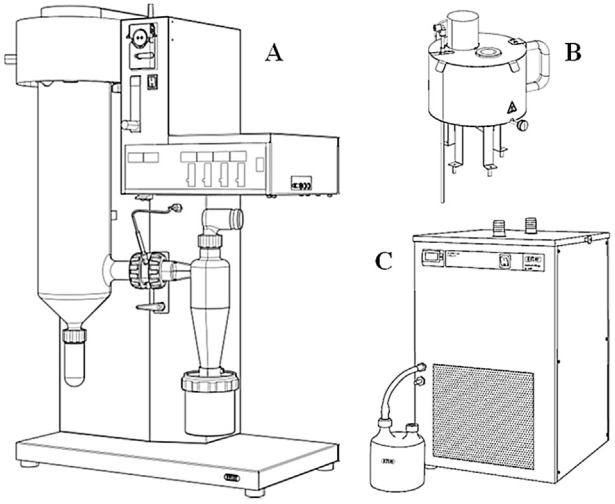

Fig. 1. Equipment configurations for microparticles production: Spray drying: A – Mini Spry Dryer B290; Spray chilling: A – Mini Spry Dryer B290 + B – Spray chilling accessory + C – Dehumidifier B296. Adapted from Mini Spray Dryer B-290 Technical data sheet, free access: http://static2.buchi.com/sites/default/files/technical-data-pdf/B-290_Data_Sheet_en_C_1.pdf.

Grosso, 2010; Sartori, Consoli, Hubinger, & Menegalli, 2015).

$$TEE = \frac{\text{Active substance quantified after processing}}{\text{Active substance used before processing}} \times 100 \quad (1)$$

2.3.2. Content of ascorbic acid

The AA content was assessed by the Tillmans method based on the titration principle with adaptations (Analytic Norms of the Adolfo Lutz Institute, 2008). Regarding the microparticles, the AA content was determined to calculate the TEE and to define the amount of microparticles to be added to the biscuits. For M-SD, 0.05 g of sample was completely dissolved in oxalic acid solution (1 g/100 mL) and transferred to a volumetric flask. For M-SC, the same amount of sample was completely melted in oxalic acid solution (1 g/100 mL), heated to 60 °C and transferred to a volumetric flask.

After preparing the samples, aliquots of 10 mL were transferred to Erlenmeyer flasks, a further 50 mL of oxalic acid solution was added, and the solution was then titrated with DCFI solution (0.2 g/100 mL). Determinations were performed in triplicate.

2.3.3. Morphology and microstructure

Visualization of the surface morphology of the arabic gum (M-SD) and lipid microparticles (M-SC) was performed using scanning electron microscopy (SEM). The samples were fixed on metal surfaces and then sputter coated with gold (four sputtering cycles; Baltec Sputter Coater SDC 50, Balzers, Liechtenstein). The observation of samples was performed in a DSM 940A FOCUS scanning electron microscope (Zeiss, Jena, Germany) with electronic capture of images at several magnifications and laser acceleration at 10 kV.

2.3.4. Mean diameter and size distribution

The mean diameter and the size distributions of the microparticles were obtained by light scattering using an LV 950-V2 equipment (Horiba, Kyoto, Japan). The M-SD samples were previously dispersed in absolute ethanol and M-SC samples in Polyoxyethylene (20) sorbitan monolaurate solution (0.5 g/100 g, Tween 20) (Matos-Jr, Di Sabatino, Passerini, Favaro-Trindade, & Albertini, 2015; Tonon, Grosso, & Hubinger, 2011).

After the initial dispersion, samples were added to the LV950-V2 sample bath with the appropriate dispersion medium (absolute ethanol: M-SD and distilled water: M-SC) until achieving transmittance levels suitable to perform the measurements. The mean particle size was expressed as the volume mean diameter ($D_{4,3}$), and the polydispersity was given by the span index, which was calculated according to Equation (2). The measurements were performed in triplicate.

$$Span = \frac{D_{0.9} - D_{0.1}}{D_{0.5}} \quad (2)$$

where $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ correspond to the diameters relative to 10, 50 and 90% of the accumulated size distribution.

2.4. Biscuits production containing the free or microencapsulated AA

Biscuits samples of short dough type (contain AA in free form, AA microencapsulated by SD and SC and control without AA) were produced following the method described by Manley (1998) with adaptations. The Table 2 describes the ingredients and the steps to produce the dough samples that were sheeting using a dough sheeter (Braslaer, Limeira, Brazil) to obtain a final thickness of 5 mm. The sheeted dough was cut in small rectangles (Mechanical cutter, Braslaer, Limeira, Brazil) and transferred to a perforated band can. Biscuits were baked in a static electric oven (0448 TRIF/Perfecta Vipinho, Curitiba, Brazil) at 170 °C for 7 min. After this the biscuits were cooled to room temperature and packed into flexible film metalized BOPP (biaxially oriented polypropylene) for further characterization.

2.5. Biscuits characterization

2.5.1. Expansion ratio

The expansion index was calculated from the ratio between the values for the volume of biscuits samples before and after baking. Each dimension of sample biscuit (thickness, width and length) was measured using an analog Vernier caliper (Mitutoyo, Suzano, Brazil) before the baking (V_{before}). After cooling step the same biscuits were measured again and the volume after baking was determined (V_{after}). The expansion index was calculated according to Equation (3). Twenty biscuits were measured for each sample (adapted from AACC 10-50-05, 2010).

$$\text{Expansion Index} = \frac{V_{before}}{V_{after}} \quad (3)$$

2.5.2. Water activity (Aw)

The biscuits samples were homogenized in a mortar and were transferred to sample cups. The Aw was determined in triplicate using a dew point water activity meter (Aqua Lab 4TEV, Decagom, Pullman, USA) at constant temperature (25.00 ± 0.30 °C).

2.5.3. Instrumental texture

Hardness of samples was evaluated using a Texture Analyser (TATX2i, Texture Technologies Corp, Hamilton, USA) with probe 3-Point bending Rig – HDP/3 PB, platform HDP/90. Twenty biscuits of each sample were used for this analysis. The hardness was represented by the maximum breaking strength (graphic Force × time) according to Mamat, Abu Hardanb, & Hill (2015) and Kaur, Sandhu, Arora, and Sharma (2015).

2.5.4. Amount of remaining AA in biscuits after baking

The Tillmans method (Analytic Norms of the Adolfo Lutz Institute, 2008) cited in 2.3.2 was used to quantify the AA content in the biscuits. Samples were ground in a mortar and sieved (mesh = 0.5 mm). Then, 5 g of sample was added to an Erlenmeyer flask, and 50 mL of oxalic acid solution (1 g/100 mL) was added. The mixture was kept under mild stirring for 5 min and then placed in an ultrasound bath for 2 min. For biscuit samples containing M-SC, the temperature of the oxalic acid solution and the ultrasound bath were kept at 60 °C. Preliminary tests showed that the samples did not need to be filtered for analysis. Samples were titrated with DCFI solution (0.2 g/100 mL). Determinations were performed in triplicate.

2.6. Statistical analyses

The results were statistically analyzed by comparison of means and by Tukey's test using the Statistic v. 10 program (Statsoft, Tulsa, OK, USA) with $p < 0.05$.

3. Results and discussion

The microparticles were successfully produced using the two processes (spray drying and spray chilling). The samples presented

Table 2
Ingredients and processing steps to biscuits production.

Ingredients	Formulation (g/100 g of dough)	Processing steps
Powdered sugar	20.8	Cream formation
Inverted sugar syrup	3.2	Conditions: Mixing speed was increased 1 to 10 with 1 min long each.
Fat	16.0	
Soy Lecithin	0.4	
Wheat flour	47.9	Adding the dry ingredients
Corn Starch	5.3	Conditions: pre-mixture of dry ingredients[a] and add them to cream.
Baking powder	0.6	Mixing speed 2 for 2 min.
Salt	0.4	
Water	5.4	Adding water
		Conditions: Mixing speed 1 for 2 min.

Equipment: Stand mixer KitAid (Classic Series 4.5-Quart Tilt, St. Joseph, USA).
[a] Free AA and microencapsulated AA were added in this step.

a free flow powder aspect, without agglomerates. The results of the microparticles characterizations are shown in Table 3.

3.1. Total encapsulation efficiency (TEE), mean diameter, size distribution and morphology

The TEE for AA was high (Table 3), indicating that the conditions used in both the SD and SC processes did not cause significant damage to the active substance. Nesterenko, Alric, Silvestre, and Durrieu (2014) observed an encapsulation efficiency near 92% using the SD technique, and Matos-Jr et al. (2015) obtained 84% of encapsulation efficiency for AA encapsulated by SC. Sartori et al. (2015) determined TEE values between 89 and 98% for particle obtained by SC. Furthermore, Comunian et al. (2013) estimated a encapsulation efficiency of approximately 98% in the encapsulation of AA by a combined method using the active substance emulsified in oil followed by complex coacervation.

Although the atomizer used and the atomizing conditions were the same in both methods, the mean diameter observed for the particles obtained by SD was lower than the diameter of the particles obtained by SC ($p < 0.05$). This difference can be attributed to the principle of microdroplet formation during the atomization in addition to the solidification process of the microparticle structure. In the SD process, the microdroplet formed can have a shrinkage associated with the solvent evaporation, which produces particles that are smaller than the initially formed droplets (Mezhericher, Levy, & Borde, 2010). In the process of SC, the particle formation is based on the hardening of the lipid microdroplets at low temperatures, which maintains the original diameter generated during atomization.

Despite the difference between the mean diameters of the particles for samples produced by SD or SC, the size distributions were within the range observed for the type of atomizer used. The mean diameters were similar to results in the literature for microparticles obtained by SD and SC. Oliveira, Santana, and Ré (2005) determined 3.42 and 6.78 μm for $D_{4,3}$ for two different SD samples. Pereira et al. (2009) used legumes seeds protein isolates to AA encapsulation by SD and showed values between 1.23 and 8.38 μm for $D_{4,3}$. Tonon et al. (2011) observed values of D_{50} between 7.95 and 17.88 μm for particles produced by SD and the values changed according to the process conditions variation. Sartori et al. (2015) showed values for $D_{4,3}$ varying between 18.00 and 67.00 μm for particles produced by SC in similar equipment. Alvim, Souza, Koury, Jurt, and Dantas (2013) produced particles by SC method encapsulating phytosterols and observed values of D_{50} between 13.80 and 32.20 μm.

Table 3
Encapsulation efficiencies, mean diameter and polydispersity index (span) for microparticles obtained by SD or SC containing AA.

Process	EE (%)	$D_{4,3}$ (μm)	Span
Spray drying	100.8 ± 0.6^a	9.3 ± 0.2^b	1.44 ± 0.03^a
Spray chilling	97.8 ± 0.7^b	31.2 ± 0.7^a	1.45 ± 0.00^a

Different lowercase letters in each column represent statistically significant difference ($p \leq 0.05$)

The samples showed high polydispersity (represented by the span), which is typical of the type of atomizer used (double-fluid), and a normal size distribution (Table 3 and Fig. 2). The microparticles produced by SD or SC had similar span indexes, with no significant difference ($p < 0.05$) within the range observed for this type of sample. The indexes were slightly lower than the results obtained by Oliveira et al. (2005) that observed 1.99 and 2.21 for their two samples analyzed, and Tonon et al. (2011) that presented span index between 2.16 and 2.65.

The surface morphology of the microparticles can be observed in the images obtained by SEM (Fig. 3). The samples for both methods showed spherical structures typical of materials subjected to atomization. Tonon et al. (2011), Chambi, Alvim, Barrera-Arellano, and Grosso (2008), Sartori et al. (2015) and Alvim et al. (2013) observed similar structures for particles obtained by SD and SC.

3.2. Production and characterization of biscuits containing free or microencapsulated AA

The biscuits were produced containing free ascorbic acid (F-AA), AA in M-SD and M-SC, and control (C, without AA), their appearance is shown in Fig. 4. The microparticles were previously mixed to dry ingredients in increasing amounts from smallest to largest constituent to promote adequate dispersion in the dough. The addition of M-SD or M-SC and free AA had no effect on the elaboration stages of biscuits and did not affect the appearance of their respective dough before baking probably because the amount added was small (0.3 g microparticles/100 g of dough and 0.1 g of free AA/100 g of dough).

After baking was observed the formation of dark spots on the surface of the biscuits containing AA in its free form (unencapsulated), which may have been due to its oxidation and thermal degradation when exposed at the surface of the biscuit (Fig. 4B, arrows indicate the dark spots formed during the baking). Degradation of AA under some process conditions, including increase of temperature, may lead to the formation of carbon dioxide, furfural and other compounds such as those produced by Maillard reactions or non-enzymatic browning, which may also have induced the formation of the dark pigment (Baiano, Marchitelli, Tamagnone, & Del Nobile, 2004; Davies & Wedzicha, 1994).

Samples of biscuits containing microencapsulated AA showed no dark spots and this may indicate that the microparticles

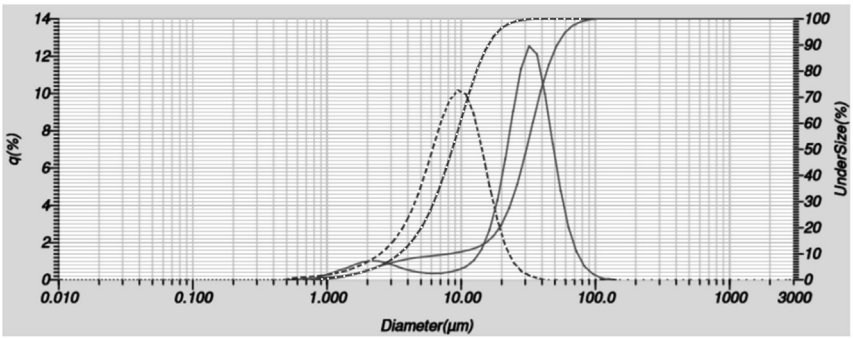

Fig. 2. Comparison between the size distribution of the microparticles obtained by SD (dotted line) or SC (continuous line).

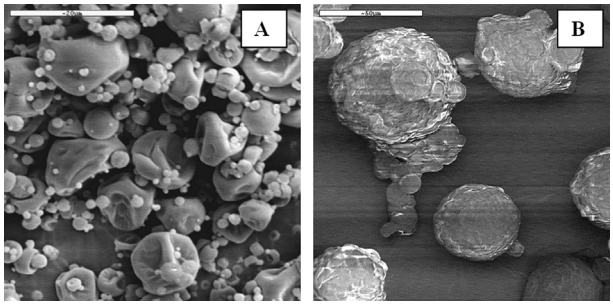

Fig. 3. Surface morphology of microparticles (Scan Electronic Microscopy – SEM) contains ascorbic acid obtained by spray drying (A; Bar = 20 μm) or spray chilling (B; Bar = 50 μm).

structures, even partially affected by the manufacture of biscuits, were still able to retain and protect the AA.

Samples F-AA and M-SC AA biscuits had similar expansion rates among themselves (Table 4). The same behavior was observed for M-SD AA and Control samples (Table 4). However, these differences did not influence the appearance of the biscuits, which in general were quite similar (Fig. 4). The biscuits produced in this work are classified as soft dough group that have the characteristics of cohesive dough with low extensibility and elasticity. The high amounts of sugar and fat give the good texture for the product without the gluten matrix formation and consequently the biscuits almost don't expand (Manley, 1998).

Regarding the strength needed to break the biscuit (Table 4) as analyzed by texturometer, the use of AA in the free form produced biscuits that were easier to break (similar to Control sample; $p \geq 0.05$) than biscuits containing microencapsulated AA, regardless of producing the microcapsules by SD or SC. This result supports the assumption that the microencapsulation of certain active substances can influence the product's characteristics, and creating a barrier may be advantageous to inhibit undesirable effects.

The water activities of biscuits containing free AA or microencapsulated AA via SD or SC showed differences among their values ($p < 0.05$), and the water activity was lower for the sample with the free AA than for the sample with SD. Despite the variation, the values are suitable for this type of product.

Based on the TEE of each microparticle sample, the addition of these microparticles to the biscuits was calculated to provide 0.6 mg AA/g biscuit, which corresponded to 40% of the RDI (Recommended Daily Intake) for adults (adult RDI = 45 mg, Brazilian Health Surveillance Agency – Anvisa). The AA values remaining in the biscuits after baking are shown in Table 4, and the biscuit samples containing free AA showed a 28% loss of AA content. For samples containing microencapsulated AA, the losses were reduced to 11% for AA SD and 15% for AA SC, representing preservation of the active substance content at over 85% following microencapsulation.

4. Conclusions

Microencapsulation of AA using the SD and SC methods was successfully performed. The particles obtained by the two methods differed in the mean diameter due to the principle of structure formation in each method but had similar and high TEE values, indicating that the processing conditions used in both methods were adequate to preserve the active substances. The microparticles showed typical polydispersity and morphology for each process. The application of free or microencapsulated AA in biscuits did not markedly change the characteristics of the product; however, microencapsulation inhibited the formation of dark spots on the biscuits that were associated with the thermal degradation of this active substance during baking. The protection of the active substance during baking was greater when AA was microencapsulated by SD, followed closely by SC, with a reduction of close to half of the

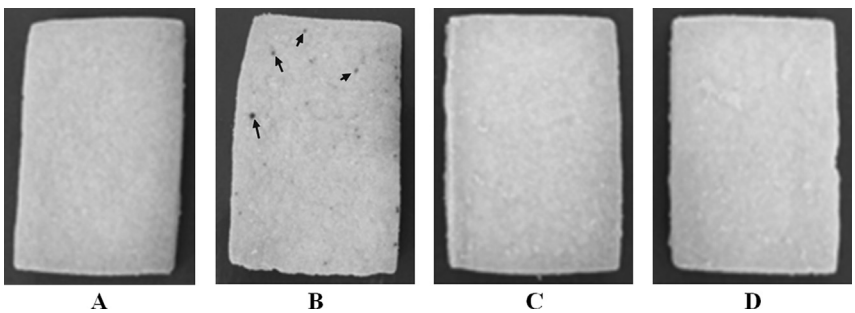

Fig. 4. Appearance of the biscuits: Control (A) – biscuit without AA; F-AA (B) – biscuit contain not encapsulated AA (arrows indicate the dark spots formed during the baking); M-SD AA (C) – biscuit contain AA encapsulated by spray drying; M-SC AA(D) – biscuit contain AA encapsulated by spray chilling.

Table 4
Characterization of biscuits containing free AA, AA SC, AA SD and Control.

	Amount of AA mg/g biscuit[1]	Expansion Index	Aw	Breaking strength (kgf)
Control	0.01 ± 0.00[d*]	1.01 ± 0.01[b]	0.271 ± 0.003[b]	3.00 ± 0.77[b]
F-AA	0.43 ± 0.02[c]	1.81 ± 0.00[a]	0.247 ± 0.009[c]	3.15 ± 0.88[b]
M-SD AA	0.53 ± 0.01[a]	1.08 ± 0.05[b]	0.328 ± 0.021[a]	4.25 ± 1.18[a]
M-SC AA	0.51 ± 0.00[b]	1.87 ± 0.00[a]	0.283 ± 0.002[b]	3.81 ± 1.55 [ab]

*Means within the column with same letter are not significantly different ($p \geq 0.05$). Control – biscuit without AA; F-AA – biscuit contain not encapsulated AA; M-SD AA – biscuit contain AA encapsulated by spray drying; M-SC AA – biscuit contain AA encapsulated by spray chilling.

loss observed for the free active substance after baking. These results indicate that both microencapsulation methods may be used for active substance protection in baked products.

Acknowledgments

The authors are grateful for the financial support provided by São Paulo Research Foundation (FAPESP), which made possible the development of this study (FAPESP 2009/10724-0, FAPESP 2010/09885-7, FAPESP 2011/11131-3 and FAPESP 2011/23442-3).

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