Effect of chemical or enzymatic crosslinking on drying of microparticles produced by complex coacervation using freeze drying and spray drying.

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Introduction

Complex coacervation is produced by phase separation based on electrostatic interactions between two polymers presenting opposite charges (Thies, 1995; Schmitt et al.,1998). Conditions as pH, ionic strength, temperature and stirring rate determine the complex formation, which allow the microencapsulation of the core material, usually hydrophobic, showing low water solubility or unsolubility. These particles are being used to protect the core material and also to control its release (Tolstoguzov, 1991; Schmitt et al., 1998). Particles produced by this process present high water content and to extend their shelf life, water must be removed. The aim of this research was to dry moist coacervated microparticles using freeze and spray dryer. Microparticles without reticulation, crosslinked with glutaraldehyde or transglutaminase were assayed. Morphological observations on the integrity of the walls before and after drying were done by optical microscopy and average size measured. Release of paprika oleoresin plus soy oil used as core material was followed using anhydrous ethanol as the release medium and spectrophotometric measures.

Materials and Methods

Microparticles production: A mixture of paprika oleoresin and soy oil (1:1 w/w; 2.5g, 25°C) was emulsified with 50 mL of gelatin (ptn) solution (2.5% w/w; 45±2°C) using ultraturrax homogeneizer (14000rpm/3min). After, 50 mL of arabic gum solution (2.5% w/w; 45±2°C) was added to the system under magnetic stirring plus 200 mL of deionized water (45±2°C). The pH was adjusted to 4.00±0.02 with HCl 2.5M. The temperature of the system was decreased slowly until 10°C using ice batch and refrigeration during 18 h (Lamprecht et al. 2001). After that, particles were sieved (∅ = 25μm) and washed with deionized water.

Particle Reticulation: Glutaraldehyde: 0.1 and 1 mmolar of glutaraldehyde/ g of protein were used during 18 hours under magnetic stirring at 25°C. ***Translgutaminase***: (Activa TG-S, Ajinomoto) 10U/ g of protein was used at the same conditions of stirring, time and temperature. After this particles were washed and sieved. (∅ = 25 μm).

Particle drying: Moist particles were dried using spray dryier (inner and out temperatures: 225°C and 125°C respectively). Samples were freezing slowly (–18°C) and the conditions used: 40°C, 0,1mmHg, end temperature 25°C/2h. Total time: 48h.

Particles Characterization: Moisture: 105°C/ 16 h (AOAC, 1998). ***Average size and size distribution***: Optical microscopy and image analyses, software Scion (www.scioncorp.com), counting at least 150 units. . ***Microstructure and Morphology***: optical microscopy (NIKON – eclipse E800 - Japan) software Image Pro Plus 4.0, dried particles/glycerol and moist particles/water. Scanning electron microscopy (SEM) was used to visualize dried particles (Jeol mod. JMS -T300, 10kv).

Release of oleoresin: 70.0 – 80.0 mg of moist particles or 17.5 – 20.0 mg of dried particles in 50 ml of anhydrous ethanol kept under stirring. Sampling at 15, 30 60 and 90 minutes were filtered and the amount of oleoresin released quantified using an analytical curve (452 nm).

Results and discussion

Core distribution: As microparticles showed monolithic and homogeneous distribution of the core material inside the particles, as can be seen on Figure 1 (optical, moist particles - left side, dried and SEM, right side).

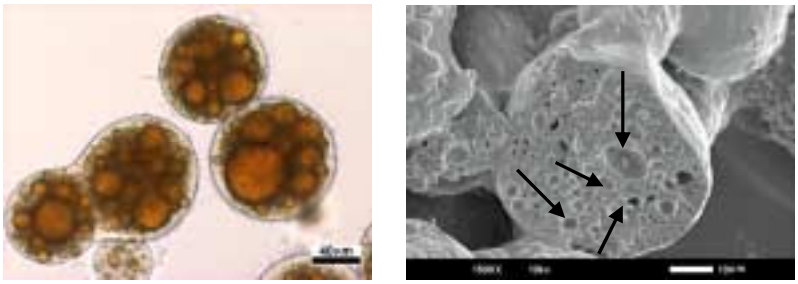


Figure 1: Micrography of the coacervated microparticles: Left side: optical microscopy particles without reticulation, bar size = 40μm. Right side: SEM, microparticles reticulated with glutaraldehyde 1,0mM/g.ptn, dried using spray dryier, bar size = 10μm. Core drops are indicated inside the micrography.

Average size and morphology: Morphology and the average size and SD of the microparticles moist, dried and swelled (water) after drying are presented Table 1. Size averages were: 43.7-96.4 μm moist particles with or without reticulation, 39.1-65.2 μm dried particles and 62.5-106.9 μm. The size of the coacervated microparticles are usually associated with the amount of core, concentration of the polymers and stirring and cooling rates (Lamprecht et al 2001, Menger et al. 2000, Bachtsi & Kiparissis, 1996).

Spray Drying and Freeze drying: Microparticles obtained by complex coacervation contain high amount of water and drying process can be usefull to extend the shelf life of the particles. On the other side water present in the matrix play one important support to the integrity of the matrix that can be loose during drying producing holes and cracks (Burgess & Ponsart, 1998). In this way, freeze drying produced whole and homogeneous walls without cracks or holes. Dried particles retained spherical format after sweeling as can see on Table 1. Sweeling occurs very fast (< 1 min) and the morphology seems like moist particles before drying. Microparticles without reticulation could not be dried using spray dryer. Only particles crosslinked with glutaraldehyde or transglutaminase could be dried. Besides this, particles crosslinked using the high amount of glutaraldehyde (1 mmolar of glutaraldehyde/ g of protein) showed much better morphological integrity. Also the yield of the spray drying process was very low, making the technique unable to this purpose as observed before by Lamprecht et al. (2001). Freeze drying allowed drying of all treatments studied including particles without reticulation (Table 1).

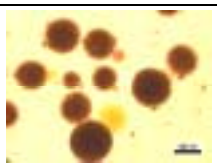
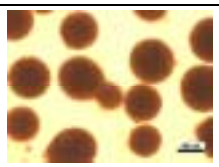
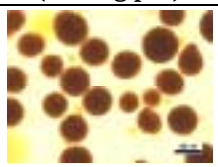
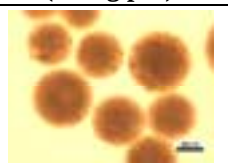
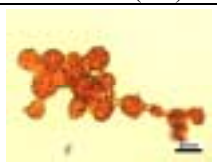
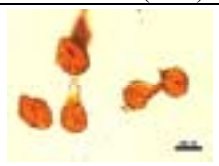
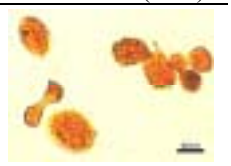
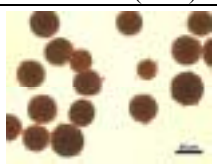
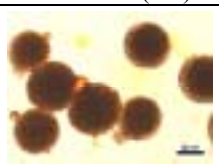
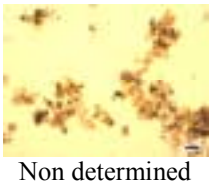
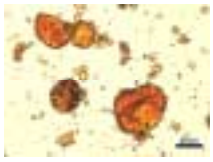
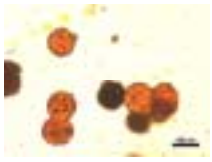
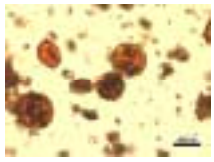
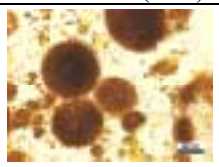
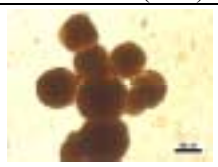
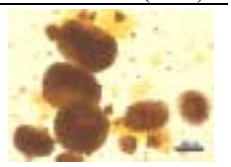
Samples	Without reticulation	Glutaraldehyde (0.1mM/g.ptn)	Glutaraldehyde (1mM/g.ptn)	Transglutaminase (10U/g.ptn)
Moist	 52.2 ± 3.7* (7.2)**	 82.2 ± 11.8 (14.3)	 43.7 ± 3.4 (7.8)	 96.4 ± 10.2 (10.6)
Freeze Dryed	 39.1 ± 3.9 (10.0)	 60.2 ± 2.3 (3.8)	 38.1 ± 5.4 (14.1)	 56.7 ± 10.8 (19.0)
Freeze Dryed After swelling	 65.1 ± 2.4 (3.6)	 101.8 ± 7.7 (7.6)	 62.5 ± 7.5 (12.0)	 106.9 ± 26.1 (24.5)
Spray dried	 Non determined	 58.4 ± 17.4 (29.8)	 56.6 ± 11.7 (20.7)	 65.2 ± 16.1 (24.7)
Spray Dried After swelling	 Non determined	 100.3 ± 23.5 (23.4)	 98.0 ± 17.9 (18.2)	 90.4 ± 15.2 (16.9)

Table 1: Average size, SD (µm), CV (%) and morphology of the coacervated microparticles: moist, dried and dried particles after swelling. Bars size = 40µm.

Core release: The release of the core material from moist and freeze dried microparticles are showed on Figure 2. For all treatments dried particles were more efficient to retain the core material. For the moist particles considering the effect of the crosslinking, the order of release obtained was: particles without reticulation > transglutaminase > glutaraldehyde (0.1) > glutaraldeheyde (1 mmolar of glutaraldehyde/ g of protein). The effect of the increasing amount of glutaraldehyde on the reticulation of gelatin and as a consequence, increasing the retention of the core material was observed before. (Iwanaga et al., 2003, Vandelli et al., 2001).

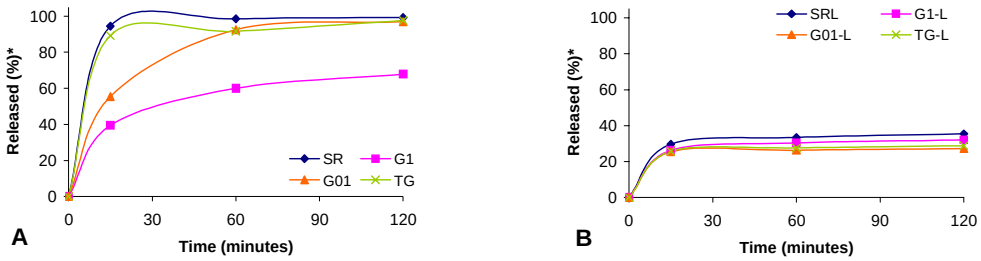


Figure 2: Release of the core material from moist (A) and freeze dried microparticles (B). SR: Without reticulation; G1: Glutaraldehyde (1mM/g.ptn); G01: Glutaraldehyde (0.1mM/g.ptn); TG: Transglutaminase (10U/g.ptn).

Conclusions: The use of the spray dyer technique to dry coacervated microparticles was only possible when the particles were crosslinked using glutaraldehyde or transglutaminase before drying. On the other side the process yield was very low, do not allowing the use of spray dryer to this purpose, at least on the conditions used to reticulate the particles. Freeze drying process was able to dry the particles, with or without reticulation, and to keep their morphological integrity. Dried particles shown swelling very fast (< 1 min) and the core release order obtained was: without reticulation > particles reticulated with transglutaminase > particles reticulated with glutaraldehyde. For all particles studied drying allowed much lower core release compared to moist particles.

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