The influence of coat to core ratio on the production of legumes proteins isolates ascorbic acid microparticles by spray drying.

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INTRODUCTION

The encapsulation of bioactive substances has become an important tool for their protection and delivery into human specific sites, to promote health and/or prevent and treat diseases. This technology has been widely explored in pharmaceutical fields, but recently foods as carriers of micro and nanostructured biomaterials has driven the attention of the food science and technology (Gouin, 2004). Spray drying is the most used technique in microencapsulation processes due to its relatively low cost, practical handling and particle formation quality (Gharsallaoui *et al.*, 2007). Lately it is important to find new alternative materials with good spray drying technological properties and efficiency in the bioavailability of active compounds and compatibility with food components and human body (Sanguansri & Augustin, 2006).

Proteins have been shown to be attractive delivery materials due to its functional properties and nutritional value (Chen *et al*, 2006). Proteins from the legumes seeds pea (*Pisun sativum*) and cowpea (*Vigna unguiculata*) have been tested in the encapsulation of tocopherol and ascorbic acid (AA) in previous studies, which showed that these biomaterials are potentials matrices for microencapsulation by spray drying in the food industry (Pierucci *et al*, 2006, 2007 and Pereira, *et al*, 2009). The well proceeded particle formation and AA retention was attributed to the seed storage protein, vicilin, present in pea and cowpea protein isolates (Pereira *et al*, 2009). The production of protein isolates with high amounts of vicilin is technologically viable (Rangel *et al*, 2003) and has been adapted for pilot scale studies (unpublished data).

AA is an essential hydrosoluble vitamin important to many physiological events, especially in the antioxidant defense system against oxidative stress (Hamilton *et al*, 2000). But AA is very instable against heat and oxidation and high degradations rate may take place in food processing, making relevant the encapsulation for such applications (Pierucci *et al*, 2004). The concentration of ascorbic acid in the spray drying feed solutions may have strong influence over protein structure and solubility; which interferes in the process yields and particle formation. Moreover, proteins from different sources may perform different behavior on such conditions. Further studies approaching the ideal proportion of protein/ascorbic acid in the production of microparticles by spray drying is needed in order to support future applications into food systems. The aim of this study was to evaluate the influence of protein: AA ratio and the type of protein source (pea or cowpea), over the drying process yield and microparticles moisture content, size distribution, morphology and AA retention.

MATERIAL AND METHODS

Materials. Pea (*Pisun sativum*) and cowpea (*Vigna unguiculata*) protein isolates, PPI and CPI, respectively were produced in laboratory scale from Brazilian seeds obtained in the local market, according to the method of Rangel *et al*. (2003) with some modifications. AA was a donated by BASF- Human Nutrition (Brazil).

Formulations of feed solutions. Feed solutions were prepared with 13% of total solids (TS), based on the protein content of the isolates, determined by the Lowry *et al* (1951) method. The coat: core ratio were 2:1, 1;1 and 1:2 for each type of protein isolate (PPI or CPI) and AA, respectively, totalizing six feed solutions in triplicate. Firstly, AA was dissolved in distilled water and mixed with pea or cowpea protein isolate solutions with an Ultraturrax T25 IKA-Labotechnik at 8000rpm, until homogenization.

Microparticles production. Feed solutions were immediately dried in a Mini Spray Dryer Büchi B-290 (Büchi Laboratoriums Technik AG) under the following conditions: inlet temperature 180 °C, measured outlet temperature 100 °C - 90 °C and solution feed rate 6 mL min^{-1}. The dried products were collected and kept in desiccators until later analysis. The process yield was calculated in dry basis, considering TS of feed solutions and the powder mass collected.

Microparticles characterization. The microparticles were analyzed as concerns moisture content, AA retention (HPLC), particle size distribution (laser diffraction) and morphology (scanning electron microscopy - SEM), according to Pierucci *et al.*, 2006.

Statistical analysis. Analysis of variance (ANOVA - one way) was used to verify differences in the microparticles moisture content and AA retention results the *post hoc* test least square difference (LSD) was used to identify the possible differences. The study admitted 0.05 as significance level.

RESULTS AND DISCUSSION

It was produced nearly 300 g of each protein isolates with final protein content of approximately 0.35g.ml^{-1}. Spray drying processes had average yields ranging from 35.7 ± 4.8 % (P1-2AA) to 58.6 ± 2.0 % (C2-1AA) (Fig. 1A); which can be considered satisfactory using the spray drying technology. The microparticles produced with CPI as coat material showed better dry product recovery than PPI. On the other hand, no statistical difference was detected concerning the coat: core ratio. The spray drying process yields are influenced by many factors related to the instrumental parameters and to the feed solutions parameters (Ré, 1998). The drying rate of particles inside the spray dryer is closely related to the process yields. The moisture content of the dried product is an important parameter to evaluate the efficiency of this process (Walton & Mumford, 1999). The moisture content of microparticles are presented on Figure 1B, which demonstrates that the increase in AA content on feed solutions led to higher moisture retention in microparticles. Probably this occurred because, in acidic media protein molecule may be partially unfolded exposing hydrophilic aminoacids residues favoring interactions with the water molecule, as demonstrated by Pedrosa *et al* (1997).

The AA retention in microparticles is demonstrated on Figure 1C; it varied from 45.2 ± 2.1 % (C1:2AA) to 88.3 ± 7.9 % (P1:1AA), which can be considered satisfactory. The formulations with lower amount of AA (P2-1AA and C2-1AA) showed significant lower core retention and no statistical significance were observed between the two types of protein isolates in the coat: core ratios 2:1 and 1:1. However, the core retention was significantly different between P1-2AA and C1-2AA. Besides, significant decrease in AA retention occurred with the increase of AA proportion (1:2) when CPI was used as coat. These results suggest that the conformational changes on protein molecule by interactions with AA, may be different between the two types of vicilin, from pea and cowpea. Previous studies have pointed that the proteolysis pattern of vicilin from pea, differently from cowpea, may promote higher AA retention (Pereira *et al*, 2009), and here it is observed that it occurs specially at high concentrations of AA in the feed solutions.

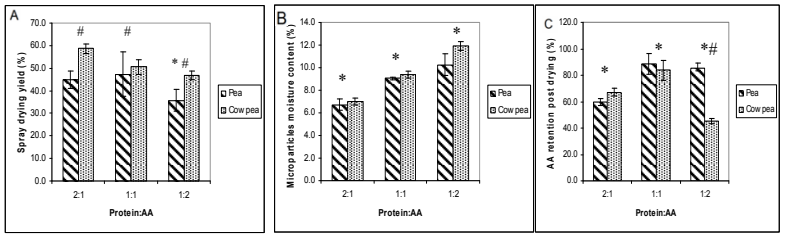

Figure 1 – (A) spray drying yield, (B) microparticles moisture content and (C) ascorbic acid retention after spray drying of P2-1AA, P1-1AA, P1-2AA, C2-1AA, C1-1AA and C1-2AA. *Significant difference between proportions; #Significant difference between legumes.

In the analysis of particle morphology it was verified that the type of protein isolates did not interfere on the morphology of microparticles. On the other hand, the micrographs on Figure 2, shows that coat: core ratio influenced the particles morphology, which were increasingly agglomerated with the increase of AA proportion. The particles presented a spherical shape and rough surfaces (fig. 2-A, 2-B and 2-C), with the exception of P1-2AA and C1-2AA. These characteristics are common for film forming materials like proteins when spray dried (Walton & Mumford 1999).

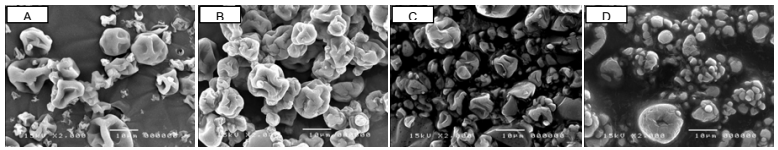

Figure 2 - Representative electron micrographs of spray dried P2-1AA (A), P1-1AA (B), C1-1AA (C) and C1-2AA (D).

Formulation	$D_{10\ (\mu m)}$	$D_{50\ (\mu m)}$	$D_{90\ (\mu m)}$
P2-1AA	0.44 ± 0.01	8.95 ± 0.73[b]	22.75 ± 0.15
P1-1AA	0.61 ± 0.08	13.07 ± 2.77[d]	27.85 ± 4.95[d]
P1-2AA	4.77 ± 1.83[a,b,d,e,f]	21.40 ± 2.58 [a,b,d,e]	45.91 ± 6.02[a,b,d,e]
C2-1AA	0.53 ± 0.00	8.82 ± 0.12	18.94 ± 0.23[e]
C1-1AA	0.44 ± 0.04	10.94 ± 0.13	27.85 ± 1.00
C1-2AA	1.00 ± 0.08	18.99 ± 0.10[a,b,d,e]	40.03 ± 0.38[a,b,c,d,e]

Table 2 – Size distribution of ascorbic acid particles produced with pea and cowpea protein isolates by laser scattering (X ± SD). [a] different from P2-1AA (p<0.05); [b] different from P1-1AA (p<0.05); [c] different from P1-2AA (p<0.05); [d] different from C2-1AA (p<0.05); [e] different from C1-1AA (p<0.05); [f] different from C1-2AA (p<0.05).

On Table 1 it is observed that the microparticles were mainly in the micro scale, although submicron particles occurred in 10% (D_{10}) of the samples analyzed. Particle sizes decreased with

the AA content on feed solutions.,The higher core ratio (1:2) induced the formation of larger particles and the increase of size distribution. The observed moisture content (Table 1) of microparticles may be related to the increase in particle size, proportionally with the increase in AA content on feed solutions.

CONCLUSIONS

Ascorbic acid (AA) microparticles were produced from different feed solutions, varying the type of legume (pea or cowpea) protein isolate and the coat: core ratio. Independently from the type of protein, the 1:1 ratio appeared to be the most efficient in retaining the active substance, and both proteins produced particles with similar size distribution. However, the cowpea protein isolates furnished better process yields than pea. More data are necessary concerning the stability of AA over a period of time and the release mechanisms when inserted in a food matrix.

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