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ENCAPSULATION OF EXTRACT FROM WINERY INDUSTRY RESIDUE USING THE SUPERCRITICAL ANTI-SOLVENT TECHNIQUE

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Abstract - Grape pomace (seed, skin and stem) is a winery byproduct with high levels of biologically active compounds, such as antioxidants and antimicrobials, that could be converted into high added-value products. Since these components are easily degraded by oxygen, light and high temperature exposure, stabilization is important, for instance, by a microencapsulation process. Therefore, the objective of this study was to investigate the influence on the particle characteristics of the operational conditions applied in the Supercritical Anti-Solvent (SAS) process for the co-precipitation of grape pomace extract and poly(-lactic-co-glycolic acid) (PLGA). The morphology and size of the particles formed, their stability and thermal profile were evaluated, and also the co-precipitation efficiency. The conditions studied allowed the production of microparticles with spherical shape for all operational conditions, with estimated particle size between 4 ± 2 and 11 ± 5 μm, and very good co-precipitation efficiencies (up to 94.4 ± 0.6%). The co-precipitated extract presented higher stability compared to the crude extract, indicating the effectiveness of the co-precipitation process and coating material against degradation processes.
Keywords: Supercritical technology; Microsize; PLGA.

INTRODUCTION

The hazard attributed to synthetic food additives for human health is leading to their replacement by natural products. Many supplies to pharmaceutical and food industries are presented as a mixture of the components of interest and a biopolymer, as encapsulated material, since this formulation facilitates the handling of the product and improves its stability (McClain, 2003; Miguel *et al*., 2008).

According to the wine industry, each 100 liters of red wine produced results in 17 kg of grape pomace - composed of seed, skin and stem - and usually it is disposed as compost. This residue still contains a high content of phenolic compounds, phytosterols, antimicrobial agents and fatty acids (Pinelo *et al*., 2006; Campos *et al*., 2008; Ul'chenko *et al*., 2009; Oliveira *et al*., 2013; Mezzomo *et al*., 2013b). Some of these compounds are associated with antioxidant activity and health benefits such as prevention of cancer and cardiovascular diseases (Pinelo *et al*., 2006; Filip *et al*., 2003). Thus, the possibility of converting the large amount of winery residue into value-added products, encourages studies related to the production of functional ingredients from grape pomace (Oliveira *et al*., 2013; Mezzomo *et al*., 2013b).

Compounds with biological activity, such as carotenoids, essential fatty acids and phenolic components, present high instability and fast degradation when exposed to environmental conditions of oxygen,

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temperature and light (Mezzomo *et al*., 2013a). Thus, the commercial use of these components requires their stabilization, for instance as microparticles protected by biopolymers (Higuera-Ciapara *et al*., 2004).

In chemistry, co-precipitation is the carrying down of target substances by a precipitation component (Patnaik, 2004). In the particles, the compounds of interest can be entrapped, chemically bound, absorbed in a biopolymer matrix or, in the particular case of the target element in a cavity surrounded by a coating (polymer membrane) encapsulated (López *et al*., 2012; Ranjit and Baquee, 2013). Among the biopolymers commercially available, poly(DL-lactide-co-glycolide) (PLGA) - a biocompatible and biodegradable, US Food and Drug Administration (FDA)-approved polymer - has been widely used to protect active ingredients from harsh environments and improve their delivery and uptake (Anderson and Shive 1997; Astete and Sabliov, 2006) without affecting the interesting components' activity (Kalantarian *et al*., 2011; Imbuluzqueta *et al*., 2011).

Some traditional particle formation techniques require relatively high temperatures, which may be inappropriate to preserve the stability of heat-sensitive bioactive substances. Hence, alternative operations such as high-pressure technology allow the production of particulate materials (powders), preserving the quality of the bioactive compound (Miguel *et al*., 2008; Varona *et al*., 2010).

Various modified supercritical techniques based on different nucleation and growth mechanisms of precipitating particles have been developed (Yeo and Kiran, 2005; Priamo *et al*., 2013). For the SAS process, supercritical carbon dioxide ($scCO_2$) and the liquid solution containing the active substance dissolved in an organic solvent are simultaneously introduced into the high-pressure vessel. The supercritical fluid is used both as anti-solvent, for its chemical properties, and as "spray enhancer", by mechanical effects. When the droplets contact the $scCO_2$ a rapid mutual diffusion at their interface takes place instantaneously, inducing phase separation and supersaturation of the precipitating solution, thus leading to nucleation and precipitation of the particles (Yeo and Kiran, 2005). The morphology and size of the particles can be controlled by employing optimum and uniform process parameters (temperature, pressure, solution and supercritical fluid flow rates) (Jung and Perrut, 2001; Adami *et al*., 2008). Besides, the $scCO_2$ can be easily and totally removed from the final product by pressure reduction, in contrast with the complex purification methods often required in traditional precipitation methods when organic anti-solvents are used. Also, the use of $scCO_2$ as anti-solvent enables the process to be carried out at near ambient temperatures and inert atmosphere, avoiding thermal degradation or oxidation of the product. For these reasons, the SAS process covers several viability requirements and has been increasingly studied lately for several applications (Miguel *et al*., 2006; Cocero and Ferrero, 2002; Miguel *et al*., 2008).

This study aimed to apply a high-pressure method for the co-precipitation of grape pomace extract in order to protect/stabilize the biological compounds present in the extract such as phenolic components and essential fatty acids. Following this objective, this work investigated the SAS operational conditions applied to the co-precipitation of grape pomace extract and poly(lactic-co-glycolic acid) (PLGA).

MATERIALS AND METHODS

Materials

Pressed grape pomace from Merlot (*Vitis vinifera*) wine production was provided by Miolo Winery (Bento Gonçalves, RS, Brazil). The pomace was dried at 32 ºC in a forced air circulation oven (A3 CARF, De Leo) to approximately 10% moisture content and, then, ground in a knife mill (Willey, De Leo). The extraction procedure was selected according to previous results (Mezzomo *et al*., 2013b; Oliveira *et al*., 2013). Despite the disadvantages of the Soxhlet process, especially the use of high temperatures, this technology produced higher extraction yield (Oliveira *et al*., 2013) and antioxidant potential (Mezzomo *et al*., 2013b) in comparison with other methods evaluated (supercritical technology and ultrasound assisted extraction). Therefore, the Soxhlet-ethanol procedure was selected for the present study. The grape pomace extract was then obtained by Soxhlet extraction using ethanol (P.A., Synth) as solvent, according to method 920.39C from A.O.A.C (2005), and the solvent removed under vacuum in a rotary evaporator.

The polymer used to form the particles in the SAS process was PLGA 50:50 (ratio of monomers PLA and PGA) with a carboxylic acid terminal group (Resomer RG 503H, Evonik). In order to prepare the SAS precipitation solution, different amounts of grape pomace extract (2, 4 and 6 mg/mL), and a fixed amount (10 mg/mL) of PLGA were solubilized in ethyl acetate (P.A., Nuclear CAQ Ind. e Com. LTDA.) at 40 ºC and constant agitation for 10 min to reach complete solubilization (determined visually). The solution was kept at room temperature for 2 hour prior to SAS

processing, in order to guarantee no solute recrystallization. The SAS process used 99.9% pure carbon dioxide (White Martins), delivered at 60 bar.

Co-Precipitation of Grape Pomace Extracts Using the Supercritical Anti-Solvent (SAS) Process

The precipitator cell used to perform the SAS process is a Supercritical Fluid Extraction (SFE) unit adapted for precipitation purposes, as described by Mezzomo *et al*. (2015). The SFE/SAS chamber is assembled in AISI 316 stainless steel with vessel dimensions: height of 31.6 cm and inner diameter of 2.012 cm, resulting in a volume of 103.28 mL. The vessel temperature is controlled by a thermostatic bath (DC30-B30, Thermo Haake). One porous frit, screen size 1 μm, is placed at the bottom of the precipitator chamber and used to collect the precipitated particles. An air-driven piston pump (M111, Maximator) and an HPLC pump (Constametric 3200 P/F, Thermo Separation Process) are used to feed the $scCO_2$ and the organic solution (ethyl acetate + grape pomace extract + PLGA) into the vessel. The two streams (CO_2 and solution) are mixed by means of a concentric tube nozzle placed at the top of the precipitation vessel (diameters of CO_2 and solution tubes: 2.012 cm and 0.159 cm, respectively). The liquid organic solvent is solubilized by the CO_2 and, through system depressurization after the precipitator, the organic solvent is deposited in a glass flask and the flow rate of gaseous CO_2 is measured by a rotameter (10A61, ABB Automatic Products). The conditions of temperature and pressure are measured by sensors directly connected to the precipitation vessel, with accuracies of ±0.5 ºC and ±2 bar, respectively. The effect of the precipitation conditions: pressure (80, 110 and 140 bar), temperature (35, 40 and 45 ºC), solution flow rate (1.0, 2.0 and 3.0 mL/min), and extract concentration in the feed solution (2, 4 and 6 mg/mL), at constant CO_2 flow rate of 1 kg_{CO2}/h, was evaluated with respect to the particle characteristics (size, morphology and thermal profile) and co-precipitation efficiency.

The experiments started by pumping pure CO_2 into the precipitator vessel. When the desired operating conditions (temperature, pressure and CO_2 flow rate) were achieved and remained stable, the solution was fed into the precipitator. After the injection of the pre-defined amount of solution (approximately 70 mL), the liquid pump was stopped and then only pure CO_2 was pumped inside the cell during 20 min in order to ensure complete drying of the particles. Subsequent to the decompression, the precipitated particles retained in the frit were collected for analysis, described as follows. All the samples were stored at temperatures below −10 ºC and protected from light to avoid the decomposition of the product.

Particles Analysis

Morphology and Size by Means of Scanning Electronic Microscopy (SEM)

Samples of the powder collected from the SAS precipitator were analyzed by a scanning electronic microscope (SEM) (JSM-63990LV, JEOL). A gold sputter was used to cover the samples with a thin layer of gold to allow the light reflection for particle evaluation. The SEM analysis uses a small sample of particles and it is very useful to evaluate the particles qualitatively (morphology and porosity) and quantitatively to estimate the particles size. The estimation of the particle size (named the estimated particle size) was determined by the Size Meter image analysis software (version 1.1), as described by Boschetto *et al*. (2013), using at least 500 particles per sample. The results of estimated particle size were expressed as mean size ± standard deviation.

Calorimetric Profiles with Differential Scanning Calorimetry (DSC)

Thermal analyses of precipitated samples were performed with a differential scanning calorimeter (DSC) (TA 4000, Mettler). Samples were analyzed under nitrogen atmosphere for temperatures between -10 and 110 ºC for the SAS particles, with a heating rate of 5 ºC/min, as described by Mezzomo *et al*. (2012). DSC analyses were conducted in order to estimate modifications in the composition, crystallinity degree and melting temperature caused by the supercritical processes.

Co-Precipitation Efficiency and Particle Stability Using UV-Vis Spectrophotometry

The crude grape pomace extract and the SAS precipitated samples were homogenized in ethyl acetate (P.A., Synth) with assistance of an ultrasound cleaner (UtraCleaner 700, 55 kHz, 40 VA, Unique) for 10 min. The resulting solution was submitted to UV-visible spectroscopy, in a UV-Visible spectrophotometer (800XI, Femto), in order to determine the grape pomace extract wavelength of maximum absorbance (Boschetto *et al*. 2013; Benelli *et al*., 2014). The maximum absorbance was observed at 350 nm, probably related to flavonoid-like compounds (Harborne, 1976; Mezzomo *et al*., 2011;

Silva *et al*., 2014a). A solution containing pure PLGA in ethyl acetate (no grape pomace extract) prepared at the same concentration used for the particle solution was also analyzed to evaluate if the polymer would affect the solution absorbance at 350 nm, and no interference was produced by the pure PLGA at this wavelength. An analytical curve that relates absorbance at 350 nm and concentration of grape pomace extract was constructed in order to identify and quantify the extract concentration in SAS produced particles. Then, the final extract concentration in the particles was calculated using the analytical curve, whereas the theoretical extract concentration was determined according to the extract mass introduced into the precipitation vessel. Finally, the extract co-precipitation efficiency was calculated by the ratio between the final and theoretical extract concentrations, in percentage.

The stability assay was conducted according to the procedure described by Gradinaru *et al*. (2003) for randomly-selected samples of SAS particles and also for the crude grape pomace extract. The samples were properly homogenized in ethyl acetate, as previously described, and the resulting solutions were maintained at room temperature under natural atmosphere and light exposure to induce their degradation. Periodic absorbance measurements were performed in order to monitor the sample degradation during 15 days of exposure.

Statistical Analysis

Results were statistically evaluated by a one-way analysis of variance (ANOVA), applied using the Software Statistica for Windows 6.0 (Statsoft Inc., USA) in order to detect significant differences among values. The significant differences at the level of 5% ($p < 0.05$) were analyzed by the Tukey test.

RESULTS AND DISCUSSION

Morphology and Size of Particles

Figure 1 presents the scanning electronic microscopies of SAS particles produced using different operational conditions, and Table 1 presents the respective estimated particle sizes.

According to Figure 1, the SAS process enabled the production of spherical microparticles in all operational conditions applied. The results presented in Table 1 indicate that the lowest estimated particle size achieved was 4 ± 2 μm**,** obtained using 4 $mg_{extract}$/mL, 3 $mL_{solution}$/min, 1 kg_{CO2}/h, 110 bar and 40 ºC (assay 9), despite being statistically equal to various other operational conditions applied.

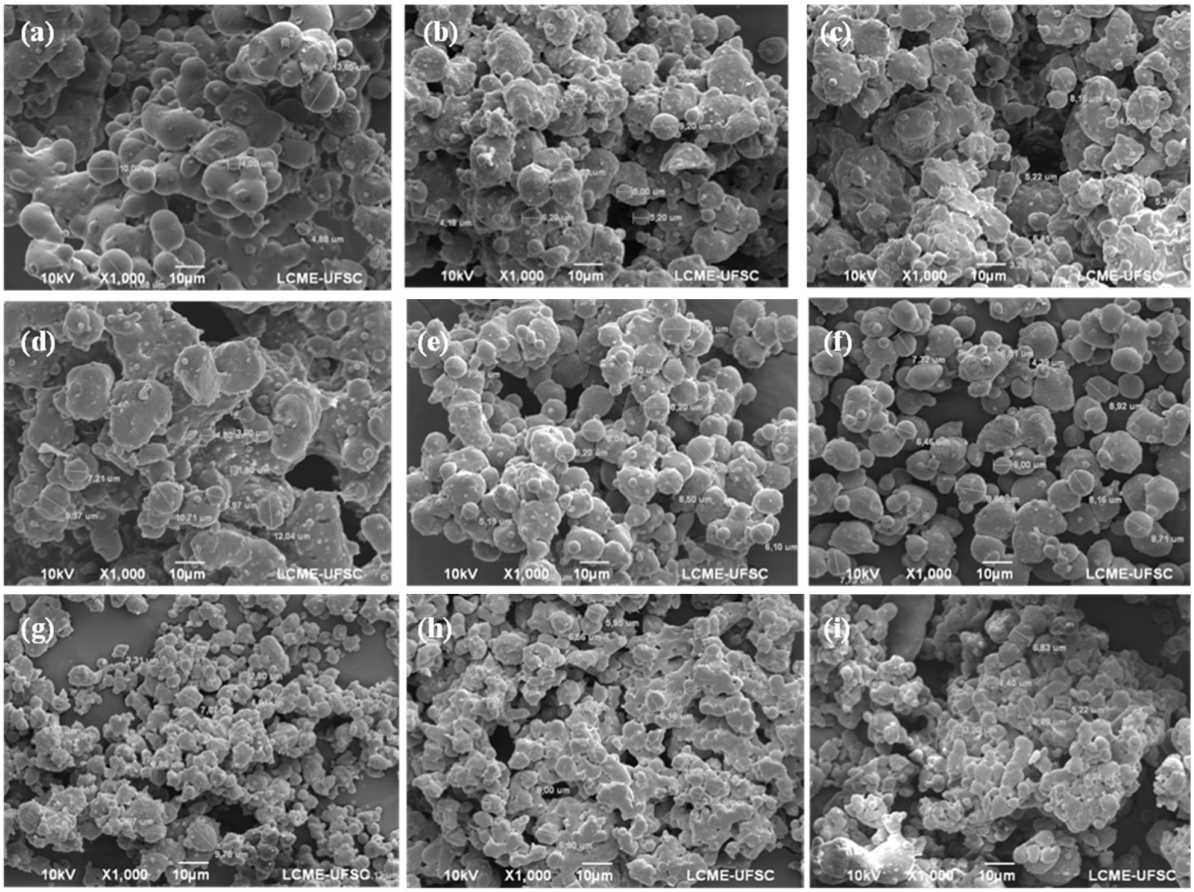
The SAS operational parameters evaluated showed slight influence on grape pomace particle size (Table 1): a) pressure and temperature did not present a significant effect ($p < 0.05$) on the estimated particle size; b) the extract concentration elevation from 2 to 4 mg/mL did not affect the estimated size, but from 4 to 6 mg/mL a reduction was statistically perceived (from 11 ± 4 to 5 ± 2 μm); c) feed solution flow showed no influence ($p < 0.05$) when comparing results obtained with 1 and 2 mL/min and with 2 to 3 mL/min, but decreased the estimated particle size significantly from 11 ± 4 to 4 ± 2 μm when increased from 1 and 3 mL/min, respectively.

Besides the particle size similarity ($p < 0.05$) among the different samples, the increase of pressure showed a tendency of reducing grape pomace estimated particle size, which can be explained by the thermodynamics aspects of the SAS system. The enhancement of pressure, at constant temperature, increases the CO_2 density, resulting in higher solvent solubility in the anti-solvent and leading to a rapid diffusion of the solvent out of the droplets (Priamo *et al*., 2010). Due to this rapid mass transfer, high levels of saturation with a high degree of nucleation can be reached, generating smaller particles (Yeo and Kiran, 2005).

As aforementioned in item b), the smaller particles produced at higher precipitation solution (extract + polymer + organic solvent) concentration can be attributed to a greater initial supersaturation. According to Franceschi *et al*. (2008), for higher concentrations an intense nucleation takes place (even more when closer to saturation) predominantly over particle growth, generating smaller particles. On the other hand, for less concentrated solutions (lower saturation) the nucleation rate decreases and the nuclei formed can grow to bigger sizes particles.

The item c) mentioned above can be explained following the same trend. When the solution flow rate is high, the ratio of CO_2 in the system is smaller (since its flow remained constant), which means slower mass transfer. As a consequence, less supersaturation occurs, promoting a low nucleation rate, resulting in bigger particles (Franceschi *et al*., 2008).

In addition, a tendency of particle agglomeration was observed, more detectable in assay 9 (4 $mg_{extract}$/mL, 3 $mL_{solution}$/min, 1 kg_{CO2}/h, 110 bar e 40 ºC) and almost not visible in assay 6 (2 $mg_{extract}$/mL, 1 $mL_{solution}$/min, 1 kg_{CO2}/h, 110 bar e 40 ºC). For the system (grape pomace extract and PLGA 50:50) and SAS conditions applied, this qualitative result can



(a) Assay 1: $4mg_{extract}/mL$, $1mL_{solution}/min$, 1 kg_{CO2}/h, 110 bar e 40 ºC; (b) Assay 2: $4mg_{extract}/mL$, $1mL_{solution}/min$, 1 kg_{CO2}/h, 80 bar e 40 ºC; (c) Assay 3: $4mg_{extract}/mL$, $1mL_{solution}/min$, 1 kg_{CO2}/h, 140 bar e 40 ºC; (d) Assay 4: $4mg_{extract}/mL$, $1mL_{solution}/min$, 1 kg_{CO2}/h, 110 bar e 35 ºC; (e) Assay 5: $4mg_{extract}/mL$, $1mL_{solution}/min$, 1 kg_{CO2}/h, 110 bar e 45 ºC; (f) Assay 6: $2mg_{extract}/mL$, $1mL_{solution}/min$, 1 kg_{CO2}/h, 110 bar e 40 ºC; (g) Assay 7: $6mg_{extract}/mL$, $1mL_{solution}/min$, 1 kg_{CO2}/h, 110 bar e 40 ºC; (h) Assay 8: $4mg_{extract}/mL$, $2mL_{solution}/min$, 1 kg_{CO2}/h, 110 bar e 40 ºC; (i) Assay 9: $4mg_{extract}/mL$, $3mL_{solution}/min$, 1 kg_{CO2}/h, 110 bar e 40 ºC.

Figure 1: Micrographs of grape pomace + PLGA particles obtained by the SAS process in different operational conditions

Table 1: Estimated particle size and co-precipitation efficiency of grape pomace extract + PLGA particles produced by the Supercritical Anti-Solvent (SAS) process for different operational conditions.

Assay	Extract concentration (mg/mL)	Solution flow rate (mL/min)	CO_2 flow rate (kg/h)	Pressure (bar)	Temperature (ºC)	Estimated particle size (μm)[(1)]	Co-precipitation efficiency (%)
1(a)							
1(b)	4	1	1	110	40	11 ± 4^{b}	85 ± 1^{bc}
1(c)							
2	4	1	1	80	40	11 ± 5^{b}	40 ± 3^{g}
3	4	1	1	140	40	8 ± 4^{ab}	52.3 ± 0.5^{e}
4	4	1	1	110	35	8 ± 5^{ab}	79.3 ± 0.7^{c}
5	4	1	1	110	45	9 ± 3^{b}	64 ± 3^{d}
6	2	1	1	110	40	8 ± 3^{ab}	94.4 ± 0.6^{a}
7	6	1	1	110	40	5 ± 2^{a}	44 ± 1^{fg}
8	4	2	1	110	40	7 ± 4^{ab}	56.4 ± 0.7^{e}
9	4	3	1	110	40	4 ± 2^{a}	48 ± 2^{f}

[(1)]Same letter in same column indicates no statistical difference between values.

suggest that particle agglomeration is directly influenced by a higher solution flow rate and extract concentration. Both aspects can promote agglomeration due to higher solution flow rate/CO_2 ratio (less supersaturation) and higher lipid content on the particle surface, respectively. Silva *et al*. (2014b) applied PLGA 50:50 to the co-precipitation of different tropical fruit (acerola, guava and passion fruit) by-product ethanolic extracts by the emulsion and solvent evaporation method and also reported an agglomeration trend, so it may be related to the PLGA 50:50 attributes, such as steric effects (Murakami *et al*., 1996) or plasticization (Pini *et al*., 2008).

Calorimetric Profiles of the Particles

Figure 2 presents the calorimetric profiles of grape pomace extract + PLGA particles obtained by the SAS process. In general, according to Figure 2, the calorimetric profiles of the SAS particles showed two main peaks (heat flow changes), one near 35 ºC and the other at 77 ºC. Some assays presented a third peak near 80 ºC and only one sample showed two more peaks around 40 and 50 ºC (assay 3: 140 bar, 40 ºC, 1 $mL_{solution}$/min, 1 $mg_{extract}$/mL). The grape pomace extract is an oily and multicomponent sample, thus some of these peaks (next to 75-80 ºC) must be associated with a large amount of phenolic compounds (Ciftci and Temelli, 2014). Since pure PLGA showed only one significant change in the heat flow at 40 ºC, that characterizes its glass transition temperature, the peaks observed for the SAS particles near 35 ºC are probably related to the polymer coating. The slight change observed in the polymer heat flow when the SAS process is used was already noticed in the literature (Mezzomo *et al*., 2012; Visentin *et al*., 2012; Santos *et al*., 2013; Mezzomo *et al*., 2015; Benelli *et al*., 2014) and it can be related to the effect of CO_2 absorption on PLGA (Pini *et al*., 2008). According to the last authors, the interaction between supercritical CO_2 and the polymer leads to a plasticization of the polymer also at low temperatures, favoring the penetration and incorporation of bioactive compounds.

Finally, according to Cocero *et al*. (2009), the calorimetric profile can give information about the interaction between the carrier and the target material after precipitation. For instance, the DSC analysis can confirm if, in a co-precipitation process, the active substance has been effectively incorporated into the carrier matrix, or if the product is merely a mixture of segregated particles of active substance and carrier: in the first case, the characteristic peaks of the active substance will not be observed in DSC analyses, while in the latter the DSC graphs will be a superposition of the diagrams of each substance, equivalent to what would be obtained with a physical mixture of particles of the two materials (Cocero *et al*., 2009). Considering that the peaks near 75-80 ºC are probably related to the grape pomace extract (Figure 2), the DSC profiles suggest that the encapsulation of the grape pomace extract effectively occurred only for the experiments performed (SAS process) in the following conditions (data according to Table 1): assay 1 (40 ºC, 110 bar, 1 $mL_{solution}$/min and 4 mg_{extrat}/mL), assay 2 (40 ºC, 80 bar, 1 $mL_{solution}$/min and 4 mg_{extrat}/mL) and assay 6 (40 ºC, 110 bar, 1 $mL_{solution}$/min and 2 mg_{extrat}/mL). Since the DSC results only suggest the degree of encapsulation, the evaluation of the co-precipitation efficiency of grape pomace extract in the biopolymer was conducted and the results presented in the next subsection.

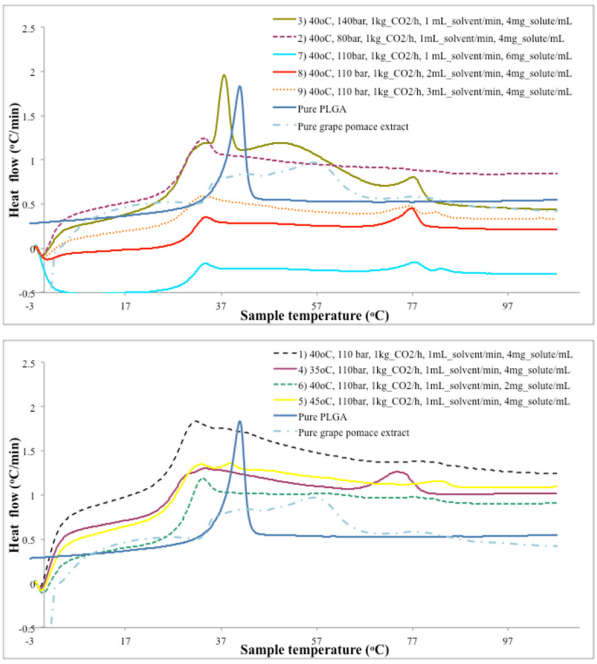


Figure 2: Calorimetric curves of grape pomace + PLGA particles obtained by the SAS process in different operational conditions.

Extract Co-Precipitation Efficiency and Particle Stability

The co-precipitation efficiencies of the grape pomace extract, at different SAS conditions, are also shown in Table 1. The SAS process permitted very good co-precipitation indices, reaching up to 94.4 ± 0.6%. The best co-precipitation efficiencies were obtained in assays 6 (94.4 ± 0.6%) and 1 (85 ± 1%),

both suggested to be effective encapsulation by the DSC results.

Regarding the influence of pressure and temperature on the co-precipitation efficiency, it was possible to observe that: a) when enhancing pressure and temperature from the lowest to the intermediate conditions applied (35-40 ºC and 80-110 bar) provoked an increase in the co-precipitation efficiency; and b) a decrease in the co-precipitation efficiency at the highest level (40-45 ºC and 110-140 bar). The optimal co-precipitation condition is related to the thermodynamic properties of the coating material and the active compound, such as the melting point (Cocero *et al*., 2009). Oliveira *et al*. (2013) showed that grape pomace extract has higher solubility in supercritical CO_2 (represented by higher global extraction yields) when higher pressure is applied in supercritical fluid extraction with pure CO_2. Consequently, since the CO_2 in the SAS process acts as anti-solvent, it was assumed that the best condition to precipitate grape pomace extract would be at lower pressures. In the present study, differently from Oliveira *et al*. (2013), the grape pomace extract was obtained by Soxhlet extraction with ethanol and, due to its polar characteristics, a reduction in its solubility in $scCO_2$ would be expected at low pressures. In contrast, some preliminary precipitation tests (using different conditions of pressure, temperature, solution and CO_2 flow rates) performed using PLGA indicated that this polymer required higher pressure levels (among the pressure conditions studied in this work) to avoid film formation, producing only particles during precipitation. Therefore, combining the mentioned pressure effects on grape pomace extract (lower solubility at low pressure) and on PLGA (better particle formation in high pressure) for their co-precipitation, the best co-precipitation efficiency was then observed at the intermediate pressure (110 bar). This same behavior was observed by Mezzomo *et al*. (2012) when co-precipitating shrimp residue extract and Pluronic F127 using the SAS process at 80, 100 and 120 bar.

The extract concentration and solution flow rate showed similar effects on the co-precipitation efficiency, the best values being obtained at the lower levels of extract concentration and solution flow rate (94.4 ± 0.6% for 2 $mg_{extract}$/mL, and 85 ± 1% for 1 $mL_{solution}$/min – assays 6 and 1, respectively). This behavior was also observed by Boschetto *et al*. (2013) when studying the co-precipitation of grape seed oil in PHBV and probably occurs due to the higher CO_2: extract ratio (g/g) that could improve the mass transfer and, consequently, the extract and polymer co-precipitation.

The stability of the SAS particles obtained from the co-precipitation of grape pomace extract and PLGA was evaluated spectrophotometrically and the results were compared to the one from the crude extract (not co-precipitated material). The extract concentration over time is shown in Figure 3. According to Figure 3, the extract released from the particle samples analyzed presented higher stability compared to the crude extract, indicating the protective effect of the SAS process and PLGA against degradation reactions. When comparing only SAS particles, the assays 2 and 7 showed the best stability results (Figure 3) and also significantly lower co-precipitation efficiencies (Table 1). This result indicates that, probably, lower extract content in the particles could improve the extract protection due to its consequently higher polymer/extract ratio.

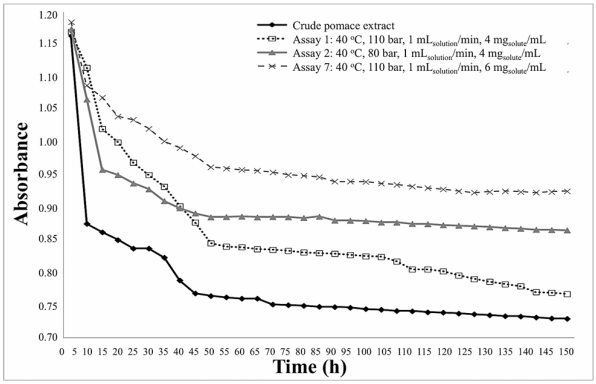


Figure 3: SAS particle/crude extract stability (extract concentration) as a function of 150-min of exposition time.

CONCLUSION

All the SAS operational conditions studied allowed the production of grape pomace extract + PLGA microparticles with spherical shape using CO_2 as supercritical anti-solvent, but with a high level of agglomeration in most of the conditions applied. The assay that can be featured among the precipitation conditions studied is number 6 (2 $mg_{extract}$/mL, 1 $mL_{solution}$/min, 1 kg_{CO2}/h, 110 bar e 40 ºC), that applied the lowest precipitation solution flow rate and intermediate values for the parameters temperature, pressure and extract concentration in the precipitation solution. These combined conditions allowed the effective encapsulation of the ethanolic grape pomace extract in PLGA 50:50 and the production of microparticles with spherical shape and little agglomeration, intermediate estimated particle size (8 ± 3 μm, statistically equal to the lowest results), and the

highest co-precipitation efficiency (94.4 ± 0.6%). The co-precipitaded extract samples presented higher stability compared to the crude extract, indicating that encapsulation in PLGA by SAS under the conditions applied shows a protective effect against degradation processes, which could also be expected from other encapsulation processes. Finally, since the SAS process applied to grape pomace extract co-precipitation uses a high-pressure technique, a complete economic analysis involving the determination of the commercial price of the final product, as well as process costs, is necessary to provide an industrial application.

NOMENCLATURE

ASES	aerosol solvent extraction systems
DSC	differential scanning calorimetry
GAS	gas anti-solvent
PGSS	particles from gas-saturated solutions
PLGA	poly(lactic-co-glycolic acid)
RESS	rapid expansion of supercritical
SAS	supercritical anti-solvent
SEDS	solution-enhanced dispersion by supercritical fluids
SEM	scanning electronic microscope
SFE	supercritical fluid extraction
$scCO_2$	supercritical carbon dioxide

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REFERENCES

Adami, R., Reverchon, E., Jarvenpaa, E., Huopalahti, R., Supercritical AntiSolvent micronization of nalmefene HCl on laboratory and pilot scale. Powder Technology, 182, 105 (2008).

Anderson, J. M., Shive, M. S., Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv. Drug Deliv. Rev., 28(1), 5 (1997).

A.O.A.C. (Association of Official Analytical Chemists), Official Methods of Analysis. 14 Ed., Washington, DC (2005).

Astete, C. E., Sabliov, C. M., Synthesis and characterization of PLGA nanoparticles. J. Biomater. Sci. Polymer Edn., 17(3), 247 (2006).

Benelli, P., Rosso Comim, S. R., Oliveira, J. V., Pedrosa, R. C., Ferreira, S. R. S., Phase equilibrium data of guaçatonga (*Casearia sylvestris*) extract + ethanol + CO_2 system and encapsulation using a supercritical anti-solvent process. Journal of Supercritical Fluids, 93, 103 (2014).

Boschetto, D. L, Dalmolin, I., Cesaro, A. M., Rigo, A. A., Ferreira, S. R. S., Meireles, M. A. A., Batista, E. A. C., Oliveira, J. V., Phase behavior and process parameters effect on grape seed extract encapsulation by SEDS technique. Industrial Crops and Products, 50, 352 (2013).

Campos, L. M. A. S., Leimann, F. V., Pedrosa, R. C., Ferreira, S. R. S., Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (*Vitis vinifera*). Bioresource Technology, 99(17), 8413 (2008).

Ciftci, O. N., Temelli, F., Melting point depression of solid lipids in pressurized carbon dioxide. Journal of Supercritical Fluids, 92, 208 (2014).

Cocero, M. J. and Ferrero, S., Crystallization of beta-carotene by a GAS process in batch. Effect of operating conditions. Journal of Supercritical Fluids, 22, 237 (2002).

Cocero, M. J., Martín, A., Mattea, F., Varona, S., Encapsulation and co-precipitation processes with supercritical fluids: Fundamentals and applications. Journal of Supercritical Fluids, 47, 546 (2009).

Chattopadhyay, P., Gupta, R. B., Protein nanoparticles formation by supercritical antisolvent with enhanced mass transfer. AIChE Journal, 48, 235 (2002).

Debenedetti, P. G., Tom, J. W., Yeo, S. D., Rapid expansion of supercritical solutions (RESS): fundamentals and applications. Fluid Phase Equilibria, 82, 311 (1993).

Elvassore, N., Parton, T., Bertucco, A., Noto, V. D., Kinetics of particle formation in the gas antisolvent precipitation process. AIChE Journal, 49, 859 (2003).

Filip, V., Plocková, M., Smidrkal, J., Pcicková, Z., Melzoch, K., Schmidt, S., Resveratrol and its antioxidant and antimicrobial effectiveness. Food Chemistry, 83(4), 585 (2003).

Franceschi, E., Kunita, M. H., Tres, M. V., Rubira, A. F., Muniz, E. C., Corazza, M. L., Dariva, C., Ferreira, S. R. S., Oliveira, J. V., Phase behavior and

process parameters effects on the characteristics of precipitated theophylline using carbon dioxide as antisolvent. Journal of Supercritical Fluids, 44, 8 (2008).

Gallagher, P. M., Coffey, M. P., Krukonis, V. J., Gas anti-solvent recrystallization of RDX: Formation of ultra-fine particles of a difficult-to-comminute explosive. Journal of Supercritical Fluids, 5, 130 (1992).

Gradinaru, G., Biliaderis, C. G., Kallithraka, S., Kefalas, P., Garcia-Viguer, C., Thermal stability of *Hibiscus sabdariffa* L. anthocyanins in solution and in solid state: Effects of copigmentation and glass transition. Food Chemistry, 83, 423 (2003).

Hanna, M., York, P., Shekunov, B. Y., Proceedings of the 5th Meeting on Supercritical Fluids, France, p. 325 (1998).

Harborne, J. B., Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd Ed., Chapman and Hall, p. 288 (1976).

Higuera-Ciapara, I., Felix-Valenzuela, L., Goycoolea, F. M., Argüelles-Monal, W., Microencapsulation of astaxanthin in a chitosan matrix. Carbohydrate Polymer, 56, 41 (2004).

Imbuluzqueta, E., Elizondo, E., Gamazo, C., Moreno-Calvo, E., Veciana, J., Ventosa, N., Blanco-Prieto, M. J., Novel bioactive hydrophobic gentamicin carriers for the treatment of intracellular bacterial infections. Acta Biomaterialia, 7(4), p. 1599 (2011).

Jung, J., Perrut, M., Particle design using supercritical fluids: Literature and patent survey. Journal of Supercritical Fluids, 20, 179 (2001).

Kalantarian, P., Haririan, I., Najafabadi, A. R., Shokrgozar, M. A.,Vatanara, A., Entrapment of 5-fluorouracil into PLGA matrices using supercritical antisolvent processes. Journal of Pharmacy and Pharmacology, 63(4), p. 500 (2011).

López, C. Ano., Delaino, L., Navarro, A. S., Martino, M., Encapsulación de compuestos bioactivos con alginatos para laindustria de alimentos. Ciencia y Tecnología Alimentaria, 59(1), p. 18 (2012). (In Spanish).

Martín, A., Mattea, F., Gutiérrez, L., Miguel, F., Cocero, M. J., Co-precipitation of carotenoids and bio-polymers with the supercritical anti-solvent process. Journal of Supercritical Fluids, 41, 138 (2007).

Mcclain, R. M., Bausch, J., Summary of safety studies conducted with synthetic lycopene. Regulatory Toxicology and Pharmacology, 37, 274 (2003).

Mezzomo, N., de Paz, E., Maraschin, N., Martín, A., Cocero, M. J., Ferreira, S. R. S., Supercritical antisolvent precipitation of carotenoid fraction from pink shrimp residue: Effect of operational conditions on encapsulation efficiency. J. of Supercritical Fluids 66, 342 (2012).

Mezzomo, N., Maestri, B., Dos Santos, R. L., Maraschin, M., Ferreira, S. R. S., Pink shrimp (*P. brasiliensis* and *P. paulensis*) processing residue: Influence of extraction method on carotenoid concentration. Talanta, 85(3), 1383 (2011).

Mezzomo, N., Martínez, J., Maraschin, M., Ferreira S. R. S., Pink shrimp (*P. brasiliensis* and *P. paulensis*) residue: Supercritical fluid extraction of carotenoid fraction. Journal of Supercritical Fluids, 74, 22 (2013a).

Mezzomo, N., Oliveira, D. A., Ferreira, S. R. S., Antioxidant potential of extracts from processing residues from Brazilian food industries. Food and Nutrition Sciences, 4, 211 (2013b).

Mezzomo, N., Rosso-Comim, S. R., Campos, C. E. M., Ferreira, S. R. S., Nanosizing of sodium ibuprofen by SAS method. Powder Technology, 270, 378 (2015).

Miguel, F., Martín, Á., Gamse, T., Cocero, M. J., Supercritical anti solvent precipitation of lycopene. Effect of the operating parameters. Journal of Supercritical Fluids, 36, 225 (2006).

Miguel, F., Martín, A., Mattea, F., Cocero, M. J., Precipitation of lutein and co-precipitation of lutein and poly-lactic acid with the supercritical anti-solvent process. Chemical and Engineering Process: Process Intensification, 47(9), 1594 (2008).

Murakami, H., Yoshino, H., Mizobe, M., Kobayashi, M., Takeuchi, H., Kawashima, Y., Preparation of poly(D,L-lactide-co-glycolide) latex for surface-modifying material by a double coacervation method. Proceedings of the International Symposium on Control. Rel. Bioact. Mater., 23, 361 (1996).

Oliveira, D. A., Salvador, A. A., Smânia Jr. A., Smânia, E. F. A., Maraschin, M., Ferreira, S. R. S., Antimicrobial activity and composition profile of grape (*Vitis vinifera*) pomace extracts obtained by supercritical fluids. Journal of Biotechnology, 164(3), 423 (2013).

Patnaik, P., Dean's Analytical Chemistry Handbook. 2nd Ed., McGraw-Hill (2004).

Pinelo, M., Arnous, A., Meyer, A. S., Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. Trends in Food Science & Technology, 17(11), 579 (2006).

Pini, R., Storti, G., Mazzotti, M., Tai, H. Y., Shakesheff, K. M., Howdle, S. M., Sorption and swelling of poly(dl-lactic acid) and poly(lactic-

co-glycolic acid) in supercritical CO_2: An experimental and modeling study. Journal of Polymer Science Part B-Polymer Physics, 46(5), 483 (2008).

Priamo, W. L., Cezaro, A. M., Ferreira, S. R. S., Oliveira, J. V., Precipitation and encapsulation of beta-carotene in PHBV using carbon dioxide as anti-solvent. Journal Supercritical Fluids, 54, 103 (2010).

Priamo, W. L., Dalmolin, I., Boschetto, D. L., Mezzomo, N., Ferreira, S. R. S., Oliveira, J. V., Micronization processes by supercritical fluid technologies: A short review on process design (2008-2012). Acta Scientiarum Technology, 35(4), 695 (2013).

Ranjit, K., Baquee, A. A., Nanoparticle: An overview of preparation, characterization and preparation, International Research Journal of Pharmacy, 4(4), 47 (2013).

Reverchon, E., Caputo, G., Marco, I. D., Role of phase behavior and atomization in the supercritical antisolvent precipitation. Industrial & Engineering Chemistry Research, 42, 6406 (2013).

Santos, D. T., Albarelli, J. Q., Beppu, M. M., Meireles, M. A. A., Stabilization of anthocyanin extract from jabuticaba skins by encapsulation using supercritical CO_2 as solvent. Food Research International, 50, 617 (2013).

Silva, L. M. R., Figueiredo, E. A. T., Ricardo, N. M. P. S., Vieira, I. G. P., Figueiredo, R. W., Brasil, I. M., Gomes, C. L., Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. Food Chemistry, 143(15), 398 (2014a).

Silva, L. M., Hill, L. E., Figueiredo, E., Gomes, C. L., Delivery of phytochemicals of tropical fruit by-products using poly (DL-lactide-co-glycolide) (PLGA) nanoparticles: Synthesis, characterization, and antimicrobial activity. Food Chemistry, 165, 362 (2014b).

Thies, J., Müller, B. W., Size controlled production of biodegradable microparticles with supercritical gases. European Journal of Pharmaceutis and Biopharmaceutics, 45, 67 (1998).

Ul'chenko, N. T., Bekker, N. P., Yunusov, O., Yuldasheva, N. K., Chernenko, T. V., Glushenkova, A. I., Lipids and lipophilic components from seeds of some fruit plants. Chemistry of Natural Compounds, 45(3), 314-317 (2009).

Varona, S., Kareth, S., Martín, Á., Cocero, M. J., Formulation of lavandin essential oil with biopolymers by PGSS for application as biocide in ecological agriculture. Journal of Supercritical Fluids, 54, 369 (2010).

Visentin, A., Rodríguez-Rojo, S., Navarrete, A., Maestri, D., Cocero, M. J., Precipitation and encapsulation of rosemary antioxidants by supercritical antisolvent process. Journal of Food Engineering, 109, 9 (2012).

Yeo, S. D., Kiran, E., Formation of polymer particles with supercritical fluids: A review. Journal of Supercritical Fluids, 34, 287 (2005).

Weidner, E., Knez, Z., Novak, Z., International Patent Publication WO 95/21688 (1995).