# CHARACTERISTICS OF GRAPE POMACE EXTRACT AND PLGA PARTICLES PRODUCED BY SAS PROCESS

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ABSTRACT – Grape pomace presents a high content of phenolic compounds, which are associated with health benefits. The encapsulation of natural extracts using polymers is important in order to facilitate the product handling and improves its stability.The objective of this work was to apply the Supercritical Anti-Solvent (SAS) process to encapsulate grape pomace extract in poly(lactic-co-glycolic) acid (PLGA). The grape pomace extract was obtained by Soxhlet with ethanol, and the encapsulation process applied ethyl acetate as primary solvent and 99.9% pure $CO_2$ as anti-solvent. Briefly, the process initiates by the adjustment of operational parameters, followed by the introduction of the extract solution simultaneously with the supercritical $CO_2$. The process was performed using $CO_2$ flow rate of 1kg/h and at different conditions of pressure (80-140bar) and temperature (35-45$^{o}$C). The scanning electronic microscopy of the particles revealed spherical shape particles with diameters next to 10μm.

## 1. INTRODUCTION

The hazard attributed to synthetic food additives in human health is conducting to their replacement by natural products. The products supplied to pharmaceutical and food industries are usually presented as a mixture of the component of interest and a biopolymer (encapsulated product), 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 engenders 17 kg of grape pomace, composed by seed, skin and stem, usually disposed as compost. This residue still contains high levels of phenolic compounds many of them reported as antioxidants (Pinelo et al., 2006). But the relatively high level of phenolic compounds in the grape pomace is a problem for its application as fertilizer because of their inhibition of germination properties (Negro et al., 2003). On the other hand, grape phenolic compounds are associated to 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 enormous amount of this residue generated by winery industry around the world into add-valued products, promotes studies using the grape pomace to obtain functional ingredients.

Compounds with biological activity, such as phenolic components, present high instability and fast degradation when exposed to environmental conditions of oxygen, temperature and light.

With the intent of employ these components in commercial products, it is necessary to stabilize them, as for example, throughout microencapsulation process in biopolymers (Higuera-Ciapara et al., 2004). In this way, the nanotechnology field has received marked attention in the development of new processes and in obtaining materials with high potential characteristics in several industries, such as of catalysts, food colors, ceramics, biopolymers, pigments, pharmaceuticals, and others (Martín et al., 2007).

The traditional encapsulation techniques are: solvent evaporation, phase separation and spray drying. These methods require relatively high temperatures, which can be inadequate for preserving the stability of a heat sensitive substance like the phenolic compounds (active principle). Hence, alternative operations such as high-pressure technology allow the production of particulated materials (powders), conserving the quality of the active component what is difficult to be achieved (Miguel et al., 2008; Varona et al., 2010).

Diverse modified supercritical techniques based on different nucleation and growth mechanisms of precipitating particles have been developed (Yeo & Kiran, 2005). The well-known techniques for particle formation using $scCO_2$ include the rapid expansion of supercritical solutions (RESS) (Debenedetti et al., 1993) and a variety of anti-solvent processes such as Gas Anti-Solvent (GAS) (Gallagher et al., 1992), Aerosol Solvent Extraction Systems (ASES) (Thies & Müller, 1998), Particles from Gas-Saturated Solutions (PGSS) (Weidner et al., 1995), Supercritical Anti-Solvent (SAS) processes (Chattopadhyay & Guplta, 2003).In the SAS process, the $scCO_2$ and liquid solution are simultaneously introduced into the high-pressure vessel. The supercritical fluid is used both as an anti-solvent for its chemical properties and as a "spray enhancer" by mechanical effects. When the droplets contact the $scCO_2$, a rapid mutual diffusion at the interface of the droplets and the $scCO_2$ takes place instantaneously, inducing phase separation and supersaturation of the polymer solute, thus leading to nucleation and precipitation of the polymer particles (Yeo & Kiran, 2005). The temperature and pressure, together with accurate metering of flow rates of solution and supercritical fluid, provide uniform conditions for particle formation. The morphology and particle size of the product can be controlled by employing optimum process parameters (Jung & Perrut, 2001; Adami et al., 2008).

The production of ultra-fine particles through supercritical anti-solvent has several advantages compared to other traditional precipitation methods, such as spray drying. The mixing between the supercritical anti-solvent and the liquid phase, containing the substance for encapsulation, is much faster than the conventional low pressure processes, which leads to higher super saturations and smaller particle diameters. The supercritical anti-solvent can be easily and totally removed from the final product by pressure reduction, in contrast with the complex purification methods often required when organic anti-solvents are used. Furthermore, a proper selection of the anti-solvent, for instance, supercritical carbon dioxide ($scCO_2$), 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, supercritical anti-solvent processes have been increasingly studied during the last years for several different applications, which include explosives, polymers, pigments, pharmaceuticals and natural compounds (Miguel et al.,

2006; Cocero and Ferrero, 2002; Miguel et al., 2008). Therefore, Supercritical Anti-Solvent (SAS) process is a very convenient method that covers several viability requirements.

The focus of this study was related to the use of high-pressure method for the encapsulation of grape pomace extract, focusing to protect/stabilize its major biological components (phenolic compounds). Following this objective, this work aimedto investigate the operational conditions of the SAS process, applied for the co-precipitation of the grape pomace extract and the poly(-lactic-co-glycolic acid) (PLGA).

## 2. MATERIAL AND METHODS

### 2.1. Material

The pressed grape pomace derived from Merlot (*Vitis vinifera*) wine production, was provided by Miolo Wine Group (Bento Gonçalves, RS, Brazil). The pomace was dried at 32 $^{o}$Cin a forced air circulation oven (De Leo, Model A3 CARF, RS, Brazil) up to approximately 10% moisture content and, then, grounded in a knife mill (De Leo, Porto Alegre/RS, Brazil). Finally, the grape pomace extract was obtained by Soxhlet extraction using ethanol as solvent, according to 920.39C method from A.O.A.C (2005), followed by concentration under vaccum in a rotary evaporator. In order to prepare the precipitation solution, different concentrations of grape pomace extract (2, 4 and 6 mg/mL), and constant concentration (10 mg/mL) of copolymer poly(lactic-co-glycolic acid) (PLGA) (Resomer RG 503H, Evonik) were solubilized by the primary solvent ethyl acetate (P.A., Nuclear, CAQ Ind. e Com. LTDA., Brazil) using constant agitation and heat application (40 $^{o}$C, 10 minutes) until reach a complete solute solubilization. The processes used 99.9% pure carbon dioxide (White Martins, Brazil), delivered at 60 bar.

### 2.2. Supercritical Anti-Solvent encapsulation

The precipitator cell used to perform the Supercritical Anti-Solvent (SAS) process is a Supercritical Fluid Extraction (SFE) unit adapted, as described by Mezzomo et al. (2013). The SFE/SAS chamber is assembled in AISI 316 stainless steel with vessel dimensions: height of 31.6cm and inner diameter of 2.012 cm, resulting in a volume of 103.28mL. The vessel temperature is controlled by 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. Anair driven piston pump (M111, Maximator) and an HPLC pump (Constametric 3200 P/F, Thermo Separation Process) are used to feed the sc$CO_2$ 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. The liquid organic solvent is solubilized by the $CO_2$ and, through system depressurization, 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 with instruments directly connected to the precipitation

vessel, with accuracies of ±0.5 $^{o}$C and ±2 bar, respectively.The effect of the precipitation conditions of pressure (80, 110 and 140 bar), temperature (35, 45 and 55 $^{o}$C), solution flow rate (1.0, 2.0 and 3.0 mL/min) and extract concentration on the feed solution (2, 4 and 6 mg/mL),at constant $CO_2$ flow rate of 1 $kg_{CO2}$/h, was evaluated in respect to the particle characteristics (size, morphology and thermal profile).

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 remain stable, the solution was feed to 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 guarantee total drying of the particles. Subsequent to the decompression, the precipitated particles retained in the frit was collected for the particle analysis (described as follow).All the samples were stored at temperatures below −10$^{o}$C and protected from light to avoid the decomposition of the product.

## 2.3. Scanning electronic microscopy (SEM)

Samples of the powder collected from the SAS precipitator were analyzed by a scanning electronic microscope (SEM) model JEOL JSM-63990LV. A gold sputter was used to cover the samples with a thin layer of gold to allow the light reflection for particle evaluation. An estimation of the mean particle size was measured by ZEISS image analysis software. The measurements were performed in quadruplicate for each sample.

## 2.4. Differential Scanning Calorimetry (DSC) analysis

Thermal analyses of the precipitated samples were performed with a Mettler TA 4000 differential scanning calorimeter (DSC). Samples were analyzed under nitrogen atmosphere for temperatures between -10 and 120$^{o}$C with a heating rate of 5$^{o}$C/min. DSC analyses were conducted in order to estimate modifications of the particles composition caused by the SAS process.

# 3. RESULTS AND DISCUSSION

The SAS process permitted to produce particles formed by grape pomace extract and PLGA in all operational conditions applied. According to SEM results from Figure 1, lower particle sizes were obtained in assay 7, which was applied the higher extract concentration (6 mg/mL), although the values are very similar. The estimated particle sizes obtained by SEM analysis (Table 1) also show the same result.

Figure 2 presents the calorimetries of grape pomace extract + PLGA particles obtained by SAS process. According to the calorimetric profiles showed in Figure 2, two main peaks were obtained, next to 35 and 77 $^{o}$C. Some assays presented a third peak next to 80 $^{o}$C and only one

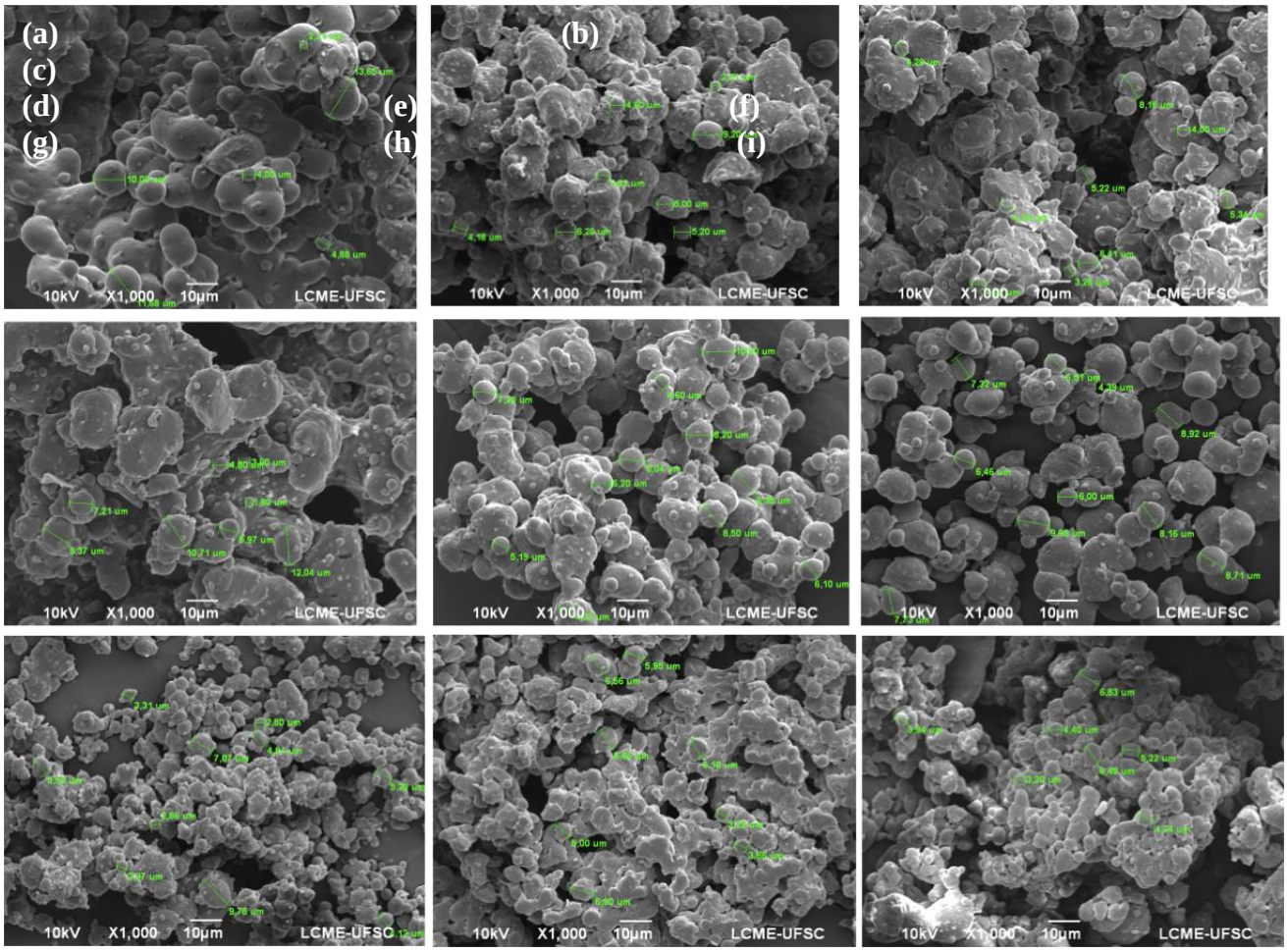
sample showed two more peaks next to 40 and 50 °C. The grape pomace extract is a multicomponent sample and, then, some of these peaks must be associated to the extract composition (phenolic compounds, fatty acids, etc.). Then, in order to conclude more remarks of process efficiency, it is necessary to investigate the extracts/particles composition.

Tabela 1 – Operational conditions applied to the Supercritical Anti-Solvent (SAS) process for encapsulation of grape pomace extract in PLGA

| Assay | Grape pomace concentration (mg/mL) | Solution flow rate (mL/min) | $CO_2$ flow rate (kg/h) | Pressure (bar) | Temperature (°C) | Particle size (µm) |
|---|---|---|---|---|---|---|
| 1(a) | | | | | | |
| 1(b) | 4 | 1 | 1 | 110 | 40 | 10 ± 5 |
| 1(c) | | | | | | |
| 2 | 4 | 1 | 1 | 80 | 40 | 5 ± 2 |
| 3 | 4 | 1 | 1 | 140 | 40 | 5 ± 1 |
| 4 | 4 | 1 | 1 | 110 | 35 | 7 ± 4 |
| 5 | 4 | 1 | 1 | 110 | 45 | 7 ± 2 |
| 6 | 2 | 1 | 1 | 110 | 40 | 7 ± 2 |
| 7 | 6 | 1 | 1 | 110 | 40 | 4 ± 2 |
| 8 | 4 | 2 | 1 | 110 | 40 | 6 ± 1 |
| 9 | 4 | 3 | 1 | 110 | 40 | 5 ± 1 |

## 4. CONCLUSION

The studied conditions allowed the production of grape pomace extract + PLGA particles using $CO_2$ as supercritical anti-solvent. The lower estimated particle size was obtained when applying the higher extract concentration (6 $mg_{extract}$/mL), at 1 $mL_{solution}$/min, 1 $kg_{CO2}$/h, 110 bar and 40 °C, although the size values of all conditions applied are very similar. The grape pomace extract is a multicomponent sample and, then, it is necessary to investigate the extracts/particles composition to give more details about the process efficiency.




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

Figura 1 – Micrographs of grape pomace + PLGA particles obtained by SAS process in different operational conditions

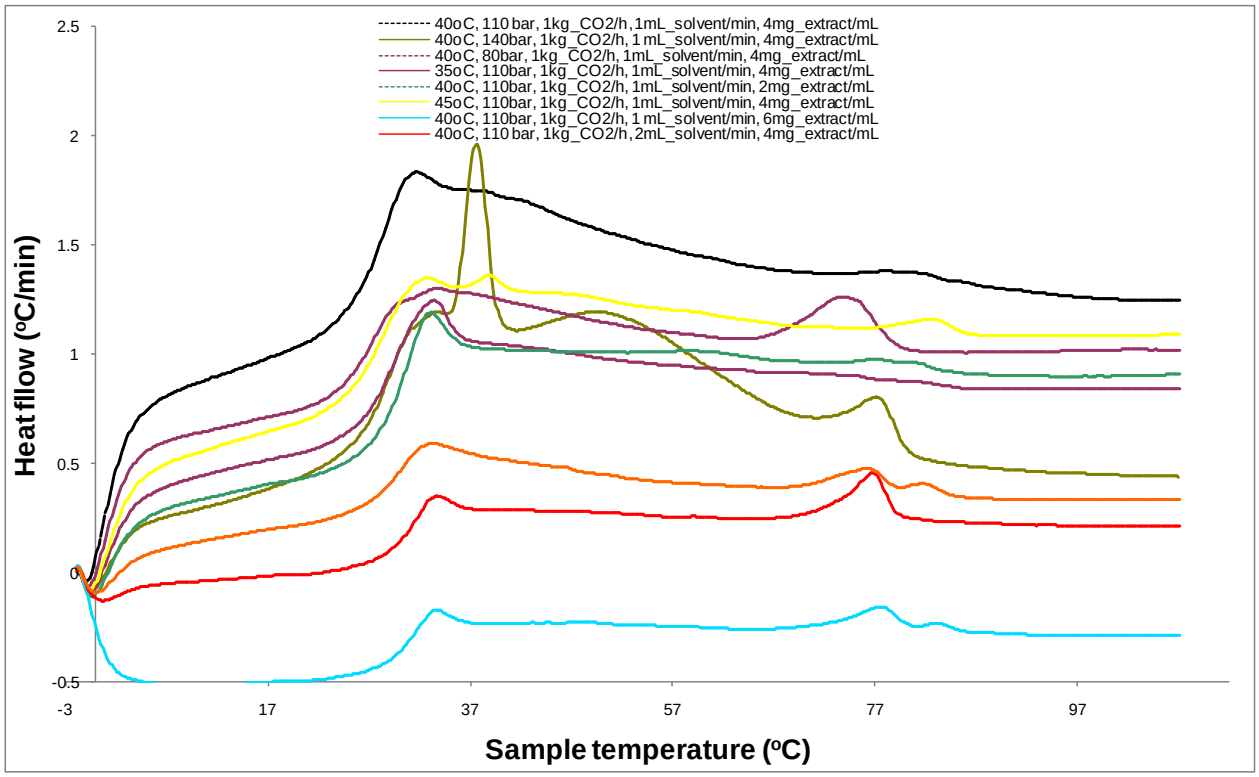



Figura2 – Calorimetries of grape pomace + PLGA particles obtained by SAS process in different operational conditions

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