Abstract

The encapsulation of antioxidants with biocompatible polymers is essential for their protection against degradation factors like light and oxygen, and facilitates its solubility in the target medium. This work presents the co-precipitation of an ethanolic extract of rosemary leaves by supercritical antisolvent (SAS) process in poloxamers in order to improve the aqueous solubility of the extract. In a first step, the precipitation of antioxidants by SAS was studied in the range of temperatures from 25 to 50°C and pressures from 8 to 12 MPa. Total content of polyphenols was quantified according to the Folin-Cicalteu method. Also HPLC analyses were performed to verify the presence of some of the major rosemary antioxidants, carnosic and rosmarinic acid. The dissolution rate of rosemary polyphenols from particles was measured in isotonic phosphate buffer solution (pH = 6.8). The encapsulation of the extract was successfully achieved with a yield up to 100%. The total polyphenolic content was dissolved from the encapsulated product, in the aqueous medium, after one hour, whereas only 15% of the antioxidants of the pure precipitate were dissolved after 8 hours.

Keywords

Antioxidants, Rosemary, Poloxamer, encapsulation, supercritical antisolvent (SAS)
1. Introduction

Rosemary (Rosmarinus officinalis) plant species has been largely studied as a source of natural products with diverse biological activities. Rosemary leaves and leaf extracts are increasingly used as food and cosmetic preservatives thanks to their content in antioxidant compounds as substitutes of synthetic antioxidants as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Etter, 2005). Moreover, rosemary antioxidants are emerging as prophylactic and therapeutic agents. They have showed antimicrobial, anti-inflammatory, antitumorigenic and chemopreventive activities which make them suitable candidates as bioactive ingredients to design functional foods (Ratnam et al., 2006; Soler-Rivas et al., 2010).

Commonly herbal extracts are marketed in the form of liquid, viscous preparations and also as powders resulting from the drying of a liquid extract. The advantages of the dried extract over conventional liquid forms are lower storage costs and higher concentration and stability of active substances (Souza et al., 2008). Additionally, and for any application, the solubility characteristics of the antioxidant in relation to the site of action must also be considered: as food preservatives, water-soluble antioxidants are very effective in muscle foods (e.g. meat) where many oxidative reactions occur in the aqueous environment, while water soluble fractions are ineffective in lipid emulsions where oxidation occurs in the lipid phase or at the lipid interface (Decker, 1998). As ingredients in functional foods, rosemary antioxidants have to be bioavailable. However, oral delivery of these antioxidants is a challenge due to various reasons such as poor solubility, instability and extensive digestion before reaching systemic circulation (Ratnam et al., 2006; Soler-Rivas et al., 2010).

In view of the above-mentioned drawbacks, encapsulation with an appropriate carrier material is necessary to obtain an effective product. Besides, encapsulated polyphenols will be protected during manufacturing processes and its palatability will be improved. (Kosaraju et al., 2008).

In this work, poloxamers were selected as encapsulating compounds. Poloxamers are triblock copolymers, type A-B-A, consisting of ethylene oxide (A: EO) and propylene oxide (B: PO) monomers
in an arrangement that allows the formation of self-assembled micelle structures in aqueous media, based on the relative difference in hydrophobicity between PO and EO (the cores of PO and water are surrounded by coronas consisting of EO and water). Therefore, they can improve the bioavailability of lipophilic compounds in aqueous media (Sharma et al., 2008; Majerik et al, 2007).

Additionally, they have generated much interest in the field of drug controlled release due to their ability to form gels in response to changes in temperature (Escobar-Chávez et al., 2006).

Recently, many ways to produce particles containing active components by using different polymers have been studied. Supercritical carbon dioxide (SC CO₂), in particular, is an advantageous processing medium for particle encapsulation because of its relatively mild critical conditions (Tc 304.1 K, Pc 7.38 MPa). Furthermore, SC CO₂ is nontoxic, nonflammable, relatively inexpensive, readily available and chemically stable.

One of the most versatile processes for particle formation with supercritical carbon dioxide is the supercritical anti solvent process (SAS), where the solute of interest is first dissolved in a conventional solvent and the solution is sprayed continuously through a nozzle, co-currently with the SC CO₂ into a chamber at moderate pressure and temperature. The high pressure CO₂ acts as an antisolvent, decreasing the solubilities of the solutes in the mixture. Therefore, a fast supersaturation takes place, leading to nucleation and formation of nano- or micro-particles. It is also possible to produce polymer co-precipitates or microcapsules in a single step using a polymer soluble in the same extract as the active compound (Cocero et al., 2009; Mattea et al., 2009).

SAS process has been already applied to the precipitation of green tea polyphenols (Mertec, et al., 2009) and to its encapsulation in polycaprolactone (Sosa et al., 2011).

A specific literature survey on the drying and encapsulation process of rosemary liquid extracts shows that research is limited, and is mainly focused on the isolation of carnosic acid (CA), one of the main antioxidant compounds in rosemary. Bailey and co-workers (1999) patented a pH controlled precipitation process for rosemary antioxidants which generates a product with mass concentration of CA between 50 to 65 %. The extraction of the antioxidants with acetone, a water-
miscible solvent, is followed by an increment of pH up to a value around 9 in order to form a salt of CA (sodium or ammonia salt). Afterwards, between four to nine volumes of buffer at the same pH are added to precipitate impurities while the salt of CA remains in solution. This solution is partially evaporated in vacuum to eliminate the organic solvent and volatile compounds, responsible for the spice odor and taste, with the steam. Then the pH is reduced to a value between 2 to 3 with phosphoric acid or acetic acid to obtain a precipitate with a high content of CA, which is recovered from the aqueous solution by filtration and finally dried at vacuum. Although this process provides a good yield of CA, it entails many purification steps, high energy consumption (evaporation and vacuum) and the use of high amount of water.

Rodríguez-Meizoso et al., 2008 presented preliminary results in precipitation of rosemary antioxidants by RESS, rapid expansion of supercritical solution, process. Firstly, the extraction of rosemary antioxidants with CO₂ using ethanol as co-solvent was performed at 15 MPa and 40 °C. Afterwards, this solution was expanded to atmospheric pressure in a chamber at 50 °C to favor the evaporation of the solvent and avoid re-dissolution of the antioxidants in the ethanol. The analysis of the particles revealed a 4 wt.% content of CA. However, the yield was not reported although considering the extraction conditions and solubility studies (Cháfer et al., 2005) it should be low. Moreover, the possibility of coupling the precipitation and encapsulation process is unlikely since few polymers are soluble in SC-CO₂ (Cocero et al., 2009).

More recently, Visentin et al. (2011) developed a two-stage fractionation process from a high viscous ethanolic oleoresin based on the solvent and antisolvent power of SC-CO₂. As a result, two fractions were obtained; the first one was a dark green powder, insoluble at 30 MPa and 50°C with low concentration of CA (< 5 g/100 g extract). The other fraction, an orange colored resinous extract with a high concentration of CA (33 wt.%), was precipitated at 10 MPa and 50°C.

Regarding the encapsulation, Souza et al., 2008 dried ethanol: water (70:30) extracts by spray drying and spouted bed dryer. They used a mixture of silicone dioxide and maltodextrine, as water-soluble carrier material, in a ratio of 2:1 with respect to the solid content of the rosemary extract.
However, the degradation of the phenolic compounds was quite high (ca. 50%) probably due to the high temperatures of the process (150°C).

The aim of this work is the study of antioxidants precipitation from ethanolic rosemary extracts by Supercritical Antisolvent Process (SAS) at mild temperatures. The encapsulation with polymers using the same process to protect the antioxidants and to improve its aqueous solubility is also evaluated.

2. Materials and Methods

2.1. Materials

Rosemary was collected in April and June 2010, in Peñafiel (Valladolid, Spain). Plants were stored at 4°C until needed for the extractions. For every experiment only the leaves were used, which were removed from the stems.

Ethanol of 96% purity, Folin-Ciocalteu reagent, gallic acid and sodium carbonate were purchased from Panreac Química (Spain). The polymers, Pluronic® F 88 (Poloxamer 238; HLB >24; $T_m = 54°C$) and Pluronic® F 127 (Poloxamer 407; HLB = 18 – 23; $T_m = 57.6°C$), were a gift from BASF. All products were used as received. Chromatographic standards, rosmarinic acid and carnosic acid, were purchased from Sigma-Aldrich. Acetonitrile, acetic acid and methanol (all HPLC gradient grade) were purchased from Panreac Quimica (Spain). Water was Milli-Q quality. These solvents were degassed and filtered through a 0.22 μm membrane (Fluoropore™, Millipore) before their utilization.

2.2. Methods

2.2.1. Preparation of Rosemary ethanolic extracts and polymer solutions

Extraction was performed according to a previous work (Navarrete et al., 2011). First, the leaves were de-oiled by solvent free microwave extraction (SFME): rosemary leaves (50 g) were put into a microwave apparatus and subjected to 450 W for 5 min. Secondly, 200 mL (96 %) of ethanol preheated at 40°C were added (ratio 4:1 v/w) and the mixture was stirred by rotation at 55 rpm. After 4 hours, the extract was filtered (MF-Millipore™, pore size 0.45 μm) by vacuum at 20 mbar, the liquid phase was recovered and stored at 4 - 6 °C, before use.
Deoiling, previous to extraction, increases the amount of extractable antioxidants in the plant material (Navarrete et al., 2011; Rodriguez-Rojo et al., 2011). Besides, essential oil monoterpenes, such as camphor or 1,8-cineole, responsible for the specific taste and odor of the spice, are eliminated since universally accepted antioxidants should be odorless, flavorless and colorless (Bailey et al., 1999).

For the co-precipitation experiments, the polymer, either Pluronic® F88 or Pluronic® F127, was dissolved in the extract in a mass ratio with respect to the dry content of the extract of 2.5:1.

### 2.2.2. SAS (Supercritical Anti Solvent): Precipitation and co-precipitation experiments

The flow diagram of the equipment used for the supercritical anti solvent precipitation is shown in Fig. 1. The CO₂ used is cooled down before being pressurized with a diaphragm pump (Dosapro, France). Afterwards, it is heated up to the required operating temperature. The CO₂ mass flow is measured with a coriolis flow meter. When the mass flow of CO₂ is constant and the working pressure and temperature remain stable, the solution is pumped by a chromatographic pump (Jasco PU 2080 - Plus) into the precipitator at the desired flow rate.

The precipitator is an insulated and jacketed AISI 316 stainless steel vessel of 1.5 L of volume. This precipitator is equipped with a Pt -100 thermoresistance with an accuracy of ± 0.1 K and a membrane digital pressure meter with an accuracy of ± 0.25 bar to measure operating conditions.

The inlet of the fluids is made through a concentric tube nozzle placed at the center top of the precipitation vessel; the nozzle consists of a 1/16 in. tube (inner diameter: 1 mm) for the solution, placed inside a 1/4 in. tube (3.2 mm i.d.) for the CO₂. At the bottom of the vessel there is a porous metallic frit with a screen size of 1 µm. There is also an external stainless steel filter, which has a screen size of 1µm.

The pressure in the precipitator is controlled by needle valves placed in parallel for safety reasons. Additionally, the valves and the outlet tube are electrically heated to prevent freezing or plugging. A vessel is used to achieve the separation of solvent and CO₂ after pressure release.
When the desired amount of solution has been injected (25 mL), the liquid pump is stopped and only pure CO₂ is fed for 10 minutes at a four times higher flow rate and the same operating conditions to ensure the complete removal of organic solvent from the precipitator. Finally, the precipitator is depressurized and the particles are recovered (10 – 1000 mg). The precipitate is stored under nitrogen atmosphere, protected from light and at temperatures below 5°C, to avoid the decomposition of the product, before their analysis.

2.2.3. Product analysis

The precipitation yield was determined by weighing the total amount of particles collected in the precipitator related to the total amount of soluble solids in the original solution.

The total soluble material concentration of the extracts was determined by drying 25 mL of extract under vacuum at 40°C on a rotatory evaporator. The mean experimental uncertainty was 5%.

As the yield of the precipitation was determined by comparison with this value, its mean uncertainty was also 5%. The dried extract was analyzed for total polyphenol, carnosic and rosmarinic acids content, as well, as reference for the SAS procedure.

The polyphenol content of the particles was measured by Folin-Cicocalteu method (Singleton et al., 1999) using gallic acid as reference compound; hence, total phenolics were determined as gallic acid equivalents (GAE). Samples were prepared by dissolution of ca. 10 mg of powder in 2 mL of ethanol for pure extract powder, or ca. 40 mg of encapsulated product in 2 mL ethanol. The analysis was carried out in triplicate and compared to the maximum loading achievable considering the initial amount of total polyphenols in the feed, pure rosemary extract or the solution of the polymer in the extract.

Additionally, major components of the rosemary extract (rosmarinic and carnosic acid) were determined by high performance liquid chromatography (HPLC) according to the method of (Wellwood & Cole, 2004) adapted from Cuvelier et al., (1996). It was performed on a reversed phase C18 Hypersil- ODS column (25 cm x 4.6 mm, 5 μm pore size; Supelco). The sample volume of injection was 20 μL; liquid samples were injected directly and for the solid samples, 20 mg of the product was
dissolved in 0.5 mL of ethanol. The mobile phase was programmed with a linear gradient elution method from 90% A (840 mL of deionized water with 8.5 mL of acetic acid and 150 mL of acetonitrile), 10% B (methanol), to 100% B in 30 min, with a flow rate of 1.5 mL/min. The system was left to stabilize for 3 min between consecutive injections. The column temperature was 25 °C. The samples were detected by UV at 284 nm. The compounds were identified by comparison with the relative retention time of standards in ethanol, calibrated between 0.2 and 20 mg/mL, and with reference to a published chromatogram (Cuvelier et al., 1996). Before HPLC analysis, the samples were filtered through a 0.2 μm nylon membrane filter (Millex GN from Millipore). The maximum uncertainty of the analysis is 4%. The presented values are the mean of three independent experiments of precipitation, to test reproducibility, and the mean error amounts to 20%.

For particle characterization of the collected precipitates scanning electron microscopy (SEM) micrographs were taken by means of a scanning electron microscope model JEOL JSM-820. Particles of representative samples were gold sputtered in an argon atmosphere at room temperature before examination.

Differential scanning calorimetry (DSC) assays of pure and encapsulated extract samples were carried out with a DSC-30 METTLER apparatus. Analyses were performed from -50 to 250°C, at a heating rate of 10 °C/min and 60 mL N₂/min.

2.3. Dissolution Test

The dissolution rate of the antioxidants from the extract precipitate and its polymer co-formulations in isotonic phosphate buffer pH 6.8 was measured. Additionally, physical mixtures of the polymer and the extract in the same ratio have been also tested to investigate whether the effect of the polymer on the dissolution rate is due just to its nature as surfactant or to interactions between the polymer and the compounds of the rosemary extract formed during the co-precipitation process. Samples of powder (ca. 200 mg) were placed in 25 mL of solution at 37°C. The mixture was stirred at 100 rpm for 8 hours and 2 mL aliquots were taken at pre-defined intervals. The sample volume was replaced with fresh buffer solution. The aliquot was filtered through a
membrane filter (0.2 μm, Millex GN from Millipore) and the filtrate was analyzed directly by Folin-Cicocalteu method to quantify the total amount of polyphenols. The presented values are the mean of two independent experiments of dissolution and are expressed in terms of % dissolved polyphenols, that means, the actual polyphenol concentration in the solution divided by the polyphenol loading of the particles (determined as in section 2.3.3) and multiplied by 100.

3. Results

3.1. SAS Precipitation

The influence of the main variables of the supercritical antisolvent process, pressure and temperature, was studied in the range from 8 – 12 MPa and 25 – 50°C. Other operational parameters such as CO₂ mass flow rate and solution flow rate were fixed according to previous experience of the group (Sosa et al., 2011) at 0.7 kg/h and 1 mL/min, respectively.

The rosemary extract used had a mean solid content of (2.7 ± 0.1) %wt. with a mean polyphenol content of (110 ± 30) mg GAE/g solid. The mean rosmarinic and carnosic acid concentration was (34 ± 8) mg/g and (58 ± 15) mg/g, respectively.

Results in terms of polyphenol, rosmarinic and carnosic acids content per mass of solids (C_{poly}, C_{ros}, C_{car}) and global yield of solids (% η_G) are displayed in Table 1.

As shown in Table 1, the recovery of antioxidants is low; the maximum is achieved at 12 MPa and 35 ºC with 13.3 %. Nevertheless, the concentration of antioxidant in the powder is, in general, increased with respect to the reference dried extract obtained by rotaevaporation; at 8 MPa and 50ºC, it is almost doubled.

Particle size of rosemary extract precipitates was analyzed by SEM micrographs (Figure 2). At all operating conditions, individual particles are below 1 µm (Figure 2.a). However, they form agglomerates up to 200 μm depending on the operating conditions. Increasing temperature at constant pressure decreases the size of agglomerates from 200 μm, at 25°C and 10 MPa (Figure 2.b), to 50 μm, at 50°C and 10 MPa (Figure 2.c).
To check if the low recovery of antioxidants was due to degradation or to loss of solids due to a small total amount of solids and individual particle size in the submicron range, the polyphenol content of the effluent was analyzed. Nevertheless the mass balance was not closed; there was a deficit of antioxidants of approximately 25%. This deficit is likely due to the difficulties in the recovery of the precipitated powder from the vessel and filter devices.

However, this means that more than 50% of antioxidants are lost within the effluent. This cannot be due to solubility of rosemary antioxidants in the CO$_2$-ethanol phase at operating conditions. According to literature (Cháfer et al., 2005), the solubility of carnosic acid in CO$_2$ with a 6 molar % of ethanol as a co-solvent, close to the concentration achieved in the precipitation vessel, at 27.5MPa and 50°C, is 0.018 mg/g. It decreases with temperature and increases with pressure and co-solvent concentration. Extrapolating to operating conditions in the SAS experiments, it would imply a loss of carnosic acid between 5 to 10 wt.%. Another plausible reason for the low yield achieved is the loss of individual particles through the filters due to its small particle size, as shown in Figure 2, hence only agglomerates could be retained. Additionally, the kinetics of the precipitation of rosemary antioxidants could be too slow and take place mainly outside of the precipitator.

To increase the yield of precipitation and the yield of recovery of antioxidants different parameters were changed: concentration of the solution and diameter of the nozzle. The concentration of the solution was increased by partially evaporating the ethanol from the extract at vacuum. The nozzle diameter was adapted by connecting a stainless steel tube of 7mm length and 0.130 mm in diameter to the 1/16 in. tube for the solution. Most significant results are shown in Table 2.

Concentration of initial solution seems to play a major role increasing the global yield of precipitation up to 90%. The increase in the initial concentration of the solution leads to a faster supersaturation, in agreement with the possible reasons for the low yield of the process. However, a purification of the extract (e.g. higher concentration of polyphenols, rosmaric and carnosic acid in the SAS powder with respect to the powder obtained by vacuum evaporation) is not achieved.
The reduction in nozzle diameter can have an **effect on** mass transfer whenever the operating conditions are inside the two-phase region of the system: solute-solvent-antisolvent. The effect of reduction in the one phase region is the decrease in particle size. In principle, the solute is considered not to have a significant **effect on** this system, so only the solvent-antisolvent (ethanol – CO$_2$) phase diagram is taken into account. According to this diagram (Chiu et al., 2008), the experiments at 40°C and 10 MPa are carried out in the 1 phase region and those at 50°C and 10 MPa are at the boundary of the two phase region. However, there is a significant increase in global yield when using the 0.130 mm diameter nozzle at 40°C and 10 MPa, whereas there is no noteworthy change at 50°C and 10 MPa. There is no clear reason for this observation; it is probably related to aforementioned mechanical limitations in the recovery of the particles due to the small amount processed.

### 3.2. Co-precipitation

The solutions of polymer in pure ethanol (96%) were processed at the most favorable operating conditions for the precipitation of the extract (50°C and 10 MPa). However, no particles were obtained even when it was processed with rosemary ethanolic extracts; similar findings were reported in literature: Poloxamer 407 processed from dichloromethane solutions or Poloxamer 188 from solutions of ethanol/ chloroform, were only successfully precipitated at 8 MPa and 35°C when drug crystals acted as seed and thus providing heteronuclei for the precipitation of polymer (Majerik et al., 2007).

In this case, pressure was increased in order to get a faster precipitation of the rosemary extracts. The extracts were successfully co-precipitated with both poloxamers at 14 MPa and 50°C. The rosemary extracts used in these experiments had a mean solid content of (3.7 ± 0.1) %wt. with a mean polyphenol content of 91 mg GAE/g solid; hence the polymer concentration in the solution was 9.2 %wt, to keep a ratio of 2.5:1 between the polymer and the dry content of the extract. This was also the concentration of pluronics used in previous experiments to precipitate the pure polymer.
Also, precipitation of extract alone was performed at the same operating conditions to verify the recovery of carnosic and rosmarinic acid, 46% wt and 13%, respectively. The global yield of solids was 48% with a mean polyphenol content of 91 mg GAE/g solid.

From DSC analysis (Figure 3), the co-precipitation of the extract and the polymer can be verified. After SAS processing the melting temperature of the polymer is decreased from 57.6 °C to 52.0°C, and also the melting peak is broader indicating that the processed polymer is less crystalline. The presence of the rosemary extract in the co-precipitated product is evidenced by the negative slope of its base line with respect to the unprocessed polymer, due to the superposition of the extract profile on the flat base line of the unprocessed polymer.

The loading of the polymer particles with the extract was determined through polyphenol content by Folin-Cicocalteu method resulting in 30 mg GAE/g in F127 and 23 mg GAE/g in F88 (the mean standard deviation was 10%). This means a ca. 100% encapsulation efficiency (110% and 90%, respectively) taking into account that the polyphenol content in the precipitation of the extract alone at the same operating conditions was 91 mg GAE/g and supposing a similar global precipitation efficiency of the polymer and the extract. Consequently, the encapsulation process avoids the loss of antioxidants that was observed during the precipitation experiments of pure extract.

Particle size rosemary extracts encapsulated in Pluronic was also below 1 µm according to SEM micrographs (Figure 2.d); moreover, the size of the agglomerates was reduced to values between 5 – 20 µm, due to the increase in pressure (14 MPa) and the presence of the polymer. Only the product obtained with Pluronic F127 is shown as it looked similar with both polymers.

Additionally, the polyphenol release from polymer co-precipitates was measured and compared to the profile from the pure extract SAS product and physical mixtures thereof with both polymers (Figure 4). It is shown that all polyphenols (F88: 100%; F127: 88%) are released from the co-formulations in the first hour, whereas only the 15% of the polyphenols are released from the pure extract product. The decrease of the amount of dissolved polyphenols with time in polymer formulations, 20% in both cases, can be due to degradation by the oxygen content in the phosphate
The improvement in the dissolution rate of the polyphenols is not only due to the effect as surfactant of both polymers, but to the co-precipitation process as physical mixtures of the processed extract and the polymers dissolved to a lower extent (F88: 60%; F127: 75%). Moreover, the degradation of polyphenols during the experiments was significantly higher in the physical mixtures; above 50% for both polymers.

4. Conclusions

Supercritical antisolvent process shows to be promising for the encapsulation of rosemary ethanolic extract with poloxamers to obtain a readily aqueous soluble powder; as shown by the polyphenol release profile, ca.100% of the polyphenols are dissolved in a phosphate buffered aqueous solution (pH = 6.8) after one hour from the encapsulated product, while ca.65% of the antioxidants are dissolved from the pure extract precipitate using the polymers as surfactants and only ca.3% of the polyphenols from the pure extract precipitate are solubilized. Besides, the protection against degradation factors during the dissolution is higher in the co-precipitated product.

The obtained particles are in the submicron range, as well as the pure precipitated particles, although they build up agglomerates between 5 to 20 µm.

This encapsulation process seems to be promising concerning its coupling with supercritical fluid techniques to enrich ethanolic extracts, such as the supercritical antisolvent fractionation process developed by Visentin and co-workers (2011).

Acknowledgments

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References


Figure captions list

Figure 1. Schematic diagram of the SAS pilot plant.

Figure 2: SEM. a) SAS precipitate with F-127 at 14MPa and 50°C b) SAS precipitate at 10 MPa and 35°C c) SAS precipitate at 10 MPa and 50°C d) SAS co-precipitate with F-127 at 14MPa and 50°C

Figure 3. DSC analysis: ■ SAS co-precipitation of extract and Pluronic® F127 at 14 MPa and 40°C. – unprocessed Pluronic® F127; --- SAS precipitation of extract at 14 MPa and 40°C.

Figure 4: Polyphenols release profiles from different co-formulations (◆ F127 and ■ P88), pure precipitated rosemary extract (▲) at the same operating conditions (14 MPa and 50°C), and physical mixtures thereof (♦ F127 – SAS and □ P88 – SAS).
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Table 1. Effect of temperature and pressure on the polyphenolic content and yield of the SAS precipitated rosemary extracts

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<th>T (°C)</th>
<th>P (MPa)</th>
<th>C_{poly} (mg GAE/ g)</th>
<th>C_{ros} (mg / g)</th>
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* The amount of sample was not enough for the analysis
Table 2. Effect of nozzle diameter and solids concentration on the polyphenolic content and yield of the SAS precipitated rosemary extracts

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<th>C_{ros} (mg/g)</th>
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