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# Micronization of vanillin by rapid expansion of supercritical solutions process

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ABSTRACT

Uniform microparticles of vanillin were precipitated by the Rapid Expansion of Supercritical Solution (RESS) process and this reduced the commercial particle size by a factor of one hundred. A design of experiments full factorial at two levels was performed. The effects that pressure, temperature, contact time and nozzle diameter had on the particle size and yield of the RESS precipitation were evaluated. Pressure and temperature were the factors that had the most marked effects on particle size and yield. The use of higher pressure and temperature is recommended to obtain the smallest particle size and the highest yield. However, at lower pressure the temperature is a crucial factor in that the use of a lower temperature led to considerably smaller particles and good yields whereas a higher temperature gave the highest particle size and the lowest yield. A higher contact time and smaller nozzle diameter led to slight improvements in the vanillin particle size and yield according to the results of the design. The crystallinity of the RESS-processed vanillin was unaltered when compared to the unprocessed material.

## 1. Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) ($C_8H_8O_3$) is the main component (80–90%) [1] of vanilla orchid pods and it has an attractive flavor and fragrance. The current global demand for vanillin is estimated to be roughly 20,000 tons per year [2] and it is becoming one of the most important aromatic substances. Vanillin is often used as a flavoring agent, a food preservative, in beverages, cosmetics and drugs due to its antioxidant [3–5] and antimicrobial [6–8] activities and it is generally regarded as a safe (GRAS) substance [9]. Vanillin is also used as a crosslinker and in the preparation of a wide range of vanillin-based renewable polymers, e.g., phenolic, epoxy and benzoxazine resins, polyesters, and acrylate and methacrylate polymers are in the focus of numerous investigations [10,11].

Vanillin has been widely used as the commercial product (~700 μm particle diameter) and this has low solubility in cold water, although the solubility increases with temperature. However, this solubility could be improved by reducing the particle size to the micrometer range. There is very little literature concerning the micronization of vanillin although composites and microencapsulates of vanillin has been obtained: microcapsules containing limonene and vanillin with a mean particle diameter of 30 μm [12] were prepared by Pitol-Filho et al. using ethyl cellulose as a coating agent by dropping the polymeric solution into a coagulation bath, which contained water, sodium dodecyl sulfate, and acetic acid in different concentrations. Noshad et al. carried out the microencapsulation of vanillin with maltodextrin using a spray dying method and this process gave a wide particle diameter range (0.7–128 μm) [13]. A maximum encapsulation efficiency of 58.3% and minimum particle size of 6.95 μm were achieved at 184 °C with a maltodextrin concentration of 8.5% and vanillin concentration of 0.36%.

The majority of the micronization methods involve the use of excessive amounts of organic solvent, high temperatures and post-processing steps and the final products often contain residues. These drawbacks could be circumvented by using supercritical $CO_2$ ($scCO_2$) technologies due to the low toxicity of this compound and the absence of organic solvent in the process, thus contributing to a substantial improvement in human health and environmental considerations.

The application of supercritical fluid technology in micronization processes requires a study of the phase equilibrium formed by the solute and the supercritical $CO_2$, i.e., the fluid-solid phase equilibrium. Binary data for the solubility of vanillin in $CO_2$ have previously been reported in the literature [14,15]. Molar fractions from 0.014 to $1.295 \times 10^{-2}$ were obtained in the pressure range 80–300 bar and the temperature range 313–353 K, which shows that vanillin is quite soluble in supercritical $CO_2$. In this way, the Rapid Expansion of Supercritical Solutions

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(RESS) process could be used to micronize this compound. In the RESS process a given amount of vanillin is placed into a solubilization chamber, which is filled with $scCO_2$ to give a supercritical solution after a certain time. The subsequent sudden expansion of this solution through a nozzle to atmospheric conditions generates a high level of supersaturation and produces rapid nucleation and the precipitation of particles on the vessel wall.

The work described here concerned a study of the micro-precipitation of vanillin by the RESS process and a design of experiments was applied in order to identify the main parameters that affect the process in terms of particle size, size distribution and yield. The resulting vanillin microparticles were analyzed by X-ray diffraction to evaluate the degree of crystallinity after the supercritical process.

## 2. Materials and methods

Vanillin (4-hydroxy-3-methoxybenzaldehyde) ($C_8H_8O_3$, 99% purity) was purchased from Sigma-Aldrich (Spain). $CO_2$ with a minimum purity of 99.8% was supplied by Linde (Spain).

### 2.1. Experimental design

A design of experiment (DOE) was carried out in order to identify the critical factors in the vanillin miconization by the RESS process. A full factorial design at two levels was performed. Four factors ($2^4$) were studied but the complete design consisted of 19 experimental points that included 16 factor points and three replications at the center point (experiments 17–19). The critical factors were selected with appropriate ranges for this design. The responses of the design were mean particle size and yield. Yield was calculated by subtracting the weight of powder precipitated from the initial weight of vanillin placed into the solubilization chamber (0.5 g). Other full factorial designs have been successfully applied for screening purposes [16–19].

Pressure (P), temperature (T), nozzle diameter ($Ø_n$) and contact time (t) were identified as possible parameters that could influence the particle size and size distribution of vanillin in the RESS process. The two levels for each factor are shown in Table 1. The levels of pressure and temperature were selected to evaluate the influence of different supercritical $CO_2$ densities and therefore solvent power. Nozzle diameters were selected according the those available from the equipment supplier. The contact times were selected in order to ensure that there was sufficient time to form a homogenous supercritical solution and to keep the costs reasonable.

### 2.2. RESS process

The experiments listed in Table 2 were carried out in a pilot plant developed by Thar Technologies® (model RESS250). A schematic diagram of this equipment is shown in Fig. 1. Pressures of 100–300 bar, temperatures of 313–343 K, nozzle diameters of 100–200 μm and contact times of 1–2 h were evaluated.

The RESS250 equipment comprises a high-pressure pump (P1) to fill a 250 mL stainless steel solubilization vessel (V1) with $CO_2$. This vessel is heated by an electrical heating jacket (V1-HJ1) that surrounds the vessel. Supercritical $CO_2$ and vanillin were continuously mixed with a magnetic stirrer (maximum speed 2500 rpm). At the end of the line there is a stainless steel collection vessel (V2) in which particles are precipitated once the supercritical solution is expanded through a stainless steel nozzle with an inner diameter in the range 100–200 μm (Thar Technologies). The equipment was described in detail in a previous publication [20].

**Table 1**
Two – Level assessment for each factor and calculated effects on PS.

| Factors | Low level | High level | PS Effects | Yield Effects |
|---|---|---|---|---|
| P (bar) | 100 | 300 | −6.49 | 53.37 |
| T (K) | 313 | 343 | 5.29 | −15.87 |
| t (h) | 1 | 2 | −1.37 | −3.62 |
| $Ø_n$ (μm) | 100 | 200 | 2.41 | −1.12 |

The experiments were performed as follows: Firstly, the commercial vanillin powder (0.5 g) was placed in the solubilization chamber. The system was held for a certain time once supercritical conditions for $CO_2$ (pressure and temperature) had been achieved in order to ensure complete equilibration. Valve MV2 was then opened and the supercritical solution was expanded through a nozzle, which was pre-heated in order to compensate for the heat loss and to prevent the nozzle from clogging during the fast expansion. The precipitated particles were collected on the wall of vessel V2 for subsequent analysis.

### 2.3. Sample characterization

Scanning electron microscopy (SEM) images of the powder precipitated on the wall of the vessel were obtained using a NOVASEM scanning electron microscope. Prior to analysis the samples were placed on carbon tape and then covered with a coating of gold using a sputter coater. Particle size distribution (PSD) and mean particle size of the processed samples were measured using DLS technology (Nano-ZS, Malvern Instruments, UK). This technique measures the diffusion of particles moving under Brownian motion, and converts this to a size and a size distribution using the Stokes–Einstein relationship. Non-Invasive Back Scatter (NIBS) technology was incorporated to give the highest sensitivity along with the highest possible size and concentration range. The measurement range was from 0.3 nm to 10 μm. Prior to analysis, the samples of vanillin were weighed and suspended in hexane (1 mg/mL) with one drop of surfactant (Tween 80).

The PSD and mean particle size of commercial vanillin could not be processed by this technique due to its large size, which gave rise to an unreliable measurement. In this case, an LS 13 320 laser diffraction particle size analyzer from Beckman Coulter was used. This system was able to measure particles up to 2000 μm. Polarization Intensity Differential Scattering (PIDS) technology was employed in which the particle size distribution could be determined by measuring the variations between the horizontally and the vertically scattered light for each wavelength. In this case the sample was introduced as a solid powder using a module (Tornado) that could handle dry powder.

X-ray diffraction (XRD) analysis was performed on Bruker D8 Advance diffractometer to determine the amorphous or crystalline nature of the precipitate obtained by both processes. All diffraction patterns were scanned from 10° to 80° in 2θ angle with a step size of 0.02° and one second as the step time.

### 2.4. Statistical analysis

Each experiment was carried out following the order established by the design. Influence of each factor on the particle size and yield of vanillin was statistically assessed by ANOVA with 95% confidence level. Data obtained from the partial least square (PLS) regression were statistically analyzed using ANOVA for the response variable in order to test the model significance ($p < 0.05$) (Table 3). The Modde (Version 5.1, Unmetrics, USA) software was employed to analyze the results and optimize the experimental conditions. Significance of pressure, temperature, nozzle diameter and contact time on particle size and yield were established at 95% of confidence level (Table 4).

## 3. Results and discussion

### 3.1. Analysis of the design of experiments

The values for the coefficient of determination $R^2$ (adjusted and experimental), degree of freedom (DF), sum of squares (SS) and mean