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# From sulfite liquor to vanillin: effect of crystallization process on the yield, purity, and polymorphic form of crystals

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**ABSTRACT**

Different approaches for vanillin crystallization were performed using an aqueous solution, with low concentrations of this potential food and pharmaceutical phenolic compound, obtained from adsorption/desorption experiments applied to an oxidized solution of lignin isolated from softwood sulfite liquor. The process yield and purity of the vanillin crystals were determined, and the thermal stability of the two resulting polymorphic forms was analyzed by DSC. Crystallization processes by swift cooling and evaporation with heat favor the nucleation of metastable vanillin crystals (Form II) with lower yields than slow evaporative and cooling processes that crystallize vanillin into the stable polymorph (Form I) with yields around 80%. Moreover, the polymorphic form of vanillin, yield, and purity were found to be highly dependent on the conditions of the crystallization process, mainly the temperature and the range of temperature variation. The melting points of polymorphic Form I and II were found to be 82.6 and 81.0°C, respectively, proving that Form I is the most thermodynamically stable vanillin crystal form. In conclusion, the process conditions for vanillin crystallization strongly affect the polymorphic form, the process yield, the contents, and the type of impurities in the final product, consequently, influencing its potential industrial applications.

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**KEYWORDS**
Sulphite liquor; vanillin; liquid-liquid extraction; crystallization; polymorphism

## Introduction

Vanillin (4-hydroxy-3-methoxy-benzaldehyde, Figure 1) is a phenolic aldehyde and a moderate hydrogen bond acceptor with reactive methoxyl, hydroxyl, and aldehyde sites.[1] It has a strong aroma and is one of the most demanded ingredients in the flavor industry. In the global market, about 60% of industrial vanillin is used as a food additive, while the remaining 40% is used as cosmetic ingredients (approximately 33%) and pharmaceuticals (roughly 7%).[2] Due to its valuable antimicrobial, antioxidant, anticarcinogenic, and antislicking properties, vanillin shows great potential as a chemical intermediate to produce pharmaceuticals such as papaverine, levodopa, ethamivan, and cyclovalone, and fine chemicals such as veratraldehyde, protocatechualdehyde.[3,4] The rising demand for vanillin for end-use industries is expected to substantially influence market growth in the next years. In 2016, the global demand for vanillin was about 18,600 tons and will grow by 6.2% by 2025.[5]

Vanillin was first isolated from Vanilla planifolia pods by Gobley in 1858 and years later was the first essence artificially synthesized by German M. Harman and Dr. Twyman in 1874.[6] The commercial vanillin market is served by three important sources namely, vanillin from natural vanilla beans, accounting for less than 1% of the total vanillin production worldwide, vanillin produced from chemical/petrochemical sources, and vanillin produced from lignin derived from wood pulping process.[7] Synthetic vanillin has been produced industrially since 1894 and is today obtained either by processing waste sulfite liquors containing lignin or by synthesis from guaiacol (roughly 85%).[8] Considering the environmental sustainability and economic relevance, lignin, the second most available renewable raw material with around 50 million tons per year produced, is identified as a promising source of vanillin production.[9] Currently, the production of vanillin from wood accounts for about 15% of the total vanillin production and Borregaard is the world's largest producer of bio-based vanillin, which produces vanillin from lignin via sulfite pulping method. Lignin-derived vanillin, which is marketed as a premium product, is priced at $100–200 per kilogram.[5] Over the years, several methods have been employed to recover the vanillin in mother liquors, e.g., repeated recrystallization from methanol-water, distillation, and fractional

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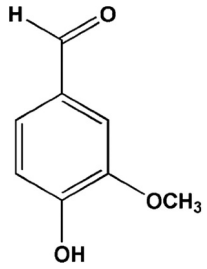


**Figure 1.** Vanillin (4-hydroxy-3-methoxy-benzaldehyde) structure.

precipitation of phenolate salts. None were successful due to the lack of purity of the resulting material, which contained more than 15% impurities, or the method was too laborious and costly to be economically feasible.[10] The main impurities of vanillin derived from lignin consist of low molecular weight compounds, such as *p*-hydroxybenzaldehyde, acetovanillone, and 5-formyl vanillin, that are difficult to separate from lignin.[10,11] Moreover, it is well established that for technical applications the vanillin purity needs to be over 97%, while the vanillin grade suitable for food applications should be higher than 99.8%.[12]

An integrated process intended to produce added-value products, such as vanillin, by directing a stream of mother liquor from a pulp and paper mill has been developed over the years in LSRE (Laboratory of Separation and Reaction Engineering).[13] In this process, vanillin obtained from lignin oxidative depolymerization is recovered using a combination of sequential steps of nano/ultrafiltration and adsorption/desorption experiments or ion-exchange processes.[12] However, the final purification step of vanillin, which includes liquid–liquid extraction and crystallization, represents a laborious task and the final product still contains impurities.[14] Crystallization is widely used as a method to isolate and purify products with specific physicochemical properties in pharmaceutical, food, fine chemicals, and bulk chemical industries. Cooling a compound from a supersaturated solution by decreasing the temperature has been the most common method of crystallization for the past 50 years, although antisolvent, reactive, and evaporative methods are also commonly employed.[15,16]

Crystallization is a complex and challenging process, where the purity, crystal size, and polymorphic form obtained need to be controlled and understood. Polymorphism is the ability of a chemical entity to crystallize into different structures where the atoms, ions, or molecules are packed differently within the crystal lattice.[17] Moreover, for each polymorph, the physicochemical properties, such as melting point, solubility, density, and stability, are influenced by the differences in crystal packing and/or structural conformation. The control of polymorphism is an important factor in maintaining high product quality and reproducibility in the food and pharmaceutical industry and it is a key issue in several scientific, technological, and industrial fields.[17,18] In 1950, W. C. McCrone reported that vanillin crystallization could occur in four different polymorphic forms: Form I, the stable crystalline form, Form II, the metastable form, Form III, and Form IV.[17] Most of the literature reports disclose only the crystallization of the stable polymorph of vanillin – Form I, which can be crystallized at normal growth conditions and has several industrial applications.[17,19] On the other hand, very few reports exist on the crystallization of metastable polymorph of vanillin – Form II, whose nucleation is more difficult to occur. Form III and Form IV are highly unstable, and their crystallization process is not completely understood.

In this work, and for the first time, a solution obtained from adsorption/desorption experiments applied to an oxidized solution of lignin isolated from softwood sulfite liquor was used for different approaches to cooling and evaporative vanillin crystallization. The main objective was to study the influence of the crystallization process on the yield, purity, and polymorphic form of vanillin crystals.

## Material and methods

### *Sulphite liquor and lignin sample*

Lignin was obtained from a softwood sulfite liquor (11.3% $w/w_{liquor}$ total solids) supplied by a pulp mill of Company A. The liquor was submitted to ultrafiltration and the resulting retentate was freeze-dried to obtain the lignin used in this work. In a previous publication, the authors detailed the ultrafiltration process, the respective operating conditions, and the main structural characteristics of lignin.[20]

### *Lignin depolymerization - alkaline oxidation with oxygen ($O_2$)*

The process of oxidative depolymerization of lignin in an alkaline medium as the high-performance liquid chromatography (HPLC) analysis, for quantification of the oxidation low molecular weight phenolic products, were performed as already described in literature.[20] Briefly, lignin oxidation in an alkaline medium using $O_2$ was performed in a Büchi AG laboratory autoclave. For the oxidation reaction, about 30 g of lignin was dissolved in a sodium hydroxide (NaOH, 99.8%, AkzoNobel) solution (500 mL, 80 $g.L^{-1}$), introduced into the reactor, heated to 120°C, and pressurized. The reaction time started when the initial temperature

reached 120°C. At this time, $O_2$ was introduced starting the data acquisition. The total pressure in the reactor was kept at 9.8 bar with a partial pressure of $O_2$ of 3.0 bar by continuous supply along the time. The reaction samples were collected at preset time intervals. The heating, temperature, $O_2$ flow rate, and total pressure inside the reactor were controlled during the reaction.

### Removal of high molecular weight lignin fractions from the oxidized mixture

The oxidized mixture of lignin oxidation, without any neutralization, was filtered using a sequence of ultra and nanofiltration. Two different flat sheet membranes with molecular weight cutoff (MWCO) of 2 kDa and 600–800 Da, respectively, were used in a GE Osmonics SEPA CF II crossflow filtration system. Both membranes are made of a thin film composite of polyamide with an effective membrane surface area of 0.014 $m^2$. Ultra and nanofiltration were performed in recirculation mode at a transmembrane pressure (TMP) of 18 and 30 bar, respectively, and a feed flow rate of 156 L/h. The permeate solution obtained from the nanofiltration was then fed to the adsorption/desorption column without further treatments.

### Adsorption/Desorption process

The experiments were performed in a jacketed glass column (Merck, Darmstadt) with an internal diameter of 26 mm and 130 mm of total height, packed with nonpolar resin SP700 with an average particle size of 250 µm, previously activated. Resin properties and activation procedure were already described elsewhere.[21] The preparative chromatography column was connected to a KNAUER pump (model Smartline P1000), with a maximum volumetric flowrate of 50 mL/min and kept at 25°C using a thermostatic bath (LAUDA, model RE 206). The permeate solution from nanofiltration, with high pH, was fed to the adsorption/desorption column for 90 minutes and a liquid volumetric flow rate of 5 mL/min. The desorption was made using two eluents: first with deionized water for 45 minutes and then ethanol (99%, Fisher Scientific) for more than 45 minutes, both at a volumetric flow rate of 5 mL/min. Water desorbs less strongly adsorbed species while ethanol desorbs more strongly adsorbed species. The concentration of all the main low molecular weight phenolic compounds in each fraction from the desorption experiments, as in the nanofiltration permeate, was determined by HPLC based on the equipment and conditions described in the literature.[22] The water desorption fraction rich in vanillin (Table 1), was selected to proceed to the crystallization.

**Table 1.** Concentration of vanillic acid, vanillin, syringaldehyde, and acetovanillone in the water desorption fraction from adsorption/desorption experiments.

| | Vanillic acid | Vanillin | Syringaldehyde | Acetovanillone |
|---|---|---|---|---|
| Concentration, g/L | 0.08 | 5.20 | 0.05 | 0.23 |

### Liquid-liquid extraction (LLE)

The aqueous fraction rich in vanillin, obtained from the adsorption/desorption experiments, was subjected to an LLE step before crystallization. A scheme with all the steps of the sequential process from sulfite liquor to vanillin is shown in Figure 2.

The LLE was performed at a controlled pH to allow the separation of phenolic ketones, acids, and aldehydes based on their acid dissociation constants (pKa). The pKa of the main low molecular weight phenolic compounds present in solution is between 4.3 and 8.3. Thus, it is expected that these compounds have a negative charge in strong alkaline solutions, as the water fraction from desorption, which has a pH around 13. Moreover, it is expected that at the end of the LLE the organic solvent, ethyl acetate, extract all the phenolate species.

Two different LLE approaches were performed, the first with two sequential extractions and another one with only one extraction step. The first LLE approach (Figure 3) started with the aqueous solution rich in vanillin (100 mL) acidified, with an aqueous solution (1:1, v/v) of sulfuric acid ($H_2SO_4$, 96%, PanReac), until pH around 8.0. The extraction was made with 100 mL of ethyl acetate (99.9%, Fischer Scientific). Then, the resulting aqueous phase from the first extraction was acidified until pH 6.0 and used for a second LLE. The second extraction was made with the same organic solvent.

The second approach was performed with only one step of LLE, with ethyl acetate, and the aqueous solution rich in vanillin (100 mL) acidified until a pH near 6.0, also with an aqueous solution of $H_2SO_4$ (1:1, v/v). After the LLE, for both approaches, the organic phase obtained was evaporated in a rotavapor, and the evaporated material was dissolved in 50 mL of water. The aqueous and organic phases and the solution resulting after evaporation and dissolution with water of the organic phase were analyzed by HPLC for the quantification of all the main phenolic compounds, using the same equipment and conditions as in the previous section.

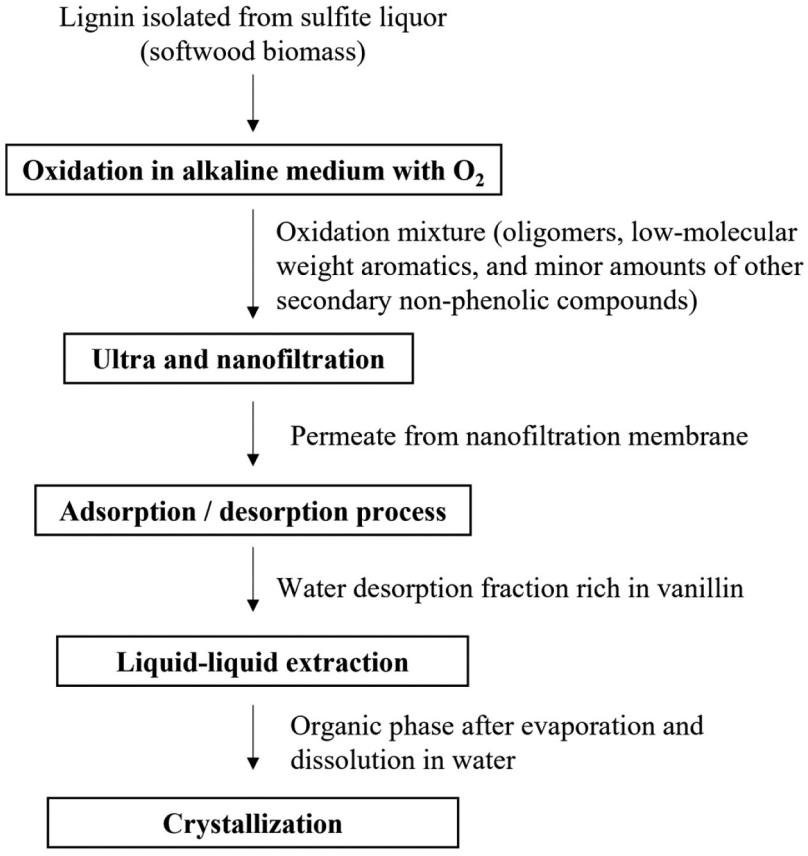


**Figure 2.** Scheme of the process from the oxidation of lignin from sulfite liquor until vanillin crystallization.

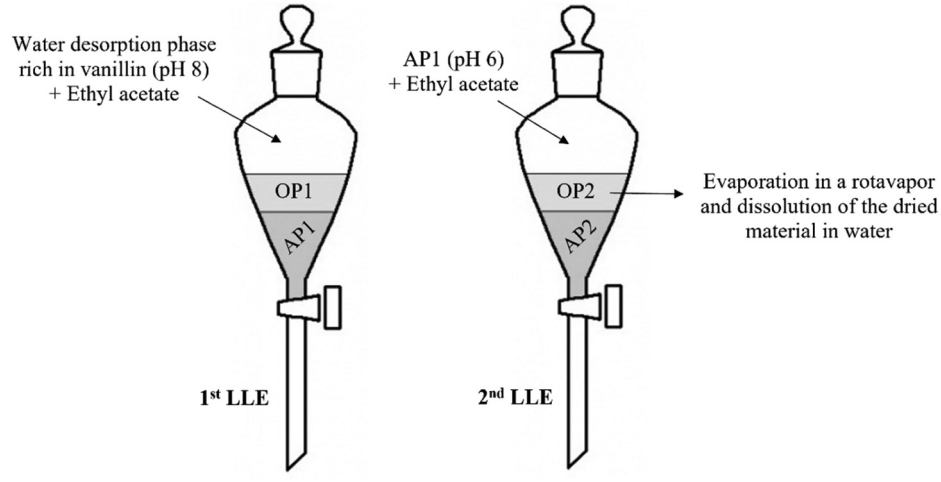


**Figure 3.** Scheme of the two-step LLE (OP1 and OP2 - organic phase from the first and second LLE, respectively; AP1 and AP2 - aqueous phase from the first and second LLE, respectively).

### *Vanillin crystallization*

In the process of vanillin purification, crystallization is considered the key step that strongly influences the type and purity of the final product. It is important to note that the aqueous solutions that proceed for crystallization, detailed in section 2.4, have low concentrations of vanillin, in contrast to most of the studies in the literature about vanillin crystallization, which work with saturated and supersaturated solutions. Vanillin is sparsely water soluble, 10 g/1000 mL water at 25°C, and its partial water solubility arises from the formation of hydrogen bonds between the surrounding water

molecules and polar groups in the vanillin molecule.[6] In this work, four different approaches to cooling and evaporative crystallization of vanillin were attempted using the aqueous solution resulting after evaporation and dissolution of the organic phase from one-step LLE. The experimental conditions of each crystallization procedure are described in the following sections.

### Cooling crystallization

For the cooling crystallization, 50 mL of the aqueous solution from LLE, after evaporation and dissolution of the organic material in water, was filled out into a 500 mL double-walled glass crystallizer (Figure 4), stirred at 250 rpm, and kept at 40°C for a few minutes. This slight heating guarantees the total dissolution of vanillin in the solution since its solubility in all solvents increases with an increase in temperature.[16] After that, the temperature was ramped from 40°C to 5.0°C at 0.1°C/min. The temperature inside of the double-walled bottle was controlled using a thermostatic circulating water bath (VWR model AP07R–40-V12V) and the agitation was maintained at 250 rpm using a magnetic stirrer impeller type. After several hours at 5.0°C, without the formation of vanillin crystals, the solution was left in a 500 mL glass crystallizer at room temperature (~25°C) for about 48 hours until the complete crystallization of the final product.

The temperature for the cooling crystallization process (5°C) was selected based on the solubility of vanillin in water.[8] In literature, it is stated that the solubility curve of this compound, in the range of 4 to 80°C, follows the polynomial equation $c = 0.0066T^2 + 0.1392T + 2.6530$, where "$c$" is the concentration of vanillin in g/L and "$T$" is the saturation temperature in °C.[14] Therefore, at 5°C the saturation is reached with a concentration of 3.5 g/L, a lower value than the vanillin concentration in the solution that proceeds for crystallization (5.20 g/L).

**Figure 4.** Glass crystallizer used for vanillin crystallization.

### Swift cooling crystallization

For swift cooling crystallization, the aqueous solution (50 mL) was heated at 40°C at a stirring speed of 250 rpm for a few minutes. Then, was swiftly transferred to the double-walled glass crystallizer of 500 mL that was kept cooled to 5.0°C and stirred at 250 rpm using a magnetic stirrer impeller type, to originate vanillin saturation in the aqueous solution. After 4 hours at 5.0°C and without vanillin crystallization, the solution was transferred to a glass crystallizer and left to evaporate slowly at room temperature (25°C) for about 48 hours.

### Slow evaporative crystallization

Evaporative crystallization was also carried out using the aqueous solution obtained after evaporation and dissolution of the organic phase from one-step LLE. Before crystallization, the aqueous solution (50 mL) was kept at 40°C for a few minutes. Then, the heated solution was left to evaporate slowly in a glass crystallizer at room temperature (~25°C). The resulting crystals were collected after the evaporation of the solvent, which took around 48 hours.

### Evaporative crystallization with heat

The aqueous solution (50 mL) was transferred into a glass crystallizer and placed on a heating plate with constant agitation at 250 rpm. The set temperature of the heating plate was 80°C. After the evaporation started, and when only about 10 mL of the solution remained, some drops of oily phase started to form. The heat was stopped, and 10 mL of water was added to the evaporated solution, to revert the oily phase. The resulting solution, about 20 mL, was left to evaporate at room temperature until complete evaporation of the solvent and crystallization of the final product.

### Purity of vanillin crystals

About 3.0 mg of the final dry product, obtained from each crystallization process, was dissolved in 5 mL of methanol until complete dissolution. The solution was analyzed by gas chromatography-mass spectrometry (GC-MS) and HPLC for the identification and quantification of contaminants, such as acetovanillone or syringaldehyde, and consequent determination of vanillin purity. The purity of the vanillin crystals obtained in each crystallization approach was defined through the ratio of the peak area value of vanillin to the total value of all contaminant's peak areas in the HPLC chromatogram obtained.

GC-MS and HPLC analyses were assessed using the equipment and experimental conditions described elsewhere.[14]

### Identification of vanillin polymorphs

The morphology of the vanillin crystals obtained from the different crystallization processes was observed using an Axiotech 100 HD optical microscope coupled to a high-resolution digital camera AxioCam 105 color. Images were processed using Zen software.

### Polymorphs characterization by differential scanning calorimetry (DSC)

The DSC thermogram was recorded to study the thermal stability and possible phase transformation of the grown polymorphic forms of vanillin. About 10 mg of each polymorphic sample was analyzed under a nitrogen atmosphere at a heating rate of 10°C/min from 40 to 200°C, using an STA 490 PC/4/H Luxx 134 Netzsch thermal analyzer.

### X-ray diffraction (XRD) analyses

X-ray diffraction (XRD) analyses were made, at room temperature, by a PANalytical X'Pert Pro diffractometer, equipped with an X'Celerator detector and secondary monochromator in θ/2 θ Bragg-Brentano geometry. The measurements were carried out using 40 kV and 30 mA, a CuKa radiation ($\lambda\alpha_1 = 1.54060$ Å and $\lambda\alpha_2 = 1.54443$ Å), in a 5–60° 2θ angular range.

## Results and discussion

### Liquid-liquid extraction (LLE)

As already described in the experimental section, two different LLE approaches, both using ethyl acetate, were performed. The first was with two consecutive extractions (Figure 3), starting with the acidification of the aqueous solution rich in vanillin until pH around 8.0. Then the resulting aqueous phase was used for another LLE at a pH around 6.0. The second LLE approach had only one extraction step with the aqueous solution rich in vanillin acidified until a pH of around 6.0. The objective of the two LLE approaches was to study the effect of consequent extractions on the recovery of phenolic compounds, mainly vanillin.

As already stated, in the aqueous fraction rich in vanillin from adsorption/desorption experiments, with a pH value around 13, all the phenolic compounds are in the phenolate form. However, the studied phenolic compounds present in alkaline media (vanillic acid, vanillin, syringaldehyde, and acetovanillone) have different pKa, between 4.3 and 8.3, and therefore the separation among aldehydes, acids, and ketones can be somewhat tuned by the pH of the aqueous solution due to differences in acid dissociation constants. When the pH of the solution decreases until a value near 8.0, acetovanillone, with pKa around 8.3, leaves the phenolate form and is extracted by the organic solvent. The acids and aldehydes, with pKa values lower than 8, remain in the phenolate form and it was expected that they continue in the aqueous phase that would proceed to the second step of LLE. However, in Figure 5 it can be observed that around 30% and 40% of the total mass of vanillin and syringaldehyde, respectively, were extracted, as about 55% of acetovanillone, by the organic solvent (ethyl acetate). Only vanillic acid, which has the lower pKa value, remains, in the totality, in the aqueous phase from the first step of LLE. In conclusion, only 70% of vanillin, 45% of acetovanillone, and 60% of syringaldehyde remain in the aqueous phase that proceeds to the second extraction. The two-step approach is not advantageous since a significant amount of vanillin is lost for the aqueous phase from the first step extraction and higher quantities of solvents are consumed.

In the procedure with only one extraction step (Figure 6), the initial fraction rich in vanillin was acidified until pH 6.0, to guarantee that all the phenolic aldehydes and ketones (vanillin, syringaldehyde, and acetovanillone) were extracted by ethyl acetate to the organic phase which will proceed to crystallization, after evaporation and dissolution of the organic material with water. Vanillic acid is the only compound with a pKa lower than the pH of the solution and consequently is the only compound that keeps in the phenolate form remaining in the aqueous phase. With this LLE approach, an extraction yield of 96%, 91%, and 99% was observed for vanillin, syringaldehyde, and acetovanillone, respectively.

Considering the results coming from the two LLE approaches, it was established to proceed with only one step of LLE, since the integrated process to obtain vanillin from lignin will benefit from a smaller number of extraction steps and a lower consumption of organic reagents. Consequently, the organic phase from the one-step LLE, after evaporation and dissolution with water, will proceed for crystallization as already described in section 2.6.

### Structural characteristics, purity, and yield of the vanillin crystals

A crystallization process could be optimized depending on its main objectives, such as crystallized mass, yield,

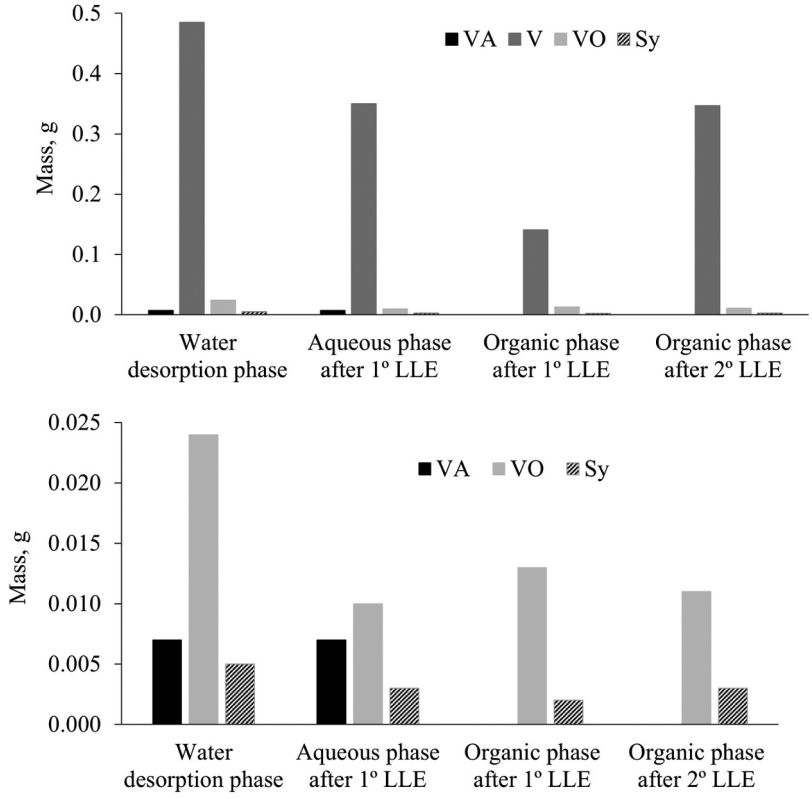


**Figure 5.** Vanillin (V), vanillic acid (VA), acetovanillone (VO), and syringaldehyde (sy) in water desorption fraction and aqueous and organic phases obtained from two-step LLE.

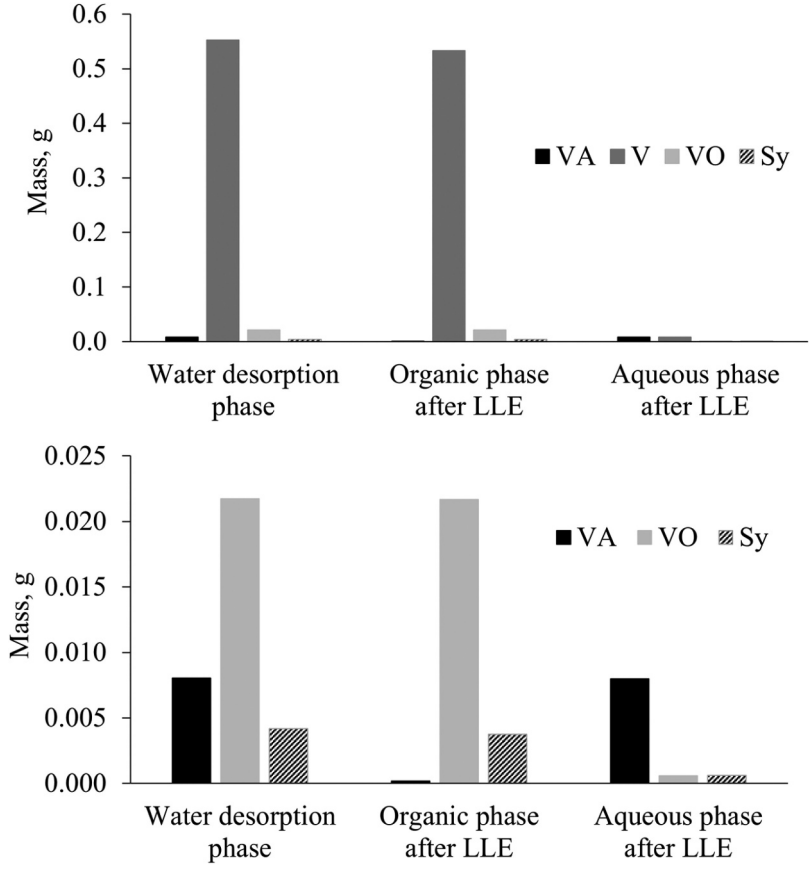


**Figure 6.** Vanillin (V), vanillic acid (VA), acetovanillone (VO), and syringaldehyde (sy) in water desorption fraction and aqueous and organic phases obtained from one-step LLE.

crystal size distribution, crystal polymorphic form, and process time. In Table 2, the form and color of the crystals, purity, and yield obtained from the different evaporative and cooling crystallization processes are detailed. The yield of vanillin crystallization was measured by taking as reference the total content of vanillin in the aqueous fraction before LLE namely, dividing the mass of vanillin crystals obtained after each crystallization process by the total amount of vanillin, in grams, in the aqueous solution from adsorption/desorption experiments, times 100 to express in percent.

A significant difference between the crystallization yield of the different processes was observed. From the slow evaporative and cooling process, vanillin crystallizes into stable polymorphic Form I with a yield of 80% and 75%, respectively. For swift cooling and evaporation with heat, the crystallization process shows a lower yield, between 52 and 55%, respectively, and gives origin to crystals with metastable Form II. It seems that, besides the influence of the crystallization process, the type of polymorphic form of vanillin formed also influences the process yield. The crystallization processes that give origin to vanillin polymorphs of Form II, the metastable form, have lower yields.

The purity of the crystals that resulted from the evaporative crystallization is slightly lower than that from the cooling crystallization processes. From evaporation, the vanillin crystals present a purity of about 95%, while for cooling processes the purity is slightly higher, with values around 99%. When the aqueous solution rich in vanillin is submitted to low temperatures (5°C), apart from the cooling range, the purity of the vanillin crystals is higher. Considering this, in opposition to that observed for yield values, it seems that the results are influenced by the crystallization process instead of the resulting polymorphic form of vanillin. Moreover, from GC-MS analyses, it could be observed that the only impurity identified was in the crystals from evaporative crystallization processes, without any type of cooling step, and corresponds to acetovanillone, in low amounts.

**Table 2.** Structural characteristics, purity, and yield of the vanillin crystals obtained from the different crystallization processes.

| Crystallization process | Form | Color | Yield, % | Purity, % |
|---|---|---|---|---|
| Slow evaporation | Form I | Yellow | 80 | 95 |
| Cooling | Form I | Yellow | 75 | 99 |
| Swift cooling | Form II | White | 52 | 98 |
| Evaporation with heat | Form II | Yellow | 55 | 95 |

## Polymorphic forms of vanillin

During the different crystallization processes, two distinct polymorphic forms of vanillin were observed: the stable Form I and the metastable Form II (Figure 7). In the stable monoclinic Form I, the molecules in each of the pairs of its asymmetric unit are linked by the hydrogen bonds between the aldehyde (C=O) and hydroxyl (O-H) groups of neighboring molecules.[18] While in metastable orthorhombic Form II, the molecules are linked by the hydrogen bonds between hydroxyl groups of the neighboring molecules. There is an interaction between O-H and O-H of two neighboring molecules of the asymmetric unit.[18]

The occurrence of a stable or metastable crystal form depends on the combination of saturation and solvent composition of the system.[23,24] In a study about the nucleation of vanillin polymorphs in different solvents, under several supersaturation ratios in the presence of different silica templates, the authors found that Form I and Form II could be

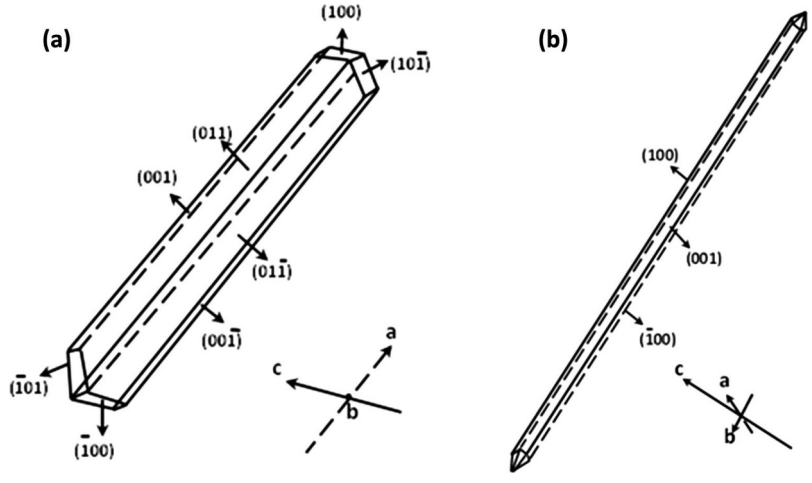


**Figure 7.** Morphology of the vanillin polymorphs: (a) stable monoclinic form I and (b) metastable orthorhombic form II.[19]

obtained in water with low and high supersaturation ratios, respectively.[25] Moreover, the high content of water in the solvent seems to increase the possibility of precipitation of metastable phase (vanillin Form II) in the system. On the other hand, in ethanol, isopropanol, and ethyl acetate, only form I was obtained.[24]

In this work, all the crystallization processes were performed using aqueous solutions. In the cooling crystallization process, the solution was cooled until 5.0°C with a slow decrease of temperature at a rate of 0.1°C/min, while during the slow evaporative crystallization, the solution was only left at room temperature until crystals were formed. In these crystallization processes, there are no sudden temperature changes, and both yielded the nucleation of the stable polymorph Form I of vanillin (Table 2). During swift cooling crystallization, the temperature is drastically changed from 30°C to 5°C, and in crystallization, by evaporation with heat, the solution is submitted to a swift increase of temperature. Considering the polymorphic form of vanillin that results from these processes, it could be concluded that the processes with high and sudden variations of temperature favor the nucleation of thinner and lengthy vanillin crystals with the metastable Form II.

It could be stated that cooling and slow evaporative crystallization give origin to vanillin crystals with Form I, which presents a rod-like shape (Figure 8(a)), while from swift cooling crystallization and crystallization by evaporation with heat vanillin crystals with a thin needlelike form were obtained (Form II, Figure 8(b)).

It is clear that a temperature variation in the order of minutes (crystallization by evaporation with heat and swift cooling crystallization), instead of hours or days (slow evaporative and cooling crystallization), promotes a sudden saturation of the solution achieving a quasi-steady state distribution of molecular crystals that favors the nucleation of metastable Form II.[17,18] In opposition, the rod-like stable monoclinic Form I crystals grew under lower saturation conditions, promoting a slow growth rate of the crystals. According to Ostwald's rule of stages, during crystallization, the system moves to equilibrium from an initial high-energy stage to a lower-energy state through minimal changes in free energy.[26] Therefore, at a higher supersaturation level, the metastable polymorph Form II nucleated possesses higher energy and transforms into the stable Form I by attaining the minimum energy.[23,27] Other authors in the literature also noticed the influence of temperature variation on the resulting polymorphic forms of vanillin. All of them reported the effect of the cooling and evaporation rate on the crystallization of vanillin in stable Form I, metastable Form II, or a mixture of both.[17,18]

### Polymorphs characterization by DSC

The thermal stability and melting point of the stable Form I and metastable Form II of vanillin crystals were analyzed through DSC. The recorded thermograms, in the temperature range from 40°C to 200°C with a heating rate of 10°C/min, are shown

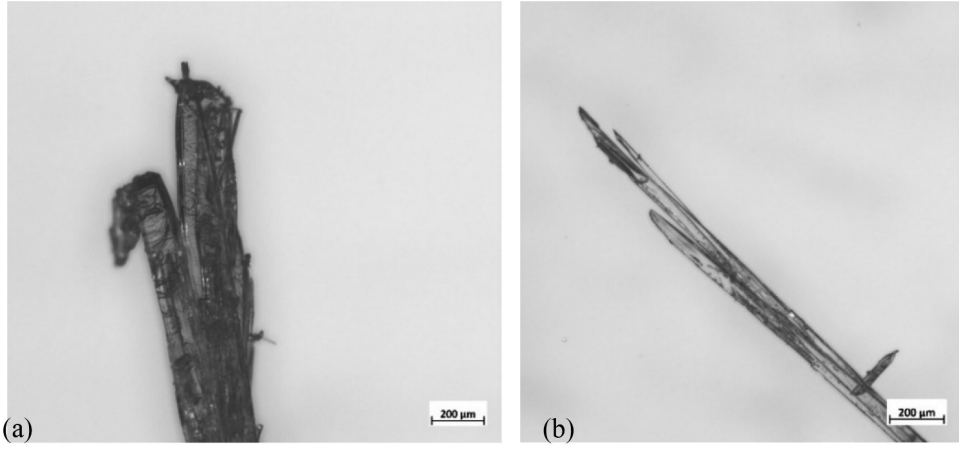


**Figure 8.** Microscope images of vanillin polymorph form I (a) and form II (b).

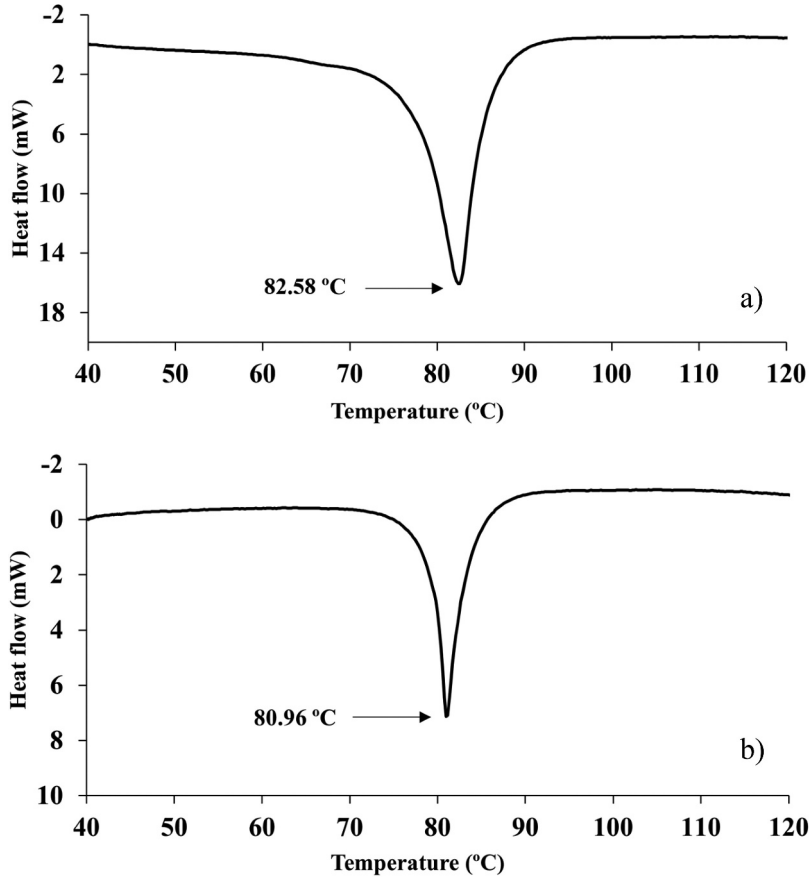


**Figure 9.** DSC thermograms of the vanillin polymorphs: a) stable form I and b) metastable form II.

in Figure 9. Pure vanillin has a melting point between 81°C and 83°C.[28]

The DSC curve obtained for the stable monoclinic Form I of vanillin crystals shows a sharp endothermic peak at 82.6°C, which corresponds to the melting point. No phase transformation was observed during the heating cycle until the melting point. What concerns the orthorhombic metastable Form II of vanillin crystals the sharp endothermic peak was noticed at 81.0°C. In this case, there was also no phase transformation observed during the heating cycle. Considering the melting point found for Form I and Form II it is evidenced that Form I is the most thermodynamically stable crystal form. According to the literature, the vanillin polymorphic stable Form I has valuable non-linear optical properties useful in industry, increasing its relevancy to materials science.[18] On the other hand, the metastable Form II has better second harmonic generation efficiency and may have other applications relevant to the food and pharmaceutical industries.[18,28]

### *X-ray diffraction (XRD) analyses*

The stable Form I and metastable Form II polymorphs of vanillin were characterized for their internal crystallographic structure by XRD analyses. The XRD pattern of the grown polymorphs of vanillin from aqueous solution is shown in Figure 10.

It was found that the intensity of the diffraction peaks shows some differences between the XRD patterns of the two polymorphic forms of vanillin, which confirms that the two forms have distinct crystal structures. Considering the presence of characteristic peaks of each polymorphic form, it is more difficult to draw valuable conclusions. However, the region between 25 and 30° shows some characteristic peaks of Form II that are not present in the same region of the Form I pattern; the presence of these peaks was also confirmed with the reported values from previous studies.[19,29]

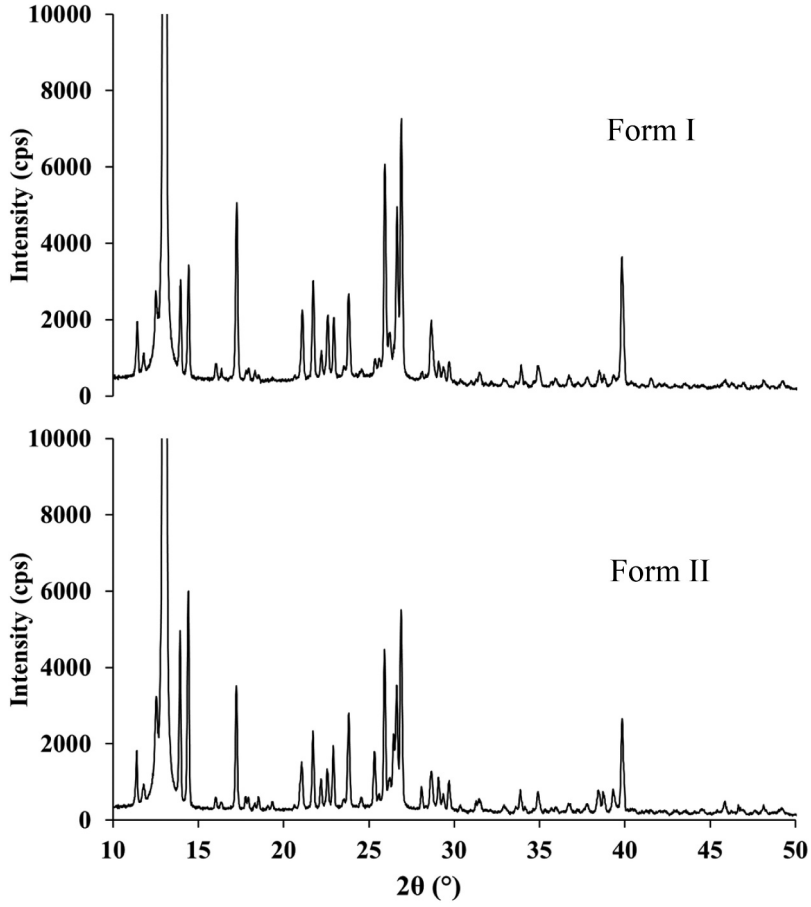


Figure 10. XRD patterns of the stable form I and metastable form II of vanillin polymorphs from aqueous solution.

## Conclusions

The influence of the crystallization process on the yield, purity, and polymorphic form of vanillin crystals was achieved. The polymorphic form of vanillin was found to be dependent on the crystallization method as well as the temperature variation during the process. The slow evaporation method and cooling crystallization give origin to the stable polymorph Form I, with a yield of 80% and 75%, respectively, while swift cooling and evaporative process with heat favor the nucleation of vanillin polymorphic Form II, with a yield of 52% and 55%, respectively. From evaporative processes, the vanillin crystals present a purity of about 95%, while for cooling processes the purity is slightly higher, with values around 99%. The melting points, observed in the DSC thermograms, of the stable Form I and metastable Form II of vanillin were found to be 82.6°C and 81.0°C, respectively. Since the metastable Form II is less stable and highly energetic, the melting point of vanillin Form II is lower than that of the stable Form I. Moreover, there was no polymorphic phase transformation obtained for both polymorphic forms of vanillin during heating until melting point. The recorded XRD patterns of the grown polymorphs are distinguished by some characteristic peaks and intensity, which confirms that the grown polymorphic forms of vanillin belong to different crystal systems. The different evaporative and cooling crystallization processes studied in this work proved to be consistent and valuable considering the attaining of vanillin crystals. Each of the polymorphic forms of vanillin may have different industrial applications due to their different physicochemical properties.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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