

Separation and Purification of Organic Acids by Gas Antisolvent Crystallization[†]

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Citric acid (CA) has successfully been separated from other organic acids, especially oxalic acid which is a byproduct of CA fermentation, by gas antisolvent crystallization with CO₂. In this process, crystal grains of CA could be obtained at high speed from an acetone solution by simple pressure regulation. The conditions for the deposition of organic acids are strongly dependent on the saturation ratio of the solute in the solution (C/C_{sat}) and on the solubilities of the acids in acetone.

Keywords: *Organic acid; citric acid; oxalic acid; malic acid; carbon dioxide; antisolvent; near critical; gas antisolvent crystallization; crystallization*

INTRODUCTION

Citric acid (CA) is an important compound used for food, confectionery, and beverages as an acidulant as well as for pharmaceutical and chemical products. CA is generally produced by surface or submerged fungal fermentation mainly with *Aspergillus niger* and is generally purified according to a firmly established process known as the method of calcium salt precipitation (Bouchard and Merritt, 1979; Matthey, 1992). CA can be isolated from fermentation broths in a series of precipitation and subsequent isolation reactions using calcium hydroxide and sulfuric acid. Further treatments include decolorization using activated carbon and recrystallization for obtaining the product (CA). However, this process includes several batch treatments which require large amounts of chemical reagents and a considerable amount of indispensable heat to drive the reactions. More calcium sulfate is formed than the weight of CA and has to be discarded as industrial waste. Though these negative factors have a significant influence on the production cost, improvements in this process have not yet been practically accomplished.

In the purification process of CA, two types of impurities have to be separated from CA. One is the impurities which are composed mainly of sugars. We previously proposed a novel and unique CA purification process using compressed carbon dioxide (CO₂) as an antisolvent (Shishikura et al., 1992). In this process, crystal grains of CA could be obtained by successive acetone extraction of CA from a condensed fermentation broth, gas antisolvent crystallization (or precipitation) of impurities with CO₂, and crystallization of CA from the supernatant (acetone solution). The impurities, mainly sugars, were selectively precipitated from the acetone solution of crude CA by introduction of CO₂ at 30 °C and 15–25 kg/cm² without the deposition of CA.

The other impurities are byproduct organic acids such as oxalic and malic acids. The yields of these byproduct acids are generally changed by the fermentation methods, materials, conditions, and microorganisms used. In a conventional purification process, oxalic acid is separated by the control of pH. The fermentation broth is adjusted with Ca(OH)₂ to pH 3, and then the precipitated calcium oxalate is removed from the supernatant before calcium citrate is produced (Bouchard and Merritt, 1979). This treatment is carried out by a batch-type reaction which requires a long time.

In the present study, to separate CA from byproduct organic acids, especially oxalic acid, gas antisolvent crystallization with CO₂ was studied. The deposition conditions of several organic acids were investigated, and the possibility of their fractional crystallization was discussed. This paper also addresses the outline of a new process for purification of CA and the results of production tests of CA based on the new process.

MATERIALS AND METHODS

Materials. The gaseous CO₂ (Nippon Oxygen Co., Japan), organic acids, and organic solvents (Wako Pure Chemicals Co., Japan) used were of commercially available grades.

CA broth was prepared by submerged fermentation with *A. niger*. The broth was produced from a medium containing potato starch cubes and rice bran powder by submerged fermentation at 37 °C for 7 days. After the filtration of microorganisms and residual substrates, the broth was concentrated to a water concentration of 15 wt %. The average compositions of fermentation broths are shown in Table 1.

Gas Antisolvent Crystallization with Compressed CO₂. The flow diagram of the experimental apparatus is shown in Figure 1. A transparent glass type level gauge (LG) was used as a settler; its volume was 250 mL (2.5 i.d. × 50 cm).

The precipitation conditions of the organic acids were measured by batch-type treatments. Acetone solutions of organic acids (50–80 mL) were previously poured into the LG. Gaseous CO₂ was introduced from the bottom of the LG (FL) and was dissolved in the acetone solution with increasing pressure. Then the pressure was increased at the rate of 5 kg/cm² per minute. The pressure and CO₂ concentration in the solution, when the solution became turbid, were regarded as the starting conditions for deposition. After the deposited particles settled, a small amount of the supernatant was

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Table 1. Average Compositions (Weight Percent) of the Fermentation Broths and Acetone Solution of Crude CA

component	broths		acetone solution of crude CA
	original	condensed	
citric acid	10.2	37.1	12.7
other organic acids	0.18	0.65	0.24
sugars	2.02	7.35	1.20
minerals	0.6	2.18	
proteins	0.1	0.36	
unknown	0.2	0.73	0.26
water content	86.7	14.2	1.46
acetone			84.14

^a All compositions were means of five batches.

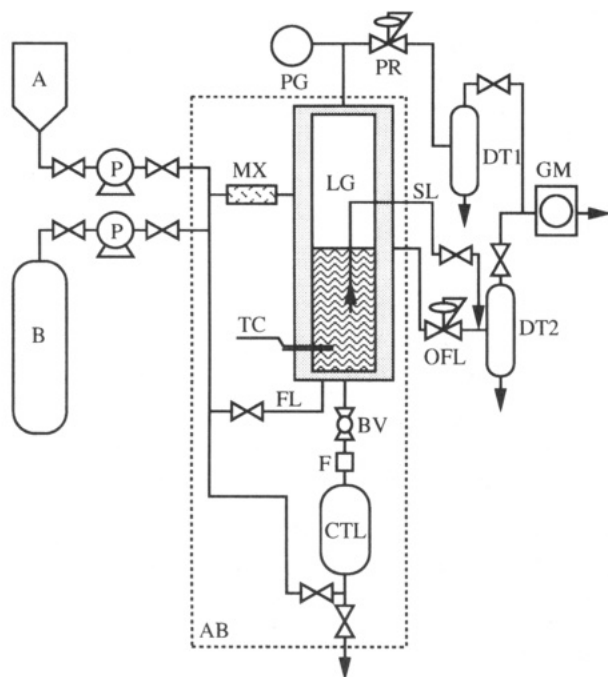


Figure 1. Flow diagram of the experimental apparatus: (A) feed tank, (B) CO₂ cylinder, (P) pump, (MX) mixing line, (LG) level gauge, (TC) thermocouple, (FL) CO₂ feed line, (PG) pressure gauge, (PR) pressure regulator, (OFL) overflow line, (SL) sampling line, (BV) ball valve, (F) filter, (CTL) supernatant receiver, (AB) air bath, (DT1,2) dry ice traps, and (GM) gas meter.

removed from the LG (SL) to the dry ice cooled tube (DT2), and the concentration and composition of the solute were analyzed. The pressure of the LG was maintained during the sampling to prevent the deposited solute from redissolving. The amount of the deposited solute and its composition were calculated from the changes in the concentration and composition of the solute in the supernatant.

The deposited particles were recovered in the following way: After the intended pressure was reached, the deposited particles were flocculated by bubble stirring for 3 min and were subsequently separated from the supernatant by settling for 3–30 min. Then the supernatant in the LG was filtered (F, 2 μm mesh) and transferred to the receiver (CTL), where the pressure was synchronized with that of LG or was controlled slightly lower than that of LG. The particles trapped by the filter (F) were dried by flowing fresh CO₂ and were recovered.

In the case of continuous precipitation, an acetone solution of crude CA (see Table 1 for the average composition and Table 2 for the preparation procedure) and CO₂ were previously mixed in the mixing line (MX; 0.32 i.d. × 8 cm), and then the mixture was fed into the LG (1 L, 8 i.d. × 20 cm); the LG was used as a settler. The supernatant was continually removed from the LG through the overflow line (OFL). After the decompression, the supernatant was collected in a dry ice trap (DT2). The flow rate of the solution was regulated at 2–30 mL/min, and CO₂ was added at 1.2 times the volume of the theoretical saturating solubility.

Analysis. Analysis of CA and other organic acids was carried out by means of HPLC on a Jasco 800 system using an Intersil C₈ column (5 m, 4.6 i.d. × 250 mm, GL Sci. Co., Ltd., Japan). The mobile phase was an aqueous solution of 0.1 M ammonium dihydrogen phosphate adjusted to pH 2.1 with phosphoric acid. The flow rate was 1 mL/min at room temperature. Detection was effected by measurement of UV absorption at 210 nm.

Sugar, minerals, and water contents were determined according to the standard methods of the Japan Food Additives Association (*Food Additives Official Hand Book*, 1986).

The size distribution of deposited particles was measured by optical microscopy.

RESULTS AND DISCUSSION

Separation of Citric Acid from Byproduct Organic Acids by Gas Antisolvent Crystallization.

Gas antisolvent crystallization was proposed as a granulating technique by Krukonis and Gallagher (Krukonis et al., 1988; Gallagher et al., 1989, 1992). This technique is applicable to a wider variety of solid materials such as polar compounds that are insoluble in supercritical fluids and also has an attractive advantage in that its operation can generally be carried out at lower pressure compared with other techniques using supercritical fluids.

Figure 2 shows the scheme of gas antisolvent crystallization. In the present study, CO₂ was used as an antisolvent to crystallize organic acids from an acetone solution. The most important factor in crystallizing or depositing solutes is the ability of gases to dissolve in organic solvents and to lower the solvent power of the organic solvent for the solutes. CO₂ is known as a nonpolar solvent which has a solvent power quite similar to that of *n*-hexane (Allada, 1988). Acetone in the liquid phase was gradually diluted with the introduction of CO₂ in which most di- and tricarboxylic acids cannot be dissolved, while the solution (liquid phase) was expanded with CO₂. As a result, the solubilities of solutes were reduced by the antisolvent effect of CO₂, and then the dissolved solutes were rapidly nucleated and precipitated from the solution. This phenomenon is analogous to recrystallization of glucose induced by the addition of ethanol to its water solution. In this case, water and ethanol are a good solvent and an antisolvent (or poor solvent) for glucose, respectively.

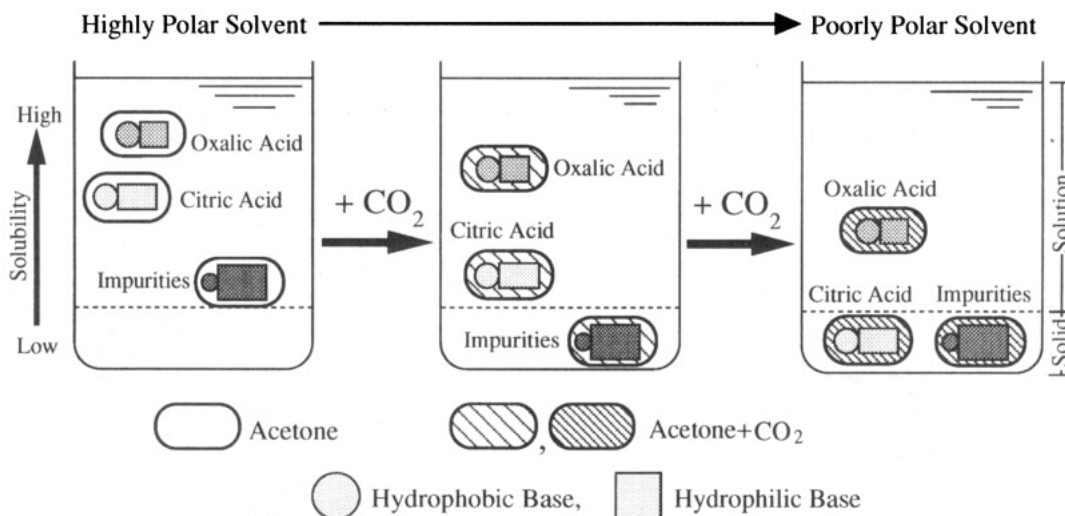
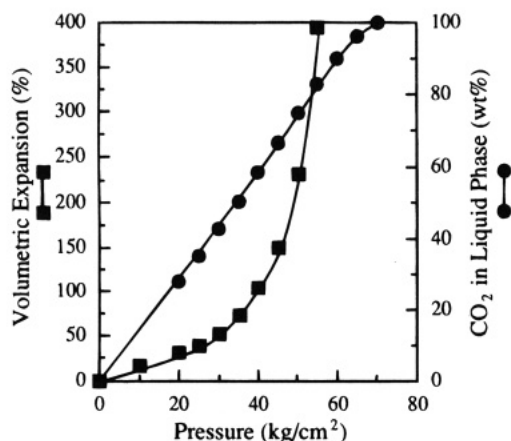
In a previous paper, we selected acetone as an extraction solvent for CA, and the impurities could be separated as precipitates from an acetone solution of CA by gas antisolvent crystallization. Therefore, to simplify the process, acetone was likewise used as a good solvent in this study. Figure 3 shows the expansion behavior of acetone by CO₂ and the weight fraction of CO₂ in the liquid phase at 30 °C. In the phase equilibrium for the CO₂–acetone binary system, the amount of CO₂ dissolved in the liquid phase increased linearly with rising pressure. At low pressure levels, the expansion slightly increased. At about 20 kg/cm², the expansion started to increase exponentially, and as the pressure approached the vapor pressure of CO₂, acetone and CO₂ became miscible. These data almost agreed with the expansion behavior and the weight fraction of CO₂ in the liquid phase calculated with the Peng–Robinson equation of state. Higher water concentration and temperature required higher pressure levels to reach similar expansion levels (Shishikura et al., 1992).

Figure 4 shows the solubilities of several organic acids in three solvents at 30 °C. The starting pressure for

Table 2. Changes in the Purity and Recovery of CA in Each Step

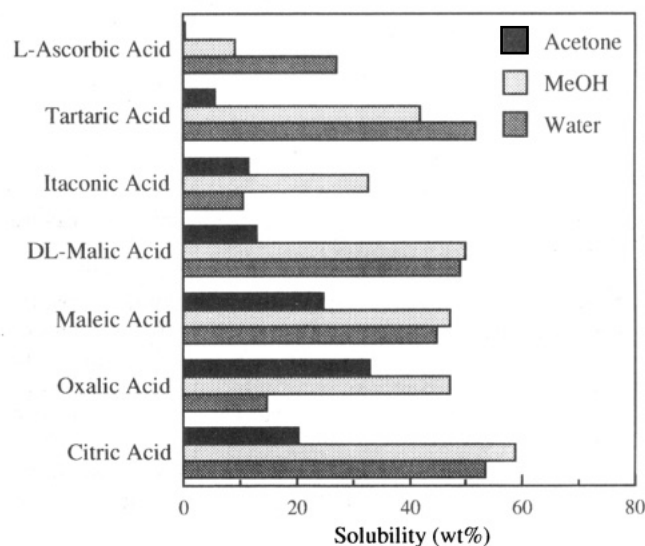
process	conditions		CA purity (wt %)	CA recovery (wt %)
	temp (°C)	pressure (kg/cm ²)		
1. fermentation ^a (submerged 20 L scale)	37		68.7–71.6	100 (100)
2. organic solvent extraction ^b	30		86.5–89.6	88.7–94.5 (88.7–94.5)
3. gas antisolvent crystallization 1 ^c	30	25	99.1–99.7	98.7–99.8 (87.5–94.3)
4. gas antisolvent crystallization 2 ^d	30	50–57	≥99.7	97.4–99.2 (85.2–93.5)
5. drying ^e	70		≥99.9	≥99.9 (85.2–93.5)

^a The fermentation broth (20 L) was concentrated to a water concentration of 14.2 wt %. ^b The CA was extracted with 15 L of acetone from the concentrated broth (2 kg). ^c The average concentrations of water and solute were 1.32 and 14.2 wt %, respectively. The settling time was set for 15 min. ^d The average concentrations of water and solute were 0.96 and 13.8 wt %, respectively. The settling time was set for 5 min. ^e Crystal grains of CA were dried by N₂ at 70 °C for 15 min.

**Figure 2.** Scheme of gas antisolvent crystallization.**Figure 3.** Expansion behavior of acetone by CO₂ and weight fraction of CO₂ in liquid phase at 30 °C.

the deposition of solute is strongly dependent on the saturation ratio of the solute in the solution (C/C_{sat}). The concentrations of oxalic and malic acids in the acetone extract we prepared were 1.29 and 0.06 wt % (solvent-free basis), respectively. These concentrations and the saturation ratios of these acids in the feed solution are very low compared with those of CA. Therefore, these byproduct acids are not expected to deposit under the pressure conditions for the deposition of CA.

Figure 5 shows the effect of the saturation ratio of several organic acids in the acetone solutions on the starting pressure for their deposition at 30 °C. The most important point to explain the possibility of the separation of CA from the byproduct oxalic and malic acids is to clarify how and where the curves of the byproduct acids overlap that of CA. The deposition of CA started at pressures near 28 kg/cm² in the range of CA concen-

**Figure 4.** Solubilities of several organic acids in three solvents at 30 °C.

tration near its saturated solubility in acetone and was completed near 55 kg/cm². On the other hand, the deposition of oxalic acid started at pressures near 38 kg/cm² in the higher saturation ratios. In the range of saturation ratios below 0.35, oxalic acid could not be deposited at pressures over 120 kg/cm². The average of the actual saturation ratio of oxalic acid in the acetone solution of crude CA we prepared (acetone extract from condensed fermentation broth of CA) was about 6×10^{-3} . Therefore, oxalic acid was suggested to be easily separated from CA. In the case of malic acid, the curve was almost similar to that of CA. This shows that malic acid simultaneously crystallized with CA over the wide

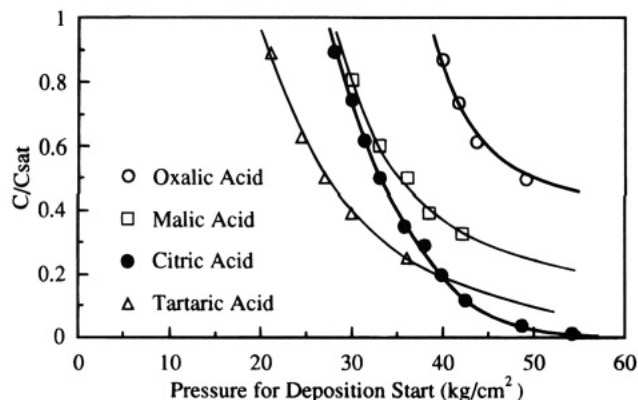


Figure 5. Effect of saturation ratio (C/C_{sat}) of several organic acids in the acetone solutions on starting pressure for their deposition at 30 °C. C , concentration in the solution; C_{sat} , saturated solubility in acetone at 30 °C.

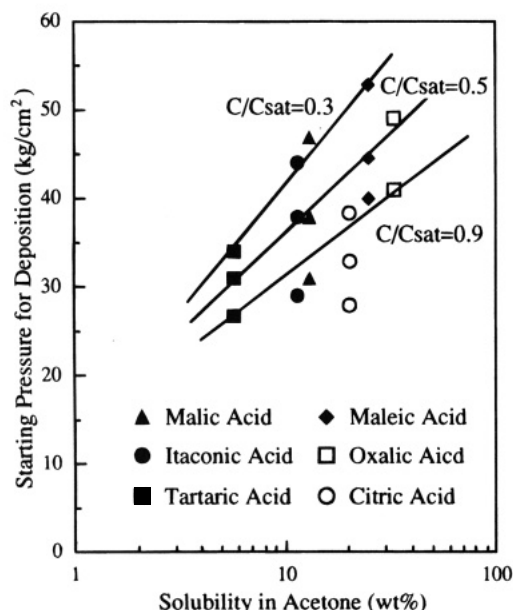


Figure 6. Effect of solubility in acetone on starting pressure for deposition of organic acids at 30 °C.

concentration range. However, this was not a serious problem because of its low concentration in the fermentation broth we prepared. The bends of the curves of dicarboxylic acids such as oxalic and malic acids were nearly similar but were different from that of the tricarboxylic acid (CA).

The starting pressures for their deposition were mutually related to their solubilities in acetone and increased with the solubilities (Figure 6). The starting pressure for their deposition increased with increasing solubilities in acetone at all saturation ratios. The range of the starting pressure for their deposition also increased with increasing solubilities in acetone. The points of the dicarboxylic acids at the same saturation ratio gave a straight line, but the points of CA did not coincide in position with those lines. Their solubilities in the solution expanded with CO_2 may be predicted by the method using regular solution theory (Dixon and Johnston, 1991).

To determine the separation efficiency of CA from the byproduct oxalic acid, a separation test was carried out using a model solution that contained oxalic acid in excess of that in the actual fermentation broth (Figure 7). As a result, CA first started to crystallize at 28 kg/cm^2 , and most of the fed CA was recovered as crystals

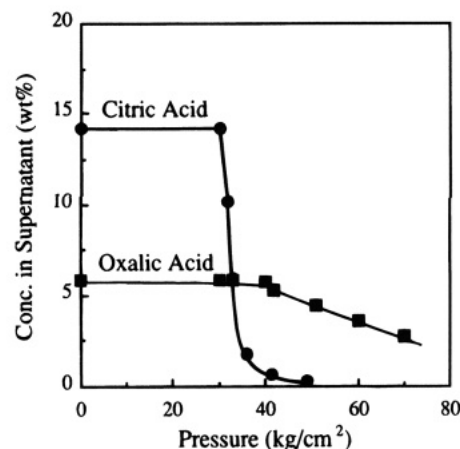


Figure 7. Effect of pressure on the concentrations of organic acids in the supernatant at 30 °C.

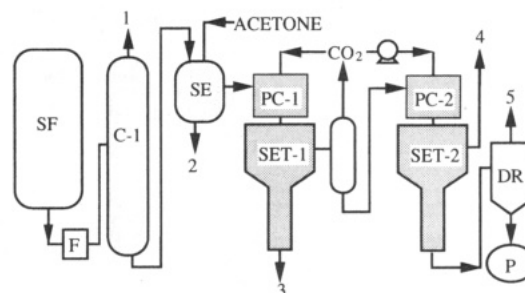


Figure 8. Flow diagram of novel CA purification process (image): (SF) submerged fermentation tank, (F) filter, (C-1) multiple effect evaporator, (SE) solvent extractor, (PC-1,2) precrystallizer, (SET-1,2) settler, (DR) dryer, (1) water, (2) impurities (residual sugars), (3) residual impurities, (4) acetone and oxalic acid, (5) acetone, and (P) product.

as the pressure rose to 42 kg/cm^2 . The purity and recovery of CA crystals were 99.8 and 96.4%, respectively. The total amount of the fed oxalic acid remained in the supernatant. In this system, fine drops of water were deposited near 21 kg/cm^2 . In the case of water concentration in the solutions above 5 wt %, the curve of oxalic acid was shifted to a lower pressure and approached that of CA. As a result, the recovery of pure CA crystal decreased with increasing water concentration in the solutions.

In this manner, CA could be separated from oxalic acid by the gas antisolvent crystallization after the separation of the impurities (mainly sugars). Generally, the microorganisms that show high productivity of CA produce large amounts of the byproduct organic acids. Even if the composition of the fermentation broth fluctuates, high-purity CA can be obtained without changing the operating conditions in this process. Therefore, the microorganisms, which show high productivity of CA, are felt to be useful in the fermentation of CA.

Image Process for Purification of CA. On the basis of the experimental results described above, we improved the novel and unique CA purification process proposed in the previous paper. Figure 8 shows the flow diagram of the new process for CA purification.

First, the fermentation broth of CA is filtered to remove microorganisms (F) and is dried to adjust its water concentration to about 10–20 wt % by multiple effect evaporation (C-1). CA is subsequently extracted with acetone from the condensed broth (SE). The residual impurities are then removed as precipitates

Table 3. Comparison of New and Conventional Processes

this purification process	calcium precipitation process
impurities are removed from CA by (1) organic solvent extraction (2) precipitation using antisolvent effect of CO ₂	CA is separated from impurities by (1) neutralization [+Ca(OH) ₂] (2) liberation (+H ₂ SO ₄)
CA is crystallized from acetone by (1) simple pressure control (2) rapid method (3–10 min)	CA is crystallized from water by (1) temperature control (2) slow method (3–14 h)
*continuous process *no chemical reagent is required *small amount of industrial waste	*process is mainly composed of batch reactions *large amounts of chemicals are required *large amount of industrial waste (CaSO ₄)

from the acetone solution of crude CA using the antisolvent effect of compressed CO₂ (PC-1). The deposited impurities are readily separated from the supernatant by a settling operation (SET-1). Finally, crystal grains of CA are obtained from the acetone solution by the antisolvent crystallization with CO₂ (PC-2, and SET-2) and dried (DR).

The operating conditions and results are shown in Table 2. The characteristics and purity of CA produced by this process fully satisfied the standards of food additives in Japan. The recovery was influenced by the extraction step of CA with acetone from the fermentation broth. All operating conditions were not dependent on the impurity concentrations in this process. CA could be obtained with a constant quality by the conditions shown in Table 2, even if the impurity concentrations in the fermentation broth increased greatly. However, the amount of the residual acetone in the CA crystals was about 14–30 ppm.

In conclusion, the characteristics of conventional and new purification processes of CA were compared and are shown in Table 3. In the case of our technique, crystal grains of CA of food additive grade can be obtained at high speed by simple pressure regulation. The control of the solute concentration and temperature to create supersaturation conditions can be omitted. We suggest that our purification process would considerably reduce energy consumption, industrial wastes, and production cost, compared with the conventional calcium salt precipitation process. Moreover, our process has wide applicability to a great variety of fermentation products. In particular, it seems profitable for the crystallization of thermolabile compounds.

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