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# Physicochemical Properties of Calcium Lactate Prepared by Single-Phase Aragonite Precipitated Calcium Carbonate.

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**ABSTRACT**

This study sought to prepare calcium lactate using precipitated calcium carbonate (PCC) by reacting lactic acid and then investigated the formation yield and physicochemical properties of synthetic calcium lactate by Fourier Transform Infrared Spectroscopy (FT-IR), Thermogravimetric-Differential Thermal Analysis (TG-DTA) and Scanning Electron Microscope (SEM). The strong OH valence band of calcium lactate prepared by PCC in the 3000~3500 $cm^{-1}$ region of the FT-IR spectra showed very low intensity. Also it was found that the characteristic carbonyl band was observed at approximately 1500~1750 $cm^{-1}$ (C=O stretching) and 1300~1400 $cm^{-1}$ (C-H bending), respectively. The diffraction profile of synthetic calcium lactate prepared by PCC showed a typical diffraction pattern for a crystalline solid. The TG-DTA curve of calcium lactate showed a rapid decrease at 200~400 °C due to the melting point of calcium lactate, which was about 240 °C. In particular, the curve had a second decrease at 600~800 °C due to the decarbonation of calcium oxide. As for SEM images, calcium lactate using PCC existed as plate-like crystals with smooth surface. These results indicated that calcium lactate pentahydrate was formed by reacting between PCC and lactic acid.

**Keywords**: Calcium lactate, Physicochemical properties, Precipitated calcium carbonate, Single-phase aragonite

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## INTRODUCTION

Calcium carbonate ($CaCO_3$) thrives throughout nature, occurring as the main mineral constituent of limestone. This mineral is also a well-known inorganic component in skeletons and tissues of several mineralization organisms [1]. In general, precipitated calcium carbonate (PCC) is synthesized through the carbonation or solution process. It is used in large amounts in the rubber, plastics, pulp, and paper industries [1, 2]. Two common polymorphs of $CaCO_3$ are aragonite (orthorhombic) and calcite (trigonal). Aragonite with a structure is less common and less stable than calcite, while calcite with trigonal symmetry is the most thermodynamically stable form of pure $CaCO_3$ at room temperature and atmospheric pressure [3]. However, owing to the low absorption rate in the body and rejection symptoms of consumers for raw material, i.e., limestone, when applied to food, however, $CaCO_3$ is not commonly used as a calcium source in the food and medical industries. In general, adequate calcium intake is important both for building peak bone mass in the first three decades of life and for making up for bone loss in later years. The average calcium intake in the Korean diet falls well below 70% of the calcium requirement [4]; hence the need for new approaches to preparing biosynthetic calcium lactate using precipitated calcium carbonate and lactic acid bacteria as an advanced calcium source with high absorption rate even in pure form.

Calcium lactate is most widely used in calcium fortification and in the food and drug industry as a calcium additive in various foods such as cookies, beverages, bread, sauces, powdered milk, and fruit juice for its high absorption rate. It is also commonly used as a tissue-reinforcing agent in various processed agricultural products and pickled foods [5, 6]. In general, calcium lactate has two racemates: L(+)-lactate and D(-)-lactate. According to recent studies in this area, calcium lactate is formed when L(+)-lactate is converted into a racemic mixture of L(+)-lactate and D(-)-lactate. In particular, L(+)-lactate is known to be more soluble than D(-)-lactate and L(+)-lactate generally produced from lactose by homo-fermentative lactic acid bacteria such as *Lactococcus spp*., *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus curvatus*, and *Pediococcus spp* [7]. Lactic acid bacteria are widely used as starter cultures in the food fermentation of sugar polymers and other raw materials from plants or animals including milk. Several lactic acid bacteria are used in probiotic products such as antimicrobial substances and useful enzymes, providing health benefits for the production of high value-added metabolites containing aromatic compounds and having smooth texture, sweeteners, and vitamins [8, 9]. Moreover, calcium lactate is generally considered in food technology as a safe food ingredient. Two different forms of calcium lactate, e.g., calcium lactate trihydrate (CLT) and calcium lactate pentahydrate (CLP), are described in the main pharmaceuticals. In particular, pharmaceutical salts frequently exist as hydrates such as calcium lactate pentahydrate, although hydrate formation and dehydration may occur during the processing or storage of pharmaceuticals [10-12]. However, in a previous study, it has been reported that calcium phosphate seems to be the better supplement for the *in vivo* system compared to the calcium lactate, probably because Ca and P rate the major constituents needed to form the endoskeleton of the vertebrates particularly mammals [13].

Therefore, this study sought to prepare calcium lactate using precipitated calcium carbonate (PCC) by reacting lactic acid and to investigate the formation yield and physicochemical properties of synthetic calcium lactate by FT-IR, TG-DTA, and SEM. Also the purpose of this study was to manufacture the biosynthetic L(+)-calcium lactate with high value-added and probiotic characteristics using lactic acid bacteria and calcium carbonate as a calcium source for application to various food industries instead of commercial calcium lactate.

## MATERIALS AND METHODS

### Preparation of single-phase aragonite precipitated calcium carbonate used as an inorganic eco-material

Figure 1 showed that the schematic diagram (a) of the apparatus used for this experimental and flow chart (b) on the processes of PCC synthesis and analysis. The reactor was used a cylinder made of Pyrex glass with a diameter of 10 cm and a volume of 1.0 L, with a 4 cm long impeller attached. The temperature in the reactor was maintained at a constant with a water bath (Jeio Tech, WB02). In order to adjust the concentration of the $CO_3^{2-}$ ions, the 0.5M $Na_2CO_3$ solution was added to $Ca(OH)_2$ at the same time at the rate of 3ml/min for a lower concentration. Furthermore, the NaOH solution generated as the by-product was reused and added to the $Ca(OH)_2$ without a re-treatment process. All of the experiments were conducted at 75 °C, below the constant agitation speed of 400 rpm. The synthesized samples were washed with ethanol two to three times in order to remove any residual ion components, then filtered and dried in an oven at 80 °C. The dry precipitates

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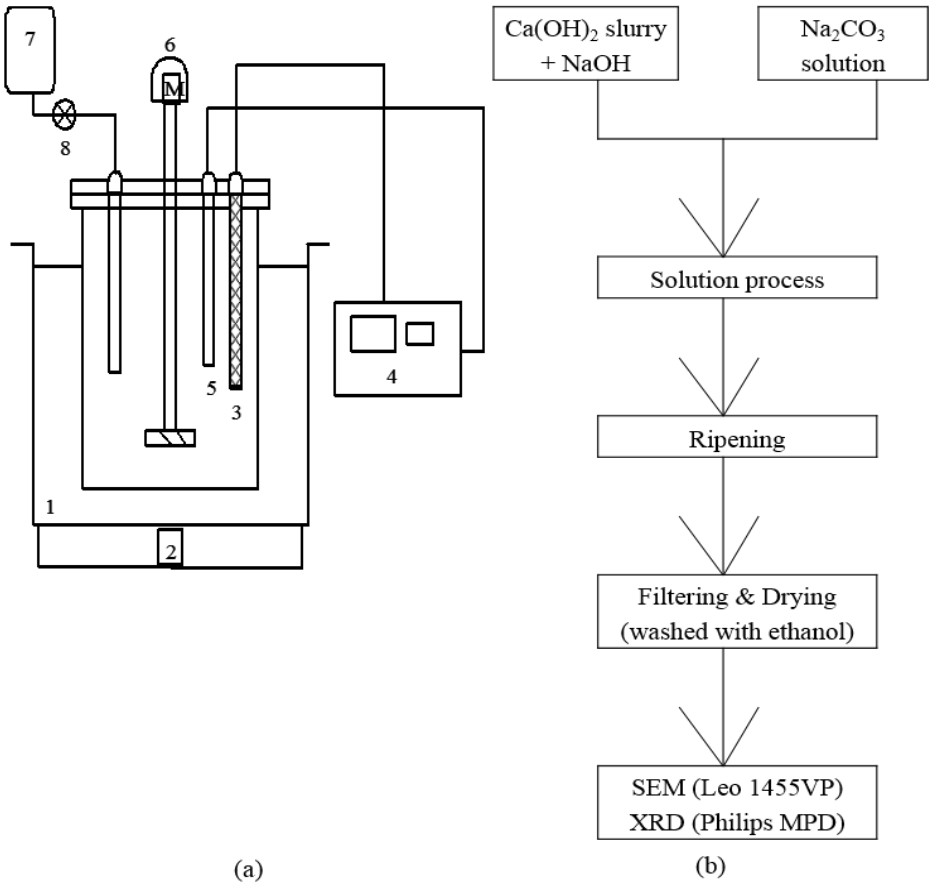
were characterized by X-ray diffraction (XRD) and the shapes of the particles were observed by scanning electron microscope (SEM).

**Figure 1: Schematic diagram(a) of the apparatus used for this experimental and a flow chart(b) on the processes of PCC synthesis and analysis : (1) Reactor (2) Water bath (3) pH electrode (4) pH meter (5) Thermometer (6) Stirrer (7) $Na_2CO_3$ solution (8) Valve.**




**Bacterial strains and selection**

The strain of L(+)lactic acid bacteria was obtained from the Korean Culture Center of Microorganism (KCCM). Four kinds of L(+)-lactic acid bacteria (*Bacillus coagulans, Lactobacillus platarum, Lactobacillus sakei, and Lactobacillus lactis*) were used for lactic acid fermentation in MRS broth for 72 hrs at 37 °C. The volumes of lactic acid production were then evaluated per lactic acid bacteria, and the lactic acid bacteria with the most efficient lactic acid production (*Lactobacillus lactis*), selected.

**Effects of calcium carbonate on the lactic acid fermentation**

An MRS medium (DIFCO 0881) was used in precultures. The basic medium for fermentations was composed of (per liter of distilled water) 10.0g Protease peptone No. 3, 10.0g Beef extract, 5.0g Yeast extract, 20.0g Glucose, 1.0g Sorbitan monoleate complex (Tween 80), 2.0g Ammonium citrate, 5.0g Sodium acetate, 2.0g $K_2HPO_4$, 0.1g $MgSO_4.7H_2O$, 0.05g $MnSO_4.4H_2O$, 0.01g, and 50g $CaCO_3$ and used to adjust the pH of the broth to 6.5. 1% precipitated calcium carbonate and 1% calcium carbonate reagent were then added to the medium, and calcium lactate production, observed 72 hrs after inoculation. Figure 2 shows the flow chart for the production of calcium lactate by lactic acid bacteria. Cultivations were carried out in 100 ml working volume in 250 ml conical flasks at cultivation temperature of 37°C and shaking speed of 100 rpm. Lactic acid was neutralized by adding two kind of calcium carbonate.

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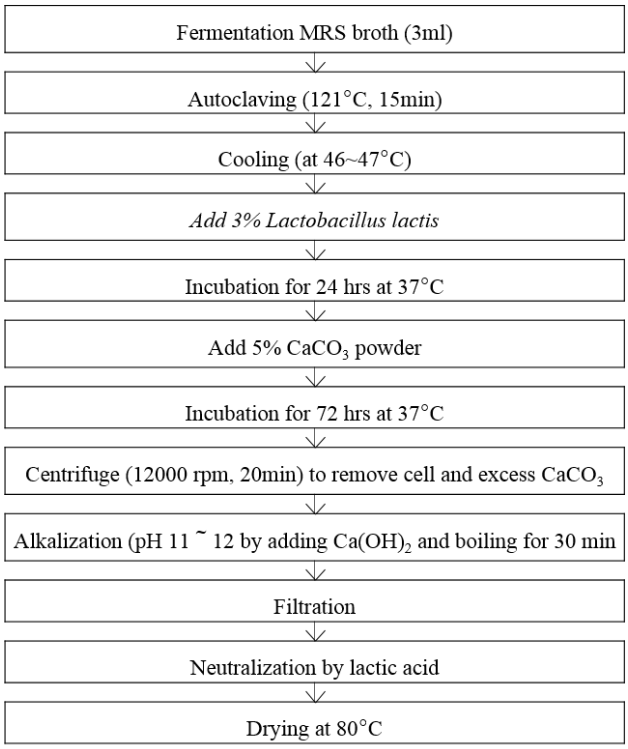
**Figure 2: Flow chart for the production of calcium lactate by lactic acid bacteria.**




**Analytical methods**

The fermentation broth (3ml) was centrifuged at 14,000 rpm for 5 min. The supernatant liquor was then diluted and finally filtered prior to injection. The resulting filtrate was used to measure lactic acid by HPLC using the RP304 column (Bio-rad) held at room temperature as the mobile phase ($H_2O$ : acetonitrile = 1 : 1, injection volume: 20 µl) at a flow rate of 0.8 ml/min. A photodiode array detector set at 250 nm in series with a refractive index detector was used for detection. Some of the supernatant of the fermentation broth was centrifuged at 12,000 rpm for 20 min, filtered using Whatman No.1 filter paper, and dried at 80 °C. Finally, calcium lactate formation by reaction between calcium carbonate and lactic acid, and hydration or dehydration behavior of the sample were investigated using digital camera, FT-IR, and TG-DTA.

***Powder X-Ray Diffraction (PXRD) analysis***

Powder X-ray diffraction was performed at room temperature with a type D/MAX 2500V/PC (Rigaku, Inc.). Measurement conditions were as follows: target, Cu; filter, Kα; voltage, 40kV; current, 450mA; time constant, 1s; step slit, 1.0°; counting time, 1.0s; measurement range, 2θ=5-60°.

***Fourier Transform Infrared Spectroscopy (FT-IR) analysis***

The infrared-ray (IR) spectrum was generated by preparing a sample according to the KBr pellet method and using FT-IR/FT-Roman and Mattson Polaris PT-IR spectrophotometer (Brucker Co., USA).

***Thermogravimetric-Differential Thermal Analysis (TG-DTA) analysis***

The TG-DTA curves were obtained by thermogravimetry differential thermal analysis (TG-DTA EXSTRA 6000 with measuring cell DSC 30E; NETZSCH Co. Ltd.). Approximately 10 mg of sample was weighed into the DSC pan. The pan was not sealed and placed in the sample side of the instrument. An identical reference pan was placed in the reference side. Scans between 25 and 250 °C were carried out at a rate of 10 °C /min, using a nitrogen gas purge at 50 ml/min.

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*Scanning Electron Microscopy (SEM)*

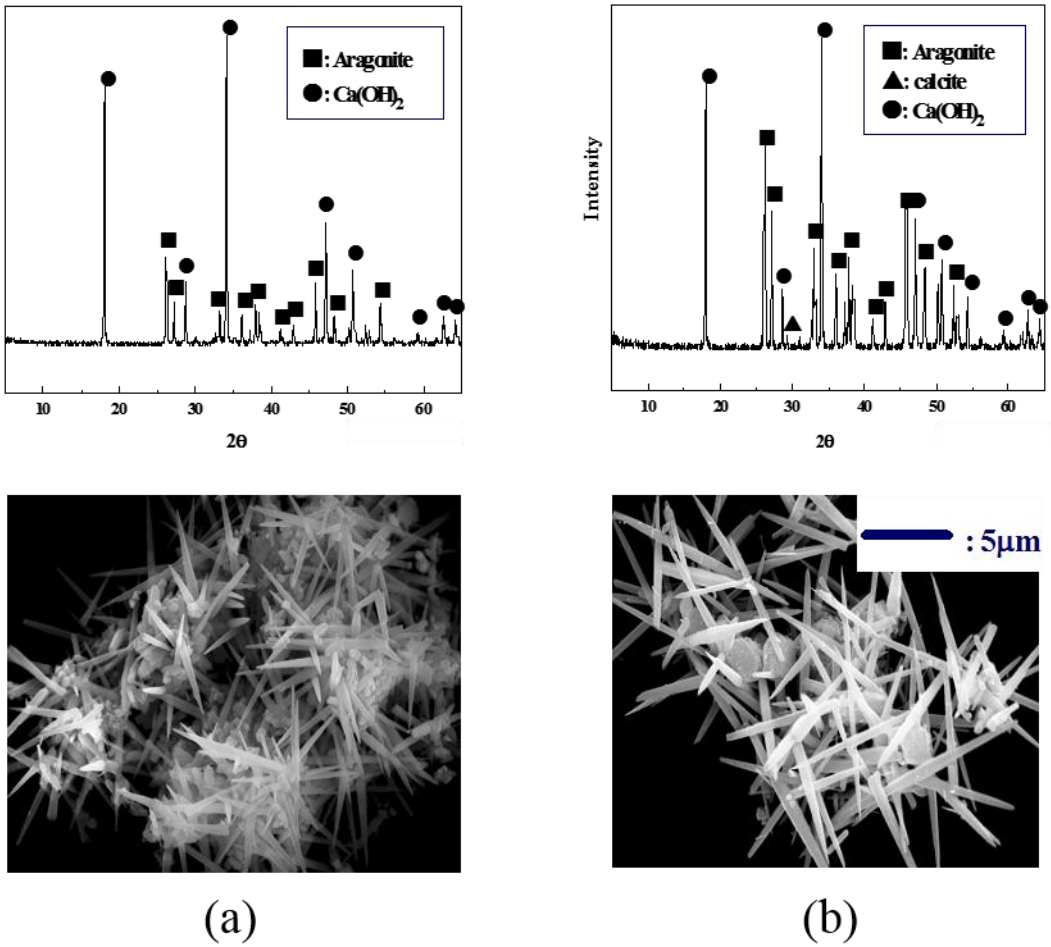
Samples were mounted onto aluminium stubs and gold coated in a sputter coater to a thickness of about 10 µm. The coated samples were then viewed at 400×magnification in the FE-SEM (HITACHI, Japan). The beam accelerator voltage was set to 20kV and the current was set to 12 µA.

## RESULTS AND DISCUSSION

### Synthesis of single-phase aragonite precipitated calcium carbonate

Figure 3 shows the XRD patterns and SEM photographic samples obtained by recycling the NaOH solution generated as a by-product in comparison with the use of reagents. Consequently, the existence of some calcite was confirmed. This result means that the purity of the by-product is lower than that of the reagent. However, particle size and morphology remained unchanged. In general, for the heat treatment at a temperature higher than 400 °C, the XRD patterns show the phase transition from aragonite to calcite. At an annealed temperature of 500 °C, it is a pure calcite phase only, suggesting that the phase transition is complete [3].

**Figure 3: XRD patterns and SEM photographs samples obtained by recycling the NaOH solution generated as a by-product. (a) reagent (b) by-product.**




### Screening of lactic acid bacteria strains with high lactic acid yield

The strains of lactic acid bacteria with high lactic acid yield are shown in Table 1. In the case of *Bacillus coagulans*, *Lactobacillus platarum*, and *Lactobacillus sakei*, lactic acid production was 34.14 mg, 176.80 mg, and 283.92 mg per ml fermentation broth, respectively. As for *Lactobacillus lactis*, however, lactic acid was the

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major product at 402.33 mg of lactic acid per ml fermentation broth. This results suggested that most of the lactic acid came from the pyruvate produced by *Lactobacillus lactis* during the fermentation process. In a previous study, it was reported that calcium carbonate was used as a neutralizing agent and the homofermentative lactic acid bacterium, *Lactobacillus sporogens*, showed fermentation yield of 99.3% and more than 98.2% of the yield was L(+)-lactic acid [14]. Also it was reported that 2065.0g of white crystal calcium lactate dihydrate prepared by *Lactobacillus sporogenes* was recovered and a yield of 84.9% was obtained [15]. On the other hand, Ferain *et al*. [16] reported that lactate dehydrogenase (LDH)-deficient *Lactobacillus plantarum*, an engineered strain that was more complicated to create, compared to *L. lactis*, because this lactic acid bacteria contains two quite different LDH-enzymes specific for L-lactate or D-lactate, respectively. Thus, *Lactobacillus lactis* was selected as a strain based on the yield of lactic acid in this study.

**Table 1: Products during fermentation by the strains of lactic acid bacteria after inoculation**

| KCCM | Strain of lactic acid bacteria | Lactic Acid (mg/ml) |
|---|---|---|
| 11712 | *Bacillus coagulans* | 34.14 |
| 11322 | *Lactobacillus platarum* | 176.80 |
| 40264 | *Lactobacillus sakei* | 283.92 |
| 41574 | *Lactobacillus lactis subsp. lactis* | 402.33 |

**Characterization of calcium lactate prepared by fermentation processes**

**Figure 4: FT-IR spectra of calcium lactate obtained by lactic acid fermentation.**
**1: Adding the 1% precipitated calcium carbonate (PCC) to the medium, 2: No adding the calcium carbonate, 3: Adding the 1% commercial calcium carbonate reagent to the medium.**

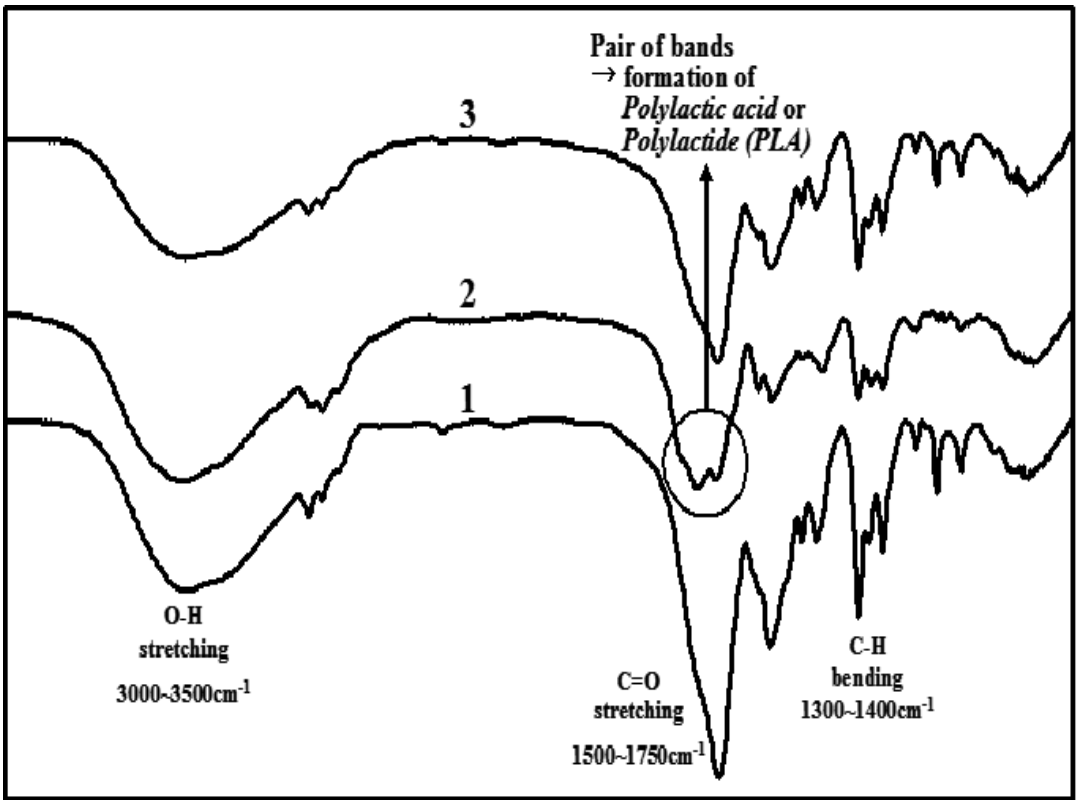


Figure 4 shows the FT-IR spectrum of L(+)-calcium lactate by precipitated calcium carbonate and *Lactobacillus lactis* in lactic acid fermentation. Compared to the FT-IR spectrum of L(+)-calcium lactate with precipitated calcium carbonate shows both differences and similarities. In all cases, the strong OH valence band of calcium lactate in the region of 3000~3500 $cm^{-1}$ of FT-IR spectra. The FT-IR spectra of L(+)-calcium lactate with precipitated calcium carbonate has the carbonyl bane with higher intensity at 1500~1750 $cm^{-1}$ than the others. We found that calcium lactate obtained by lactic acid fermentation, characteristic signals

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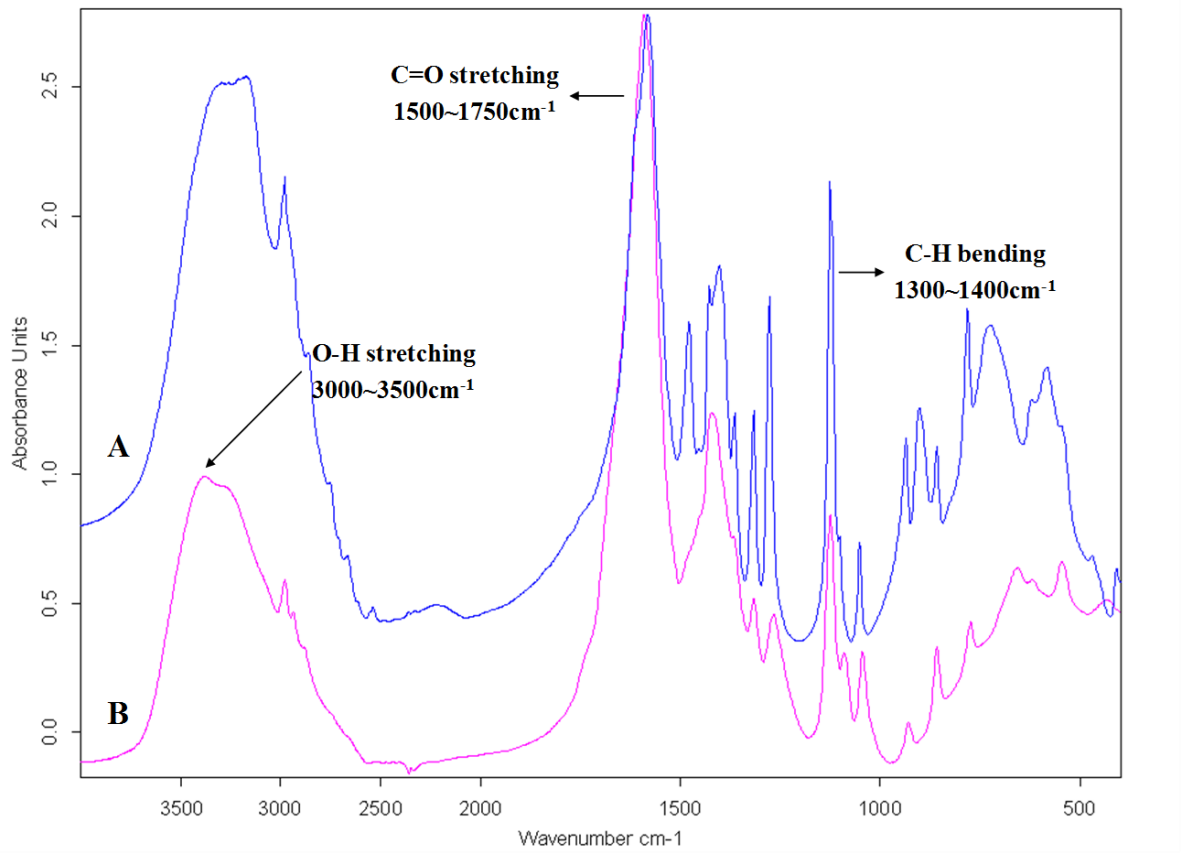
were found at approximately 3000~3500 $cm^{-1}$ (OH band) with a very high density, 2950~3000 $cm^{-1}$ (C-H stretching), 1500~1750 $cm^{-1}$ (C=O stretching), as well as 1300~1500 $cm^{-1}$ (C-H bending). Also in the case of the sample without calcium carbonate reagent or precipitated calcium carbonate, the pair of bands appearing near 1500~1750 $cm^{-1}$ contrary to the sample added calcium carbonate reagent or precipitated calcium carbonate due to formation of poly lactic acid (PLA) around 1720 $cm^{-1}$. In general, *lactobacillus lactis* have been developed into food-grade cell factories for the production of hydrolytic and other enzymes, protein or peptide ingredients and therapeutic substances. Therefore, lactic acid bacteria are used for the production of high-value metabolites involved in flavor, texture or health applications [8]. In a previous study, it was reported that the carboxylate peaks 1592 and 1430 $cm^{-1}$ (symmetry) and C=O stretching 1700 $cm^{-1}$ in IR spectra of the anhydrous calcium lactate [17].

**Characterization of calcium lactate pentahydrate prepared by lactic acid bacteria**

Figure 5 shows the FT-IR spectrum of L(+)-calcium lactate prepared by calcium carbonate reagent and *Lactobacillus lactis* in lactic acid fermentation. As comparing to the FT-IR spectrum of L(+)-calcium lactate reagent, it shows both differences and similarities. The FT-IR spectra of L(+)-calcium lactate prepared by calcium carbonate reagent and *Lactobacillus lactis* with lower intensity at approximately 3000~3500 $cm^{-1}$ (OH band) than the reagent. Also we found that calcium lactate obtained by lactic acid fermentation, characteristic signals were found at approximately 1500~1750 $cm^{-1}$ (C=O stretching), and 1300~1400 $cm^{-1}$ (C-H bending). However, the FT-IR spectra of L(+)-calcium lactate prepared by calcium carbonate reagent and *Lactobacillus lactis* show the OH band and C-H bending with lower intensity compared to the calcium lactate reagent.

**Figure 5: FT-IR spectra of calcium lactate pentahydrate (CLP) by lactic acid bacteria.**
**A : Calcium lactate pentahydrate reagent, B : Calcium lactate prepared by *L. lactis*.**




**Hydration and dehydration characteristics of calcium lactate pentahydrate**

The TG-DTA curve of calcium lactate pentahydrate was obtained through thermogravimetry differential thermal analysis. The solid line of the TG-DTA profile for calcium lactate are shown in Fig. 6. The curve showed a rapid decrease at 200~400 °C due to the melting point of calcium lactate, which was about

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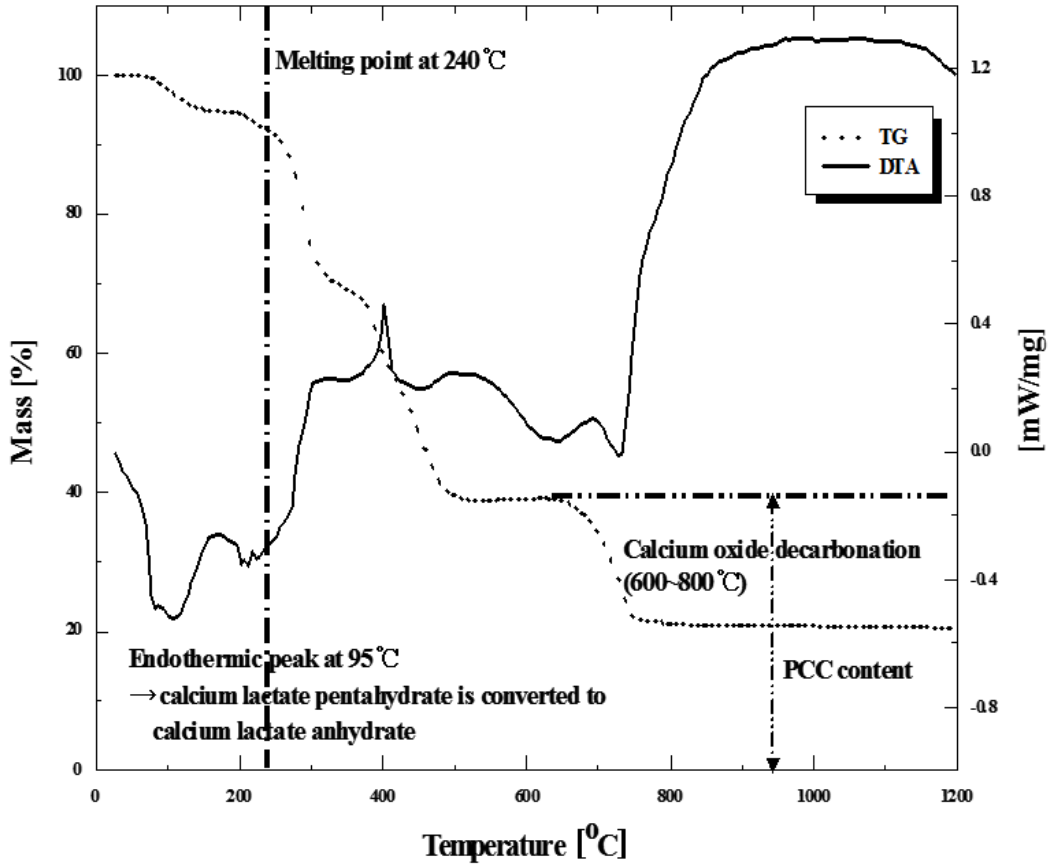
240 °C. In particular, the curve had a second decrease at 600~800 °C due to the decarbonation of calcium oxide. When the calcium lactate sample was subjected to DSC in an open pan under the same experimental conditions as those for TG-DTA, the peak maximum temperature in the DSC curve was consistent with that for the dehydration step in TG-DTA. The dotted line shows the DSC curve for calcium lactate pentahydrate, displaying an endothermic peak at about 95 °C. In a previous study, the DSC curves of unpulverized calcium lactate pentahydrate and its tablets showed endothermic peaks due to dehydration at 79.5 °C and 54.6 °C, respectively, which were differ from our results [18]. On the other hand, it was reported that thermal decomposition kinetic mechanisms of calcium carbonate do not vary with decreasing of their average diameters [19]. In general, calcium lactate pentahydrate is converted into calcium lactate anhydrate [12]. Also it is known that the hydration and dehydration behaviors of drug and filler is essential to develop stable pharmaceutical formulations, because the physiochemical and mechanical properties and biological effects of hydrates could differ from those of anhydrates [18].

**Figure 6: TG-DTA curve thermograph of calcium lactate pentahydrate.**




**SEM image of calcium lactate pentahydrate using precipitated calcium carbonate**

Figure 7 shows the SEM images of calcium lactate and unreacted precipitated calcium carbonate crystals. Calcium lactate using precipitated calcium carbonate existed as plate-like crystals with a smooth surface. These results suggested that the particles of calcium lactate using precipitated calcium carbonate fused together and formed large, aggregate particles. Sakata *et al*. [12] reported that the changes in surface morphology, the particle size of calcium lactate pentahydrate was also affected by dehydration. In a previous study about several calcium sources, it was reported that calcium lactate and the combination of calcium carbonate and xylitol were superior to pure calcium carbonate or calcium citrate for bone health [20]. On the other hand, Heaney *et al*. [21] reported that three forms of calcium sources including calcium carbonate, encapsulated calcium carbonate and calcium citrate were equally absorbed and had equivalent bioavailability. However, given the equivalent bioavailability of the calcium carbonate and calcium citrate, the cost benefit analysis favors the less expensive carbonate product.

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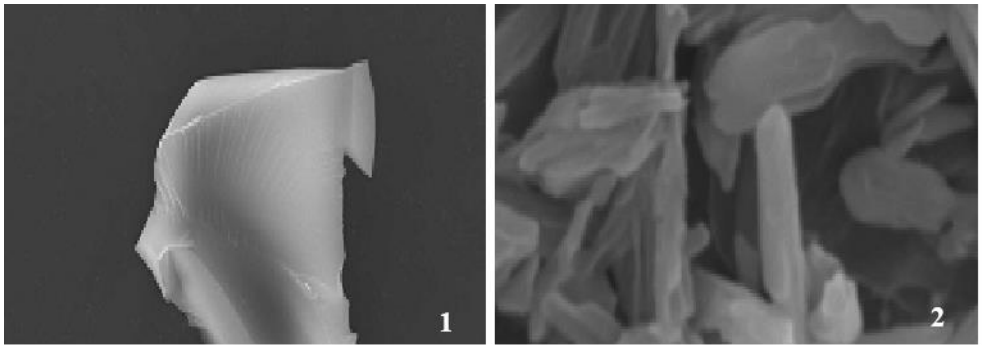
**Figure 7: Scanning electron microscope (SEM) images of calcium lactate pentahydrate and un-reacted precipitated calcium carbonate.**
**1: calcium lactate pentahydrate prepared by PCC.**
**2: Un-reacted PCC after the end of reaction.**




## CONCLUSIONS

In conclusion, we prepared calcium lactate pentahydrate as a calcium additives using biological method. Our results confirmed that the possibility for the formation of calcium lactate pentahydrate prepared by single-phase aragonite PCC. Therefore, these results may be have substitution effect of the imported calcium lactate and increasing effect of make efficient use of resources inside of the country.

## ACKNOWLEDGEMENT

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