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Preparation and Characteristics of Calcium Lactate from Black Snail

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Abstract

Calcium lactate (CL) prepared from powdered black snail (PBS) or its ashed powder (ABS), was investigated for ideal manufacturing conditions to optimize color, solubility and sensory quality. Based on the amount of PBS and 100 mL lactic acid (LA), the yields of PBS-CL were 300% and 15 g in 10% LA and 260% and 20 g in 20% LA. Yields of ABS-CL based on the amount ABS and 100 mL LA were 400% and 60 g in 10% LA and 329% and 66 g in 20% LA. Both of the yields were decreased with an increase of the LA concentration on the basis of PBS and ABS amounts, but proportionally increased with the increment in the LA concentration on the basis of LA volume. Optimal preparation times of the dehydrated PBS-CL and ABS-CL were, respectively, 4 hr and 5 hr at 100°C, 3 hr and 4 hr at 120°C, and 1 hr and 2 hr at 150°C, which showed shorter time in preparing the dehydrated ABS-CL. PBS-CL and ABS-CL were confirmed to be $Ca(CH_3CHOHCO_2)_2$ by the analysis results of IR and ^{1}H-NMR. Calcium contents of the anhydrous PBS-CL and ABS-CL were individually 15.4% (w/w) and 17.3% (w/w) representing 84.2% and 94.5% of each theoretical value. Colors of PBS-CL and ABS-CL were light yellow and light-greenish white each. Solubilities of PBS- CL and ABS-CL in distilled water at pH 3~8 were 5.43 and 6.11 g/100 mL, respectively, which demonstrated higher mean solubilities rather than the 4.74 g/100 mL of standard CL. Solubilities of PBS-CL (3.14~5.03 g/100 mL) and ABS-CL (4.69~6.05 g/100 mL) against soup soy sauce, 3% brine, *Soju* (Korean distilled liquor), thick soy sauce, grape juice and orange juice were higher than those of standard CL (2.94~5.84 g/100 mL). ABS-CL was believed to have a wide use range due to its low sourness while different applications of PBS-CL in food are expected due to its mild astringent taste and strong savory taste despite its strong bitter taste as estimated by sensory evaluation.

Key words: black snail, calcium lactate, preparation, characteristics

INTRODUCTION

Calcium is the most plentiful substance among internal minerals and an adult contains about 1,200 g of calcium. While its 99% of body calcium is deposited in bones and teeth, the remaining 1% is present in cell sap and implements a variety of functions on neural transmission, muscle contraction and relaxation, myocardial contraction, cellular metabolism, villus movement, blood coagulation, bacterium engulfment of lymphocyte, excitement, and metabolism of hormone and nutrients (1). The excretion of internal calcium has been shown to be promoted by the increased protein intake, which increases the risk of osteoporosis (2,3), and the development of a calcium supplement having high internal adsorption would be beneficial for preventing hypertension, diabetes and brain disease (4). A number of studies have reported that natural foods which are rich sources of calcium, are seashell (5), sea tangle (6), crustacean (7), pig bone (8), fish bone (9), cuttle bone (10), egg shell (11,12), etc. However, most studies have been principally concerned with the ingredient characteristics of calcium resources and the manufacture and characteristics of calcium supplements; only a few studies using egg shell (11) and ostrich eggshell (12) have investigated the manufacture of calcium lactate having high internal adsorption. Calcium lactate, a nontoxic water-soluble (13) food additive for calcium supplement (14,15), is currently used for making bread (16), soy milk (17), orange juice (18) and yogurt (19). Calcium lactate is also reported to function as an anti-microbial (20), enhancer of bone mineral density (21), anti-caries (22), anti-carcinogen (23,24). Calcium lactate is manufactured by adding calcium hydroxide or calcium carbonate to a lactic acid solution and heating them, which has the ability to easily effloresce into a colorless crystalline pentahydrated salt (14). When it is heated at 100°C, the calcium lactate becomes dehydrated calcium lactate, which can have the disadvantage of dully stimulating the stomach as calcium chloride does (14).

Black snail, called Godung (gastropod), fresh water Godung, Golbengyi, Godi, conch or snail in Korea, is

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widespread in Asian countries (including Korea, China, Japan, Taiwan, etc.), Central and South America, and Africa. It is 35 mm long on average with a diameter of 15 mm. It is hard-shelled in such diverse shapes as sleek, rough, bumpy, vertically linear or horizontally linear ones (25). Colors are also diversified, e.g., yellow, yellowish brown, dark brown, brown or black. Black snail has been widely used since ancient times to make a broth because blue-colored substances that leaches out during boiling are known to be good for liver diseases such as hepatitis, cirrhosis, liver cancer, etc. There are 14 species and 4 subspecies that are indigenous to Korea (25).

This study investigated the manufacturing characteristics and solubility of calcium lactate regardless of ashed and non-ashed, to evaluate the potential of the shell for use as a high value-added calcium resource.

MATERIALS AND METHODS

Materials

Black snail (*Semisulcopira bensoni*), having averages about 35 mm in length and 15 mm in diameter, were originated from North Korea. A 72% ultrapure lactic acid (Daejung Co. Ltd., Korea) was used and standard calcium lactate (CL) was prepared with pentahydrated DL-calcium lactate (Duksan Pharm. Co. Ltd., Korea), which were dehydrated to be anhydrous at 120°C for 3 hours. Samples for measuring solubility were 25% *Soju*-Korean distilled liquor (Charm Soju, Kumbokju Co., Korea), soup soy sauce (Samhwa Co., Korea), thick soysauce (Samhwa Co., Korea), 3% brine (Refined salt, Hanju Co., Korea), orange juice (Wungjin Co., Korea) and grape juice (Wungjin Co., Korea).

Preparation of black snail powder and ashing

The black snails were triplicately washed with tap water and steamed at 121°C for 20 min in an autoclave. The snails were then dried at 60°C and pulverized to pass through a 100 mesh sieve to obtain powdered black snail (PBS) using a laboratory mill (Model 4, Thomas Scientific, USA). The PBS was ashed in a furnace (Hwashin Co., Korea) at 600°C for 4.5 hours.

Preparation of dehydrated calcium lactate

Calcium lactate was prepared from PBS and ashed black snail (ABS) according to the manufacturing procedure illustrated in Fig. 1. One hundred milliliters of lactic acid solution adjusted to be 10～50% was placed in a 500 mL Erlenmeyer flask with a cooling device-attached and was heated at 70°C while adding a predetermined amount of PBS or ABS constantly stirring with a magnetic stirrer/hot plate (Misung, MS-300, Korea) and finally neutralized at pH 7.0. Then, the neutralized lactate solution was passed

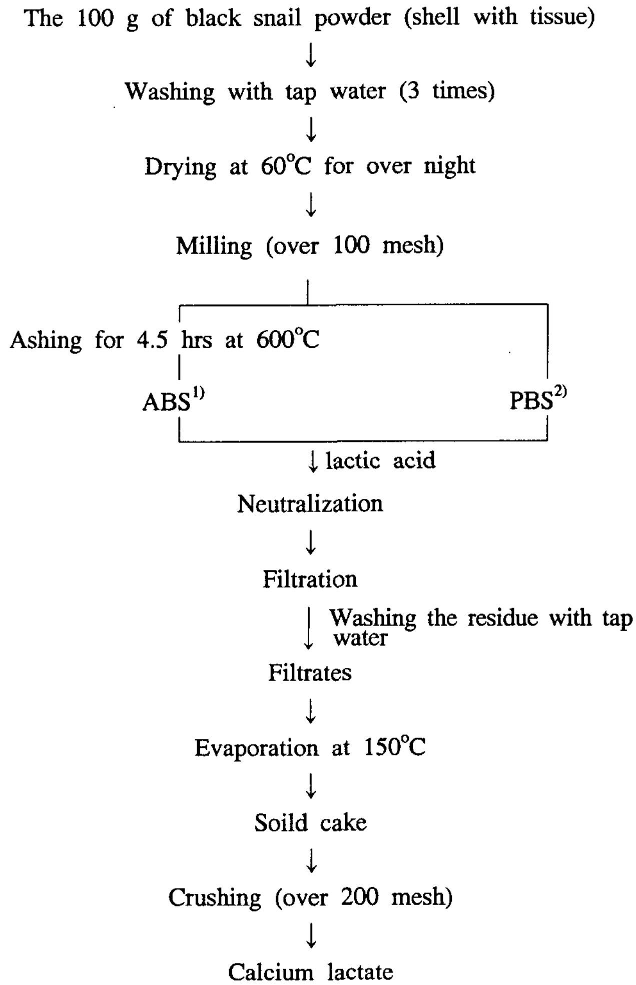

Fig. 1. Preparation procedure of calcium lactate from black snail. [1]ABS, ash of black snail; [2]PBS, powder of black snail.

through a glass filter to eliminate the residue and was then dehydrated at 100～150°C to obtain anhydrous calcium lactate.

Analysis of mineral contents

The mineral content of calcium lactate was analyzed by the method of Seo et al. (26). Briefly, 100 mL of 6 N HCl added to 500 mg of samples, which was dissolved over night, filtered through filter paper (Whatman No. 2), brought to 1000 mL volume with distilled water, and then analyzed by ICP-AES (JY 38 Plus, France). The analyzing conditions were set to the 40.66 MHz frequency, 12 L/min plasma gas flow, 0.2 L/min sheath gas flow, 0.1 L/min auxiliary gas flow and 1 L/min sample flow rate. The measurements were performed in each natural frequency.

Analysis of IR and ^1H NMR

The infrared-ray (IR) spectrum was generated by preparing a sample according to the KBr pellet method (27) and using FT-IR/FT-Raman and Mattson Polaris PT-IR spectrophotometer (Brucker Co., USA). The nuclear magnetic resonance (^1H NMR) spectrum was retrieved from a 200 MHz spectrophotometer (Varian Gemini-200, USA). D_2O enabled to estimate values of a chemical shift (δ) for tetramethylsilane (TMS), an internal standard.

Measurement of solubility

The solubility of calcium lactate was measured at different pHs by using distilled water whose pH was adjusted within the range of pH 3~pH 8. To calculate the solubilities in tap water, *Soju*, soup soy sauce, thick soy sauce, 3% brine, orange juice and grape juice, sample solution was added into 1~2 g calcium lactate to be 20 mL, allowed to sit at room temperature for 30 min and centrifuged at 2000 × g for 15 min. Supernatant-eliminated insoluble residue was dried at 105°C and the dissolved was calculated to determine the solubility (%) based on 100 mL.

Sensory evaluation

Sensory tests on sour, bitter, astringent, fishy and savory tastes were evaluated by twenty five panelists selected from graduate and undergraduate students in the Department of Food Science and Technology, using a five-point scale method (28) as very strong (5 points), strong (4 points), medium (3 points), weak (2 points) and very weak (1 point).

Statistical analysis

All experiments except sensory evaluation were triplicately determined and represented as mean±standard deviation (SD). The result from the sensory evaluation was calculated from the mean value and SD of the 25 panelists. Significance was verified by performing Duncan's multiple range test using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) software package.

RESULTS AND DISCUSSION

Yields of calcium lactate on different lactic acid concentrations

Fig. 2 shows the amounts of PBS and ABS required for neutralizing the lactic acid solution controlled to 10, 20, 30, 40 and 50%. The amounts of PBS for neutralizing 100 mL lactic acid solution were 38 g in a 10% solution, 64 g in a 20% solution and 90 g in a 30% solution, but the solution could not be neutralized at concentration of 40% or higher due to its high viscosity. For neutralizing the lactic acid solution, the amounts of ABS were 15 g in a 10% solution, 20 g in a 20% solution, 35 g in a 30% solution, 55 g in a 40% solution and 70 g in a 50% solution, which proves that the ABS amount was proportionally augmented with the increment of the lactic acid concentration. Fig. 3 shows the yields of calcium lactate (CL) from each lactic acid concentration on the basis of

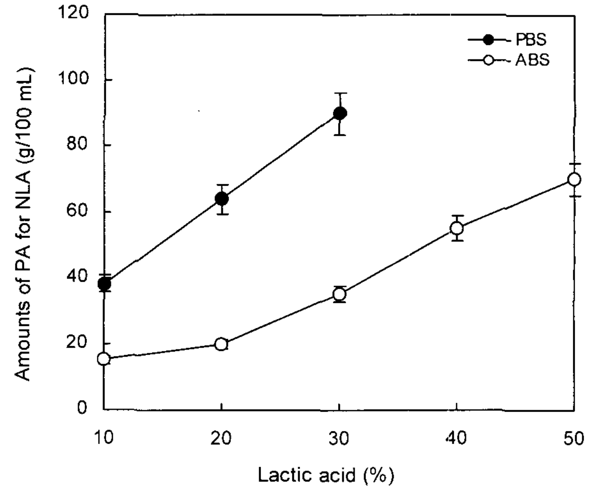

Fig. 2. Relationship between lactic acid concentration and amounts (g) of powder of black snail (PBS) and ash of black snail (ABS) consumed for neutralization of 100 mL lactic acid. Values are mean±SD of triplicate determinations. Abbreviations of PA is PBS and ABS, and NLA is neutralization of 100 mL lactic acid.

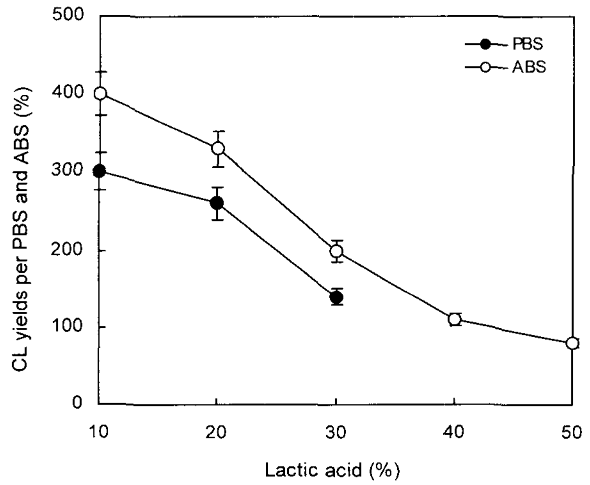

Fig. 3. Effect of lactic acid concentration on yields (%) of *calcium lactate (CL) per powder of black snail (PBS)* and ash of black snail (ABS). Values are mean±SD of triplicate determinations.

ABS and PBS. ABS yielded 400% CL in a 10% solution, 329% CL in a 20% solution, 195% CL in a 30% solution, 109% CL in a 40% solution and 64% in a 50% solution. PBS produced 300%, 260% and 140% of CL in 10%, 20% and 30% solution, respectively. Accordingly, the yields of CL were decreased with the increase of the lactic acid concentrations in the cases of both ABS and PBS. Fig. 3 shows the yields of CL on the basis of 100 mL lactic acid solution. While PBS yielded CL 12 g, 19 g and 24 g of CL in 10%, 20% and 30% solution, respectively. ABS resulted in the highest yield in 30% solution, as evidenced by CL yields of 60 g, 66 g, 68 g, 60 g and 45 g in the order of the lactic acid concentrations from 10% to 50%. According to these results, a 10% lactic acid solution yielded the highest CL after considering all bases of the ABS and PBS and the amount of lactic acid solution. Although ABS showed the highest yield, it may still be more efficient to manufacture CL using non-ashed PBS due to the low yield of PBS and the ashing costs for ABS. Zhao and Song (11) and Ko and No (12) have used ABS in manufacturing calcium lactate with egg shells and ostrich eggshells, respectively, and have reported that the yields were not attributed to particle size (0.15~0.84 mm), temperature in the neutralization, and stirring speed and time. When molar ratios of eggshell ash to lactic acid were changed from 1 : 1.44 to 1 : 3.12, the lactate yields were augmented as lactic acid concentrations were increased, which was consistent with our results (Fig. 4) based on the amounts of lactic acid.

Preparation temperature and time of dehydrated calcium lactate

Pentahydrated calcium lactate salts were prepared by reaction of the 10% lactic acid solution and PBS or ABS, which have the ability to easily effloresce (14). The CL then needs to by dried and converted to a dehydrated salt.

Fig. 5 illustrates the dehydrating temperature and time for preparing anhydrous salt with PBS-CL and ABS-CL. Each time needed to manufacturing anhydrous PBS-CL and ABS-CL was 4 h and 5 h at 100°C, 3 h and 4 h at 120°C, and 1 h and 2 h at 150°C, respectively. Anhydrous CL can be generally obtained by heating at 100°C (14), however Ko and No (12) manufactured it by heating at 150°C.

Identification of calcium lactate

IR and ^{1}H-NMR spectra of the anhydrous PBS-CL and ABS-CL were identical, so only PBS-CL results are shown

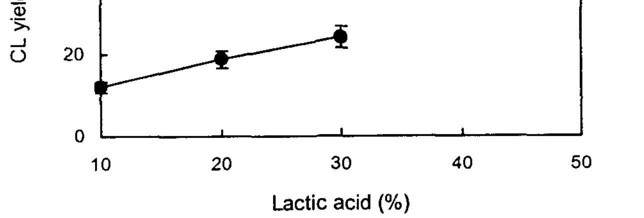

Fig. 4. Effect of lactic acid (LA) concentration on yields of calcium lactate (CL) prepared from powder of black snail (PBS) and ash of black snail (ABS). Values are mean ± SD of triplicate determinations.

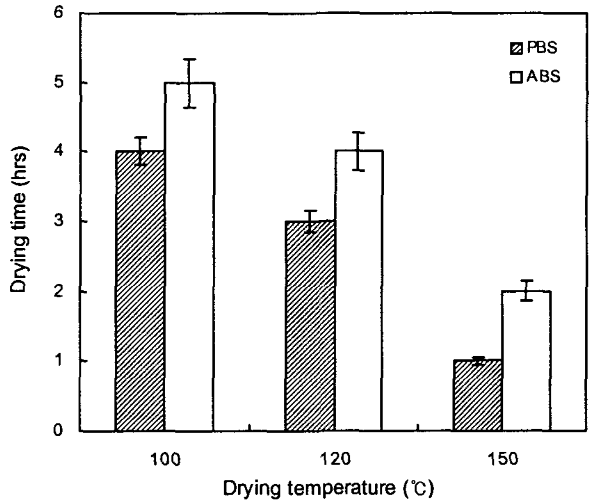

Fig. 5. Effect of drying temperatures on the drying time of calcium lactate prepared from powder of black snail (PBS) and ash of black snail (ABS). Values are mean ± SD of triplicate determinations.

in Figs. 6 and 7. The carboxylate peaks 1592 (asymmetry) and 1430 cm^{-1} (symmetry) which can show in the lactate were confirmed in the IR spectrum. Due to the resonance structure, the 1700 cm^{-1} stretch frequency of C=O in the CL prepared with PBS or ABS was assumed to be shifted from the value of literature (29). The ^{1}H-NMR spectrum using D_2O as a solvent confirmed the quartet at 3.96 ppm as a CH proton peak of $CH_3CH(OH)COO^-$ and the doublet at 1.14 ppm as a peak of CH_3 (29). And, the OH proton was replaced with the D_2O solvent, and was naturally not seen on the spectrum (29). Therefore, PBS-CL and ABS-CL were verified to be $Ca(CH_3CHOHCO_2)_2$ by the IR and ^{1}H-NMR spectra (30).

Minerals content of the calcium lactate

Table 1 shows the mineral contents of PBS-CL and ABS-CL manufactured by regulating the ratios of 10% lactic acid solution to PBS and to ABS as 100 : 38 (v/w) and 100 : 15 (v/w), respectively. Calcium lactate [Ca ($CH_3CHOH\ CO_2)_2$] had one Ca molecule and two lactic acid molecules with a molecular-weight of 218, 18.3% of which was calcium. The percentages of calcium in PBS-CL and ABS-CL were 15.4% (w/w) and 17.3% (w/w), respectively, which were 84.2% and 94.5% of each theoretical value. Other minerals in PBS-CL and ABS-CL, respectively, included 219.0 and 76.0 mg% (w/w) of Fe and 150.2 and 102.5 mg% of Na, and the contents of Mn and Zn in the range of 5.1~56.0 mg% (w/w). Ko and No (12) reported that the calcium lactate manufactured with ashed ostrich eggshells contained 40.98% (w/w) of Ca, and 0.2~0.4 mg% (w/w) of trace elements such as P, Mg, Na, Fe, Cu, Zn, Mn and Al.

Color of calcium lactate

L* values of PBS, ABS, PBS-CL, ABS-CL and standard

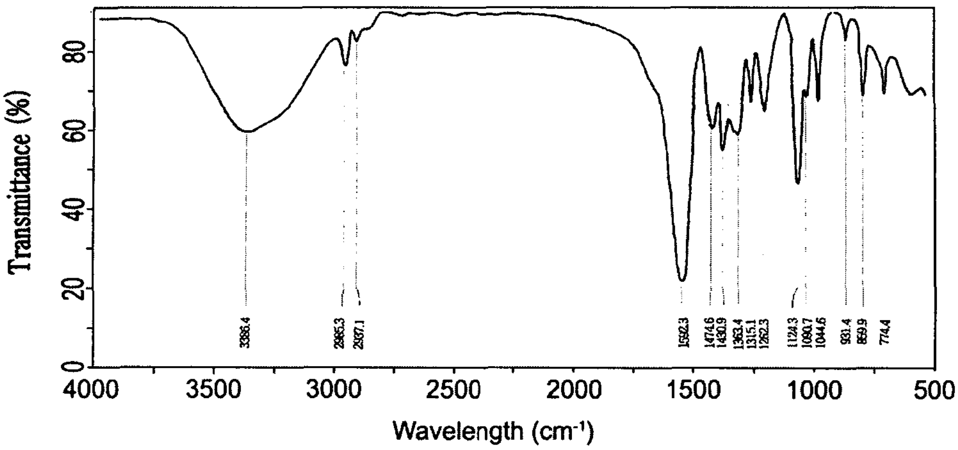

Fig. 6. IR spectrum of dehydrated calcium lactate prepared from powder of black snail (PBS).

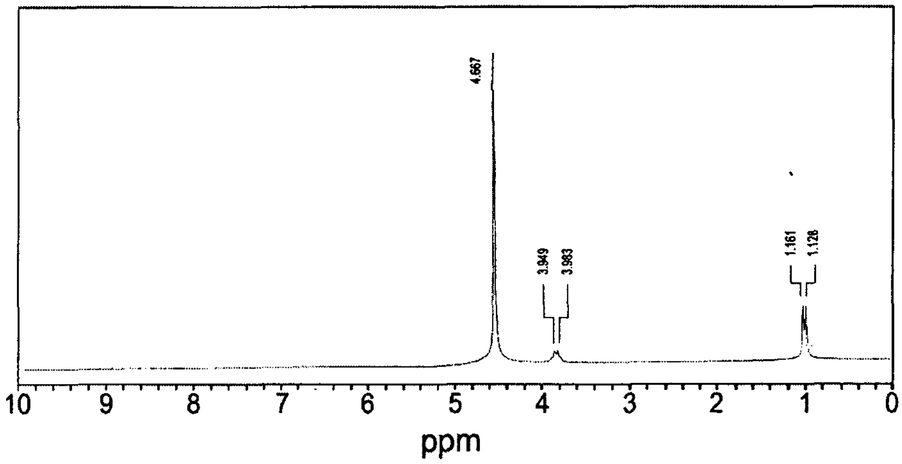

Fig. 7. ^{1}H-NMR spectrum of dehydrated calcium lactate prepared from powder of black snail (PBS).

Table 1. Mineral compositions of dehydrated calcium lactate prepared from powder of black snail (PBS) and ash of black snail (ABS) (mg/100 g)

Minerals	PBS-CL$^{1)}$	ABS-CL	Standard CL$^{2)}$
Ca	15,400.5$^{b3)}$	17,305.0^{a}	17,930.7^{a}
Fe	219.0^{a}	76.0^{b}	nd
Na	150.2^{a}	102.5^{b}	nd
Mn	23.0^{b}	56.0^{a}	nd
Zn	5.1^{a}	6.2^{a}	nd
Cu	nd$^{4)}$	nd	nd

$^{1)}$CL: Dehydrated calcium lactate.
$^{2)}$Standard CL: Calcium *D,L*-lactate (pentahydrate) was dehydrated at 120°C for 3 hrs.
$^{3)}$Values are means of triplicate determinations, different superscripts within a row indicate significant differences at $p<0.05$.
$^{4)}$Nd: Not detected.

Table 2. Color of dehydrated calcium lactate prepared from powder of black snail (PBS) and ash of black snail (ABS) (mg/100 g)

Calcium lactates	L*	a*	b*
PBS	73.69$^{d3)}$	-1.23^{e}	6.48^{b}
ABS	58.55^{e}	0.76^{b}	3.70^{d}
PBS-CL$^{1)}$	79.57^{c}	2.77^{a}	16.51^{a}
ABS-CL	96.90^{b}	-0.97^{d}	6.17^{c}
Standard CL$^{2)}$	99.88^{a}	-0.43^{c}	0.23^{e}

$^{1)}$CL: Dehydrated calcium lactate.
$^{2)}$Standard CL: Calcium *D,L*-lactate (pentahydrate) was dehydrated at 120°C for 3 hrs.
$^{3)}$Values are means of triplicate determinations, different superscripts within a row indicate significant differences at $p<0.05$.

CL were 73.69, 58.55, 79.57, 96.90 and 99.88, respectively (Table 2). While the color of standard CL was white, PBS-CL and ABS-CL manufactured with PBS (light-greenish gray color) and ABS (light-bluish dark gray color) were light yellow and light-greenish white. Moreover, PBS-CL and ABS-CL individually had a* values of 2.77 and -0.97, and b* values of 16.51 and 6.17, which explained that the b* values were increased in manufacturing anhydrous CL while the a* values were increased in using PBS but decreased when using ABS. These results may be due to the dark pigmented substances on shell of the black snail not being completely burned at 600°C but remaining.

Solubility of calcium lactate

Table 3 shows the solubilities of PBS-CL and ABS-CL in distilled water at different pHs from pH 3~pH 8. The mean solubilities of PBS-CL and ABS-CL were each 5.43 g/100 mL and 6.11 g/100 mL, which were higher than 4.74 g/100 mL of standard CL; ABS-CL showed the highest solubility. From the results by each pH, PBS-CL had the higher solubility 6.08~6.22 g/100 mL at pHs 6 and 7 whereas the lower solubility of 4.72~4.76 g/100 mL was found at pHs 4 and 8. ABS-CL resulted in the higher solubility of 6.30~6.44 g/100 mL at pHs 5~7 and the lower one of 5.77~5.94 g/100 mL at pHs 3, 4 and 8. However, the solubilities of standard CL had insignificant differences within the range of 4.60~4.91 g/100 mL at pHs 3~8. The solubilities of PBS-CL, ABS-CL and standard CL in liquid foodstuffs are shown in Table 4. Most of the liquid foodstuffs, with the exception of soup soysauce, had higher solubilities for PBS-CL and ABS-CL manufactured with black snail than for standard CL. Especially, ABS-CL was more soluble in those foodstuffs, i.e., its solubility was as high as 60% in soup soysauce, 41~46% in 3% brine, Soju (alcohol conc. 25%) and grape juice, and 27% in orange juice.

Sensory quality

Table 5 shows the sensory evaluation of the anhydrous PBS-CL and ABS-CL manufactured with black snail comparing the results with that of standard CL. Sour tastes were in an ascending order of PBS-CL<ABS-CL<standard CL. Bitter taste earned a high score, 4.15-points for PBS-CL which had a low score of 2.01-points for astringent taste. Also, PBS-CL got a higher score on savory taste than did ABS-CL or standard CL. The fact that some fishy taste was noted in PBS-CL is possibly a result of using the shell of black snail along with flesh-tissue in manufacturing PBS. Moreover, the comparatively higher bitterness in ABS-CL may be because the flesh-tissue of black snail were eliminated and consequently the minerals were concentrated by the ashing process, and therefore more diverse minerals were presented in ABS-CL than in standard CL manufactured from calcium carbonate.

Table 3. Solubility of calcium lactate prepared from powder of black snail (PBS) and ash of black snail (ABS) in distilled water with different pH

pH	PBS-CL[1)]	ABS-CL	Standard CL[2)]
3	5.10 (104)[3)]^{bC}[4)]	5.77 (118)^{aB}	4.91 (100)^{cA}
4	4.72 (99)^{bD}	5.94 (125)^{aB}	4.76 (100)^{bA}
5	5.65 (123)^{bB}	6.30 (137)^{aA}	4.60 (100)^{cB}
6	6.08 (129)^{bA}	6.44 (136)^{aA}	4.72 (100)^{cA}
7	6.22 (130)^{aA}	6.34 (132)^{aA}	4.80 (100)^{bA}
8	4.76 (103)^{bD}	5.85 (126)^{aB}	4.63 (100)^{bB}
Mean solubility	5.43 (115)^{b}[4)]	6.11 (129)^{a}	4.74 (100)^{c}

[1)]CL: Dehydrated calcium lactate.
[2)]Standard CL: Calcium *D,L*-lactate (pentahydrate) was dehydrated at 120°C for 3 hrs.
[3)]Parenthesis are percent against solubility of standard CL.
[4)]Values are means of triplicate determinations, different superscripts within a row (a-c) and column (A-F) indicate significant differences at $p<0.05$.

Table 4. Solubility of calcium lactate prepared from powder of black snail (PBS) and ash of black snail (ABS) in the various liquid foods (g/100 mL)

	PBS-CL[1)]	ABS-CL	Standard CL[2)]
3% salt water	4.73 (119)[3)]^{b}[4)]	5.81 (146)^{a}	3.99 (100)^{c}
Soju (25%)	4.70 (116)^{b}	5.87 (145)^{a}	4.04 (100)^{c}
Thick soysauce	3.14 (106)^{b}	4.69 (160)^{a}	2.94 (100)^{c}
Soup soysauce	3.26 (56)^{c}	5.40 (93)^{b}	5.84 (100)^{a}
Orange juice	5.03 (116)^{b}	5.50 (127)^{a}	4.33 (100)^{c}
Graph juice	4.37 (102)^{b}	6.05 (141)^{a}	4.28 (100)^{b}

[1)]CL: Dehydrated calcium lactate.
[2)]Standard CL: Calcium *D,L*-lactate (pentahydrate) was dehydrated at 120°C for 3 hrs.
[3)]Parenthesis are percent against solubility of standard CL.
[4)]Values are means of triplicate determinations, different superscripts within a row indicate significant differences at $p<0.05$.

Table 5. Sensory evaluation of dehydrated calcium lactate prepared from powder of black snail (PBS) and ash of black snail (ABS) (g/100 mL)

Attributes	PBS-CL[1)]	ABS-CL	Standard CL[2)]
Sour taste	4.21^{a}[3)]	2.53^{c}	3.53^{b}
Bitter taste	1.23^{c}	4.15^{a}	2.50^{b}
Astringent taste	2.01^{b}	2.53^{a}	2.52^{a}
Fishy taste	2.65^{a}	1.05^{b}	1.02^{b}
Savory taste	3.80^{a}	1.23^{b}	1.08^{b}

[1)]CL: Dehydrated calcium lactate.
[2)]Standard CL: Calcium *D,L*-lactate (pentahydrate) was dehydrated at 120°C for 3 hrs.
[3)]Sensory evaluation was conducted by 25 panelists using a 5-point scale (1 point: very weak to 5 points: very strong), and values are means of 25 panels, different superscripts within a row indicate significant differences at $p<0.05$.

CONCLUSION

In manufacturing PBS-CL and ABS-CL from PBS and ABS, the proper concentration of lactic acid was 10~20% and the yields for PBS and ABS were 260~300% and 329~400%, respectively. The time taken to manufacture the dehydrated PBS-CL and ABS-CL was 3~4 h at 120°C. The anhydrous PBS-CL and ABS-CL contained 84.2% and 94.5% calcium, and showed light yellow and light-greenish white colors, separately. In distilled water at pH 3~8, the mean solubilities of PBS-CL and ABS-CL were 5.43 g/100 mL and 6.11 g/100 mL, respectively which were higher than 4.74 g/100 mL of standard CL. Most liquid foodstuffs including 3% brine, Soju-Korean distilled liquor, thick soysauce, grape juice and orange juice (but not soup soysauce) showed higher solubilities, 3.14~5.03 g/100 mL and 4.69~6.05 g/ 100 mL, respectively for PBS-CL and ABS-CL as opposed to 2.94~5.84 g/100 mL for standard CL. According to the sensory evaluation, ABS-CL was considered to have a wide range of applications due to its low sourness, and PBS-CL should also have valuable applications in food processing because of its low astringent taste and strong savory flavor, despite its very high bitter taste.

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