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Study on the characteristics of γ-Aminobutyric acid(GABA) salt produced by various processes

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Abstract

Background/Objectives: GABA (γ - Aminobutyric acid) salts were prepared by spray-drying and superheated steam using a mixture of culture broth, salt water derived from deep seawater and commercial salt, and several characteristics of those were studied.

Methods/Statistical analysis: The morphology of GABA salts were observed by SEM (Scannig Electron Microscopes). The chromaticity and GABA contents of the GABA salt prepared by various methods were shown as an average for three or more repeated experiments. For statistical processing for sensory evaluation, analysis of variance was performed using the SPSS software package (Version 22.0, SPSS Inc., Chicago, IL, USA) and Duncan's multiple range test ($p<0.05$) was performed.^{o}

Findings: According to the raw materials and processing methods, GABA salt showed different crystal form, but it was possible to produce GABA salt containing 3-4% (weight of GABA/weight of salt) GABA without changing the mineral contents of the raw salts. The color changes of GABA salts according to the addition ratio of the culture broth could be minimized by the discoloration of the culture medium. Morphological differences were found between the commercial sun-dried salt, spray-dried salt and salt dried by superheated steam, and no separated particles were detected which indicates that the various processes could be applied to make GABA salts. Sensory evaluations of GABA salts compared with refined salt showed low scores in saltiness, bitterness, color and overall preference so commercialization of GABA salts needs not combining process with GABA and salt but solve the organoleptic problems.

Application/Improvements: With the GABA salts in this study, intake of 150-200 mg GABA per 5 g of daily recommended salt intake that the physiological activity to prevent hypertension can be expected.

Key Words : GABA salt, spray drying, fermentation, superheated steam, sensory characteristics.

1 Introduction

Salt is an inorganic substance essential for maintaining the physiological function of human beings, and it is not only essential for maintenance of vital phenomena such as maintaining muscle excitability, promoting metabolism and maintaining osmotic pressure of cell membranes, but also is a component that plays an important role in adjusting the seasoning of food and improving the manufacturing and storage of processed foods1. According to the 2011 National Nutrition Survey of the Korea Centers for Disease Control and Prevention, the average Korean salt intake is 12.16 g (sodium 4,791 mg) which was slightly lower than in 2010 (12.27 g) but more than 2 times higher than that of the World Health Organization recommendation of 5 g (1,969 mg of sodium), and higher than Japan, which has relatively high salt intake, as well as the United Kingdom and the United States. In particular, the average intake

of salts of men between 30s to 40s in Korea enjoy eating kimchi, fermented foods, and ramen is about 5,400 mg, which is a major cause of chronic diseases such as hypertension, heart disease, cerebrovascular disease and kidney disease due to sodium overload[2,3]. γ-Aminobutyric acid (GABA) is a nonprotein amino acid that exists mostly in the bone marrow of brain and increases neurotransmitters. In addition to physiological functions such as promoting brain function, it is also known to be effective in lowering blood pressure, diuretic action, antioxidant activity, regulating secretion of growth hormone, and also in pain relief[4,5,6]. In the 2000s, experiments using lactic acid fermentation to produce GABA[7,8] in the culture medium by the decarboxylation of glutamic acid present in the medium proceeded to produce a culture medium including high GABA content[9,10]. Superheated steam is steam that is heated to a temperature higher than the boiling point, and even if the temperature is kept above the boiling point, water drops are not generated[11]. When the superheated steam is used in a drying process, the drying is done through contact between the superheated steam and the material[12] and the superheated steam provides heat for evaporation of moisture to the material and transfers the evaporated steam to the outside. The superheated steam drying is limited to the food and paper fields but there are many fields to be studied and has the advantage that drying speed is improved and the sterilization and deodorization process can be performed at the same time[13,14]. Previous studies have confirmed the possibility of producing GABA salt by crystallization of culture broth containing GABA15 and sea water. In this study, GABA was coated on the salt by spraying the GABA containing culture broth with superheated steam, the deep sea water in which the fresh water was removed and the culture broth were spray dried, to propose a process for the preparation of GABA salt which has the function of solving the health problem due to excessive consumption of salt and comparing the characteristics of the prepared GABA salt with the conventional commercialized salt. The sensory characteristics of GABA salt and common salt were compared for conforming the possibility of commercialization of GABA salt.

2 Materials and Methods

2.1 Materials

2.1.1 Raw materials

Sea water which some fresh water removed from Goseong deep sea water agricultural industrial complex was used as salt water and the rice bran used as a raw material for the GABA production medium supplied within 24 hours from polishing in Gimpo Nonghyup rice processing complex (RPC) and was stored at -18^0C. Flower salt (refined salt, Hanju Corporation, Ulsan, Korea), roasted salt (roasted salt, sannaedle Co., Ltd, Chungbuk, Korea), sun-dried salt (sun drying salt, taepyeongsaltfarm, shinan, korea), and pepper salt (processed salt, taepyeongsaltfarm, shinan, korea) were purchase from supermarkets.

2.1.2 Fermentation strain

Lactobacillus sakei B2-16 isolated from kimchi received from Biovan Co., Ltd. (Bucheon, Korea) was used for the fermentation. Lactobacillus sakei B2-16 activated 24 hrs in Lactobacilli MRS broth at 30^0C as a seed culture and stored at -70^0C in a form of 30% glycerol stock

2.1.3 Preparation of GABA salt by crystallization

A 25% concentration of salt water and a culture broth of 3% GABA content were mixed in a titanium crystal plate (4 m 1.5 m 0.05 m) located in Donghae Sun-dried Salt (Gosung, Korea) at 2-3 cm thickness, and crystallization proceeded to produce GABA salt containing 200 mg of GABA per g of salt. The crystals of the mixture of salt water and culture broth entering the crystal plates at 9 am on a clear day with a maximum temperature of 30^0C or higher were confirmed visually after 1 pm and salt harvesting was possible at 5 pm.

2.1.4 Preparation of GABA salt by spray drying

Mixtures of GABA-containing culture broth and sea water were spray dried to prepare GABA salts of various GABA contents. A

spray dryer (SD-Basic, LabPlant, UK) capable of drying up to 100 ml per hour using a 0.5 mm nozzle was used. The inlet temperature was adjusted to 120 to 200^0C for the mixed solution introduced into the dryer was sufficiently dried. The amount of air entering the spray dryer was 0.2-0.65 m^3/min and the nozzle pressure was 0.5-2.5 kg/m^3.

2.1.5 Preparation of GABA salt using superheated water vapor

Using an oven-type superheated steam generator (DC Quto QF-5200C, Naomoto, Japan), GABA was coated on commercial flower salt (sun dried salt) and roasted salt by spraying a culture broth containing GABA followed by drying using superheated steam. The temperature of superheated steam was adjusted to 200^0C and the culture broth containing GABA to be added was sprayed onto the salt, followed by drying for 30 seconds with superheated steam, and cooling for 1 minute. Heating and drying process repeated 5 times to completely dry the culture broth so that the salt was coated with the GABA-containing culture broth completely.

2.2 Methods

2.2.1 Measurement of salinity

Salinity of salt water and salt was measured using a digital salt meter (ES-421, ATAGO, Tokyo, Japan). The sample was dissolved in an appropriate amount of distilled water to measure the salinity, and then the salinity was calculated by multiplying the dilution factor.

2.2.2 Production of GABA by fermentation

10 times (v/w) of water was added to rice bran and stirred at 55 to 60^0C for 18 hours that the supernatant obtained by centrifugation at 5000 rpm. 2% sucrose (Daejung, Ansan, Korea), 1% yeast extract (BD difco, New Jersey, USA), 0.5% sodium acetate (Daejung, Ansan, Korea) and 5% glutamic acid (Sigma-aldrich Co., St. Louis, MO, USA) were added to supernatant and sterilized at 80^0C for 30 minutes and 3% seed culture was inoculated.1% MSG

(Daesang Co., Seoul, Korea) was added to lactobacilli MRS broth (BD difco, Franklin Lakes, NJ, USA) and standing cultured for 48 hours at 30^0C for seed culture. GABA producing fermentation carried at 50 rpm, 30^0C without adjusting pH using a 2 L working volume fermenter (FMT ST-S05, Fermentec, Cheongju, Korea) and the content of GABA produced in the medium was checked for 72 hours.

2.2.3 Analysis of GABA content in culture medium

RP-HPLC (Waters 2487 Dual λ Absorbance detector, Waters 1525 Binary HPLC pump, Waters 717 plus Autosampler, Milford, MA, USA) was used to analyze GABA contents in the culture medium referring to reverse HPLC methods[16,17].

2.2.4 Analysis of GABA content in processed salt

LC-MS was used to measure the GABA content of GABA salt prepared by various methods. Ultimate 3000 RS UHPLC (Thermo scientific Co. Ltd., Hudson, NH, USA) was used as HPLC, LTQ Orbitrap XL (Thermo scientific Co. Ltd., Hudson, NH, USA) ESI Ion source was used as Mass spectrometry, and acquity uplc BEH C18 (1.7 mum, 2.1100 mm, Waters, Milford, MA, USA) column was used. SIM mode, [+] mode were used for measurement and sheath gas was 50, and spray voltage was 5 kV. For HPLC, solvent A was distilled water and solvent B was dissolved 0.1% formic acid in acetonitrile, the flow rate was fixed at 0.25 mL/min, and at 3 minutes, the ratio of solvent A was changed from 95% to 5%, the ratio of solvent B was changed from 5% to 95%. Solvent ratio returned to the original ratio at 5.5 minutes and maintained for 6 minutes and standard curve was determined using γ-aminobutyric acid (MW 103.12, Sigma-aldrich Co., St. Louis, Mo., USA) in a purity of 99% or more at a concentration of 500-50,000 ppb and the injection volume of the sample was 5 muL.

2.2.5 Mineral content measurement

Na, Ca, K and Mg contents of GABA salt were measured using ICP/OES (Optima 8300, PerkinElmer Inc., Waltham, MA, USA). 2 ml of 0.5 N HNO_3 (Merck, Darmstadt, Germany) was added to

the sample to be measured, heated at 90^0C for one hour in a hot plate (VS-130SH, Vision scientific, Daejeon, Korea), then diluted to an analytical range and analyzed in the wavelength range of 163-782 nm.

2.2.6 Discoloration and chromaticity measurement of culture medium

5.0% (w/v) of food grade activated carbon (SGS-100, Shingi chemical Co., Ltd., Yongsan, Korea) was added to the culture broth. Culture broth heated to 80^0C and stirring for 3 hours to prevent sedimentation of activated carbon, activated carbon was removed by centrifugation at 8,000g for 15 minutes. After removing the activated carbon, the O.D. value was measured at wavelength range of 260 nm using distilled water as a control.

2.2.7 Chromaticity measurement of GABA salts

The L, a, and b values of GABA salts with different GABA contents by spray drying with different mixing ratios of GABA-containing culture broth and salt water were measured. A colorimeter (CM-3500D, Konica Minolta, Japan) using a pulsed xenon arc lamp as a light source which have a wavelength range of 400 to 700 nm in the wavelength interval of 20 nm, and measurement was done 3 times each then the average value was shown.

2.2.8 Morphology observation

The morphology and surface of GABA salt coated with GABA containing culture broth by superheated steam and spray-dried with GABA-containing culture broth and salt water mixtures were observed using a field emission scanning electron microscope (JSM-6701, JEOL Co., Ltd, Tokyo, Japan).

2.2.9 Sensory evalueationt

Sensory evaluation was performed on 30 college students majoring in food for GABA salt prepared with crystallization and superheated steam to contain 20 mg of GABA per gram of salt and commercially available flower salt (refined salt, Hanju Corporation,

Ulsan, Korea). Refined salt and two kinds of GABA salt were dissolved in distilled water to prepare 1% salinity. Each salt solution was given in an appropriate amount in a plastic cup and the mouth was rinsed with warm water before the sensory test. Then, the salt solution was evaluated with an appropriate amount, and resting 3 minutes after evaluation one item, the sensory evaluation of the next test group was carried out. The sensory evaluation was based on a 5-point scale. The items were scored for the intensity of salty taste, bitter taste, savory taste, and overall preference.

2.3 Statistical analysis

The chromaticity and GABA contents of the processed salt prepared by various methods were shown as an average for three or more repeated experiments. For the statistical processing for sensory test, analysis of variance was performed using the SPSS software package (Version 22.0, SPSS Inc., Chicago, IL, USA) and Duncan's multiple range test ($p < 0.05$) was performed.

3 Results and Discussion

3.1 Salinity of salt water derived from deep sea water

The salinity of the salt water used as a sample varied depending on the removal rate of fresh water, but the salinity was similar to the salinity of the sea water producing the sun-dried salt with the salinity between 22-24%.

3.2 Mineral content of processed salt

The minerals content of commercial salts and GABA salt prepared by various methods were compared. The mineral contents of sun-dried salt (taepyeongsaltfarm, shinan, korea), salt prepared by spray drying of sea water derived deep sea water, GABA salt produced by spray-drying the mixture of sea water and GABA-containing culture broth to obtain 100 mg of GABA per gram of salt, coating commercial refined salt with GABA containing culture broth by superheated steam include 100 mg of GABA per

gram of salt finally, and processed salt granulated with garlic and salt are shown in Table 1. Refined salt showed higher Na content than sun-dried salt and commercial processed salt, and in the case of GABA salt prepared by various methods by adding GABA-containing culture broth, there was little change in the mineral contents as compared with the salts before addition of the culture broth. Na content of spray dried salt produced from salt water derived from deep sea water which some fresh water removed, but K and Mg contents were very high, therefore it is considered that the mineral contents change in the process of removing fresh water from the deep sea water[18] and commercial garlic salt showed Na content similar to that of sun-dried salt. GABA salt prepared by spray drying the mixture of salt water derived from deep sea water and GABA containing culture broth was characterized by high contents of K and Mg similar to those before addition of culture broth and there was little change in mineral contents during processing. Also in case when GABA was coated on the surface of salt using superheated steam, there was almost no change in the mineral contents of the raw salt, and various methods of producing GABA salt were confirmed while minimizing the compositional change of the raw salt.

Table 1. Mineral content of various salts

(unit: mg/kg)

Sample	Process	Na	Ca	K	Mg
Sun-dried salt		333,287	1,429.0	3,067.3	9,797.0
Garlic salt		341,719	166.73	1,907.0	649.17
Roasted salt		374,524	234.71	1,527.8	672.53
Deep sea water	Spray drying	313,995	1,076.4	15,560	30,919
GABA salt	Spray drying	300,215	927.12	14,120	25,783
GABA salt	Crystallization	303,298	1,004.2	14,397	28,956
Refined salt		386,014	164.52	1,807.9	642.19
GABA salt	Coating	330,841	180.33	1,805.4	660.82

3.3 Decoloration of culture broth

In order to minimize the change of chromaticity of GABA salt, the culture broth showing absorbance of 0.425 ± 0.31 at 260 nm was bleached by activated carbon to 0.017 ± 0.02 that a transparent culture broth was prepared. Final color of culture broth could not be distinguished with distilled water by the naked eye and used in the process of producing GABA salt.

3.4 GABA content of salt

The GABA contents of GABA salts which made of spray drying and drying by superheated steam were measured for the verifying amount of GABA supplied in the form of culture broth all added to the GABA salts. GABA contents of GABA salts controlled by varying the mixing ratio of salt water and GABA containing culture broth and spraying amount of culture broth. GABA contents of three spray dried GABA salts and the two kinds of GABA salts which drying by super heated steam after sprayed GABA containing culture broth on two commercial salts were shown in Table 2. In the crystallization process of GABA-containing culture broth and salt water, it was confirmed that a part of GABA added did not crystallize at a GABA concentration of 3% (w/w) or more of the salt weight15 but in the case of 3% GABA coating with superheated steam and GABA salt mixed with GABA up to 4% by spray drying of culture broth, there were no significant differences in the addition amount and the analytical value, so that the processes of producing GABA salts did not cause loss of GABA contents by spray drying and coating method using superheated steam.

Table 2. GABA contents of co-crystallization salt with salt water and fermentation broth containing various GABA content

Process (base salt)	Base salt	GABA added (mg GABA/g salt)	GABA analyzed (mg GABA/g salt)	Theoretical yield (%) (Analyzed amount/ Theoretical amount)×100
Superheated steam	Sun-dried salt	30	29.93±1.40	99.78±4.67
Superheated steam	Roasted salt	30	30.17±1.23	100.56±4.11
Spray drying		10	10.10±0.20	101.00±2.00
Spray drying		20	19.83±0.25	99.17±1.26
Spray drying		40	40.37±1.07	100.92±2.67

3.5 Chromaticity of GABA salts

The chromaticity of commercial salts and GABA salts prepared by various processes were measured and shown in Table 3. The L, a and b values were 98.55±0.02, -0.24±0.01, -0.04±0.01 respectively when spray drying the salt water only showed a bright white color compared with L, a and b values of 91.13±0.01, -0.26±0.01, 3.53±0.01 of commercial sun-dried salt. As the amount of the non-bleached culture broth increased, the L value decreased and the

b value increased, indicating that darkness and yellowish color increased. When bleached culture broth was added, L and b values were significantly higher than those of the non-bleached culture broth, and it was confirmed that when the GABA salt was prepared by spray drying, the color of the added culture broth affected the color of the final product. GABA salt where the culture broth was added to the commercial sun-dried salt at the same concentration as that of spray drying, and GABA was coated using superheated steam or manufactured using a crystallization process were somewhat dark and yellowish compared to the spray dried GABA salt. In the case of long-term exposure to sunlight in the crystallization process and when superheated steam is used, it is regarded that the time of exposure to high temperature is longer than that of spray drying, and maillard browning of culture broth affects the final color of the product. In case of GABA salt prepared using superheated steam was the darkest and yellowish in the GABA slat group of this study, the color of light yellowish as compared with that of pepper salt, which is a typical commercial salt, so that the marketability due to colors of GABA salts are determined to be not a problem.

Table 3. Chromaticity of commercial salts and GABA salts prepared by various processes

Method	GABA conc. (mg/g salt)	Color			fermented broth
		L	A	B	
Spray drying	0	98.55±0.02	-0.24±0.01	-0.04±0.02	No bleaching
	2	98.70±0.12	-0.21±0.02	1.35±0.02	
	10	96.86±0.03	-0.24±0.01	3.25±0.01	
	20	95.74±0.05	-0.26±0.01	3.78±0.06	
Spray drying	20	98.07±0.05	-0.55±0.02	0.69±0.01	Bleaching
	30	98.16±0.02	-0.69±0.01	2.06±0.01	
	40	97.96±0.03	-0.76±0.01	2.85±0.02	
Superheated steam	40	80.33±0.06	-1.12±0.01	10.12±0.03	
Crystallization	40	84.23±0.02	-1.58±0.01	9.25±0.02	
Sun-dried salt	0	91.13±0.01	-0.26±0.01	3.53±0.01	
Pepper salt	0	76.99±0.01	0.36±0.02	12.24±0.02	

3.6 Morphology observation

The results of observing the morphology of GABA salts processed by various methods using electron microscopes are shown in Fig.1. In case of sun-dried salt, like the research results[19,20] hexagonal

crystals found in refined salt were not observed and GABA salts by crystallization of deep sea water and culture broth containing GABA[15] did not reveal any particular crystals, and it was found that the spray-dried GABA salt was composed of very small, round-shaped crystals. In case of spray drying salt water from deep sea water only, the size of the crystals was similar to that of the case of mixing with the culture broth, but it showed a quadrangular crystal shape rather than a circular shape as confirmed at a magnification of 500 times. This suggests that various mineral contents seem to affect the crystal structure of salts and future research is required. Pepper salt, which is one of the processed salts commercially distributed in markets, appears in the form of a large crystal structure with amorphous small particles att0ached, and it differs from processed salt produced by spray drying and crystallization process, and it is considered that the structure of the processed salt is changed depend on the manufacturing process. In case of processed salt coated with GABA by spraying culture broth to sun-dried salt and drying by superheated steam, the size of the particles is smaller than that of the sun-dried salt and appears to represent some regular form, it is considered that the shape change of crystals due to initial moisture absorption and rapid drying occurs in the action of superheated steam. In the case of all suggested processes for producing GABA salts, the added GABA did not form crystals separately or segregate fractions from GABA salt.

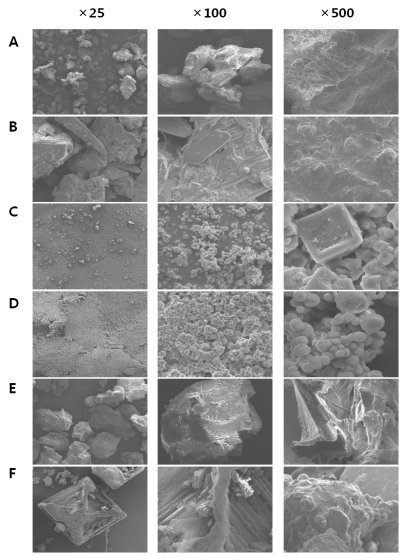

Figure 1: Comparison of surface image between GABA salts, commercial sun-dried salt and pepper salt in the scanning electron microscope. (A; sun-dried salt, B; sun-dried salt containing 20 mg GABA/g salt, C; spray-dried salt containing 20 mg GABA/g salt, D; spray-dried salt, E; superheated steam dried salt containing 20 mg GABA/g salt, F; pepper salt)

3.7 Sensory evaluation of GABA salts

Table 4 summarizes the sensory evaluation results of commercial refined salt and GABA salts (20 mg GABA/g-salt) prepared by two different methods. Control is commercial refined salt and sample 1 was crystallized GABA salt using GABA containing culture broth and salt water. Sample 2 was coated with GABA containing culture broth to refined salt using superheated steam. As a result of the sensory evaluation, it was shown that there was a significant difference between all three samples except the savory taste. First, the salty taste of GABA salt was less than that of refined salt at the same salinity. It is considered that there is difference of a relative taste due to the difference in the content of minerals other than Na ions. On the other hand, the bitter taste was rather low that the saltiness and bitter taste of GABA salt were lower

than refined salt. In spite of GABA added, it was found that the processed salt had a low savory taste, indicating that the residual nitrogen source in the added GABA containing culture broth inhibited the original taste of the salt. In color preference and overall acceptance, the superheated steam dried GABA salt did not show any significant difference compared to the refined salt, but the crystallization products showed low sensory values that it is necessary to study how to increase organoleptic preference in process as well as sensory evaluation.

Table 4 Sensory characteristics of functional salts

Sample	Sensory characteristics				
	Saltiness	Bitterness	Savory taste	Color	Overall acceptance
Control[1)]	3.37[a2)]±1.12[3)]	3.05±1.08	2.95±1.31	3.63[a]±0.83	3.32[a]±1.34
Sample 1	2.53[b]±1.12	2.79±1.27	2.74±1.37	2.42[b]±1.22	2.26[b]±1.24
Sample 2	2.95[ab]±1.03	2.68±0.75	2.68±1.06	3.63[a]±1.16	2.89[ab]±1.20

Strongly liked : 5 points, Neutral : 3 points, Strongly disliked : 1 point
[1)]Refined salt.
[2)]Means with different letters within a row are significantly different from each other determined by Duncan's multiple range test. α=0.05.
[3)]Mean±SD.

3.8 Discussion

γ-Aminobutyric acid (GABA) containing salts were prepared through crystallization, spray drying and coating processes. Salt from deep sea water with fresh water removed by osmotic pressure has higher mineral contents and fermentation broth by lactic acid bacteria that contained GABA were combined using various processes. Spray drying of the mixtures of sea water and fermentation broth produced GABA containing salts at various GABA contents. In addition, spraying the culture broth on the surface of the commercial salt and drying by superheated steam effectively produced the GABA salt. Depending on the process and contents of GABA, the color of GABA salts changed. The appearance and surface of the prepared GABA salts were examined with a scanning electron microscope. Morphological differences were found between the commercial sun-dried salt, spray-dried salt and salt dried by superheated steam, and no separated particles were detected which indicates that the various processes could be applied to make GABA salts. Sensory evaluations of GABA salts compared with refined

salt showed low scores in saltiness, bitterness, color and overall preference so commercialization of GABA salts needs not combining process with GABA and salt but solve the organoleptic problems.

4 Conclusion

Various studies have been conducted to solve the problem of excessive intake of salt. Methods for directly reducing the intake of salt, replacing some components of the salt, and studied on various functional salts have been carried out. GABA is that has been recognized as a function of blood pressure lowering, was produced through fermentation, and the GABA-containing culture broth was added to the salt by spray drying and surface coating using superheated steam to prepare GABA salt, and several characteristics were compared with the GABA salt prepared by the crystallization process and the commercial salt. Although different crystal forms were shown depending on the GABA addition process, it was possible to produce 3-4% GABA-containing salt per weight of salt without changing the composition of the main ingredient. Therefore, ingestion of 5 g of salt per day would allow 200 mg of GABA to be consumed and thus sufficient physiological activity could be expected. On the other hand, in case of GABA salt, the sensory characteristics get lower scored than that of refined salt in the market, and it is considered that the research on increasing the organoleptic preference can effectively solve the problems caused by excessive consumption of salt.

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