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Comparison of NaCl and D-Pinitol Content of Freeze-Dried Ice Plant, Natural and Purified Commercial Salts and their Radical Scavenging Activity

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ABSTRACT - This study investigated the suitability of freeze-dried ice plant (*Mesembryanthemum crystallinum*) as the source of an edible healthy salt alternative by examining the content of NaCl, D-pinitol, total phenols, total flavonoids, and DPPH radical scavenging activity compared with those of natural and purified salts. Our results showed that the NaCl content of freeze-dried ice plant, natural and purified salts was 19, 94 and 99%, respectively. The D-pinitol content of freeze-dried ice plant was 777 mg/100 g, whereas D-pinitol was not detected in either natural or purified salts. The total phenol and flavonoid contents of freeze-dried ice plant were 115 mg GAE/100 g and 985 mg RE/100 g, respectively. The DPPH radical scavenging activity of freeze-dried ice plant was markedly higher than that of natural and purified salts. Altogether, these results show that freeze-dried ice plant contains both NaCl and bioactive compounds and can be used as a source of edible salt with positive health effects.

Key words : Ice plant, NaCl, D-pinitol, Antioxidant activity, Natural salt alternative

Salt intake is indispensable for the physiological functioning of the human body. It not only provides various minerals but also contributes to the taste of food. However, when people consume excessive amounts of salt, the osmolality of blood vessels increases, thereby contributing to blood pressure rise and hypertension[1)]. Therefore, the recommended allowance of salt consumption is limited worldwide. In Japan, the daily salt intake for adults is limited to 10 g/day[2)] and in Korea, the Korea Food and Drug Administration (KFDA) recommends a 2 g/day intake of sodium through processed foods[3)]. However, due to preferences for foods such as kimchi, salted fish, Koreans are reported to have a daily salt intake of more than 10-16 g[4)]. Therefore, it is necessary to make efforts to reduce the intake of salt through dietary control and the use of salt alternatives.

The ice plant (*Mesembryanthemum crystallinum*) is native to southern and eastern Africa, and nowadays widespread along the coastal areas of Europe, USA, Mexico, Chile, the Caribbean and western Australia[5)]. The ice plant main particularity is that its entire above ground surface is covered with unicellular trichomes, called bladder cells[6)]. They are filled with a water solution and function as peripheral salinity and water reservoirs providing protection from short term high salinity of water deficit stress[7)]. The ability to accumulate saline in it from the soil can be a potential alternative for salt[8)]. Also, ice plant not only contains useful ingredients such as inositol and β-carotene, but also a significant amount of pinitol, which can lower blood glucose levels. It is, therefore, emerging as a healing food suitable for diabetic patients[9)]. It is also known that the ingestion of natural products containing phenolic compounds and flavonoids is helpful for prevention of various adult diseases[10)]. Even if many studies have been conducted on the ice plant antioxidant activity, and even if ice plant has already been considered as an alternative[11)], ice plant was never compared with natural and purified salts in terms of their contents in NaCl, D-pinitol and the antioxidant activity.

Therefore, this study was to investigate the contents of NaCl, D-pinitol, total phenols, and flavonoids, and the DPPH radical scavenging activity of freeze-dried ice plant, natural and purified salts and to examine the suitability of freeze-dried ice plant powder as an edible healthy salt alternative.

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Materials and Methods

Sample and reagent preparation

The ice plant was purchased from a farm (Goryeong, Korea). Natural and purified salts were purchased from an ordinary market. The ice plant was freeze-dried (Bondiro; Il Shin Lab Co. Ltd., Seoul, Korea) and stored at -20°C. Buffer and analytical reagents used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Measurement of NaCl content

$$\mathrm{NaCl}(\%, \mathrm{w/w}) = \frac{\mathrm{b} \times \mathrm{f} \times \mathrm{D} \times 0.00117}{\mathrm{a}} \times 100$$

a : Sample weight (g)

b : Consumption of 0.02 N $AgNO_3$ solution (mL)

f : Titer of 0.02 N $AgNO_3$ solution

D : Dilution factor

0.00117 : NaCl (g) number corresponding to 0.02 N $AgNO_3$ 1 mL

The NaCl contents of the freeze-dried ice plant, natural and purified salts were measured using the Mohr method[12]. First, 1 g of sample was diluted 100 times and filtered (Whatman No. 1, Springfield, UK). Then 10 mL of the diluted sample was taken into a conical tube and added 1 mL of a 2% potassium chromate solution to it. Titration was then performed using a 0.02 N silver nitrate solution to calculate as follows.

Measurement of D-pinitol content

Analysis of D-pinitol content using HPLC was determined with some modifications[13]. To analyze the D-pinitol content, samples were extracted. The freeze-dried ice plant, natural and purified salts were dissolved in a 50% solution of acetonitrile in distilled water and were sonicated for 30 min at 25°C. HPLC analysis was performed using Agilent 1260 Infinity series HPLC system (Agilent Co., Santa Clara, CA, USA) with an RID 1260 detector (Agilent Co., Santa Clara, CA, USA). The used column was an Agilent carbohydrate column, 5 μm, 4.6 × 250 mm (Agilent Co., Santa Clara, CA, USA) and maintained at 35°C. The mobile phase consisted of distilled water and acetonitrile (20:80, v/v) flowing during 15 min at a flow rate of 1.0 mL/min. The injection volume was 20 μL.

Determination of total phenol and flavonoid contents

The total phenol content was determined according to the Folin-Denis method. The sample solution (1 mL) was placed in a test tube with distilled water (7 mL) and Folin-Denis reagent (0.5 mL) saturated with a sodium carbonate solution (1 mL), and was allowed to stand for 30 min. The absorbance was measured at 715 nm. The total phenol content (mg/100 g) was calculated as gallic acid equivalents[14].

To measure the content of total flavonoids, the sample solution (1 mL) was placed in a test tube with diethylene glycol (10 mL) and 1 N-NaOH solution (1 mL) and allowed to stand for 30 min. The absorbance was measured at 420 nm. The total flavonoid content (mg/100 g) was calculated as rutin equivalents[15].

DPPH radical scavenging activity

The freeze-dried ice plant, natural and purified salts were dissolved in DMSO and then evaluated the DPPH radical scavenging activity on the basis of the electron donating ability (EDA) of the stable DPPH free radical. The DPPH solution in each sample was vigorously vortexed and made to stand for 30 min at 25°C. The DPPH radical scavenging activity was measured using a spectrophotometer at 517 nm, and EDA was calculated using the following formula[16].

$$\mathrm{EDA}(\%) = \left[1 - \left(\frac{\mathrm{A}}{\mathrm{B}}\right)\right] \times 100$$

A : absorbance value of testing solution

B : absorbance value of control solution

Statistical analysis

All experiments were repeated three times. Results were statistically analyzed via ANOVA and Duncan's multiple range test using SAS software 9.4 (SAS Institute Inc. Cary, NC, USA). A P-value of < 0.05 was considered statistically significant.

Results and Discussion

Contents of NaCl and D-pinitol in freeze-dried ice plant

This study was to investigate a natural salt alternative by comparing the NaCl and D-pinitol contents of freeze-dried ice plant with natural and purified salts. The NaCl contents of freeze-dried ice plant, natural and purified salts were 19±0.9%, 94±1.1% and 99±0.1% respectively, which is shown in Table 1. Traditionally, fermented foods have been

Table 1. Contents of NaCl and D-pinitol in freeze-dried ice plant, natural and purified salts

	Freeze-dried ice plant	Natural salt	Purified salt
NaCl (%)	19±0.9[c,1)]	94±1.1[b]	99±0.1[a]
D-pinitol (mg/100 g)	777±15.9[a]	N.D[b,2)]	N.D[b]

1)Results are presented as the mean±SD from 3 independent experiments set in triplicate. Means with different letters showed significant difference at $P < 0.05$ using Duncan's multiple range test.

2)N.D; Not detected (LOD : 53.8 μg/mL, LOQ : 163.2 μg/mL

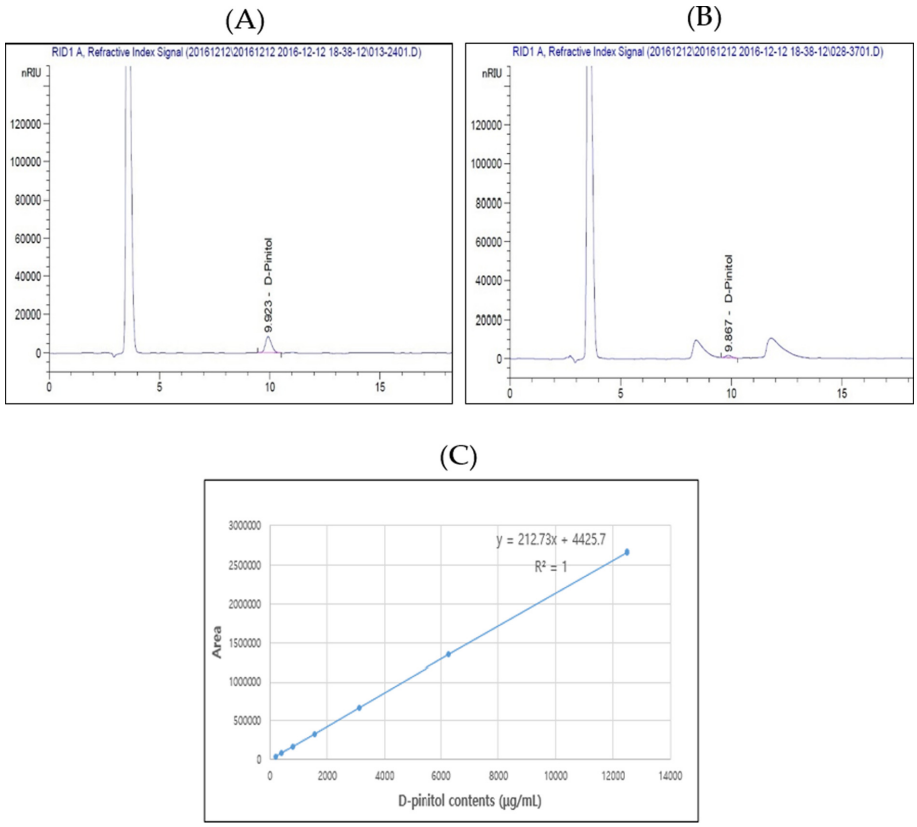

Fig. 1. HPLC chromatograms of the standard solution (A), and freeze-dried ice plant (B), and standard curve (C) of D-pinitol in freeze-dried ice plant.

widely used in an effort to reduce salt intake[17]. A variety of fermented foods such as doenjang (Korean fermented soybean paste) and soy sauce are made based on natural salt, so they have been used for reducing salt intake with a relatively low content of NaCl, minerals, and improved functionalities through the fermentation[18]. In the study comparing the quality characteristics of conventional soy sauces, the salinities of the soy sauces were found to be 17-23%[19] indicating that the NaCl content of the freeze-dried ice plant was similar to that of the soy sauces. Also recently, there have been many strategies on sodium reduction by developing salty flavor using yeast extracts, ribonucleotides, spices. Then complex natural mixtures with unexplained desired taste effects are fractionated in order to identify taste enhancers. Most replacers belong to the alternative minerals, amino acids, salty peptides, organic acids, and aroma compounds[20]. Studies on plant aqueous extracts with retaining salty taste of NaCl and low sodium content have been conducted[21]. In this respect, it is also desirable to study materials mixed with the extract of ice plant and other plant extracts.

D-pinitol is converted into chiro-inositol in the body, and it reduces blood glucose by allowing glucose in the blood to enter the cells. Therefore, D-pinitol can be said to be effective in the treatment of diabetes, because it regulates glucose uptake in the body[22]. In addition, domestic venture companies received approval for ingredients of health functional foods (KFDA No. 2005-15, 2007-12) in August 2005 and August 2007)[23]. As shown in Table 1, the D-pinitol content of freeze-dried ice plant was 777 mg/100 g, but it was not detected in the natural and purified salts. It is known that D-pinitol is one of the bioactive compounds of soybean. A previous study on the blood glucose lowering effect of D-pinitol isolated from soybeans showed that the domestic soybean has about 1.3-1.7 g/kg of D-pinitol[24]. It showed that the content of D-pinitol in the ice plant is 4.5-5.9 times even higher than fresh soybeans. It is well known that the higher the salt intake, the higher the incidence of hypertension[25]. Therefore, freeze-dried ice plant which has a large amount of D-pinitol content when compared with natural and purified salts, can be considered as a natural salt alternative and material to prevent diabetes.

The antioxidant activity of freeze-dried ice plant

This study measured the antioxidant activity of freeze-

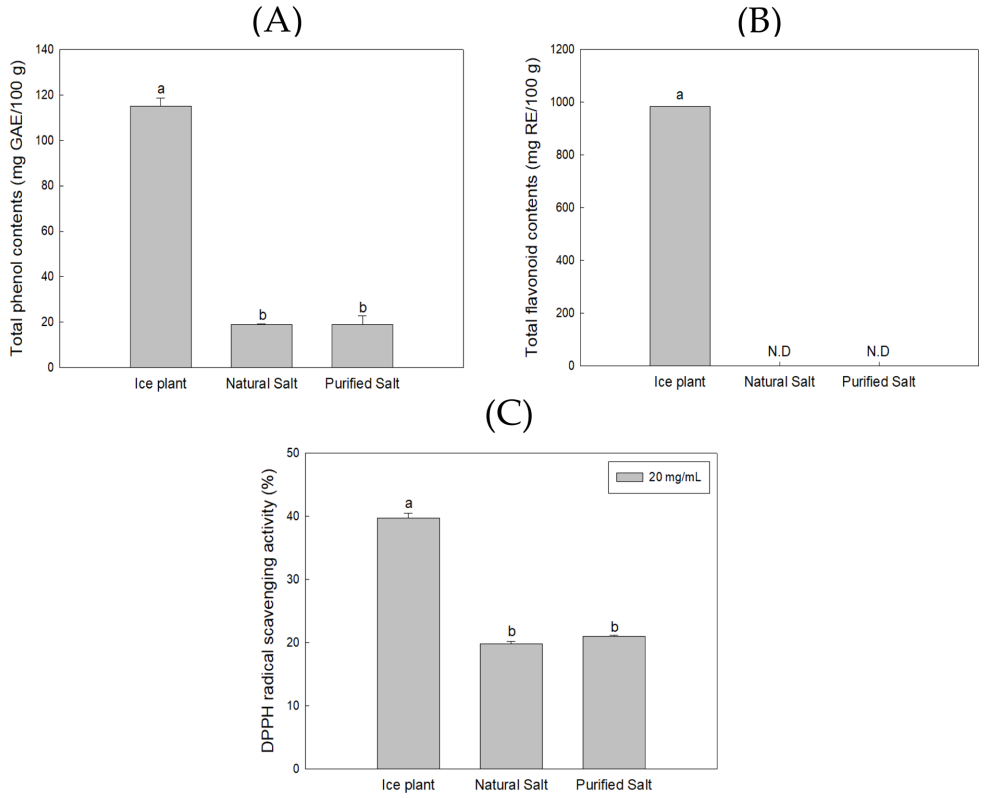

Fig. 2. Total phenol contents (A), total flavonoid contents (B), and DPPH radical scavenging activity (C) of freeze-dried ice plant, natural and purified salts. Results are presented as the mean±SD from 3 independent experiments set in triplicate. Bars with different letters show significant difference at $P < 0.05$ using Duncan`s multiple range test. N.D; not detected, GAE; gallic acid equivalent, RE; rutin equivalent.

dried ice plant compared with natural and purified salts. As shown in Fig. 2, the phenol content of freeze-dried ice plant was 115 mg GAE/100 g, which was significantly higher than that of natural and purified salts, 18 mg GAE/100 g. The flavonoid content of freeze-dried ice plant was 985 mg RE/100 g and it was not detected in the natural and purified salts. Natural salt contain minerals a lot such as calcium, magnesium and purified salt has a high NaCl content of 99% or more[26]. However ice plant, the natural plant, has more antioxidant content than commercial salts just obtained by natural and chemical processing of seawater. Also, bioactive components could be changed depending on sample pretreatment such as drying method or sanitation technology, and freeze-dried plants may have higher antioxidant content such as flavonoids than the raw materials, which is considered as change in the tissue structure[27,28]. Further research is needed to occupy the bounds between biochemistry and food quality management. Meanwhile, phenolic compounds are a secondary metabolite widely distributed among plants, and the phenolic hydroxyl group can easily bind with other macromolecules, resulting in various physiological activities[29]. Due to the action of antioxidant compounds in the freeze-dried ice plant, it appears to be an antioxidant and antidiabetic agent.

The DPPH radical scavenging activity of freeze-dried ice plant, natural and purified salts is shown in Fig. 2. Freeze-dried ice plant showed a DPPH radical scavenging activity of 40% at a concentration of 20 mg/mL, and the DPPH radical scavenging activities were 20% and 21% in the natural and purified salts, respectively. The study measuring the DPPH radical scavenging activity of the methanolic ice plant extract was similar at 44.8% at a concentration of 400 μg/mL[30]. Overall, the freeze-dried ice plant was confirmed that it has a potential for use as an edible healthy salt alternative.

국문요약

본 연구는 아이스플랜트의 기능성 소금 대체제로서의 가능성을 조사하고자 천연 및 정제소금과의 NaCl, D-pinitol, 총 페놀, 총 플라보노이드 및 DPPH 라디칼 소거능에 대한 기초자료를 비교 조사하였다. 아이스플랜트 동결건조분말의 NaCl 함량을 분석한 결과 19%이었고 D-pinitol의 함량은 777 mg/100 g으로 천일염 및 정제염에 비해 NaCl 함량은 유의적으로 낮으나 D-pinitol을 함유하

고 있어 건강기능성에는 도움이 될 것으로 사료된다. 또한, 아이스플랜트 동결건조분말의 총 페놀 및 플라보노이드 함량은 각각 115 mg/100 g 및 985 mg/100 g을 함유하고 있어 DPPH라디칼 소거능의 항산화효과를 갖는 것으로 나타났다. 따라서 아이스플랜트 분말의 bioactive compound을 함유한 대체 소금 천연소재로서 가능성을 확인한 기초 연구를 제공하였다.

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