Thesis for the degree of
Doctor of Philosophy

Anticancer and Antiinflammatory Effects of Bamboo Salt and *Rubus coreanus* Miquel Bamboo Salt

August 2011

The Graduate School
Pusan National University

Department of Food Science and Nutrition

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ABBREVIATIONS

Bamboo salt (1×) / BS (1×)	1 time baked bamboo salt
Bamboo salt (3×)	3 times baked bamboo salt
Bamboo salt (9×) / BS (9×)	9 times baked bamboo salt
B	Bamboo salt
P	Purified salt
S	Solar salt
PB	Purple bamboo salt (9 times baked bamboo salt)
RB	*Rubus coreanus* Miquel bamboo salt
N	Normal
C	Control
O	Omeprazole

Ⅰ. INTRODUCTION

Salt is one of absolutely necessary nutrients in human life and takes part in many physiological processes of human body. Sodium chloride ensures the balance of acid and alkali, electrolytic balance in human body as well. The essential components of salt are sodium and chlorine, which is the necessity for the human body. The sodium (Na^+) is the main positive ion circulated outside the cell. Our body is composed by 6 thousand billion cells. Each cell floats in the solution of NaCl. The human body produces many acidic materials; it is sodium cushioning effect that maintains the body of weak alkalinity (1). There are many kinds of salt found in the market such as purified salt, solar salt, Guwun salt, bamboo salt, etc (2).

Bamboo salt as a functional food was originated from Korea and has been well-known in East Asia. The solar salt was put into young bamboo joint case, which only grew for three years, using the natural red clay to seal at both ends, and using pine for the fuel, baked at 1000℃~1500℃ of high temperatures (3, 4). Bamboo has been used as a drug from its roots to its leaves (5). According to various oriental medical literatures, bamboo is highly efficacious in curing stomachache, stopping stomach bleeding, discharging phlegm, and controlling stroke, diabetes, headache, hypertension, dizziness, prenuptial anemia, child

convolutions, epilepsy, insomnia, overdrinking and eyesore, and relieving fatigue (6, 7). Bamboo salt is well known as one of the most famous folk medicines. Also pharmaceutical scientists are researching bamboo salt on the health benefits effects such as anti-cancer effect and anti-virus effect (8). *Rubus coreanus* Miquel contains significant amounts of polyphenol antioxidants such as anthocyanin pigments linked to potential health protection against several human diseases (9).

Bamboo salt has been reported four main functions. First, detoxification efficacy: removal heavy metal toxins, bacteria, pathogenic bacteria, virus, blood-fat, cholesterin and so on (10, 11). Second, bamboo salt can balance human body function: it not only enriches the mineral composition, but also balance trace elements, and its structure is natural and reasonable (12, 13). Third, bamboo salt is a high energy material: it can continue to have far-infrared radiation, because it was made in a special way by firing at a high temperature above 1000℃ (14). Fourth, bamboo salt is a kind of antioxidant and free radical scavenging food (15-19). It can release a large number of electrons, and it not only blocks the predatory behavior by the free radical, but also can promptly repair the cells which are damaged by free radical (20-23). Bamboo salt was recorded both in traditional medicine and common life as having a good functional role, but it still lack of scientific research data. The 9 baked bamboo salt

called purple bamboo salt, is a special processed salt according to the traditional recipe. It is known to have various therapeutic effects on diseases such as inflammations, viral disease, diabetes, circulation organ disorder and cancer. However, physicochemical properties of the bamboo salt and its mechanisms of the therapentic effects of the diseases were not well studied (24-31).

In this study, it was tried to understand the physicochenmical properties of the bamboo salt and then was focus on the antioxidation, anticancer and antiinflammation effects of bamboo salt and *Rubus coreanus* Miquel added bamboo salt. To investigate the physicochemical properties of purified salt, solar salt and bamboo salt, it was determined pH, and mineral contents. The structural characteristics analysis by XRD, XPS, FT-IR methods were carried out. Bamboo salt was evaluated for the antioxidation, anticancer and anti-inflammatory effects on human cancer cells and their molecular mechanisms were also studied. *Rubus coreanus* Miquel was added to the bamboo salt to increase medicinal function and prepared *Rubus coreanus* Miquel bamboo salt. It was evaluated the increased functionalities and also determined preventative effects on hepatitis and gastritis in SD rats. It was proved that bamboo salt and *Rubus coreanus* Miquel bamboo salt were as functional foods which showed the health benefits.

Ⅱ. LITERATURE REVIEW

1. Salt and functional salt

1) Types of salt

(1) Purified salt

Purified salt, which is most widely used presently, is mainly sodium chloride. Food grade salt accounts for only a small part of salt production in industrialised countries (3% in Europe) although worldwide, food uses account for 17.5% of salt production (32). The majority is sold for industrial use. Salt has great commercial value because it is a necessary ingredient in many manufacturing processes. A few common examples include: the production of pulp and paper, setting dyes in textiles and fabrics, and the making of soaps and detergents. The manufacture and use of salt is one of the oldest chemical industries. Salt can be obtained by evaporation of sea water, usually in shallow basins warmed by sunlight; salt so obtained was formerly called bay salt, and is now often called sea salt or solar salt. Rock salt deposits are formed by the evaporation of ancient salt lakes, and may be mined conventionally or through the injection of water. Injected water dissolves the salt, and the brine solution can be pumped to the surface where the salt is

collected. After the raw salt is obtained, it is refined to purify it and improve its storage and handling characteristics. Purification usually involves recrystallization. In recrystallization, a brine solution is treated with chemicals that precipitate most impurities (largely magnesium and calcium salts). Multiple stages of evaporation are then used to collect pure sodium chloride crystals, which are kiln-dried.

(2) Solar salt

Solar salt, obtained by the evaporation of seawater, is used in cooking and cosmetics (33). Generally more expensive than table salt, it is commonly used in gourmet cooking and specialty potato chips, particularly the kettle cooked variety. Mineral salt has long been mined wherever it was available; the salt mines of Hallstatt go back at least to the Iron Age. However, there are many places where mineral salt is not present, and the alternative coastal source has also been exploited for thousands of years. The principle of production is evaporation of the water from the sea brine. In warm and dry climates this may be accomplished entirely by using solar energy, but in other climates alternative and often expensive fuel sources must be

used. For this reason, modern solar salt production is almost entirely found in Mediterranean and other warm, dry climates.

(3) Processed salt

Fired salt which refers to the salt made at the top of 400°C but baked salt regarded as the bottom of 400°C. Boiled solar salt under sunshine confined the hog water impossibility evaporated, which impact on the ingredients change the pernicious ingredients. Evaporating process take good advantages to solar ingredients that eliminate perniciousness and benefits to people's health determined by lock mode keeping in storage. Then bitter taste eliminated and general taste appeared. Compared to other salts, salty taste significantly decreased and suitable to make salado, boiled dishes and adjusting raw dishes materials (34).

(4) Other salt

① Table salt

In western cuisines, salt is used in cooking, and also made available to diners in salt shakers on the table. Table salt is

purifed salt, which contains about 97% to 99% sodium chloride (35). It usually contains substances that make it free-flowing (anticaking agents) such as sodium silicoaluminate or magnesium carbonate. Some people also add a desiccant, such as a few grains of uncooked rice, in salt shakers to absorb extra moisture and help break up clumps when anticaking agents are not enough.

② Salty condiments

In many East Asian cultures, salt is not traditionally used as a condiment. In its place, condiments such as soy sauce, fish sauce and oyster sauce tend to have a high sodium content and fill a similar role to table salt in western cultures. They are most often used for cooking rather than as table condiments (36).

2) Characteristics of salt

(1) Chemical properties of salt

Salt occurs naturally all over the world as the mineral halite, as well as in seawater and salt lakes. Some salt is one the surface, the dried-up residue of ancient seas like the famed Bonneville

Salt Flats in Utah. Surface salt depositions and man-made saltworks can be seen from space. In ocean coastal areas, saltwater can "intrude" on underground freshwater supplies, complicating the lives of those who provide our drinking water supplies. Scientists have also found salt in meteors and on Mars where its presence signals the possibility of extra-terrestrial life (37). According to chemistry, the term "salt" is used for ionic compounds that is composed of positively charged cations and the negatively charged anions, so that the product remains neutral and without a net charge. The ions may be inorganic (Cl^-) as well as organic ($CH3\text{-}COO^-$) and monoatomic (F^-) as well as polyatomic ions (SO_4^{2-}). Salt's solution in water is called electrolytes. Both, the electrolytes and molten salts conduct electricity. Zwitterions are salts which has an anionic center and a cationic center in the same molecule. Few examples of it are the amino acids, many metabolites, peptides, and proteins. Normal salt is a salt which do not contain a hydroxide ion (OH^-) or a hydrogen ion (H^+). Salts with OH^- are basic salts and with H^+ are acid salts. The salt which has lost its saltiness is known as impure salt. It also refers to natron (38).

Tight ionic bonding unites the elements sodium and chloride to make the chemical compound sodium chloride. Man has discovered a vast variety of ways to harness the chemical properties of salt to improve our lives. Chemically, there are

many “salts;” the resulting compound created by reacting an acid and a base; positively charged metal atoms (the sodium ion in the case of common salt) replacing the negatively charged hydrogen atoms of an acid, leaving the chloride ion. Sodium chloride crystals are cubic in form and salt crystals are commonly used to exemplify crystalline structure and many science students are familiar with the process of growing salt crystals. Its color varies from colorless, when pure, to white, gray or brownish when in the solid, halite, form. Salt dissolves readily in water. Salt crystals can be grown in various sizes and salt companies prepare particles in a wide variety of sizes to meet customer needs (39).

(2) Analysis of chemical properties

① Chemical composition determined by ICP-OES

ICP-OES is a fast multi-element technique with a dynamic linear range and moderate-low detection limits (0.2-100 ppb). The instrument uses an ICP source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light of a characteristic wavelength. Up to 60 elements can be screened per single sample run of less than one minute and the samples can be analysed in a variety of aqueous or organic

matrices. There are fewer chemical interferences than with FAAS, but some spectral interferences are possible and there are some element limitations (40). ICP-OES is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample. ICP-OES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances. ICP-OES is a good method used for analysis of trace elements in salt, the trace elements likes Na, K, Mg, Fe, Mn, P, S can be analysed (41).

② Structure analysis method of XRD

X-ray diffraction (XRD) is a versatile, non-destructive technique that reveals detailed information about the chemical composition and crystallographic structure of natural and manufactured materials (42, 43).

Based on the principle of X-ray diffraction, a wealth of structural, physical and chemical information about the material investigated can be obtained. A host of application techniques

for various material classes is available, each revealing its own specific details of the sample studied (44). Salts can be studied using the XRD system. When these mineral constituents are visible in the salt profile, they can be physically extracted using dental tools and analyzed separately. Important salt mineral phases within the salt may be detected by XRD analysis because many of these minerals have distinct peaks (45).

A special humidity chamber for a conventional X-ray diffractometer was designed. The application of this method for the direct observation of phase transition reactions such as hydration-dehydration and deliquescence crystallization not only in bulk samples but also, for the first time, within confined spaces of a porous material was a major objective of the present thesis. Glass frits with pore sizes similar to common building materials, e.g. natural stone or brick, were used for the direct observation of phase transitions of sodium sulfate, magnesium sulfate and sodium chloride within narrow pores. In these experiments RH-XRD was used to study both the rates of the respective phase transition reactions and the characterization of the corresponding equilibrium states. Another objective of the work was to prove the capability of RH-XRD for an accurate determination of equilibrium humidities of hydration and deliquescence reactions of salt crystals (46). For that purpose, a new procedure was adopted for the determination of equilibrium

humidities that is based on kinetic measurements. This procedure was used to determine the deliquescence humidities of NaCl and $Ca(N0_3)_2 \cdot 4H_20$ and the equilibrium humidity of the $Ca(N0_3)_2$-$Ca(N0_3)_2 \cdot 2H20$ transition. Comparison with commonly accepted literature data for these phase equilibria confirmed the accuracy of the method, which also offers good precision. The same technique was then applied to investigate the NaCl deliquescence in the confined pore space of glass frits with median pore diameters in the range of 1.4-70 μm.

③ Mineral content analysis method of XPS

X-ray photoelectron spectroscopy (XPS) is a quantitative spectroscopic technique that measures the elemental composition, empirical formula, chemical state and electronic state of the elements that exist within a material. XPS spectra are obtained by irradiating a material with a beam of X-rays while simultaneously measuring the kinetic energy and number of electrons that escape from the top 1 to 10 nm of the material being analyzed. XPS requires ultra high vacuum (UHV) conditions (47, 48). XPS is a surface chemical analysis technique that can be used to analyze the surface chemistry of a material in its "as received" state, or after some treatment, for

example: fracturing, cutting or scraping in air or UHV to expose the bulk chemistry, ion beam etching to clean off some of the surface contamination, exposure to heat to study the changes due to heating, exposure to reactive gases or solutions, exposure to ion beam implant, exposure to ultraviolet light. XPS is also known as ESCA, an abbreviation for Electron Spectroscopy for Chemical Analysis (Fig. 1). XPS detects all elements with an atomic number (Z) of 3 (lithium) and above. It cannot detect hydrogen (Z = 1) or helium (Z = 2) because the diameter of these orbitals is so small, reducing the catch probability to almost zero. Detection limits for most of the elements are in the parts per thousand range. Detections limits of parts per million (ppm) are possible, but require special conditions: concentration at top surface or very long collection time (overnight). XPS is routinely used to analyze inorganic compounds, metal alloys, semiconductors, polymers, elements, catalysts, glasses, ceramics, paints, papers, inks, woods, plant parts, make-up, teeth, bones, medical implants, bio-materials, viscous oils, glues, ion modified materials and many others (49-51). The potential of the technique in solving problems relevant to salt systems is highlighted by discussing selected examples among the applications, thus developing the applied concepts of the aqueous salt processes in cases of bamboo salts and solar salt.

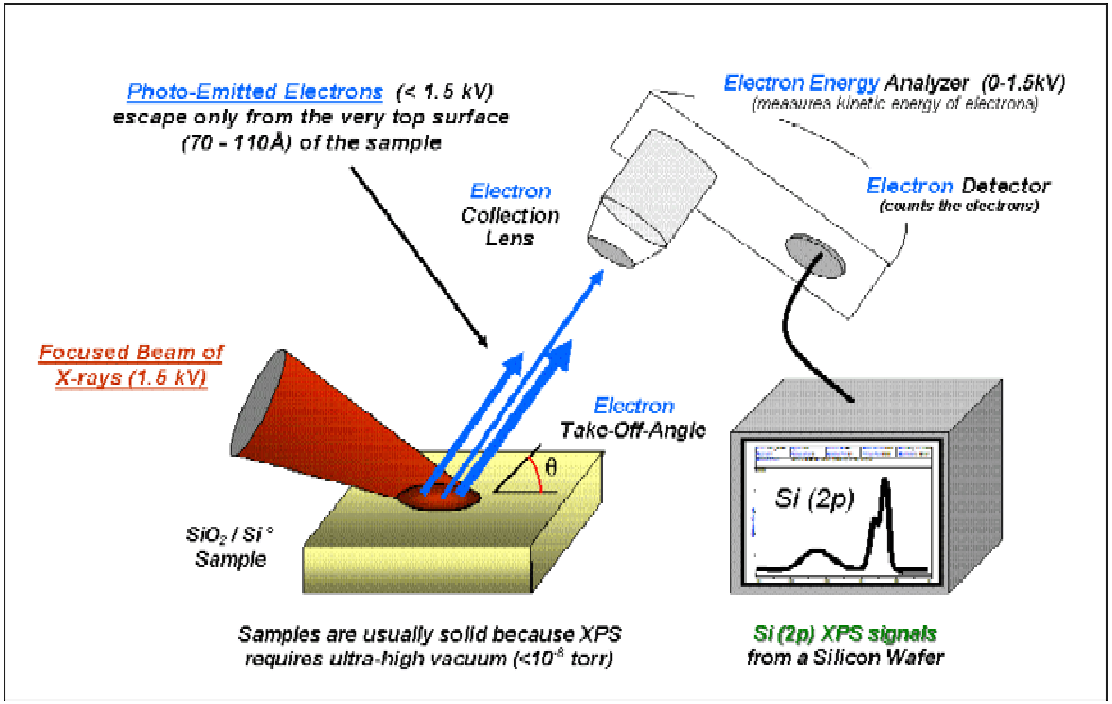


Fig. 1. Basic components of a monochromatic XPS system

(BV Crist, *Annotated Handbooks of Monochromatic XPS Spectra*, 2005)

④ Fourier transform infrared (FT-IR) spectrometry

FT-IR stands for Fourier Transform InfraRed, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

Fourier Transform Infrared (FT-IR) spectrometry was developed in order to overcome the limitations encountered with dispersive instruments (Fig. 2). The main difficulty was the slow scanning process. A method for measuring all of the infrared device called an interferometer. The interferometer produces a unique type of signal which has all of the infrared frequencies frequencies simultaneously, rather than individually, was needed. A solution was developed which employed a very simple optical "encoded" into it. The signal can be measured very quickly, usually on the order of one second or so. Thus, the time element per sample is reduced to a matter of a few seconds rather than several minutes. FT-IR can identify unknown materials,

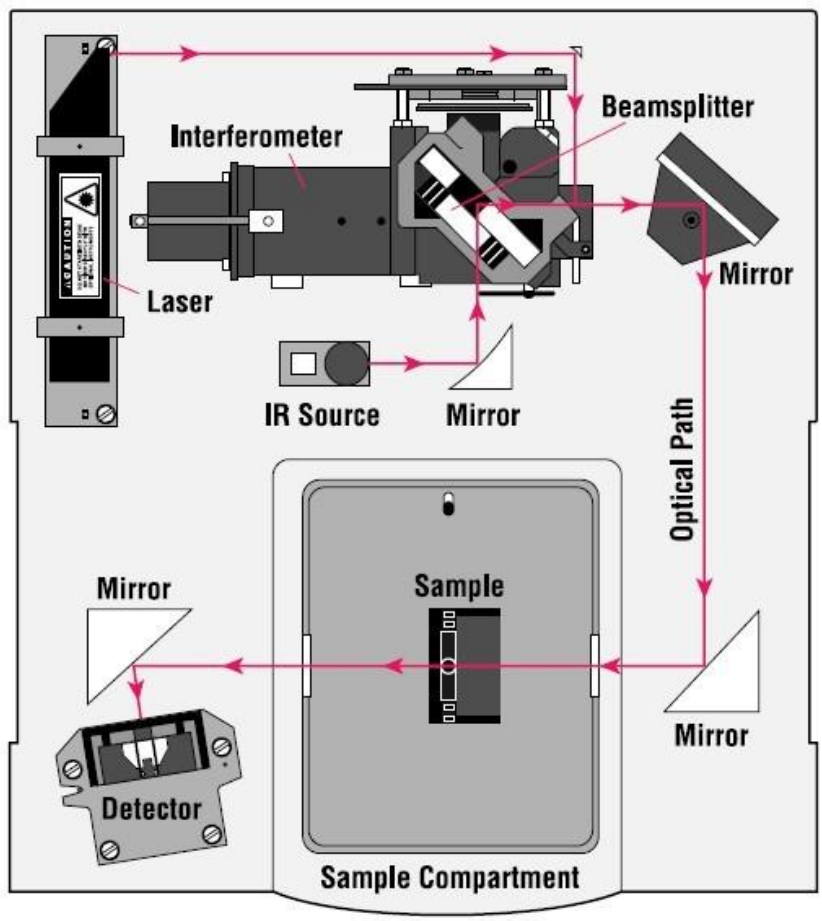


Fig.2. The FT-IR simple spectrometer layout

(Yan HJ *et al*, *Cellulose*, 2009)

determine the quality or consistency of a sample and determine the amount of components in a mixture (52-54).

(3) Taste

Gourmets often believe solar salt to have a better taste and texture than ordinary table salt, although one cannot always taste the difference when it is dissolved (55). In applications where solar salt's coarser texture is retained, it can provide a different mouth feel and changes in flavor due to its different rate of dissolution. The mineral content also affects the taste. It may be difficult to distinguish solar salt from other untreated salts, such as pink Himalayan salt, or grey rock salt. Solar salt is less purified than high mineral content pink salt. All solar salts profiled at equal sodium content had a significant increase in salty taste intensity compared to at equal weight per volume salty taste intensities. This was expected since the concentration of sodium cations in solution was increased. Solar salts at equal sodium content were also different in salty taste intensity suggesting that the other minerals do play a role in salty taste (56).

The ocean from which solar salts originated had a definite effect on the mineral content and possibly the flavor. Solar salt is

typically 98% sodium chloride and 2% minerals such as magnesium, potassium, iron, calcium, zinc and many other elements. There was a study evaluated magnesium, potassium, iron, calcium and zinc content because we expected that these cations had the most potential impact on mineral content, salty taste and flavor. The study demonstrated that mineral content varied based on harvest location. The impact of trace minerals on flavor directly appeared to be minimal, but flavors such as mineral and metallic and sometimes stronger intensities of umami and astringent mouthfeelwere noted in solar salts compared to table salt. Several studies have shown that zinc salts have higher astringency and umami sensation (57), while bitterness has been associated with magnesium and calcium salts (58, 59). Metallic flavor has been associated with iron compounds (60).

(4) Health related effects

① Acute effects

Too much or too little salt in the diet can lead to muscle cramps, dizziness, or electrolyte disturbance, which can cause neurological problems, or death. Drinking too much water, with

insufficient salt intake, puts a person at risk of water intoxication (hyponatremia). Salt is sometimes used as a health aid, such as in treatment of dysautonomia. Death can occur by ingestion of large amounts of salt in a short time (about 1 g per kg of body weight). Deaths have also resulted from attempted use of salt solutions as emetics, forced salt intake, and accidental confusion of salt with sugar in child food (61).

② Long term effects

The effects of salt consumption on long term health outcomes is controversial. While it has been linked with a number of conditions its causal effect on mortality and morbidity is unknown. Some associations include: a) stroke and cardiovascular disease (62). b) high blood pressure: Evidence shows an association between salt intakes and blood pressure among different populations and age range in adults. Reduced salt intake also results in a small reduction in blood pressure. c) left ventricular hypertrophy (cardiac enlargement): evidence suggests that high salt intake causes left ventricular hypertrophy, a strong risk factor for cardiovascular disease, independently of blood pressure effects. Excessive salt (sodium) intake, combined with an inadequate intake of water, can cause hypernatremia. It

can exacerbate renal disease. d) edema: a decrease in salt intake has been suggested to treat edema (fluid retention). e) stomach cancer: it is associated with high levels of sodium, but the evidence does not generally relate to foods typically consumed in the UK.

The risk for disease due to insufficient or excessive salt intake varies because of biochemical individuality. Some have asserted that while the risks of consuming too much salt are real, the risks have been exaggerated for most people, or that the studies done on the consumption of salt can be interpreted in many different ways (63). Some isolated cultures, have been found to consume little salt and capable of an active physical activity, this may indicate an adaptation of humans to low levels of sodium that originated in the predominantly vegetarian diet of human primate ancestors (64).

3) Functional salt

(1) Matcha (green tea) solar salt

Matcha is a premium green tea powder harvested in the famous tea fields of Japan. A lengthy harvesting technique produces the gorgeous color, a high level of amino acids, and a sweet flavor (65). The tea leaves are stone ground to the fine

powder which uses to create Fusion Matcha Salt. Matcha is renowned for its health benefits but is now making a splash in gourmet cooking world wide. Traditional uses include finishing for Asian foods such as tempura, edamame and tofu dishes. Matcha salt is perfect for lighter dishes that will allow the delicate aroma to come through. Try on scrambled eggs, steamed fish, chocolate and desserts (66).

(2) Glasswort (Hamcho) salt

Glasswort is one of the most salt tolerant plants growing in the salt marshes and tidal flats along the western coast of Korea. It has not only been eaten as a food, but also used in folk medicine for treating constipation, obesity and diabetes (67). Selected solar salt in purified sea restrict where located products in Shinangun. After cleaning and purifying, all of them should be made in processing of powders and mixing.

(3) Chitosan salt

Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. It is is

currently the focus of much medical research, as it is a polyglucosamine (the second-most-common dietary fiber, after cellulose) (68). Regarding as chitosan salt, adopt solar salt products are better for people to absorbed in terms of combining process of lower molecular in chitosan (69).

2. Bamboo salt

1) History

Bamboo salt was first produced by Jin-pyo Yul-sa (famous Buddhist priest) during his meditation period at the famous Busa-Uibang (Buddhist retreat located atop Mt. Byun-san) about 1,300 years ago and passed on mainly by Buddhist priests through generations as folk medicine (70). In the history of oriental medicine, Korean has developed many kinds of excellent traditional medical treatments. Many natural ingredients have been utilized and transform into traditional medicine that can help in relief illness. Bamboo was used by Korean as an important medical material. It has many different medical efficacy varies from its stub, roots, and leaves. About 1000 years ago, some Korean medicine doctors and monks started to make medical salts by inserting sea salt in a thick bamboo stub and baking them together with pine tree firewood.

The ancient bamboo salt was baked only twice or trice. They used the salt as special medicine treatments. Finally they found out that bamboo salt could have its complete medical efficacy if it is baked over 9 times repeatedly. And they also noticed that if bamboo salt is completely melted, the toxic characteristic disappeared. Now bamboo salt is well known as one of the most famous traditional medical treatments, not only in Korea, but also in many Asia countries. Many pharmaceutical scientists all around the world are researching bamboo salt's special therapeutics like anti-cancer effect and anti-virus effect.

2) Manufacture method

Bamboo salt is made from solar salt, bamboo, pine tree resin and loess. The solar salt was put into young bamboo joint case, which only grew for three years, using the natural red clay to seal at both ends, and using pine for the fuel, baked at 1000℃~1500℃ of high temperatures (2).

3) Health benefits

(1) Bamboo

Bamboo is aggressively anti-bacterial. It has a natural property called bamboo kun that kills up to 70% of any bacteria attempting to incubate on the bamboo, be it in its natural form or fabric (71). Bamboo shoots have been found to be low in fat and calories, which people are always concerned with when trying to eat healthy. Bamboo shoots are also good for you because they are an excellent source of two nutrients that human bodies really need, fiber, and potassium. One serving of bamboo shoots can provide 10% of the fiber a human needs a day (72).

(2) Bamboo salt

Bamboo salt contains more than 70 essential minerals and micro nutrients. High sulfur from bamboo tree trunk, may helps purifying blood thus lowering the blood pressure. Bamboo salt has high oxidation reduction potential (ORP) and alkalinity. ORP helps to prevent ageing through antioxidation acitivity. Alkaline nature helps to neutralize the acidic toxins in our body. Bamboo was used as an important medicine material. It has many different medical efficacy varies from its stub, roots, and leaves. Bamboo salt could have its complete medical efficacy if it is baked over 9 times repeatedly. And they also noticed that if bamboo salt is completely melted, the toxic characteristic

disappears. Bamboo salt is prepared by roasting salt at temperatures between 800 and 2000℃ (73) in a bamboo container plugged with mud at both ends. This product absorbs minerals from the bamboo and the mud, and has been shown to increase the anticlastogenic and antimutagenic properties of the fermented soybean paste known in Korea as doenjang (74). Bamboo salt is well known as one of the most famous traditional medical treatments. Also many pharmaceutical scientists all around the world are researching bamboo salt's special therapeutics like anti-cancer effect and anti-virus effect.

3. *Rubus coreanus* Miquel (raspberry)

1) Origin of *Rubus coreanus* Miquel

The *Rubus coreanus* Miquel is the edible fruit of a multitude of plant species in the genus Rubus, most of which are in the subgenus Idaeobatus; the name also applies to these plants themselves. Raspberries are perennial. The name originally referred to the European species Rubus idaeus (with red fruit), and is still used as its standard English name (75).

2) Nutrients and health benefits

Raspberries contain significant amounts of polyphenol antioxidants such as anthocyanin pigments linked to potential health protection against several human diseases. The aggregate fruit structure contributes to its nutritional value, as it increases the proportion of dietary fiber, placing it among plant foods with the highest fiber contents known, up to 20% fiber per total weight. Raspberries are a rich source of vitamin C, with 30 mg per serving of 1 cup (about 50% daily value), manganese (about 60% daily value) and dietary fiber (30% daily value). Contents of B vitamins 1-3, folic acid, magnesium, copper and iron are considerable in raspberries (76). Raspberries rank near the top of all fruits for antioxidant strength, particularly due to their dense contents of ellagic acid (from ellagotannins), quercetin, gallic acid, anthocyanins, cyanidins, pelargonidins, catechins, kaempferol and salicylic acid. Yellow raspberries and others with pale-colored fruits are lower in anthocyanins. Due to their rich contents of antioxidant vitamin C and the polyphenols mentioned above, raspberries have an ORAC value (oxygen radical absorbance capacity) of about 4900 per 100 grams, including them among the top-ranked ORAC fruits. Cranberries and wild blueberries have around 9000 ORAC units and apples average 2800 (77). Although there are no clinical studies to date proving these effects in humans, antioxidant and antiproliferative (chemopreventive) effects against cancer have

been linked to the amount of phenolics and flavonoids in various foods including raspberries.

4. Oxidation and antioxidation

In many countries, nutritionists are obliged to use fats and oils or fat rich ingredients, such as fishmeal, carcass meal, poultry byproduct meal and vegetable-origin products to achieve a balanced least-cost formulation. The nutrient value of feed containing these ingredients can be extremely variable, as the fat content may deviate from tabular values and rancidity frequently degrades the energy content and the availability of amino acids. In addition, the formation of free radicals during peroxidation of fats destroys critical nutrients, such as vitamins A, D and E. The quality of products may be affected by the evolution of aldehydes and ketones, which impart off-flavor to poultry meat, and the pigmentation of broiler skin and egg yolks is invariably reduced (78-80).

1) Oxidation process

Auto-oxidation is a spontaneous reaction of atmospheric oxygen with fatty acids, which leads to cmplex chemical

changes that eventually manifest themselves in the development of off-flavours in food/feed. Oxidation process is divided nto three stages Induction, Propagation and Termination. Heavy metals present at low levels in fats, oils and other fatty ingredients can promote autoxidation. In oxidation process oxygen reacts with unsaturated fatty acids giving rise to free radicals. Initially, peroxides are formed which in turn break down to hydrocarbons, ketones, aldehydes, and smaller amounts of epoxides and alcohols. The result of the oxidation of fats, oils and fat rich ingredients is the development of objectionable flavors and odors characteristic of the condition known as "oxidative rancidity" (81).

2) Physiological effect of oxidation in human

The process of oxidation in the human body damages cell membranes and other structures including cellular proteins, lipids and DNA. When oxygen is metabolised, it creates ‘free radicals’ which steal electrons from other molecules, causing damage. The body can cope with some free radicals and needs them to function effectively (82). However, an overload of free radicals has been linked to certain diseases, including heart disease, liver disease and some cancers. Oxidation can be

accelerated by stress, cigarette smoking, alcohol, sunlight, pollution and other factors. Antioxidants are found in certain foods that neutralise free radicals. These include the nutrient antioxidants, vitamins A, C and E, and the minerals copper, zinc and selenium (83). Other dietary food compounds, such as the phytochemicals in plants and zoochemicals from animal products, are believed to have greater antioxidant effects than either vitamins or minerals. These are called the non-nutrient antioxidants and include phytochemicals, such as lycopenes in tomatoes, and anthocyanins found in cranberries. A diet high in antioxidants may reduce the risk of many diseases, including heart disease and certain cancers. Antioxidants scavenge the free radicals from the body cells, and prevent or reduce the damage caused by oxidation. The protective effect of antioxidants continues to be studied around the world. For instance, men who eat plenty of the antioxidant lycopene (found in tomatoes) may be less likely than other men to develop prostate cancer. Lutein, found in spinach and corn, has been linked to a lower incidence of eye lens degeneration and associated blindness in the elderly. Flavonoids, such as the tea catechins found in green tea, are believed to contribute to the low rates of heart disease in Japan (84).

3) Antioxidants

Antioxidants are those substances that extend the induction period of autoxidation process and thus help to reduce destruction of the fats/oils/fat rich ingredients. Antioxidants delay rancidity by binding to oxygen molecules and therefore making them unavailable to free radicals. They also function by reacting with initiating and propagating free radicals and forming non-reactive substances that lay dormant within the substrate (85, 86). The remarkable characteristic of antioxidants is that they have optimum effectiveness in low concentrations in oil. This can be attributed to the fact that when great quantities of antioxidants are added to an oil, they act as prooxidants (87-90). Antioxidants can be classified into four groups: Primary, Synergistic, Secondary, and Miscellaneous. Primary antioxidants function by terminating the free-radical chain reaction. They donate hydrogen or electrons to free radicals and then convert them into more stable products (91-93). They may also function in reactions with lipid radicals, forming lipid antioxidant complexes. Most primary antioxidants are chemical antioxidants, such as phenols (e.g. nordihydroguaiaretic acid), ‘hindered' phenols (e.g. BHA and BHT), and other miscellaneous primary antioxidants (e.g. ethoxyquin). Phenols are benzine rings, compounds that contain hydroxyl groups and

that are highly reactive (94-96). Synergistic antioxidants are broadly classified as oxygen scavengers and chelators. Synergists may act as hydrogen donors to free radicals, which regenerate the primary antioxidants (97). Synergists may also provide an acidic medium that improves the stability of primary antioxidants. Oxygen scavengers include ascorbic acid (vitamin C), ascorbyl palmitate, sulfites, and erythorbates (98, 99). These react with free oxygen and remove it from a closed system. Chelators, such as citric acid, phosphates, and tartaric acid, are not true antioxidants but are highly effective as synergists with both primary antioxidants and oxygen scavengers. They react with prooxidant metals like iron and which raises the activation energy of initiation reactions. These compounds enhance the effectiveness of primary antioxidants or inhibit the effect of pro-oxidants. Secondary, or preventive, antioxidants include esters (the product of a reaction between an alcohol and an acid to form a solid that is soluble in oil), which function by decomposing the lipid peroxides into stable end products. Ascorbic acid can be reacted with alcohol so that it forms a salt, or ester (100, 101). Miscellaneous antioxidants such as flavonoids (metabolites in plants that occur in almost all plant parts) and related compounds and amino acids function as both primary antioxidants and synergists. Both natural as well as

synthetic antioxidants have been studied for their antioxidant effect and are used in the feed (102-106).

5. Cancer and its prevention

1) Carcinogenesis process

Humans are exposed to a wide variety of carcinogenic insults, including chemicals, radiation, physical agents and viruses. The majority of human cancers are thought to arise from exposure to naturally occuring or man-made chemicals. In addition, it has become increasingly apparent that an individual's genetic background can dramatically affect the level of susceptibility to a carcinogenic exposure (107, 108). The ultimate goal of chemical carcinogenesis research is to achieve an understanding of the process involved in the induction of human cancer to an extent that will allow intervention to prevent the disease, either in the general population or in susceptible subpopulations.

The purpose of this review is to provide an understanding of the basic mechanisms of carcinogenesis, with an emphasis on the potential targets for mechanism-based prevention strategies. Strategies for the nutritional modulation and chemoprevention of cancer should focus on stopping the carcinogenesis process at the earliest possible point in the pathway. Targets for interfering

with tumor initiation events include modifying carcinogen activation by inhibiting the enzymes responsible for that activation or by directly scavenging DNA-reactive electrophiles; enhancing carcinogen detoxification by altering the activity of detoxifying' enzymes; and (enhancing DNA repair processes. Targets for blocking the processes involved in the promotion/progression stages of carcinogenesis include altering the expression of genes involved in cell signaling, particularly those regulating cell proliferation, apoptosis, and differentiation; and (109) decreasing inflammation. Examples of dietary factors and chemopreventives interacting with each of these processes were provided.

Progress in the field of carcinogenesis has revealed multiple targets for the nutritional modulation and chemoprevention of cancer, and additional targets will be identified as more is learned about the carcinogenesis process. Capitalize on the availability of new tools such as transgenic mice, to develop and test mechanism-based strategies for preventing cancer.

2) Mechanisms of causing cancer

The association between environmental factors, such as diet, irradiation, infection, and cancer, have long been recognized

through epidemiology studies. Host susceptibility to cancer has been increasingly recognized through the studies of familial cancer syndromes, single nucleotide polymorphism etc (110). Carcinogenesis probably results from the interaction between environmental factors and host susceptibility, and is a multi-steps process, involving a number of somatic genetic alterations through a series of morphological changes. These include activation of oncogenes, silencing of tumor suppressor genes through mutation, loss of heterozygosity or CpG island methylation. The epigenetic change, CpG island methylation, is increasingly being recognized to be an early and important mechanism for tumor suppressor gene silencing. Epigenetic silencing does not involve the changes of nucleotide sequence, hence it is potentially reversible and may potentially offer early chemoprevention (111).

4) Protective mechanism from cancer

(1) Apoptosis in cancer cell

There was a presentation focused on PTEN, a phosphatase (112) that plays a critical role in controlling cancer cell survival and metabolism (113, 114). Cancer is a genetic disease that may be brought about by dysregulation of 4 different pathways: 1.

increased activity of the cell cycle; 2. decreased activity of differentiation pathways; 3. decreased DNA repair; 4. decreased cell death. DNA repair is, however, tightly linked to cell death. In fact, activation of ATM by ionizing radiation leads to activation of chlk2 and p53, an internal switch whose recruitment may lead to cell cycle arrest, cell stabilization, or apoptotic death. P53-induced apoptosis requires the effector activity of caspase 9 and Apaf 1, and follows the intrinsic pathway with mitochondrial involvement and apoptosome formation. It is a well-accepted tenet of cancer biology that clonal evolution of cancer selects for cells that are characterized by enhanced proliferation and survival. How can this be reconciled with the paradox that dysregulation of cellular homeostasis is usually associated with induction of apoptosis? Cellular stress, as well as oncogene activation and deregulation of cell growth, all lead to activation of p53, which in turn "chooses" between cell cycle arrest and apoptosis. In normal cells, deregulation of cellular proliferation triggers apoptosis, but in tumors, noxa such as radiation and chemotherapeutic drugs trigger even more apoptosis (115).

(2) Mechanisms of apoptosis

The importance of apoptosis is widely recognised in many fields of medicine and it is till now one of the hottest fields in biomedical research. Apoptosis is a fundamental cell event, and the understanding its mechanisms of action will give a lot of expectations in terms of its future use in tumor diagnosis and therapy (Fig. 3). In a healthy cell, the outer membrane of mitochondria expresses the anti-apoptotic protein Bcl-2 on its surface (116). Bcl-2 is bound to a protein named Apaf-1 (Apoptotic Protease Activating Factor-1). Cell internal damage (e.g. from reactive oxygen species) causes Bcl-2 to release Apaf-1 (117); in the meantime, a Bcl-2-related protein (Bax) penetrates the mitochondrial membranes, causing cytochrome-c to leak out into the cytosol (118). Then, released cytochrome-c and Apaf-1 bind, in an ATP-dependent manner, to a third protein, named caspase 9. The resulting complex of cytochrome c, Apaf-1, caspase 9 (and ATP) is called the "apoptosome", which executes the apoptotic death. Indeed, activated caspase 9 cleaves and, in so doing, activates other caspases. The sequential activation of one caspase by another creates an expanding cascade of proteolytic activity, which leads to digestion of structural proteins in the cytoplasm, degradation of chromosomal DNA, and phagocytosis of the cell (119).

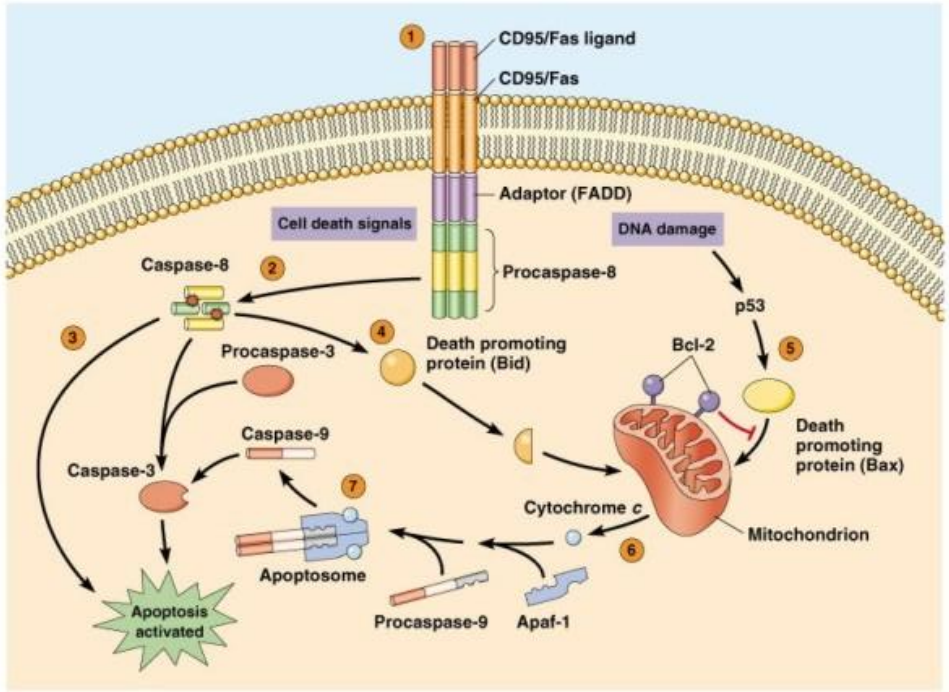


Fig. 3. Statin induced apoptosis

(K Engelhard *et al*, *European J Anaesthesiology*, 2004)

Cytotoxic T cells recognize (and bind to) their target through a ligand-receptor mechanism and, in this way, kill dangerous cells via apoptosis. Many cytotoxic agents iduce apoptosis as they trigger CD95 and the TNF (120). CD95 and the TNF receptor are integral membrane proteins with their receptor domains exposed at the surface of the cell. Binding of the complementary death activator (CD95L and TNF, respectively) transmits a signal to the cytoplasm leading to activation of caspase 8. Caspase 8 (like caspase 9) initiates a cascade of caspase activation, leading to phagocytosis of the cell (121).

Neurons, and perhaps other cells, have another way to self-destruct that unlike the two pathways described above, it does not use caspases. Apoptosis-inducing factor (AIF) is a protein that is normally located in the intermembrane space of mitochondria. AIF is released from the mitochondria (like the release of cytochrome c in the first pathway) upon the arrival of an apoptotic signal: then, it migrates into the nucleus and binds to DNA, triggering its destruction and cell death (122). Other factors that are released from the mitochondria, are the endonuclease endoG, Smac/Diablo, and Htr/Omi (123-125).

6. Inflammation

1) Inflammation

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Fig. 4) (126). Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. ithout inflammation, wounds and infections would never heal. Similarly, progressive destruction of the tissue would compromise the survival of the organism. However, chronic inflammation can also lead to a host of diseases, such as hay fever, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). It is for that reason that inflammation is normally closely regulated by the body (127, 128). Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues (129). A cascade of biochemical events

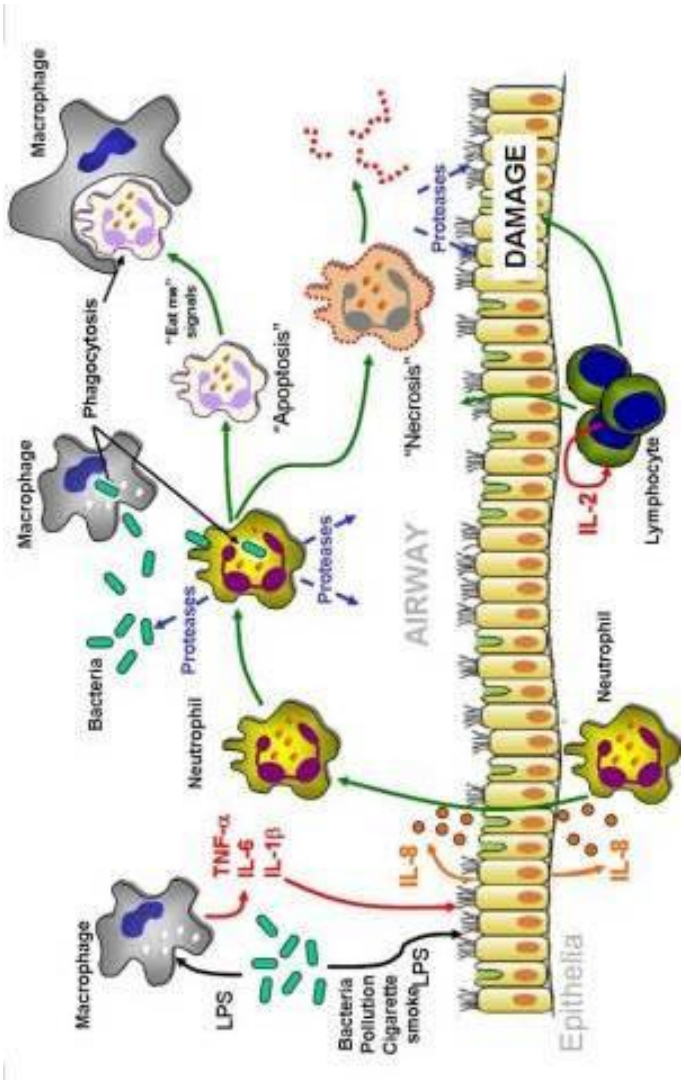


Fig. 4. Basis of inflammation

(Peter Libby MD *et al*, *Circulation*, 2002)

propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue (130). Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

2) Mechanisms of inflammation

Inflammation, a complex reaction due to the activation of the immune system, is aimed to defend the host against foreign invaders and to heal injured self-tissues. When this reaction is excessive, it may be noxious to the host due to a cascade of deleterious events. Two main stimuli of inflammation, bacteria and cerebral ischemia, are studied in our group. A first line of research is aimed at defining the physiopathological factors involved in sepsis and studying the role of inflammatory cytokines, oxidative stress and of different immune cell populations (neutrophils, macrophages and Kupffer cells) and their pharmacological modulation. A second line of research studies the inflammatory mediators and molecular mechanisms

of the damage induced by cerebral ischemia, which is among the leading causes of mortality and disability (131).

3) Oxidation and inflammation

Chronic diseases like cancer, heart disease and diabetes are leading causes of death in modern life. A huge amount of time and money is thrown at these diseases. Even so, modern medicine has not come up with successful treatments. At best, they are able to manage some of the symptoms. Less well known by most people is the fact that all of these problems have two things in common. They share the traits of inflammation and oxidation. Inflammation and oxidation are very closely related. And each has an important role in keeping us healthy. Both oxidation and inflammation create free radicals. Because oxidation causes damage to cells and tissues, it can trigger inflammation. And when inflammation creates free radicals, it can trigger oxidative stress (132).

4) Inflammation related diseases

(1) Cancer

Inflammatory cells produce various cytokines and chemokines that attract leukocytes and have powerful effects on tumour development (133). Early in the neoplastic process, these cells are powerful tumour promoters, producing an attractive environment for tumour growth, facilitating genomic instability and promoting angiogenesis. The chemokines and cytokines influence the whole tumour organ, regulating the growth, migration and differentiation of all cell types in the tumour micro environment, including neoplastic cells, fibroblasts and endothelial cells (134-136). Later in the tumorigenic process, neoplastic cells also divert inflammatory mechanisms such as selectin–ligand interactions, MMP production and chemokine functions to favour neoplastic spread and metastasis (137-139). This may be part of an attempt by the tumour to subvert immune cell functions, so favouring tumour development (140-143). Yet, the recruitment of inflammatory cells may also be counterproductive for tumour development, and also may represent an attempt by the host to suppress tumour growth (144-146). The pro-tumour actions of inflammatory cells include releasing growth and survival factors, promoting angiogenesis (147) and lymphangiogenesis, stimulating DNA damage, remodelling the ECM to facilitate invasion, coating tumour cells to make available receptors for disseminating cells via lymphatics and capillaries, and evading

host defence mechanisms. Although inflammatory responses should also be anti-tumour, cancer patients are often defective in their inflammatory responses. One approach is to evaluate whether functional polymorphisms in genes that regulate inflammatory processes, for example, genes encoding MMPs, cytokines, chemokines or selectins, harbour altered risk for developing cancer or are indicators of prognosis (148, 149). Yet for all the local inflammation in tumours, in many cases the overall innate immunity of the host is blunted. The challenge for the future is to normalize the inflammatory network to regain a normal host response overall: decreasing the high levels of tumour-promoting properties of the infiltrating cells, such as pro-inflammatory cytokines, while increasing their tumour-suppressing properties, such as antiinflammatory cytokines.

(2) Hepatitis

Hepatitis is a medical condition defined by the inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. The name is from the Greek hepar, the root being hepat-, meaning *liver*, and suffix *-itis*, meaning "inflammation" (150). The condition can be self-limiting (healing on its own) or can progress to fibrosis (scarring)

and cirrhosis. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia (poor appetite) and malaise. Hepatitis is acute when it lasts less than six months and chronic when it persists longer. A group of viruses known as the hepatitis viruses cause most cases of hepatitis worldwide, but it can also be due to toxins (notably alcohol, certain medications, some industrial organic solvents and plants), other infections and autoimmune diseases.

(3) Gastritis

Gastritis is an inflammation of the lining of the stomach, and has many possible causes. The main acute causes are excessive alcohol consumption or prolonged use of nonsteroidal anti-inflammatory drugs (also known as NSAIDs) such as aspirin or ibuprofen. Sometimes gastritis develops after major surgery, traumatic injury, burns, or severe infections. Gastritis may also occur in those who have had weight loss surgery resulting in the banding or reconstruction of the digestive tract. Chronic causes are infection with bacteria, primarily *Helicobacter pylori*, chronic bile reflux, stress and certain autoimmune disorders can cause gastritis as well. The most common symptom is abdominal upset or pain. Other symptoms are indigestion,

abdominal bloating, nausea, and vomiting and pernicious anemia. Some may have a feeling of fullness or burning in the upper abdomen. A gastroscopy, blood test, complete blood count test, or a stool test may be used to diagnose gastritis. Treatment includes taking antacids or other medicines, such as proton pump inhibitors or antibiotics, and avoiding hot or spicy foods. For those with pernicious anemia, B_{12} injections are given (151-153).

Ⅲ. MATERIALS AND METHODS

1. Preparations of bamboo salt and *Rubus coreanus* Miquel bamboo salt

Bamboo salts (1, 3, 9 times baked bamboo salt) and solar salt were provided by Taesung food company (Gochang, Jeonbuk) (Fig. 5) and purified salt was provided by Hanju Corporation (Ulsan). ***Rubus coreanus*** Miquel bamboo salt was prepared as shown in Fig. 6 with 74% of 9 times baked bamboo salt, 25% of ***Rubus coreanus*** Miquel extracts and 1% of medicinal herbs. The salts were dissolved in the distilled water before experiment.

2. Physicochemical analysis of bamboo salt

1) Scanning electron microscopy (SEM) analysis

The salt surface morphology and crystal structure of the dendrites were characterized using a scanning electron microscope (JSM-U3, JEOL, or HSM-2B, Hitachi) with an accelerating voltage of 10kV (154, 155).

Selecting the bamboo [1)]

↓

Stuffingsolar salt [2)] into the bamboo tube

↓

Sealing the bamboo tube with loess

↓

Baking the salts at 1,000-1,500℃ with a pine firewood
and pine resin as a fuel source bamboo salt (1 time bamboo salt)

↓

Repeating the above processes for 9 times to obtain the
9 time bamboo salt (Purple bamboo salt)

Fig. 5. The method of preparing bamboo salts at manufacturer (Taesung food company)

[1)] Bamboo: 3 years old king bamboo

[2)] Solar salt: from Korean West Coast

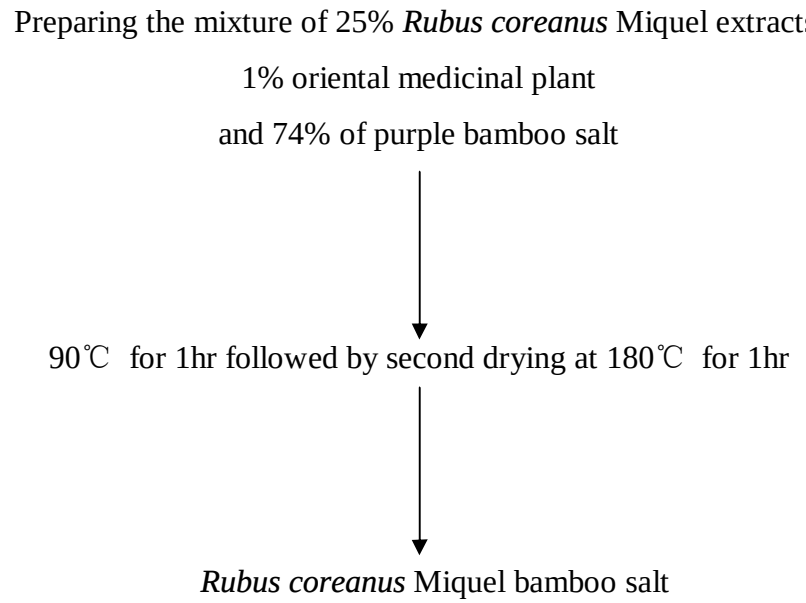


Fig. 6. The method of preparing *Rubus coreanus* Miquel bamboo salt at manufacturer (Taesung food company)

2) Analysis of mineral elements (ICP-OES)

Minerals were determined by ICP-OES 6010C (Inductively Coupled Plasma-Atomic Emission Spectrometry; Perkin-Elmer, Waltham, MA, USA) following EPA (Environmental Protection Agency of the USA, 1986) method 6010. Samples of mineral supplements were collected every 3mo for a total of 6 samples for each supplement and analyzed (156, 157).

3) X-ray diffraction (XRD) analysis

XRD analyses were performed on a PANalytical X'Pert PRO X-ray diffractometer (radius: 240.0 mm). Incident X-ray radiation was produced from a line focused PW3373/10 Cu X-ray tube, operating at 45 kV and 35 mA, providing Kα1wavelength of 1.540596 Å. The incident beam passed through a 0.04 rad, Soller slit, a ½° divergence slit, a 15 mm fixed mask and a 1° fixed anti scatter slit. After interaction with the sample, the diffracted beam was detected by an X'Celerator RTMS detector. The detector was set in scanning mode, with an active length of 2.022 mm. Samples were analysed utilising Bragg-Brentano geometry over a range of 3 – 75° 2θ with a step size of 0.02° 2θ, with each step measured for 200 seconds (158).

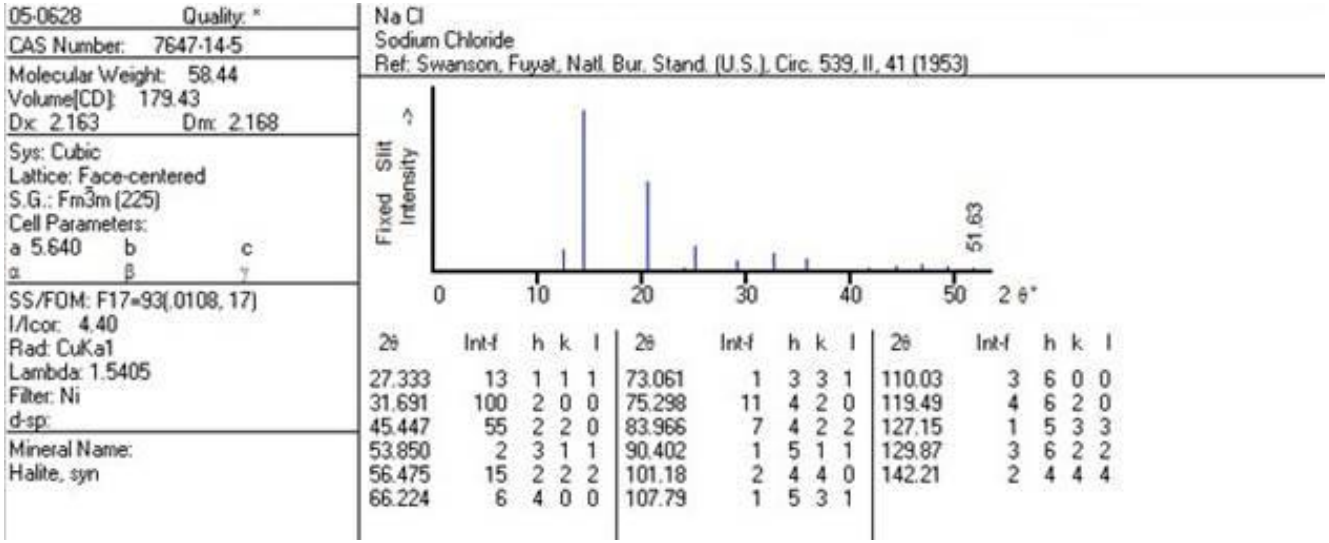


Fig. 7. Chromatograms of XRD in NaCl

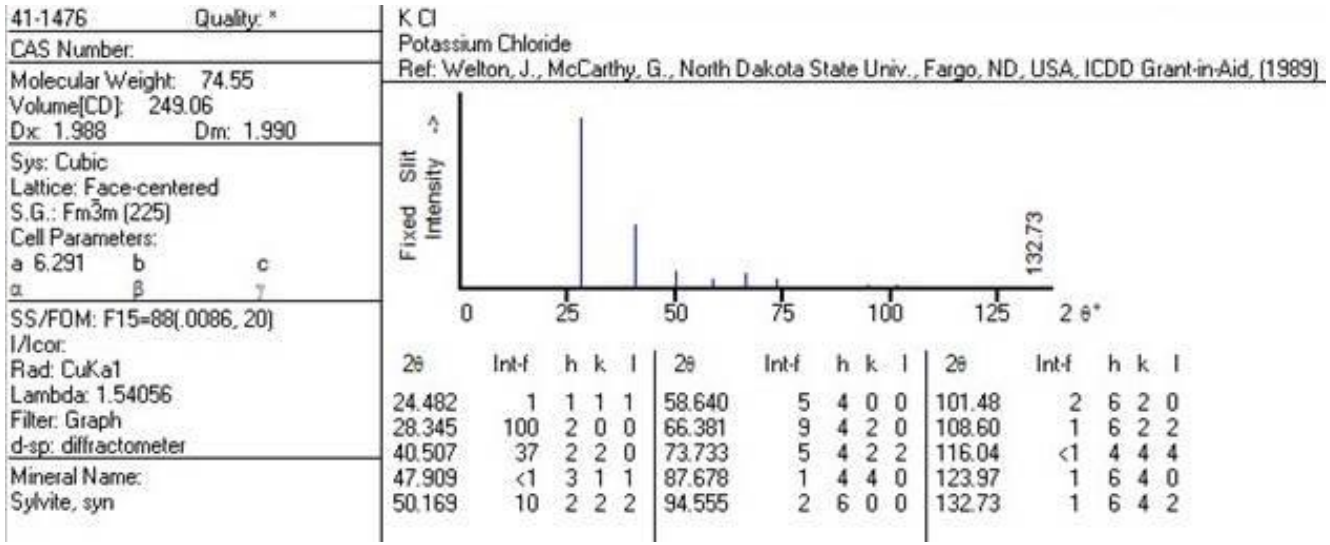


Fig. 8. Chromatograms of XRD in KCl

4) X-ray photoelectron spectroscopy (XPS) analysis

X-ray photoelectron spectroscopy (XPS) is a quantitative spectroscopic technique that measures the elemental composition, empirical formula, chemical state and electronic state of the elements that exist within a material. XPS spectra are obtained by irradiating a material with a beam of X-rays while simultaneously measuring the kinetic energy and number of electrons that escape from the top 1 to 10 nm of the material being analyzed. XPS requires ultra high vacuum (UHV) conditions. XPS experiments were performed using a VG Scientific ESCALAB 250 XPS spectrometer with monochromatic Al Kα source including charge compensation at KBSI (Busan, S. Korea). XPS is a surface chemical analysis technique that can be used to analyze the surface chemistry of a material in its received state, or after some treatment, for example: fracturing, cutting or scraping in air or UHV to expose the bulk chemistry, ion beam etching to clean off some of the surface contamination, exposure to heat to study the changes due to heating, exposure to reactive gases or solutions, exposure to ion beam implant, exposure to ultraviolet light (159, 160).

3. Assay for antioxidant effect of bamboo salt

1) Determination of pH

The pH of the sample was measured with 10 folds diluted solution using pH meter (Corning220, USA).

2) Fourier transform infrared (FT-IR) spectrometry

IR spectra were collected as absorbance spectra using an IRScope II IR microscope (Bruker Optics, Billerica, MA) equipped with a small sized MCT (HgCdTe) detector. The microscope was linked to a Vector 22 FT-IR spectrometer (Bruker) and was equipped with a software controlled x,y-stage. A specially designed microscope box was purged by dry air reducing spectral contributions from atmospheric water vapor and CO_2. FT-IR spectra were recorded using a 45×45-μm square aperture defined by knife-edges. Nominal spectral resolution was 8 cm^{-1}. Eight interferograms were averaged per pixel spectrum and apodized using a Happ–Genzel apodization function for Fourier transformation. Interferograms were zero-filled by a factor of 4. The background spectrum was recorded at a position of the IR window outside the tissue sample. 91×91 spectra were collected at a step size of 20 Am in x and y

direction for a total sample area of 1800 ┌ 1800 μm^2. Data acquisition was carried out by means of the OS/2 based OPUS software package supplied by Bruker (161).

3) Redox potential amperometric analysis

The OH^- immunosensor, Ag/AgCl (in saturated KCl), and a Pt wire were used as working, reference, and counter electrodes respectively (162).

4) DPPH radical scavenging effect

The DPPH radical scavenging activity was determined according to the method of Blois. Four milliliters of different concentration of the sample solutionwere added to 1.0 ml DPPH methanol solution (1.5×10^{-4} M). After storing at room temperature for 30 mins, the absorbance of solution was determined at 520 nm using a spectrophotometer and the remaining DPPH was quantified. The results were expressed as the means of trip licates (163).

4. Assessment of inhibitory effects of cancer progress

1) Assay for antimutagenic effect

(1) Mutagens

N-Methyl-N-nitro-N-nitrosoguanidine (MNNG) was obtained from the Aldrich Chemical Co. (Milwaukee, WI, USA) and dissolved in the distilled water.

(2) Test for antimutagenicity

Salmonella typhimurium strain TA100 histidine requiring mutants was used as a test baterial strain. Mutagenicity/ antimutagenicity test was carried out by a modified plate incorporation test (164). A 0.5 ml of phosphate buffer containing MNNG was distributed in sterile capped-tubes. Then 0.1 ml of the tester, from an overnight culture ($1\sim2\times10^9$ cells/ml) and 0.1 ml of test compounds (50 μl of mutagen and 50 μl of the salt aqueous solution) were added. The tubes were gently vortexed and preincubated at 37℃ for 30 min. The top agar (2 ml) kept at 45℃ were added to each tube and vortexed for 3 seconds. The entire mixture was overlaid on the minimal agar plate. The plates were incubated at 37℃ for 48 hrs and then the revertant baterial colonies on each plate were counted.

2) Assay for *in vitro* anticancer cancer effect

(1) Cell culture

The HCT-116 human colon adenocarcinoma cell line was purchased from American Type Culture Collection (ATCC, Rockbille, MD, USA). AGS human gastric adenocarcinoma cells and the HT-29 human colon adenocarcinoma cells were obtained from the Korean Cell Line Bank（Seoul, Korea）.. Cancer cells were cultured in RPMI-1640 medium (Gibco Co., Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Gibco Co.) at 37 ℃ in a humidified atmosphere containing with 5% CO_2 (Forma, model 311 S/N29035, USA). The medium was changed two or three times aweek.

(2) 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenylte-trazolium bromide bromide (MTT) assay

The anticancer effects of salt samples were assessed by MTT assay. The planting cancer cells were put into the 96 well plate, and 180 μl was divided by 2×10^4 cells/ml per well. According to some proportion, 20 μl of specimen was added into the 37℃, 5% CO_2 vessel to culture for 72 hrs. Based on this, MTT

solution was added and cultured for another 4 hrs under the same conditions. After removing the supernatant medium, 150 μl of DMSO was added per well and mixed for 30 minutes. Finally the ELISA reading was used to measure the absorbance at 540 nm (165,166).

(3) RNA extraction and reverse transcription-polymerase chain reaction

Total RNA of the cell was isolated using a Trizol reagent (Invitrogen Co., CA, USA) according to the manufacture's recommendations. Total RNA was digested with RNase-free DNase (Roche, IN, USA) for 15 min at 37℃ and repurified using a RNeasy kit according to the manufacture's protocol (Quiagen, CA, USA). One μg of total RNA was used for first-strand cDNA synthesis using Superscript II reverse transcriptase (BD Bioscience, Palo Alto, CA). Reverse transcription was performed at 30℃ for 10 min, 42℃ for 30 min, and 99℃ for 5 min, respectively, to inactivate the avian myeloblastosis virus RTXL. Primers (Table. 1) specifically prepared to amplify the genes of interests are shown in Table 1. Amplification was performed in a master-cycler (Eppendorf, Hamburg, Germany) with denaturing at 94℃ for 1 min, annealing at 54℃ for 1 min,

Table 1. Primer sequence

Gene	Direction	
Bax	Forward	5’-CCCAGTTGAAGTTGCCATCA-3’
	Reverse	5’-TCCCCCCGAGAGGTCTTCT-3’
Bcl-2	Forward	5’-TGGATCCAGGATAACGGAGG-3’
	Reverse	5’-CAAACAGAGGTCGCATGCTG-3’
iNOS	Forward	5′-GCAGCTGGGCTGTACAAA-3′
	Reverse	5′-AGCGTTTCGGGATCTGAAT-3′
COX-2	Forward	5’- GAATCATTCACCAGGCAAATTG- 3’
	Reverse	5’- TCTGTACTGCGGGTGGAACA-3’.
TNF-α	Forward	5’-GACCCTCAGACTCAGATCATCCTTCT-3’
	Reverse	5’-ACGCTGGCTCAGCCACTC-3’
IL-1β	Forward	5’-CTCCATGAGCTTTGTACAAGG-3’
	Reverse	5’-TGCTGATGTACCAGTTGGGG-3’

extension at 72℃ for 30 sec for 25 cycles and finally 72℃ for 7 min. The amplified PCR products were run in 1.0% agarose gels and stained with ethidium bromide (EtBr), and visualized under UV light. The intensities of the bands were estimated by densitometry (Multi Gauge V3.0 software, Fujifilm Life Science, Tokyo, Japan) (167).

(4) Western blot analysis

The apoptosis and inflammation expressions and actin were determined by western blot analysis. Western blotting was performed according to the following procedures. In brief, the cells were lysed in RIPA buffer containing 50 mM Tris-HCl (pH 8.0), 0.4% Nonidet P-40, 120 mM NaCl, 1.5 mM $MgCl_2$, 2 mM phenylmethylsulfonyl fluoride, 80 μg/mL leupeptin, 3 mM NaF, and 1 mM dithiothreitol at 4℃ for 20 mins. The cell lysates (25 μg) were separated by 12% sodium dodecyl sulfatepolyacrylamide gel electrophoresis, transferred onto a polyvinylidene fluoride membrane (Amersham Pharmacia Biotech, Little Chalfont, UK), blocked with 5% skim milk, and hybridized with the primary antibodies (diluted 1:1,000). After incubation with the horseradish peroxidase-conjugated secondary antibody at room temperature, immunoreactive

proteins were detected using a chemiluminescent enhanced chemiluminescence assay kit (Amersham Pharmacia, Arlington Heights, USA) according to the manufacturer's instructions. The western blot bands were visualized using a LAS3000 luminescent image analyzer (Fujifilm Life Science, Tokyo) (168).

3) Experimental of lung metastasis

A highly metastatic line of colon 26-M3.1 carcinoma cell was maintained as monolayer cultures in Eagle's Minimal Essential Medium supplemented with 7.5% fetal bovine serum, vitamin solution, sodium pyruvate, non-essential amino acids, and L-glutamine. Cultures were maintained in a humidified atmosphere of 5% CO_2 at 37℃.

Experimental lung metastasis was assessed by injecting colon 26-M3.1 cells into the lateral tail vein of 6-wk-old female Balb/c mice. The indicated dose of salt solution was injected to the mice subcutaneously, and then colon 26 M-3.1 cells (2.5×10^4/mouse) was inoculated intravenously after 2 d. The mice were killed 2 wk later and their lungs were fixed in Bouin's solution (saturated picric acid: formalin: acetic acid, 15:5:1,

v/v/v). Metastasis was assessed by counting the lung tumor colonies under a dissecting microscope (169).

5. Assessment of *in vivo* inflammatory effect

1) Animals

Male Sprague-Dawley rats (n=36, 7-week-old) were purchased from Hyochang Science (Daegu, Korea). They were maintained in a temperature controlled (temperature 25 ± 2°C, relative humidity 50 ± 5%) facility with a 12-h light/dark cycle and free access to a standard rat chow diet and water.

2) Hepatitis experiment

(1) Induction of hepatitis

To investigate the preventive effect of the various salts against CCl_4-induced hepatitis, the animals were divided into six groups of six rats each. The experimental design was as follows: the normal control group administered distilled water for 14 days and a single dose of vehicle (2 ml/kg bw of olive oil, p.o.); the CCl_4 control group received a 14-day repeated oral administration of distilled water followed by single administration of CCl_4 (2 ml/kg bw dissolved in the olive oil,

1:1, v/v) on the last day to induce the liver damage. ;, the salt + CCl_4 groups received a 400 mg/kg bw of salt dissolved in water for 2 weeks and hepatitis was induced as the same manner as mentioned the above. Twenty-four hours after the administration of CCl_4, the rats were anaesthetized by carbon dioxide and killed. Their blood and livers were collected and preserved at -70°C until biological assays were performed (170).

(2) AST, ALT and LDH

Aspartate aminotransferase (AST) and ALT Alanine aminotransferase (ALT) levels of the serum were determined using commercially available kits (Asan Pham, Seoul, Korea). LDH levels of the serum were determined using specific, commercially available kit (Cayman Chemical Company, Ann Arbor, USA)

(3) Triglyceride and total cholesterol

Triglyceride (TG) and total cholesterol (TC) concentration of plasma were determined using specific, commercially available kits (Asan pham, Seoul, Korea).

3) Gastritis experiment

The experimental design was as follows: the normal control group received 7-day repeated oral administration of distilled water and a single dose of vehicle (2 ml/kg bw of olive oil, p.o.); the rats were given 1 ml of HCl/ethanol (60% in 150 mM HCl) p.o. through esophageal intubation and killed 1 h later under deep ether anesthesia. The stomachs were removed, inflated by injecting 10 ml of 1% formalin for 10 min to fix the tissue walls, and opened along the greater curvature. The area (square millimeters) of hemorrhagic lesions developed in the stomach was measured under a dissecting microscope with a square grid (10×) (171).

6. Statistic analysis

Data are expressed as mean ± standard error values (n = 3). Means with different letters are significantly different ($p<0.05$) by Duncan's multiple range tests. Each experiment was replicated at least 3 times.

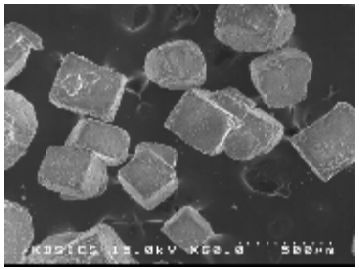
Ⅳ. RESULTS AND DISCUSSION

Part 1. Characteristics and functional effects of bamboo salt

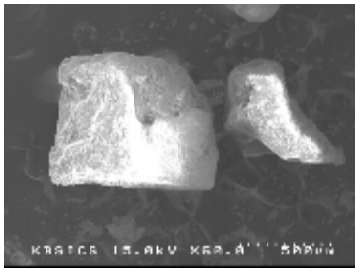
1. Physicochemical properties

1) Morphological structure by SEM microscope

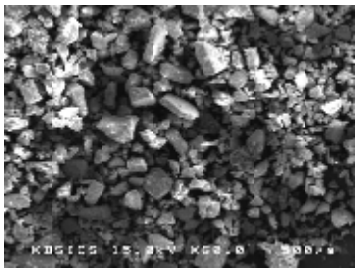
Fig. 9 showed SEM micrographs of purified salt, solar salt, and bamboo salt obtained at different number of times baked. The crystal structure of purified salt was very ordered, with the crystal size diameter at about 400 μm. In the case of solar salt, the structure was composed of irregular crystals and the crystal size diameter was 600 μm with a 3 times bigger volume than purified salt. It could be seen that the powder obtained at 9 baked times was very fine, with a particle size diameter of about 5 μm. This indicates that when the number of baking times increased, the particle size decreases. The morphology of the powder was well dispersed due to the salt locating between the particles and preventing their aggregation during the synthesis course (172).



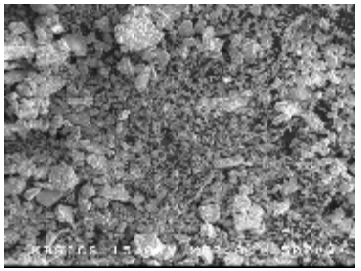
Purified salt



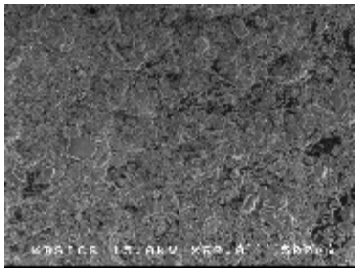
Solar salt



Bamboo salt (1×)



Bamboo salt (3×)



Bamboo salt (9×)

Fig. 9. The photograph of 5 different kinds of salts in SEM method (×60)

2) Content of mineral elements by ICP-OES

To compare mineral elements in different kinds of bamboo salt, we measured Na, Ca, Mg, Fe, Mn, P, S and K (Table 2). Mineral content such as K, Mn, Fe and P of bamboo salt (9×) were higher than those of other salt samples. Mineral absorption is an essential process for the human beings, therefore mineral deficiency is very important. The health benefits of potassium include stroke, anxiety and stress, muscular strength, metabolism, heart and kidney disorders, water balance, electrolytic functions, nervous system and other general health benefits of potassium. Health benefits of manganese ensure healthy bone structure, bone metabolism, helping in building essential enzymes for building bones. Phosphorus is an essential mineral that is usually found in nature which combined with oxygen. Calcium assists in the process of blood clotting and help to maintain the acid-alkaline balance in the blood. There was a study reported that bamboo salt contains many kinds of mineral, the element constitution was different from purified salt and solar salt (173).

Table 2. Comparison of mineral contents in different kinds of salts by ICP-OES

[W/W (%)]

Test item	Purified salt	Solar salt	Bamboo salt (1×)	Bamboo salt (3×)	Bamboo salt (9×)
Na	38.6600	32.3000	38.3200	38.4000	38.9200
Ca	0.0332	0.1079	0.2764	0.3167	0.2176
Mg	0.0004	0.9986	1.0052	1.2460	0.6969
Fe	N.D.	N.D.	N.D.	N.D.	0.0165
Mn	N.D.	0.0006	0.0002	0.0008	0.0025
P	0.0002	0.0002	0.0015	0.0023	0.0791
S	0.0153	0.5232	0.6976	0.8040	0.5419
K	0.1317	0.3933	0.4096	0.5182	0.8470

N.D. = Not detected

3) Structure analysis by XRD

The XRD pattern for purified salt showed high peaks at 31.9 and 45.6 with respective intensities of 2901 and 253, indicating the presence of NaCl salt crystal structure. The XRD pattern for solar salt shows high peaks at 32.2 and 45.9 with respective intensities of 322.1 and 18.9, also indicating the presence of NaCl salt crystal structure (Fig. 10). XRD patterns of purified salt and solar salt presented the salt diffraction patterns collected on these materials synthesized only by NaCl. The XRD patterns for bamboo salt show high peaks at 31.7 and 45.4, indicating the presence of NaCl crystal structure. However, there is an extra peak at 27.4 which shows the presence of KCl salt crystal structure. As shown in Fig. 11, the XRD patterns of powders obtained at different calcined times are similar. The major phases are all KCl salt (Fig. 11). It also can be found that the intensity of diffraction peaks of the as-prepared powder synthesized in salt is higher than that synthesized in NaCl-KCl salt at the same temperature, which is probably because the viscosity is smaller than that of NaCl-KCl melt at the same temperature; and the diffusive rate is higher, and the particle growth is easier (174).

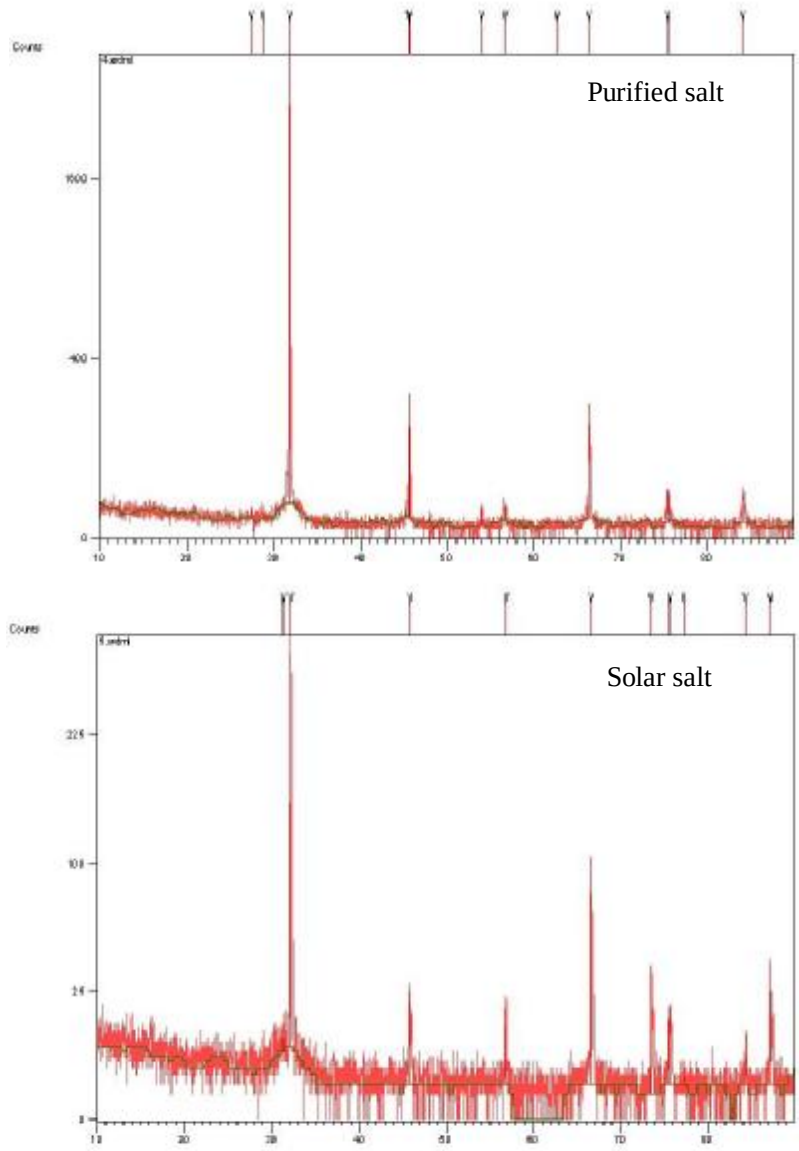


Fig. 10. Chromatograms of XRD in purified salt and solar salt

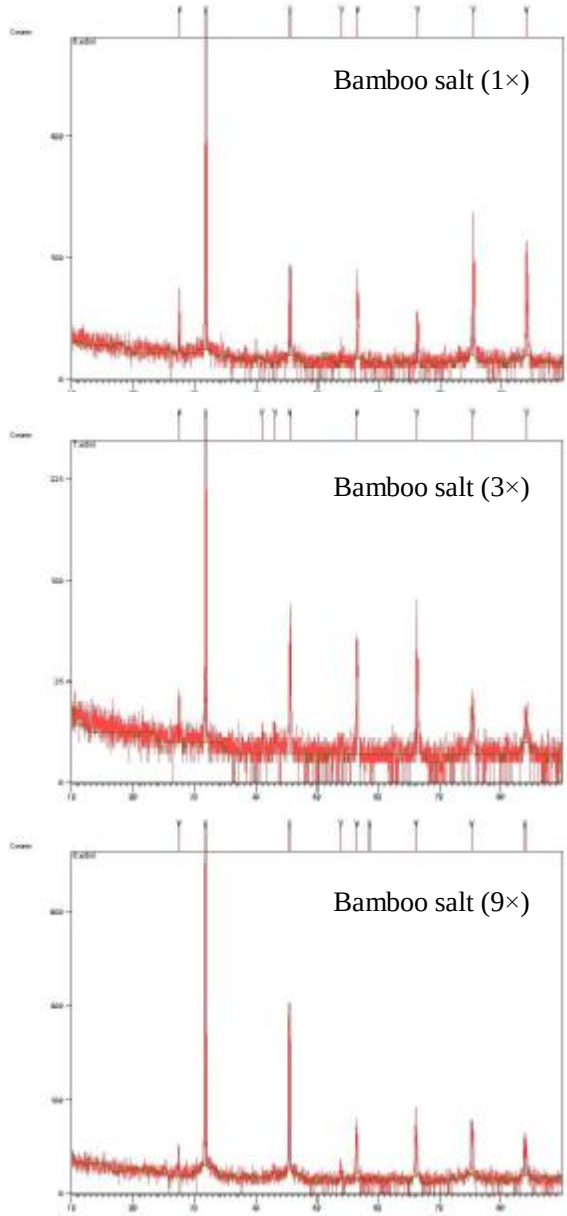


Fig. 11. Chromatograms of XRD in bamboo salt

4) Mineral content of salt surface by XPS

Electron Spectroscopy for Chemical Analysis (ESCA) experiments were done using a VG Scientific ESCALAB 250 XPS spectrometer with monochromated Al Kα source and charge compensation (175). The ESCA Cl2p spectra for three surfaces were observed at 196.3 eV, while the Na1s peak was shown at 1070.9 eV. These peak of purified chromatogram due to the presence of deposited NaCl crystals. In the chromatogram of solar salt (Fig. 12), the Cl2p peak shown at 197.1 eV and Na1s peak were shown at 1070.4 eV. There was a little Mg1s peak at 1302.3 eV. This indicates that the purified salt surface areas was NaCl and surface of solar salt was NaCl and a little of $MgCl_2$. The spectra in Fig. 12 showed peaks at 196.6 and 1069.5 eV, corresponding to Cl2p and Na1s, respectively, and these were K2p, Mg1s, and S2p peaks at 291.0, 1303.3 and 165.9. The spectra in Fig. 13 also showed similar peaks, corresponding to Cl2p, Na1s, K2p, Mg1s, S2p, respectively. These peaks were due to the presence of deposited NaCl, KCl and $MgCl_2$ crystals. The presence of the S2p peak in the spectra was due to the presence of sulfur which was present in all three surfaces. The quantities of Na, K, Mg, and Cl were normalized at 27.8%, 0.9%, 7.8%, and 31.3% for bamboo salt (1×). Na, K, Mg, and Cl quantities are normalized at 1.9%, 0.1%, 0.23%, and 7.7% for

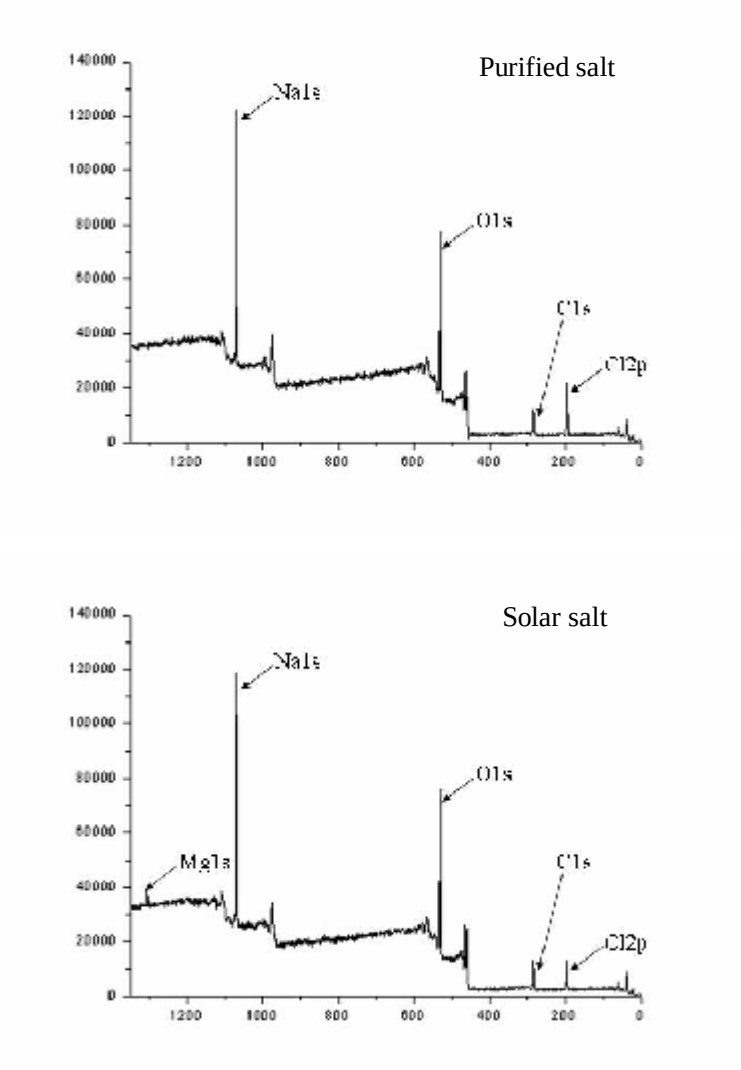


Fig. 12. Chromatograms of XPS in purified salt and solar salt

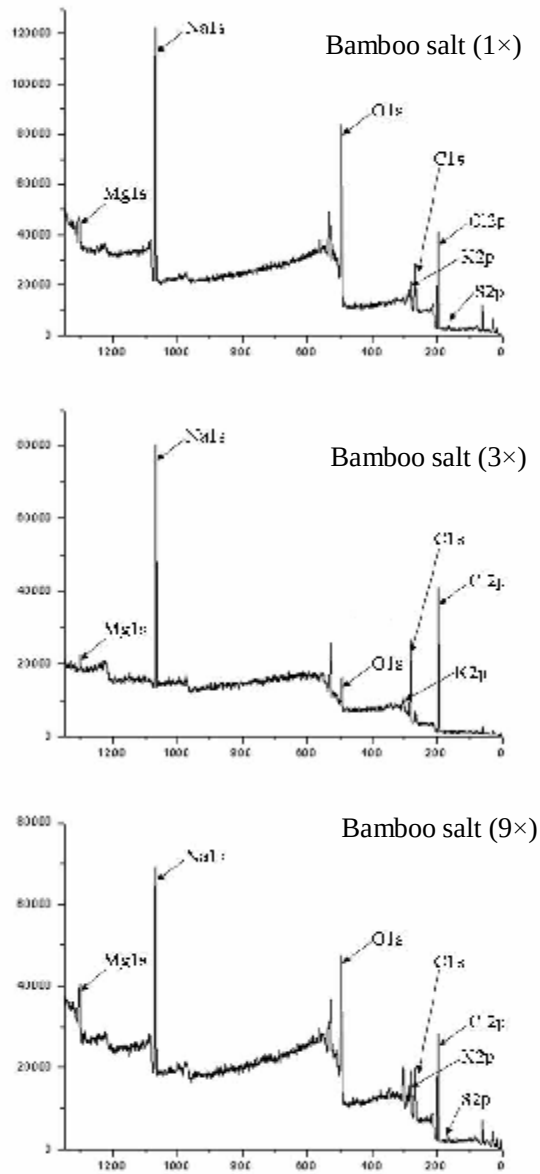


Fig. 13. Chromatograms of XPS in bamboo salt

bamboo salt (3×). The quantities of Na, K, Mg, and Cl were normalized at 19.7%, 1.4%, 5.8%, and 28.3% for bamboo salt (9×). This indicates that bamboo surface areas were covered by the NaCl-KCl-$MgCl_2$ complex structure and included many mineral compositions. Purified salt and solar showed the more contents of Cl2p, Na1s than bamboo salt and these were other mineral peaks of bamboo salt showed more contents than solar salt. This indicates that bamboo salt has complex structural materials including NaCl, KCl, $MgCl_2$, and other materials like sulfur.

2. Antioxidant effects

1) Determination of pH

The pH of purified salt, solar salt, and bamboo salt (1×), (3×), (9×) were 6.29, 9.13, and 10.5 respectively. The pH of bamboo salt is generally higher than that of the previous two salts. This indicates that bamboo salt was alkaline matter and there were more OH^- and Cl^- in bamboo salt (Table 3). The equation, pH = $[H^+]$ + $[OH^-]$ states the relationship between OH^- concentration and pH which means the higher of the pH, the higher of the OH^- concentration.

Table 3. pH of 5 different kinds of salts

Sample	pH
Purified salt [1)]	6.29 ± 0.02
Solar salt [2)]	9.13 ± 0.01
Bamboo salt (1×) [3)]	10.50 ± 0.02
Bamboo salt (3×) [4)]	10.66 ± 0.02
Bamboo salt (9×) [5)]	11.04 ± 0.01

[1)] Purified salt Producer: Hanju Corporation, Korea

[2)] Solar salt Producer: Taesung Food Company, Korea

[3)] Bamboo salt (1×) Producer: Taesung Food Company, Korea

[4)] Bamboo salt (3×) Producer: Taesung Food Company, Korea

[5)] Bamboo salt (9×) Producer: Taesung Food Company, Korea

2) OH group measuring by FT-IR

The FT-IR spectra of the compound OH in different kind of salts were given in the Fig. 14. The FT-IR peak of bamboo salt was observed at the wavelength 3429 cm^{-1} with an intensity of 0.323 absorbance unit. The FT-IR peaks of purified salt and solar salt were observed at the wavelength 3429 cm^{-1} as well with an intensity of 0.05 and 0.138 absorbance unit respectively. The bamboo salt showed the highest OH FT-IR peak, and solar salt showed the higher peak than purified salt. This indicates that there was most OH in bamboo salt. This result proved that bamboo salt has the best antioxidant effects. Due to the fact that bamboo salt has a higher concentration of OH group, it has a higher antioxidative effect (176).

3) Redox potential amperometric analysis of salt

The current response of OH^- was examined by Versa STAT 4 (Princeton Applied Research, USA) as shown in Fig. 15. Cyclic voltammograms (CVs) were recorded for the salts in a 0.1 M phosphate buffer solution at pH 7.4 by cycling the potential between -2 V and 2 V (scan rate 100 mV/s). The reduction peak of bamboo salt was observed to be about 3 times broader than that for solar salt. As seen, there was a reduction peak at 1.8 V

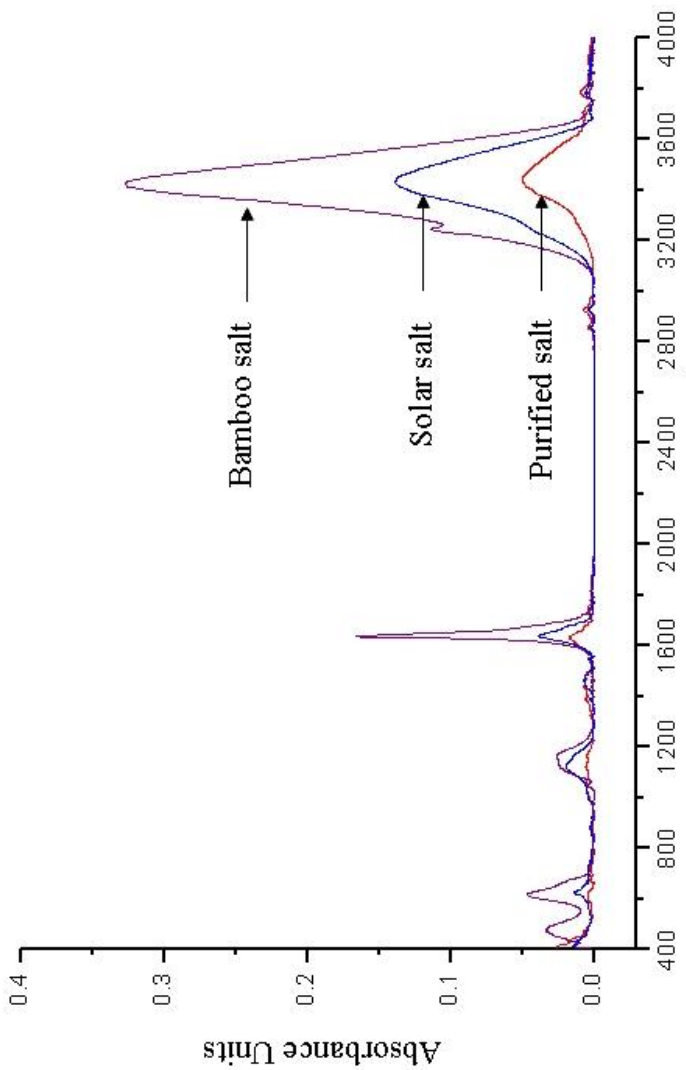


Fig. 14. Chromatograms of FT-IR in salt samples

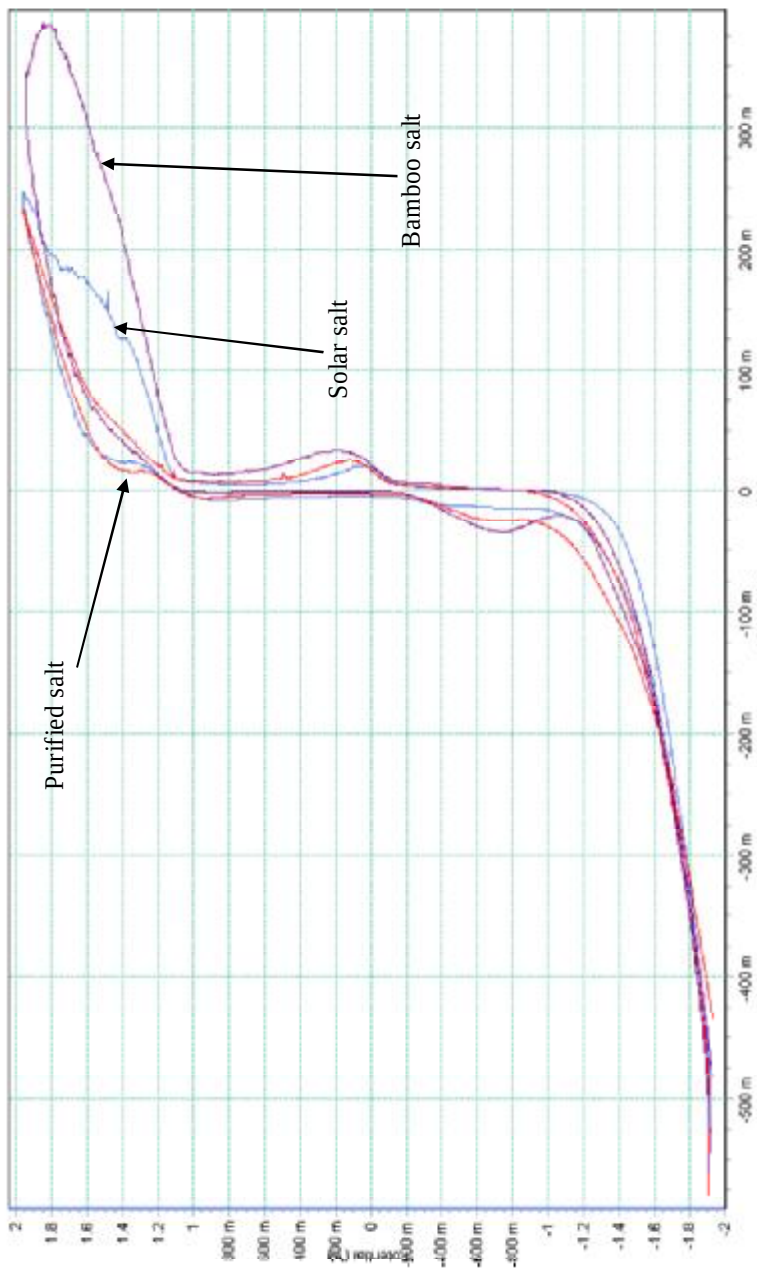


Fig. 15. Redox potential amperometric analysis of salt samples

in each case whereas there was no significant reduction peak in the CV recorded with the purified salt. The reduction peak potential was related to the direct electron transfer reaction of OH^-. This showed that the presence of OH^- increased the reduction potential current and shifted the OH^- reduction potential slightly towards the negative direction. Moreover, the OH^- might have an effect in decreasing the reduction potential difference in addition to stabilizing the salt crystalline structures on the electrode surface. These results prove that bamboo salt has the best antioxidant effects.

4) DPPH radical scavenging effect

The radical scavenging effects of salt samples on DPPH radical were tested (Fig. 16). Purified salt, solar salt, bamboo salt (1×), bamboo salt (3×) and bamboo salt (9×) showed scavenging activity on DPPH radical at concentrations of 10% and 20%, respectively. At concentration of 10%, the radical scavenging activities of purified salt, solar salt, bamboo salt (1×), bamboo salt (3×) and bamboo salt (9×) were 0.8%, 1.2%, 7.6%, 11.4% and 18.4%, respectively. At concentration of 20%, their activities showed similar results (0.8%, 2.1%, 16.5%, 21.2% and 28.5%) as those at concentrations of 10% (Fig. 21). The radical

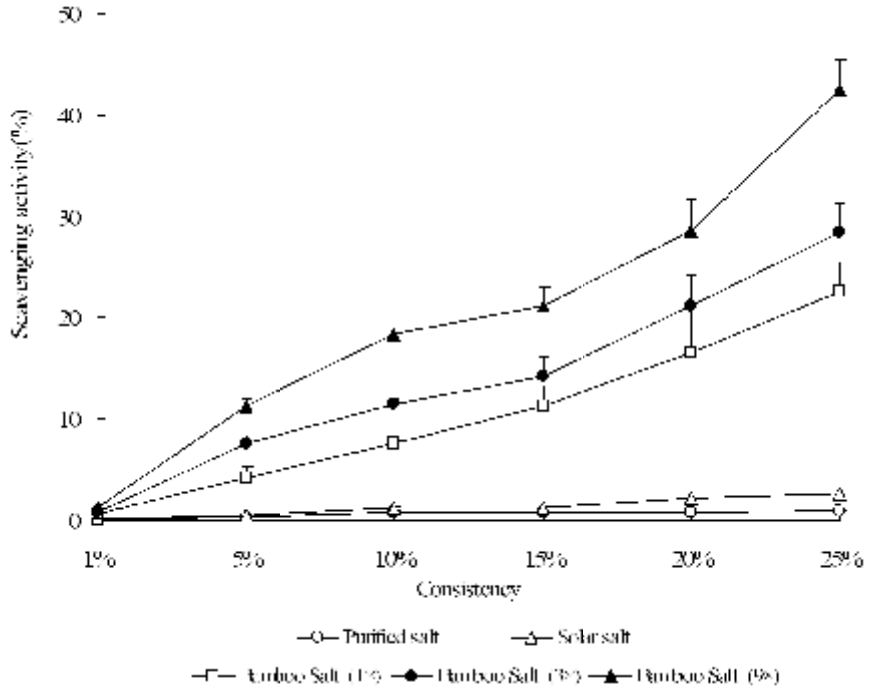


Fig. 16. DPPH radical scavenging activity of salt samples

scavenging activity of bamboo salt (9×) was more potent than that of bamboo salt (3×) and bamboo salt (1×), the three kinds of bamboo salt samples showed much better radical scavenging activity than solar salt and purified salt. This indicates that the radical scavenging activity of bamboo was more potent than that of purified salt and solar salt. Bamboo salt is a functional food, added to kimchi could increase the the antioxidative effect (177).

3. Inhibitory effects of cancer progress

1) Antimutagenic effect

Ames test is an important fast experimental method that can screen for chemical carcinogens. Biological genetic mutation is regarded as the key factor of cancer. Therefore, chemical mutation of some microorganism is used to test the carcinogenic activity. The bamboo salt (×9) showed inhibition effects of spontaneous in the *Salmonella typhimurium* TA100 strain (Table 4). At 1.25 mg/plate, the inhibition rates against mutagenicity of purified salt and solar salt were -11% and -6%. Bamboo salt (×1), bamboo salt (×3) and bamboo salt (×9) showed the inhibition rates at -2%, 2% and 19% ($p<0.05$). At 2.5 mg/plate, the inhibition rates of purified salt and solar salt were -20% and -15%. Bamboo salt (×1) and bamboo salt (×3) showed the

Table 4. Effect of aqueous solutions from different kind of salt products on the mutagenicity induced in *Salmonella typhimurium* TA100

Treatment (level of sample, mg/plate)	Revertants/plate	
	1.25	2.5
Spontaneous	121±11 $^{c\ 1)}$	121±11 ^{D}
Purified salt	134±18 ^{a} (-11) $^{2)}$	145±32 ^{A} (-20)
Solar salt	128±17 ^{b} (-6)	139±15 ^{B} (-15)
Bamboo salt (1×)	123±26 ^{bc} (-2)	127±29 ^{C} (-5)
Bamboo salt (3×)	118±17 ^{d} (2)	121±14 ^{D} (0)
Bamboo salt (9×)	98±18 ^{e} (19)	84±12 ^{E} (31)

1) Values are mean ± SD of revertants/plate

2) The values in parentheses are the inhibition rates (%).

$^{a\sim e,\ A\text{-}E}$ Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

inhibition rates at -5% and 0% and bamboo salt (×9) showed the best inhibition rate of 31% ($p<0.05$). This results indicated that most of salts showed mutagenicity in *Salmonella typhimurium* TA100, however the bamboo salt, especially 9 times baked bamboo salt exerted antimutagenic effect.

Bamboo salt, solar salt salt and purified salt showed co/anti-mutagenic effects of spontaneous in the *Salmonella typhimurium* TA100 strain (Table 5). At 1.25 mg/plate, the inhibition rates of purified salt and solar salt were -30% and -15% ($p<0.05$) which showed the comutagenic effect. Compare to purified salt and solar salt, bamboo salt (×1) also showed the comutagenic effect (-6%). However bamboo salt (×3) and bamboo salt (×9) showed the antimutagenic effect (1% and 9%). When the concentration was 2.5 mg/plate, purified salt and solar salt also showed a high comutagenic effect at the inhibition rates of -50% and -42%, respectively. 1 time baked bamboo salt (×1) showed slight comutagenic effect (-12%) less 30% than solar salt and 3 times baked bamboo salt (×3) also showed a little comutagenic effect (-4%), however, 9 times baked bamboo salt (×9) showed antimutagenic effect (17%). Salt was a cotimutagenic material which was not good for human health, but bamboo salt could reduce the cotimutagenic effect. In particular, 9 times baked bamboo salt showed the best antimutagenic effect because of 9 baked times courses and chemical changes (178).

Table 5. Effect of aqueous solutions from different kind of salt products on the mutagenicity induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 0.4 μg/plate) in *Salmonella typhimurium* TA100

Treatment (level of sample, mg/plate)	Revertants/plate	
	1.25	2.5
Spontaneous	121±11 [1)]	
MNNG(control) [2)]	874±26 [d]	874±26 [E]
Purified salt	1021±34 [a] (-30) [3)]	1247±23 [A] (-50)
Solar salt	988±33 [b] (-15)	1188±24 [B] (-42)
Bamboo salt (1×)	922±6 [c] (-6)	965±26 [C] (-12)
Bamboo salt (3×)	868±21 [d] (1)	902±13 [D] (-4)
Bamboo salt (9×)	805±18 [e] (9)	744±12 [F] (17)

[1)] Values are mean ± SD of revertants/plate

[2)] MNNG: N-Methyl-N-nitro-N-nitrosoguanidine

[3)] The values in parentheses are the inhibition rates (%).

[a~e, A-F] Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

2) *in vitro* anticancer effects

Anticancer effects of purified salt, solar salt and bamboo salt were evaluated using the MTT assay in HT-116 and HT-29 colon carcinoma cells and AGS human gastric adenocarcinoma cells. It is further investigated whether the inhibitory effect of salt was associated with apoptosis and inflammation related genes using RT-PCR and western blot analysis.

At the concentrations of 0.1% to 1.0%, along with the increase of concentrations, the growth inhibitory rates of salt samples were increased. Since above the concentrations of 2%, the growth inhibitory rate of bamboo salt was higher than 100% under preliminary result, the concentrations of 0.5% and 1% were chosen to do the experiment (Fig. 17).

(1) *in vitro* anticancer effects in human colon carcinoma cells

① *in vitro* growth inhibition of cancer cells

Antiproliferative effects of purified salt, solar salt and bamboo salt against HCT-116 and HT-29 colon carcinoma cells were evaluated using the MTT assay. As the concentrations increased, the growth of the HCT-116 and HT-29 cells was increasingly retarded in a sample-dependent manner. The

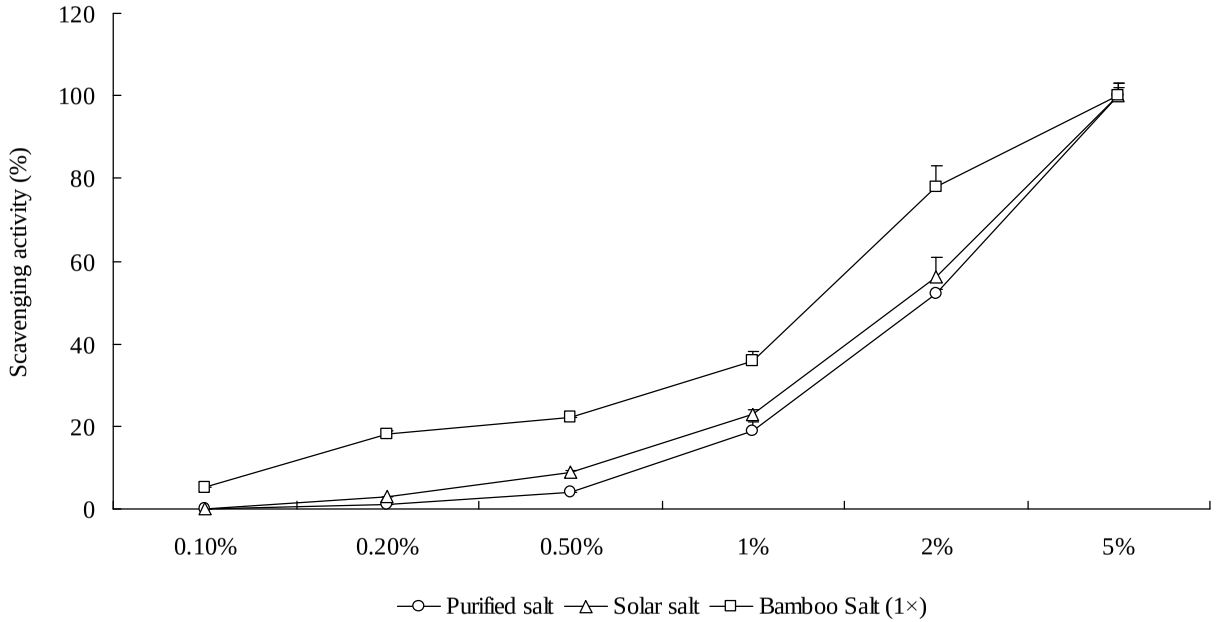


Fig.17. Effect of 3 kind of salts with different consistencies on the growth of HCT-116 human colon carcinoma cells in MTT assay

growth inhibitory rates of HCT-116 and HT-29 human colon carcinoma cells by the treatment of salt samples was shown in Table 6. When the aqueous solutions of salt samples (0.5%) were used to experiment with HCT-116 human colon carcinoma cells, and the growth inhibitory rates of purified salt, solar salt, bamboo salt (1×), bamboo salt (3×) and bamboo salt (9×) were 6%, 8%, 16%, 21% and 29%, respectively. At the concentration of 1%, the growth inhibitory rates of bamboo salt (9×) was 53%, higher than that of bamboo salt (3×) and bamboo salt (1×) (44% and 41%) on the other hand, solar salt and purified salt showed relatively low inhibitory effect of 22% and 18%, respectively ($p<0.05$). The anticancer effect on the HT-29 human colon carcinoma cells by the aqueous solutions of salt samples was basically consistent with HCT-116 human colon carcinoma cells growth inhibitory effect (Table 7). When the experimenting with 0.5% of HT-29 human colon carcinoma cells, the growth inhibitory rates of purified salt, solar salt, bamboo salt (1×), bamboo salt (3×) and bamboo salt (9×) were 8%, 13%, 18%, 22% and 31%, respectively. At the concentration of 1%, bamboo salt (9×) showed the highest growth inhibitory rates of 57%, the growth inhibitory rates of bamboo salt (3×), bamboo salt (1×), solar salt and purified were 46%, 42%, 25% and 21%, respectively ($p<0.05$).

These results demonstrated that the bamboo salt was more

Table 6. Inhibitory effect of aqueous solutions from different kinds of salt products on the growth of HCT-116 human colon carcinoma cells in 3-(4,5-dimetyl-thiazol)-2,5-diphenyl tetrazolium (MTT) assay

Treatment	OD_{540} (Level of sample, %)	
	0.5	1
Control	0.485±0.005 $^{a\,A}$	
Purified salt	0.460±0.022 ^{b} (6) [1)]	0.401±0.009 ^{B} (18)
Solar salt	0.447±0.004 ^{c} (8)	0.381±0.011 ^{C} (22)
Bamboo salt (1×)	0.413±0.007 ^{d} (16)	0.290±0.008 ^{D} (41)
Bamboo salt (3×)	0.385±0.017 ^{e} (21)	0.271±0.013 ^{E} (44)
Bamboo salt (9×)	0.348±0.016 ^{f} (29)	0.228±0.005 ^{F} (53)

[1)] The values in parentheses are the inhibition rates (%).

$^{a\sim f,\ A\text{-}F}$ Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 7. Inhibitory effect of aqueous solutions from different kinds of salt products on the growth of HT-29 human colon cancer cells in 3-(4,5-dimetyl-thiazol)-2,5-diphenyl tetrazolium (MTT) assay

Treatment	OD_{540} (Level of sample, %)	
	0.5	1
Control	0.517±0.003 $^{a\,A}$	
Purified salt	0.476±0.011 ^{b} (8) $^{1)}$	0.408±0.006 ^{B} (21)
Solar salt	0.450±0.008 ^{c} (13)	0.388±0.004 ^{C} (25)
Bamboo salt (1×)	0.424±0.005 ^{d} (18)	0.300±0.007 ^{D} (42)
Bamboo salt (3×)	0.403±0.008 ^{e} (22)	0.279±0.006 ^{E} (46)
Bamboo salt (9×)	0.357±0.010 ^{f} (31)	0.222±0.007 ^{F} (57)

$^{1)}$ The values in parentheses are the inhibition rates (%).

$^{a\sim f,\ A\text{-}F}$ Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

effectively impeded the proliferation and survival of HCT-116 and HT-29 human colon carcinoma cells than purified salt and solar salt. In addition, the more times bamboo salt baked, the better anticancer effects exerted.

② Apoptosis-related genes of Bax and Bcl-2

To determine the protective mechamisms from growth of cancer cells by the bamboo salt samples, expressions of Bax and Bcl-2 gene in HCT-116 and HT-29 human colon carcinoma cells were determined by RT-PCR and western blot after 48 hrs incubation with treatment of 1% salt solutions. As shown in Fig. 18 and 19, in the presence of 1% bamboo salt (9×), the pro-apoptotic Bax and the anti-apoptotic Bcl-2 showed significant changes. Accordingly, the results suggested that the salt induced apoptosis in HCT-116 and HT-29 human colon carcinoma cells via a Bax and a Bcl-2 dependent pathway. Thus, apoptosis induction by bamboo salt (9×) was related to increase Bax and decrease Bcl-2 in terms of mRNA and protein expressions compared to 1 and 3 times baked bamboo salt samples, solar salt and purified salt samples. From the result, 9 times bamboo salt showed the best anticancer effects in two kinds of colon carcinoma cells, and 1 time and 3 times baked bamboo salt showed the better anticancer effects than purified and solar salt.

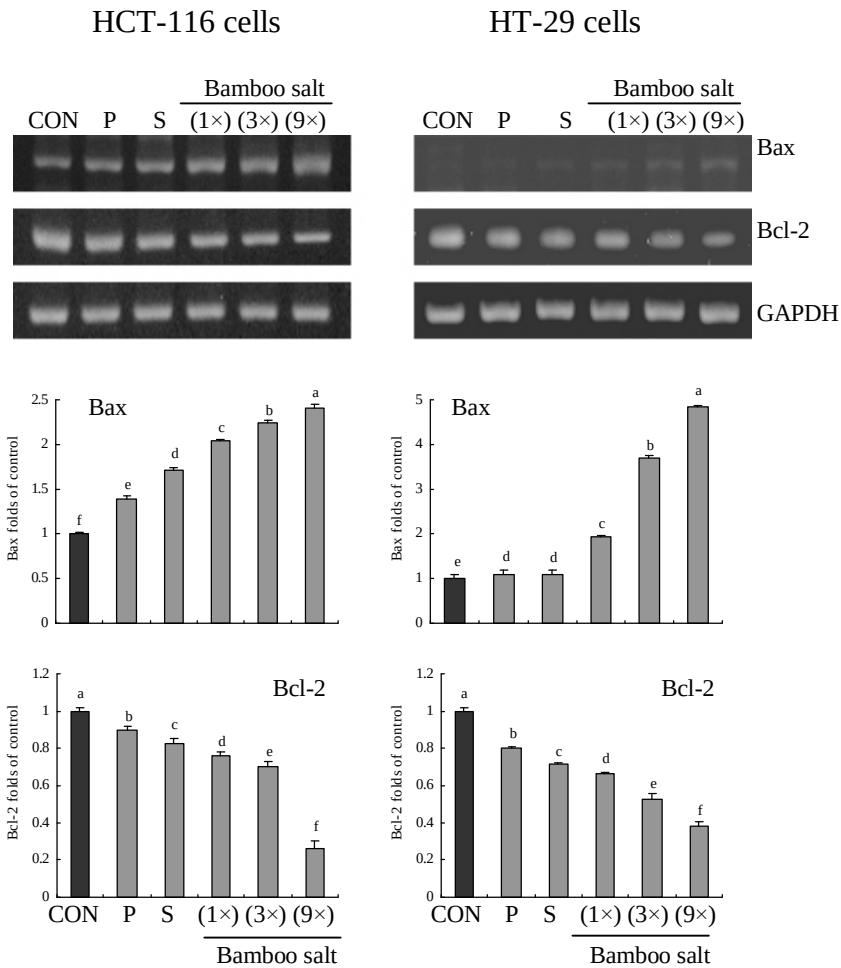


Fig. 18. Effects of salt samples (1%) on mRNA expressions of Bax and Bcl-2 in HCT-116 and HT-29 cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH × control numerical value (control fold ration: 1)

[a~f] Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

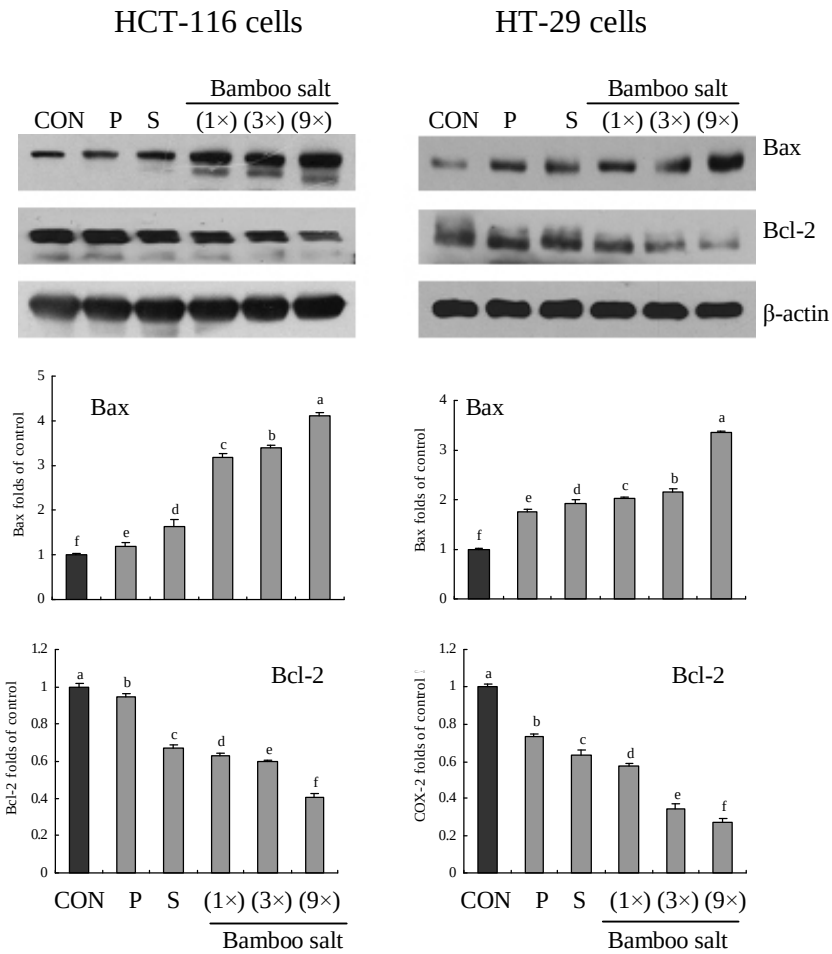


Fig. 19. Effects of salt samples (1%) on protein expressions of Bax and Bcl-2 in HCT-116 and HT-29 cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / β -actin × control numerical value (control fold ration: 1)

[a~f] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

③ Inflammation-related genes of iNOS and COX-2

The inhibition of iNOS and COX-2 activity exposes a number of human cell lines to inflammation stimuli. Therefore, it is examined whether the anticancer actions of the bamboo salt were associated with inhibition of iNOS and COX-2 activation gene expression. The expression level of iNOS and COX-2 was obviously down-regulated by bamboo salt (9×). Bamboo salt (1×) and bamboo salt (3×) were also effective in modulating iNOS and COX-2 (Fig. 20, 21). Cyclooxygenase-2 has been suggested to play an important role in colon carcinogenesis and NOS including iNOS also could be a good target for chemoprevention of colon cancer (181). Overall, the results revealed that treatment with bamboo salt showed the better antiinflmmation effect in colon cancer than solar salt and purified salt.

(2) *in vitro* anticancer effects of AGS human gastric adenocarcinoma cells

① *in vitro* growth inhibition of cancer cells

To evaluate the effects of bamboo salt on cell proliferation of AGS human gastric adenocarcinoma cells, the cells were cultured in the absence or presence of salt samples. After

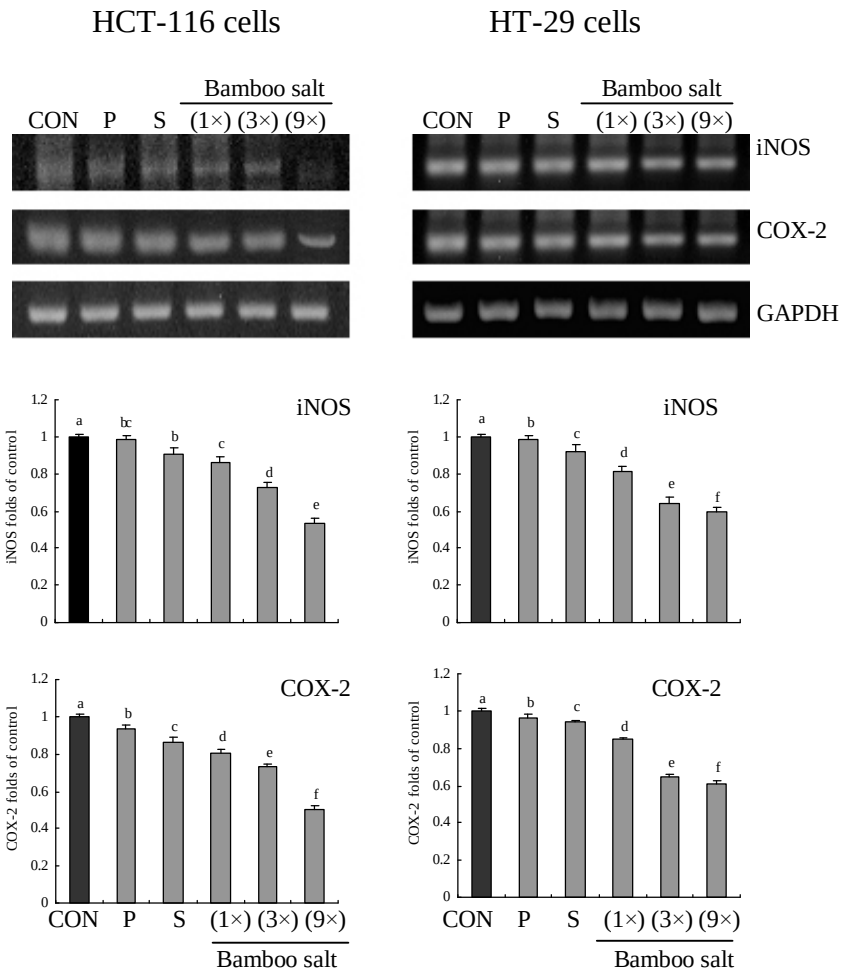


Fig. 20. Effects of salt samples (1%) on mRNA expressions of iNOS and COX-2 in HCT-116 and HT-29 cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH × control numerical value (control fold ration: 1)

$^{a\sim f}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

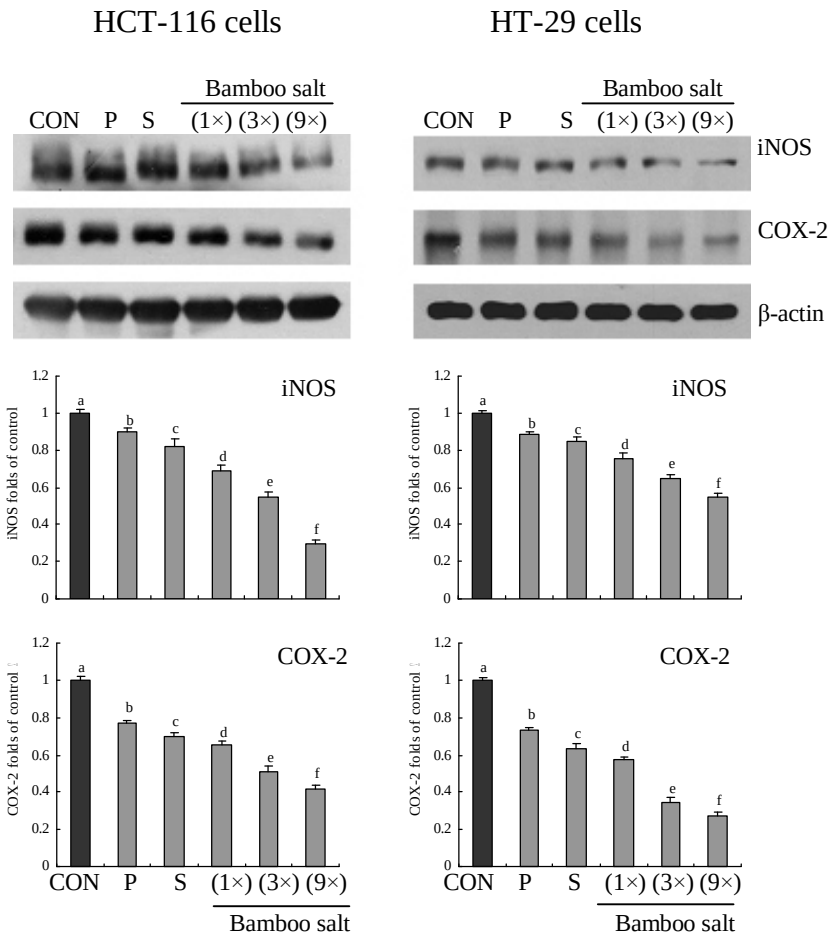


Fig. 21. Effects of salt samples (1%) on protein expressions of iNOS and COX-2 in HCT-116 and HT-29 cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / β -actin × control numerical value (control fold ration: 1)

[a~f] Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

treatment for 48 hrs, MTT assay were performed. As shown in Table 8, bamboo salt had a marked dose-dependent inhibitory effect on AGS cell growth. The growth of AGS cells by treatment of 0.5% concentration of purified salt, solao salt, bamboo salt (1×), bamboo salt (3×) and bamboo salt (9×) were inhibited by 5%, 8%, 14%, 20% and 25%, respectively. In addition at 1% of the concentration, the growth inhibitory rates of them were 15%, 20%, 36%, 41% and 51%, respectively ($p<0.05$).

It was obvious that the anticancer effect on AGS human gastric adenocarcinoma cells of bamboo salt (9×) was better than that of other bamboo salt. On the other hand, solar salt and purified salt showed relatively low effect on AGS cells growth inhition.

② Apoptosis-related genes of Bax and Bcl-2

The expression levels of members of the Bcl-2 family such as Bax and Bcl-2 were characterized using RT-PCR and western blotting analysis. Bamboo salt, solar salt and purified salt induced down-regulation of anti-apoptotic Bcl-2 protein levels as well as up-regulation of pro-apoptotic Bax protein levels. Bamboo salt (9×), bamboo salt (3×) and bamboo salt (1×), were more effective in the down-regulation of Bcl-2 and up-

Table 8. Inhibitory effect of aqueous solutions from different kinds of salt products on the growth of AGS human gastric adenocarcinoma cells in 3-(4,5-dimetyl-thiazol)-2,5-diphenyl tetrazolium (MTT) assay

Treatment	OD_{540} (Level of sample, %)	
	0.5	1
Control	0.498±0.002 $^{a\,A}$	
Purified salt	0.473±0.006 ^{b} (5) [1)]	0.423±0.006 ^{B} (15)
Solar salt	0.458±0.007 ^{c} (8)	0.398±0.005 ^{C} (20)
Bamboo salt (1×)	0.428±0.010 ^{d} (14)	0.319±0.007 ^{D} (36)
Bamboo salt (3×)	0.398±0.009 ^{e} (20)	0.294±0.014 ^{E} (41)
Bamboo salt (9×)	0.374±0.011 ^{f} (25)	0.244±0.008 ^{F} (51)

[1)] The values in parentheses are the inhibition rates (%).

$^{a\sim f,\ A\text{-}F}$ Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

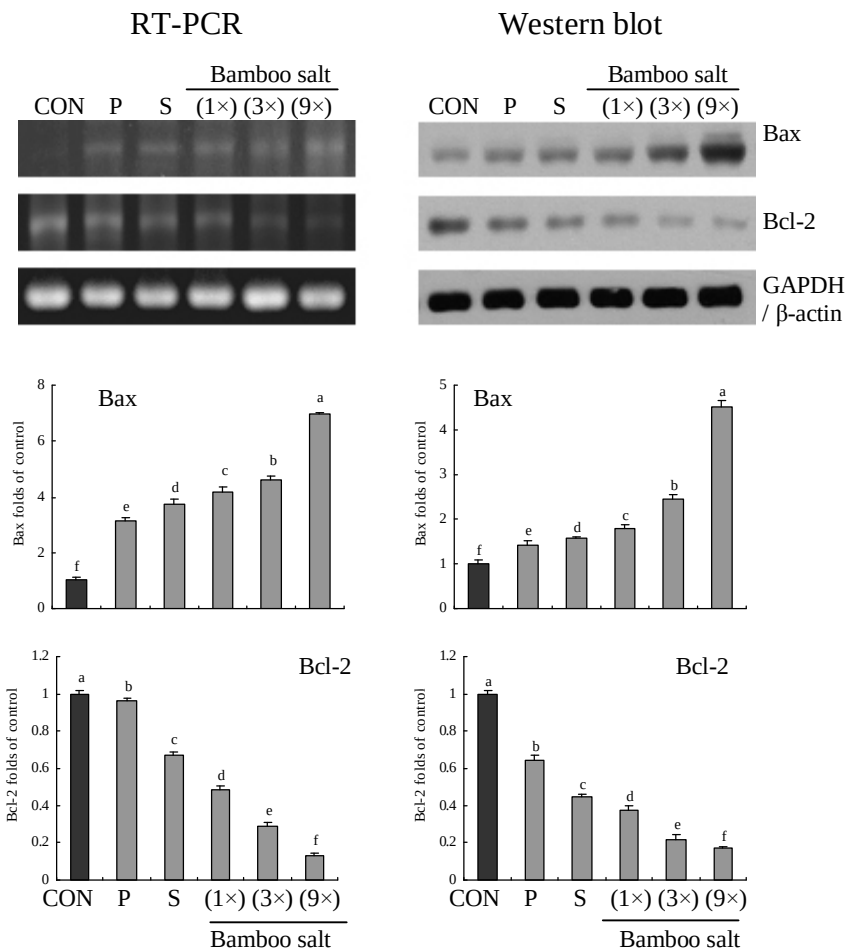


Fig. 22. Effects of salt (1%) samples on mRNA and protein expressions of Bax and Bcl-2 in AGS cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH (β -actin) × control numerical value (control fold ration: 1)

$^{a\sim f}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

regulation of Bax than the solar salt and purified salt. These results indicated that a portion of the antiproliferative activity of the bamboo salt was related to apoptosis mediated by up-regulation of Bax and down-regulation of Bcl-2 pathways and in particular, 9 times baked bamboo salt showed the most obvious effect.

③ Inflammation-related genes of iNOS and COX-2

As shown in 23, in the presence of 1% salt, mRNA expressions of COX-2 and iNOS were detected in untreated control AGS human gastric adenocarcinoma cells. However, bamboo salt (9×), bamboo salt (3×) and bamboo salt (1×) down-regulated effectively the expressions of iNOS and COX-2 which compared to the solar salt and purified salt. Accordingly, bamboo salt (9×) can be expected to contribute to the prevention of cancer by preventing inflammation as well as antiprolifertion of the cells.

These results suggested that bamboo salt (9×) had the best anticancer effect and bamboo salt (3×) showed the better effect than bamboo salt (1×) that had a slightly stronger effect than solar salt and purified salt, the solar salt and purified salt had a little stronger effect than untreated control carcinoma cells.

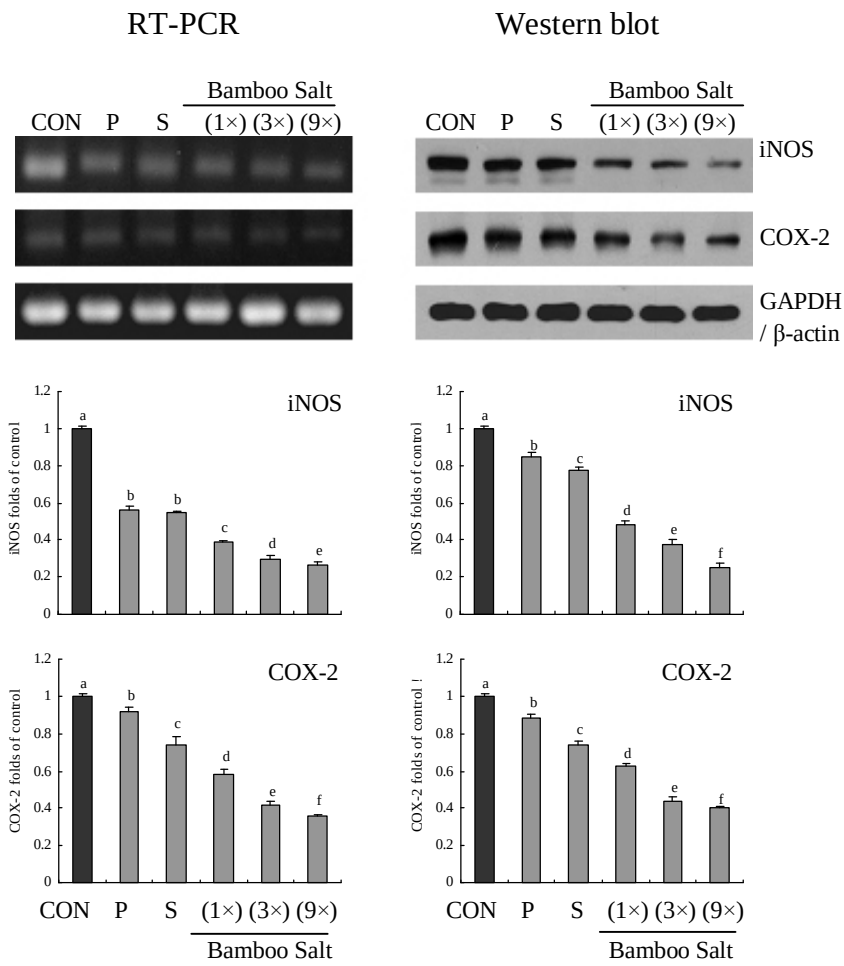


Fig. 23. Effects of salt samples (1%) on mRNA and protein expressions of iNOS and COX-2 in AGS cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH (β -actin) × control numerical value (control fold ration: 1)

$^{a\sim f}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

3) *in vivo* antimetastatic effect

The antimetastatic activities of bamboo salts with respect to the prophylactic inhibition of tumor metastasis were investigated by using an experimental metastasis model in mice (Fig. 24). All bamboo salt-treated mice had significantly fewer lung metastatic colonies than solar salt, purified salt-treated and control mice. The bamboo salt (9×) was the most effective salt in inhibiting lung metastasis of colon 26-M3.1 cells at the filling volume of 400 mg/kg and 800 mg/kg, which demonstrated that bamboo salt (9×) (16% and 20%) inhibits tumor formation and lung metastasis more than bamboo salt (3×) (8% and 16%) and bamboo salt (1×) (4% and 6%). Solar salt (-2% and -10%) and purified salt (-4% and -14%) showed the promotion effects, and purified salt was more potent than that of solar salt. The research of antimetastatic effect of solvent fractions from functional food add in bamboo salt that showed bamboo salt can increase antimetastatic than *doenjang* add in solar salt (179, 180).

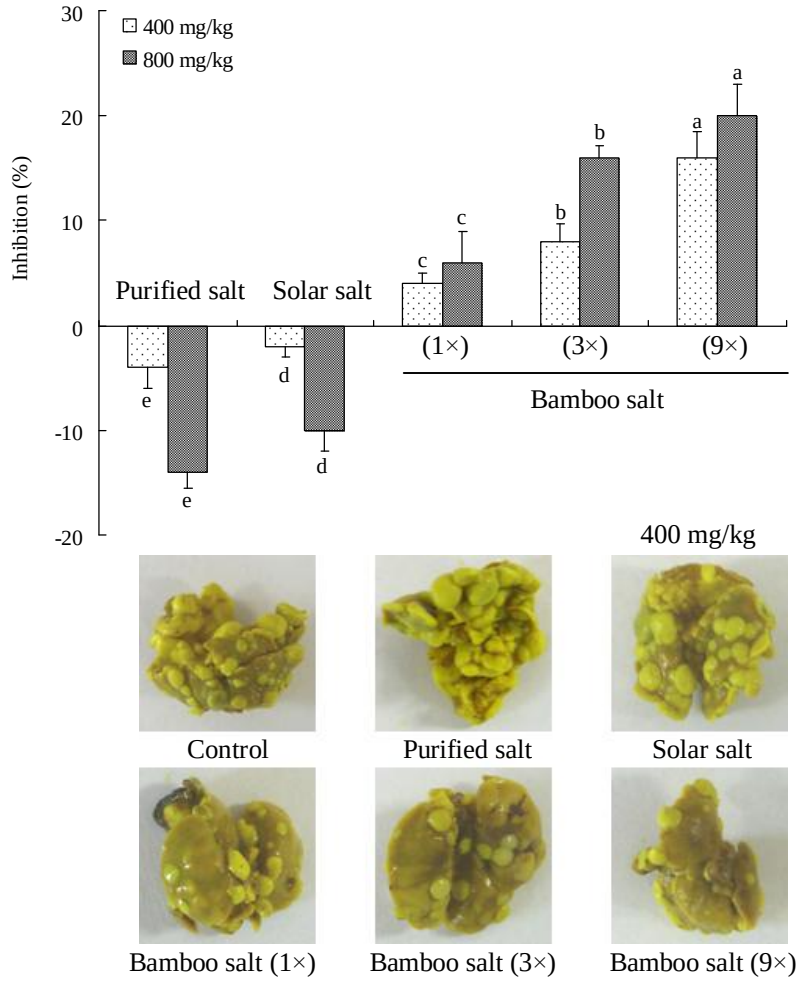


Fig. 24. Inhibitory effect of salt samples on tumor metastasis produced by colon 26-M3.1 cells in Balb/c mice*.

[a-e] Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test

* The mice were administered with the indicated dose of salt by SC injection and then inoculated intravenously with colon 26 M-3.1 cells 2.5×10^4/mouse) after 2d. Eight mice were assigned to each group.

4. Antiinflammatory effects in rats

1) Protective effect of hepatitis induced by CCl_4

(1) Plasma AST and ALT levels

Aspartate aminotransferase (AST) and alanine aminotransferease (ALT) are enzymes located in liver cells that leak out into the general circulation when liver cells are injured. AST is found in many body tissues including the heart, muscle, kidney, brain, and lung. ALT is found predominately in the liver, with lesser quantities found in the kidneys, heart, and skeletal muscle (181). The AST levels of rats fed with different kinds of salt were 169.5 IU/L in the normal rats, 1693.8 IU/L in the CCl_4 control rats 1553.1 IU/L in the purified salt rats, 1285.3 IU/L in the solar salt rats, 1077.0 IU/L in the bamboo salt (1×) rats and 1038.1 IU/L in the bamboo salt (9×) mice (Fig. 25). The ALT levels of these six groups were 45.7, 1438.2, 998.4, 804.9, 790.2 and 634.4 IU/L, respectively (Fig. 26). Raju reported that the plasma AST and ALT levels in CCl_4 group rats dramatically increased when compared with the normal group rats, which indicated the liver damage obviously (182). In this study, it was estimated that bamboo salt showed hepatitis preventive effects and 9 times bamboo salt showed the best effect.

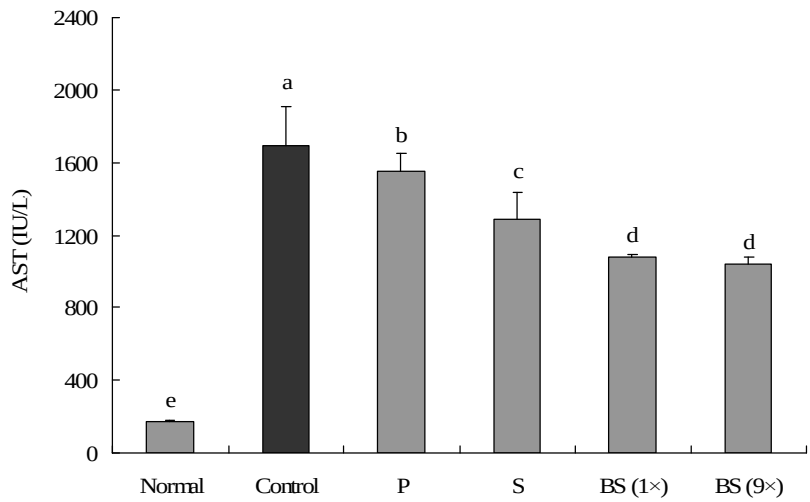


Fig. 25. Effect of bamboo salt (400 mg/kg) on AST (Aspartate aminotransferase) levels in rats treated with CCl_4

P: Purified salt

S: Solar salt

BS: Bamboo salt

[a~e] Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

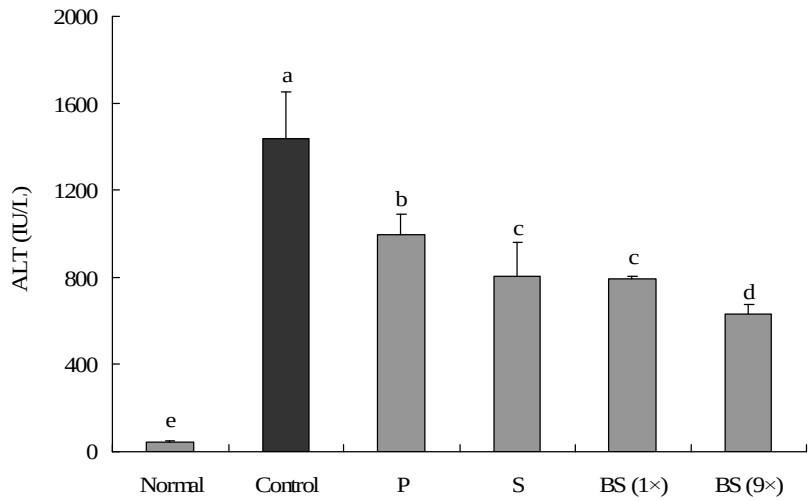


Fig. 26. Effect of bamboo salt (400 mg/kg) on ALT (Alanine aminotransferease) levels in rats treated with CCl_4

P: Purified salt

S: Solar salt

BS: Bamboo salt

[a~e] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

(2) Plasma LDH levels

Lactate dehydrogenase (LDH) is an enzyme found in many body tissues, including the liver. Elevated levels of LDH may indicate liver damage (183). In the present study, CCl_4 was used to induce liver damage in rats. It significantly elevated hepatic enzyme activity of total LDH (184). The LDH levels of these six groups [normal, control, purified salt, solar salt, bamboo salt (1×), bamboo salt (3×), bamboo salt (9×)] were 1173.5 IU/L, 6742.6 IU/L, 5875.2, 5432.9, 4776.4 and 3877.8, respectively (Fig. 27). The LDH levels in sample group rats were decreased when compared with the CCl_4-induced liver injury rats. Significant decrease in LDH levels when the silymarin treatment of hepatitis, silymarin is considered to a flavonolignan mixture which is an unique type of bioflavonoid and an antioxidant that scavenges damaging free radicals. Scientific studies show that oral supplementation with silymarin can supporting liver function (185). In this study, bamboo salt decreased in LDH levels than other salts, therefore the results also estimated 9 times bamboo salt showed the best hepatitis preventive effect.

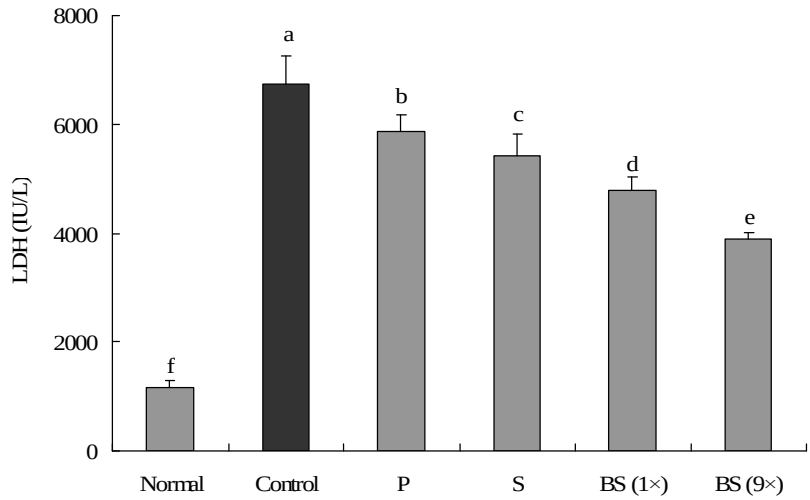


Fig. 27. Effect of bamboo salt (400 mg/kg) on LDH (Lactate dehydrogenase) levels in rats treated with CCl_4

P: Purified salt

S: Solar salt

BS: Bamboo salt

[a~f] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

(3) Plasma TG and TC levels

Plasma triglyceride (TG) levels of rats fed with different kinds of salt, showed similar results, but the bamboo salt (9×) showed significantly lower TG levels than other sample groups (Fig. 28). The plasma levels of TC in CCl_4-treated rats markedly increased when compared with control rats, the bamboo salt (9×) showed the highest TC levels of sample groups (Fig. 29). There was a study suggested that TC levels were related hepatitis as AST, ALT and LDH levels, but were not best standard (186). TG levels were not related with hepatitis in other studies as same as related study (187).

(4) Expressions of inflammation realated genes of iNOS, COX-2, TNF-α and IL-1β in liver

RT-PCR and western blot analysis were conducted to investigate whether the inhibitory effect of salts on inflammation was due to gene regulation of inflammatory mediators, such as iNOS, COX-2, TNF-α, and IL-1β. As shown in Figs. 30 and 31, these inflammatory mediators were hardly detected in the normal group. However, CCl_4 treatment sharply increased both mRNA and protein expressions. Bamboo salt (9×)

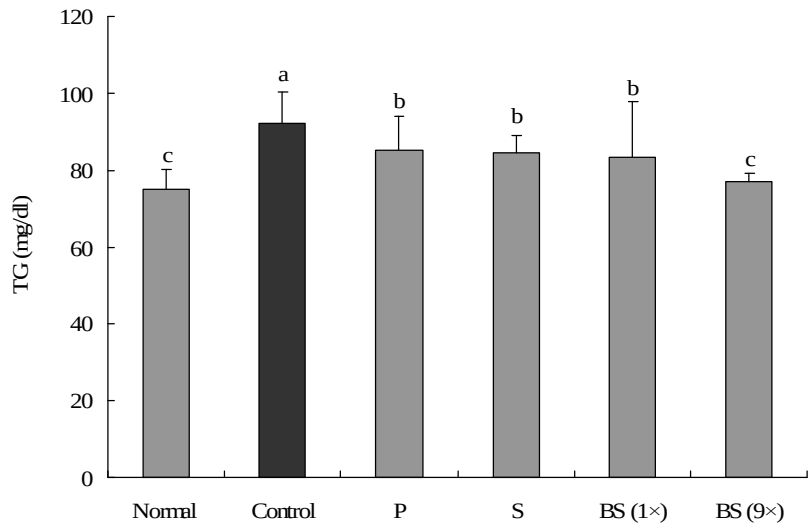


Fig. 28. Effect of bamboo salt (400 mg/kg) on TG (Plasma triglyceride) levels in rats treated with CCl_4

P: Purified salt

S: Solar salt

BS: Bamboo salt

[a~c] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

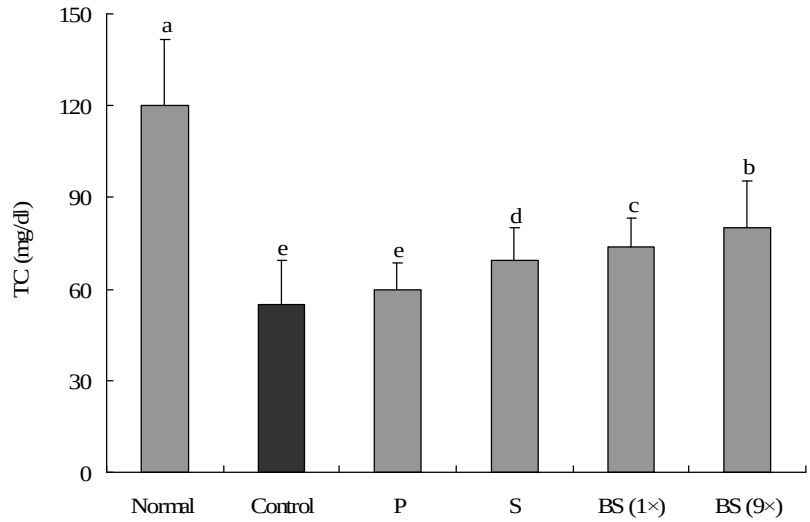


Fig. 29. Effect of bamboo salt (400 mg/kg) on TC (Total cholesterol) levels in rats treated with CCl_4

P: Purified salt

S: Solar salt

BS: Bamboo salt

[a~e] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

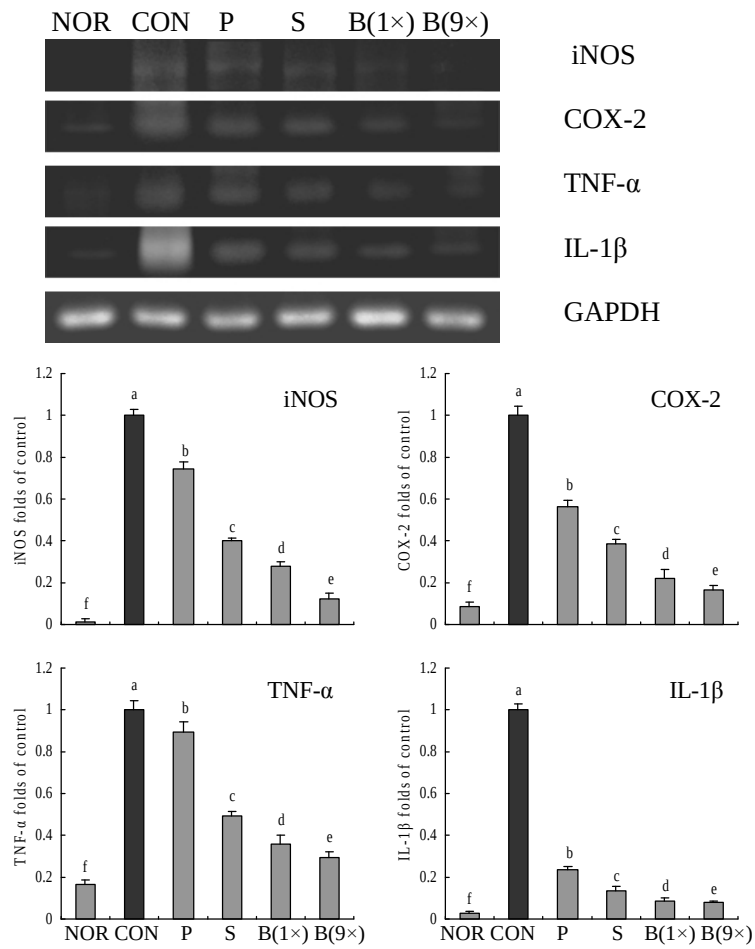


Fig. 30. Effects of bamboo salt (400 mg/kg) on mRNA expressions of iNOS, COX-2, TNF-α and IL-1β in liver of rats treated with CCl_4

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH × control numerical value (control fold ration: 1)

$^{a\sim e}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

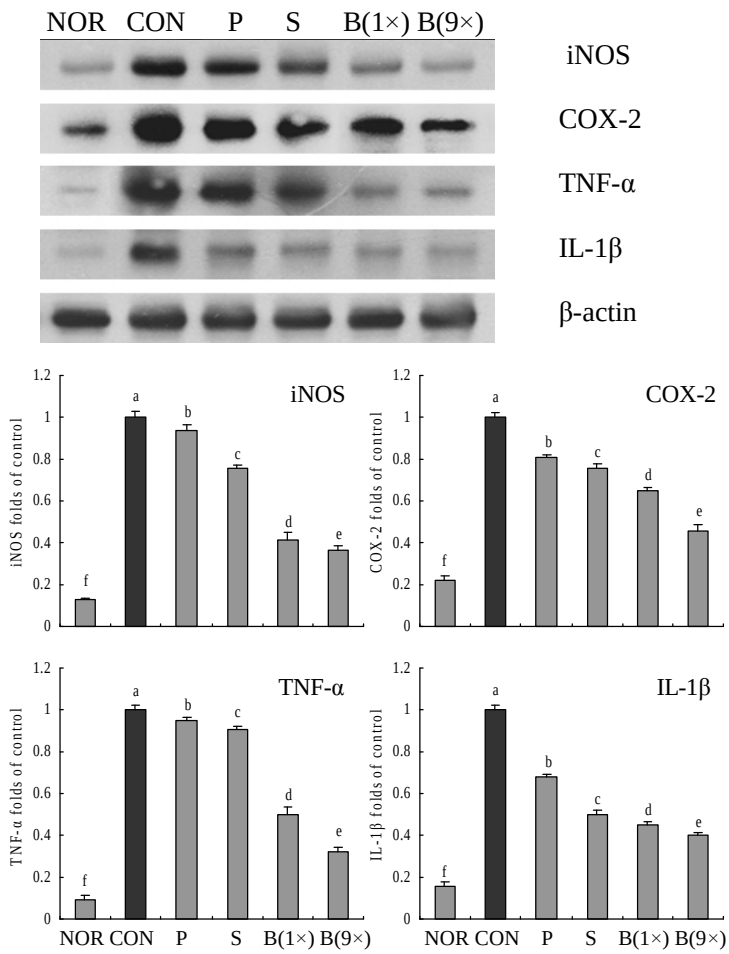


Fig. 31. Effects of bamboo salt (400 mg/kg) on protein expressions of iNOS, COX-2, TNF-α and IL-1β in liver of rats treated with CCl_4

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / β -actin × control numerical value (control fold ration: 1)

[a~e] Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

significantly decreased mRNA and protein expressions of COX-2, iNOS, TNF-α and IL-1β. The more decreased mRNA and protein expressions showed the better antiinflammtion effects. In inflammatory process, activated inflammatory cells (neutrophils, eosinophils, mononuclear phagocytes and macrophages) secrete increased amounts of nitric oxide (NO), prostaglandin E_2 (PGE_2) and cytokines, such as interleukin IL-1β, IL-6, and tumor necrosis factor TNF-α. These substances not only induce cell and tissue damage, but also activate macrophages in rheumatoid arthritis, chronic hepatitis, etc (188). Accordingly, bamboo salt (9×) can be expected to contribute to the prevention of hepatitis and bamboo salt (3×), bamboo salt (1×) revealed the better hepatitis prevention effect than solar salt and purified salt.

Part 2. Functional effects of *Rubus coreanus* Miquel bamboo salt

1. Antioxidant effects

1) DPPH radical scavenging effects

The radical scavenging effects of ***Rubus coreanus*** Miquel bamboo salt on DPPH radical were tested. The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening of many samples for radical scavenging activity. These advantages make the DPPH method interesting for testing microalgae as a natural source to scavenge radicals (189). ***Rubus coreanus*** Miquel bamboo salt and purple bamboo salt showed scavenging activity on DPPH radical at concentrations of 10% and 20%, respectively. At concentration of 10%, the radical scavenging activities of ***Rubus coreanus*** Miquel bamboo salt and purple bamboo salt were 18.4% and 55.5%, respectively. At concentration of 20%, their activities showed similar results (28.5% and 75.6%) as those at concentrations of 10% (Fig. 32). The radical scavenging activity of ***Rubus coreanus*** Miquel bamboo salt was more potent than that of purple bamboo salt.

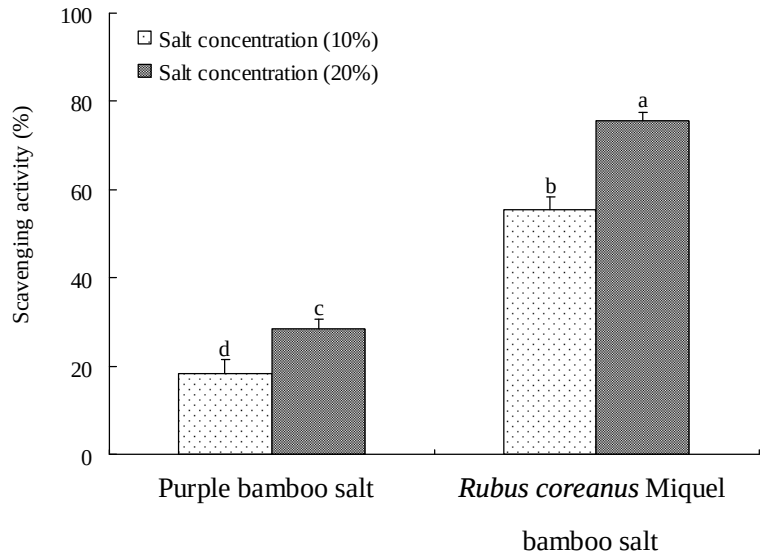


Fig. 32. DPPH radical scavenging activity of salt samples

[a~d] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Purple bamboo salt = bamboo salt (9×)

2. Inhibitory effects of cancer progress

1) Antimutagenic effects

The *Rubus coreanus* Miquel bamboo salt and purple bamboo salt showed inhibition effects of spontaneous mutagenicity in the *Salmonella typhimurium* TA100 strain (Table 9). At 1.25 mg/plate, the inhibition rates of *Rubus coreanus* Miquel bamboo salt and purple bamboo salt were 41% and 19%, respectively ($p<0.05$). At 2.5 mg/plate, the inhibition rates of them were 55% and 31% ($p<0.05$).

Antimutagenic activities of *Rubus coreanus* Miquel bamboo salt and purple bamboo salt against MNNG in *Salmonella typhimurium* TA100 were compared. *Rubus coreanus* Miquel bamboo salt and purple bamboo salt exhibited antimutagenic activities, *Rubus coreanus* Miquel bamboo salt exhibited an antimutagenic effect of more than 30%, two fold increase as compared to purple bamboo salt, at the high concentration (2.5 mg/plate) tested (Table 10). This result indicates that *Rubus coreanus* Miquel bamboo salt and purple bamboo salt exhibited antimutagenic effects against the MNNG. Ames test is the assay for identifying substances that can produce genetic damage that leads to gene mutations. For salts, the predictivity results for cancer induction is important for this study (190).

Table 9. Effect of salt samples on the inhibition of spontaneous revertant in *Salmonella typhimurium* TA100

Treatment (level of sample, mg/plate)	Revertants/plate	
	1.25	2.5
Spontaneous	121±11 [a 1)]	
Purple bamboo salt	98±18 [b] (19) [2)]	84±12 [c] (31)
Rubus coreanus Miquel bamboo salt	71±12 [d] (41)	54±11 [e] (55)

[1)] Values are mean ± SD of revertants/plate

[2)] The values in parentheses are the inhibition rates (%).

[a~e] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 10. Effect of salt samples on the antimutagenicity in *Salmonella typhimurium* TA100 with MNNG (0.4 μg/plate)

Treatment (level of sample, mg/plate)	Revertants/plate	
	1.25	2.5
Spontaneous	121±11 [1)]	
MNNG [2)]	874±26 [a]	
MNNG +Purple bamboo salt	805±18 [b] (9) [3)]	744±17 [c] (17)
+*Rubus coreanus* Miquel bamboo salt	738±22 [c] (18)	633±15 [d] (32)

[1)] Values are mean ± SD of revertants/plate

[2)] MNNG: N-Methyl-N-nitro-N-nitrosoguanidine

[3)] The values in parentheses are the inhibition rates (%).

[a~d] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

2) *in vitro* anticancer effects

(1) *in vitro* growth inhibition of cancer cells

To investigate anti-proliferative effects of ***Rubus coreanus*** Miquel bamboo salt, purple bamboo salt against HT-29 human colon cancer cells and AGS human gastric adenocarcinoma cells, cytotoxicity assays was performed in the cells treated with salts using MTT assay. As the concentrations increased, the growth of the HT-29 and AGS cells was retarded in a dose-dependent manner. Growth inhibitory effects of purple bamboo salt and ***Rubus coreanus*** Miquel bamboo salt samples on HT-29 human colon cancer cells are shown in Table 11. When the aqueous solutions of salt samples (0.5%) were treated to HT-29 human colon cancer cells, the growth inhibitory rates of ***Rubus coreanus*** Miquel bamboo salt and purple bamboo salt were 48% and 37%, respectively. At the concentration of 1%, the growth inhibitory rates of ***Rubus coreanus*** Miquel bamboo salt was 72%, higher than that of purple bamboo salt (54%) ($p<0.05$), and solar salt showed 11%. Table 12 demonstrated that ***Rubus coreanus*** Miquel bamboo salt and purple bamboo salt treatment resulted in a concentration-dependent inhibition of AGS cells growth, as compared to untreated control. The ratio of growth inhibitory effects were 68% and 51% by the treatment of 1% ***Rubus***

Table 11. Inhibitory effect of salt samples on the growth of HT-29 human colon cancer cells in 3-(4,5-dimetyl-thiazol)-2,5-diphenyl tetrazolium (MTT) assay

Treatment	OD_{540} (Level of sample, %)	
	0.5	1
Control	0.462±0.002 [a]	
Purple bamboo salt	0.292±0.011 [b] (37) [1)]	0.212±0.008 [d] (54)
Rubus coreanus Miquel bamboo salt	0.241±0.009 [c] (48)	0.129±0.007 [e] (72)

[1)] The values in parentheses are the inhibition rates (%).

[a~e] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

Table 12. Inhibitory effect of salt samples on the growth of AGS human gastric adenocarcinoma cells in 3-(4,5-dimetyl-thiazol)-2,5-diphenyl tetrazolium (MTT) assay

Treatment	OD_{540} (Level of sample, %)	
	0.5	1
Control	0.481±0.004 [a]	
Purple bamboo salt	0.327±0.006 [b] (32)	0.237±0.010 [d] (51)
Rubus coreanus Miquel bamboo salt	0.207±0.008 [c] (43)	0.154±0.009 [e] (68)

[1)] The values in parentheses are the inhibition rates (%).

[a~e] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

coreanus Miquel bamboo salt and purple bamboo salt.

It was obvious that the anticancer effect on HT-29 human colon cancer cells of ***Rubus coreanus*** Miquel bamboo salt was better than that of purple bamboo salt, and common bamboo salt samples were better than that of solar salt.

(2) Apoptosis-related genes of Bax and Bcl-2

To determine which type of apoptotic gene was related to camcer cells growth by salt samples, activations of Bax and Bcl-2 gene in HT-29 cells and AGS cells were examined by RNA extraction and a RT-PCR and western blot. As shown in Fig. 33 and 34, in the presence of 1% salt, the pro-apoptotic Bax and the anti-apoptotic Bcl-2 showed significant changes. The induction of apoptosis by ***Rubus coreanus*** Miquel bamboo salt was related to increased Bax and decreased Bcl-2 in terms of mRNA and protein expression compared to purple bamboo salt. These results suggested that ***Rubus coreanus*** Miquel bamboo salt regulated the apoptosis-related gene expressions in cancer cells showing anticancer effect, ultimately.

Bcl-2-family proteins are central regulators of cell life and death. At least three major classes of Bcl-2-family proteins have been delineated, including proapoptotic proteins that contain

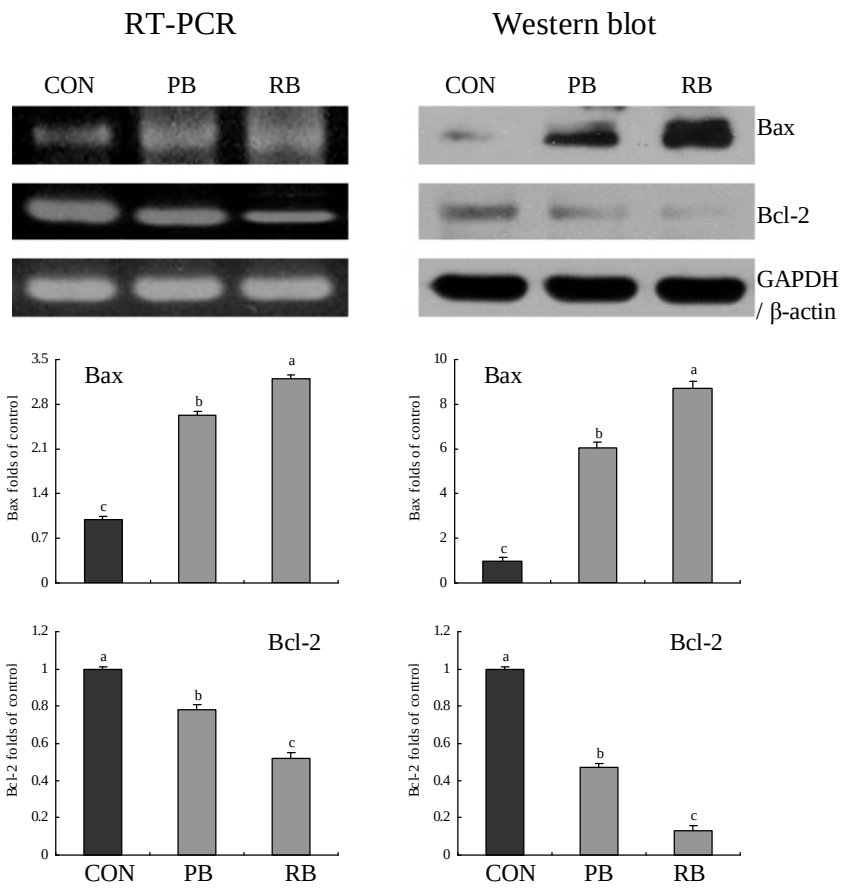


Fig. 33. Effects of bamboo salt samples (1%) on mRNA and protein expressions of Bax and Bcl-2 in HT-29 cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH (β -actin) × control numerical value (control fold ration: 1)

a~c Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

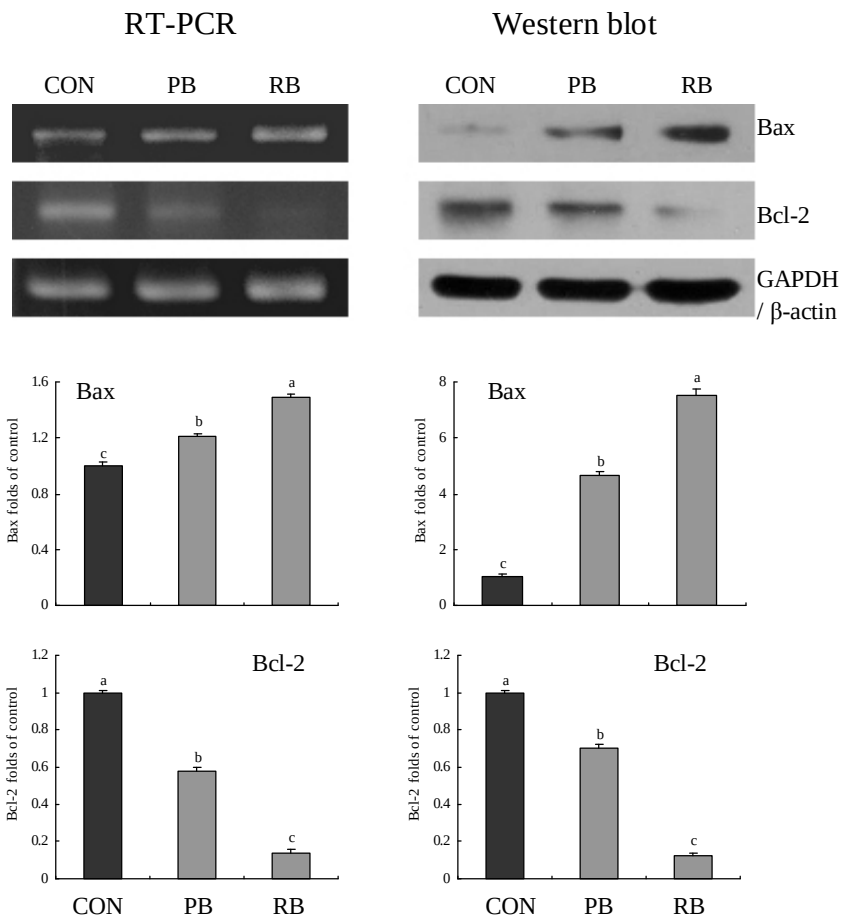


Fig. 34. Effects of bamboo salt samples (1%) on mRNA and protein expressions of Bax and Bcl-2 in AGS cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH (β -actin) × control numerical value (control fold ration: 1)

[a~c] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

several conserved regions of sequence similarity (191). Using Bcl-2-family genes could check the anticancer effects of salts.

(3) Inflammation-related genes of iNOS and COX-2

As shown in Fig. 35 and 36, the mRNA and protein expressions of inflammation-ralated genes, iNOS and COX-2, were down-regulated in HT-29 cells and AGS cells by treatment of ***Rubus coreanus*** Miquel bamboo salt. In the presence of 1% salt, mRNA and protein expression of COX-2 and iNOS were gradually reduced. Accordingly, ***Rubus coreanus*** Miquel bamboo salt can be expected to contribute to the prevention of cancer by regulation of inflammation-related genes. The results suggested that bamboo salt showed strong functional effects and ***Rubus coreanus*** Miquel bamboo salt with ***Rubus coreanus*** Miqueladdition showed a stronger anticancer activity than common bamboo salts. In addition of ***Rubus coreanus*** Miquel could increase functional effect for processed food (192). Eating ***Rubus coreanus*** Miquel bamboo salt may be helpful in reducing cancer risk.

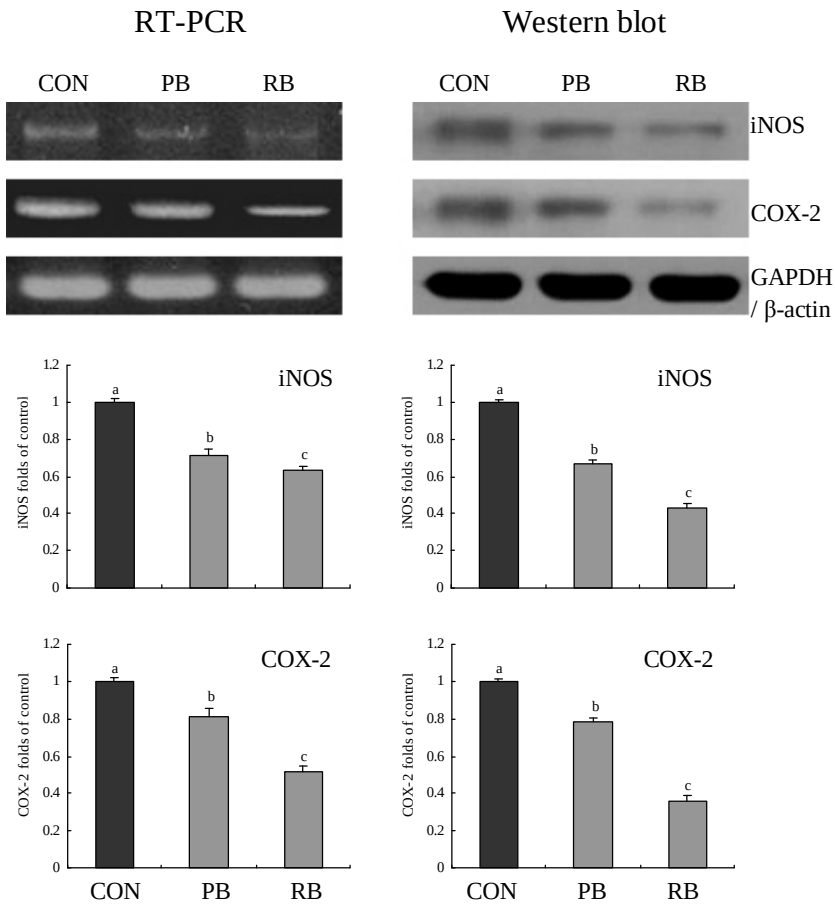


Fig. 35. Effects of bamboo salt samples (1%) on mRNA and protein expressions of iNOS and COX-2 in HT-29 cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH (β -actin) × control numerical value (control fold ration: 1)

a~c Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

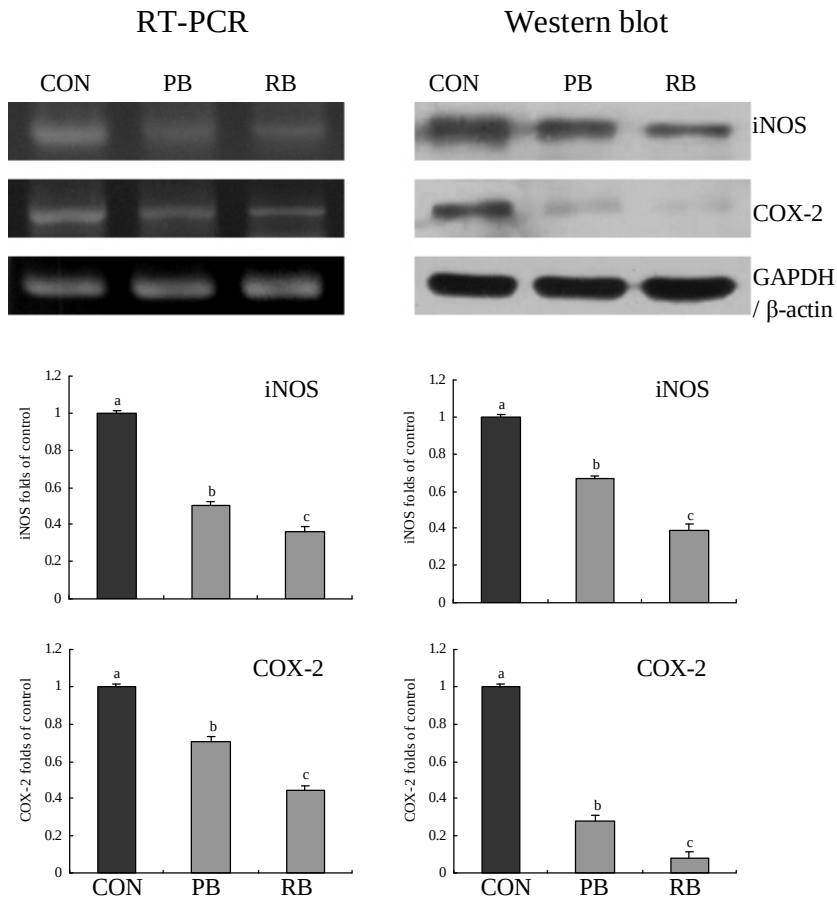


Fig. 36. Effects of bamboo salt samples (1%) on mRNA and protein expressions of iNOS and COX-2 in AGS cells

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH (β -actin) × control numerical value (control fold ration: 1)

[a~c] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

(4) *in vivo* antimetastatic effect

In order to investigate if ***Rubus coreanus*** Miquel bamboo salt has antitumor activity, it is examined the prophylactic effect of ***Rubus coreanus*** Miquel bamboo salt on the experimental lung metastasis produced by colon 26-M3.1 cells. Fig. 37 showed that administration of salts (400 mg/kg) for 2 days before tumor inoculation significantly inhibited lung metastasis of colon 26-M3.1 cells in a dose-dependent manner. Treatment with ***Rubus coreanus*** Miquel bamboo salt before tumor inoculation reduced significantly lung metastasis of colon 26-M3.1 cells. This suggested that ***Rubus coreanus*** Miquel bamboo salt is able to induce prophylactic as well as therapeutic effect against lung metastasis produced by colon 26-M3.1 tumor cells.

3. Antiinflammatory effects in rats

1) Protective effect of hepatitis induced by CCl_4

(1) Plasma AST and ALT levels

Because of hepatitis in the liver by a single oral dose of CCl_4, It is estimated that hepatic dysfunction by measuring the activities of AST and ALT that serve as markers of hepato-

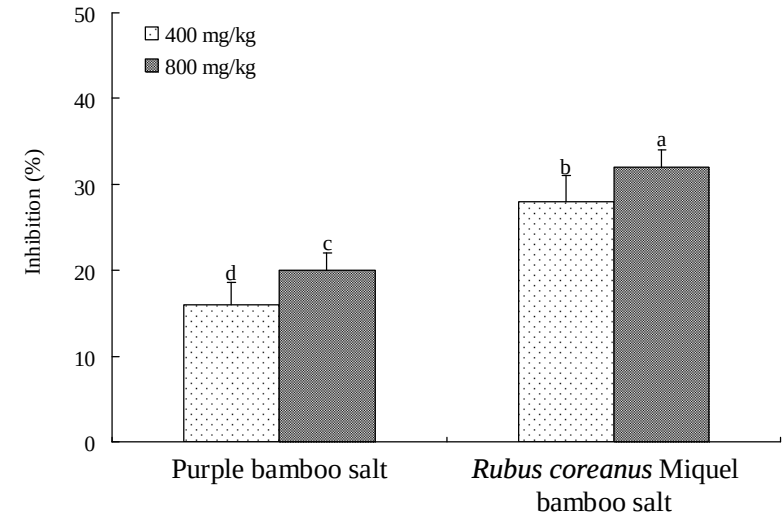


Fig. 37. Inhibitory effect of salt samples on tumor metastasis produced by colon 26-M3.1 cells in Balb/c mice*

[a-d] Means with different letters are significantly different ($p < 0.05$) by Duncan's multiple range test

* The mice were administered with the indicated dose of salt by SC injection and then inoculated intravenously with colon 26 M-3.1 cells 2.5 $\times 10^4$/mouse) after 2d. Eight mice were assigned to each group.

cellular necrosis after their release into the plasma in response to membrane adamage. The AST levels of rats fed with different kinds of salt were 169.5 IU/L in the normal mice, 1693.8 IU/L in the CCl_4 control rats, 1038.1 IU/L in the purple bamboo salt rats, 939.5 IU/L in the ***Rubus coreanus*** Miquel bamboo salt rats (Fig. 38). The ALT levels of these four groups were 45.7, 1438.2, 634.4 and 570.8 IU/L, respectively (Fig. 39). The AST and ALT levels of ***Rubus coreanus*** Miquel bamboo salt showed lower than control group, therefore ***Rubus coreanus*** Miquel bamboo salt showed the best protective effect of hepatitis. ***Rubus coreanus*** Miquel has been employed as a traditional antiinflammation medicine for centuries (193). Adding ***Rubus coreanus*** Miquel could increase the inflammation activity in bamboo salt as a new functional food.

(2) Plasma LDH levels

The LDH levels of normal, control, purple bamboo salt and ***Rubus coreanus*** Miquel bamboo salt were 1173.5 IU/L, 6742.6 IU/L, 3877.8 IU/L and 3212.2 IU/L respectively (Fig. 40). The results also suggested ***Rubus coreanus*** Miquel bamboo salt showed the better hepatitis preventive effect than purple bamboo salt.

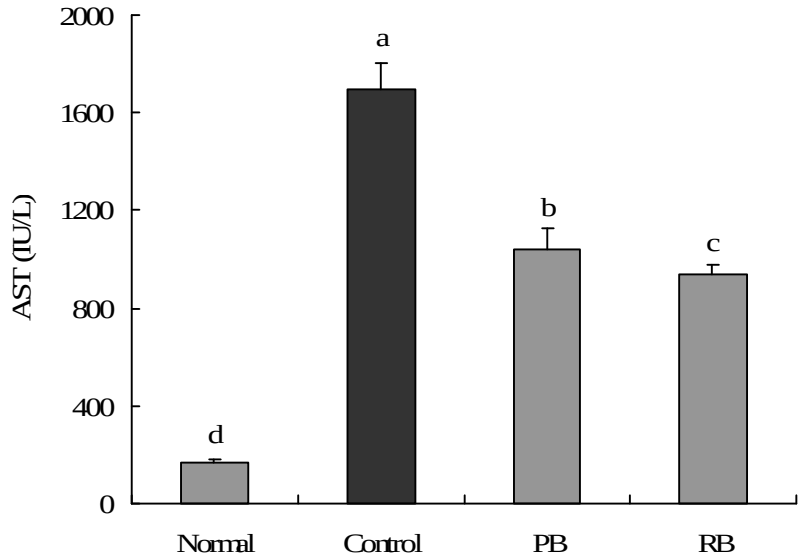


Fig. 38. Effect of ***Rubus coreanus*** Miquel bamboo salt (400 mg/kg) on AST (Aspartate aminotransferase) levels in rats treated with CCl_4

PB: Purple bamboo salt

RB: ***Rubus coreanus*** Miquel bamboo salt

[a~d] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

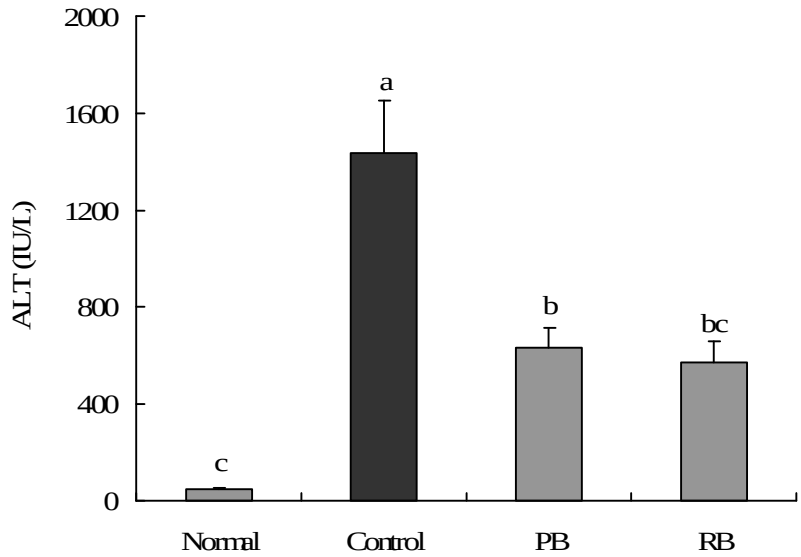


Fig. 39. Effect of *Rubus coreanus* Miquel bamboo salt (400 mg/kg) on ALT (Alanine aminotransferease) levels in rats treated with CCl_4

PB: Purple bamboo salt

RB: *Rubus coreanus* Miquel bamboo salt

[a~c] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

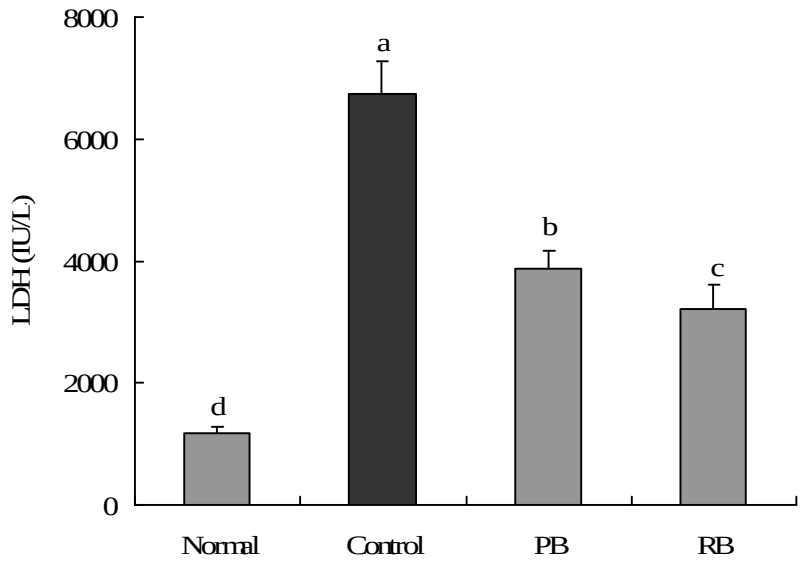


Fig. 40. Effect of ***Rubus coreanus*** Miquel bamboo salt (400 mg/kg) on LDH (Lactate dehydrogenase) levels in rats treated with CCl_4

PB: Purple bamboo salt

RB: ***Rubus coreanus*** Miquel bamboo salt

$^{a\sim d}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

(3) Plasma TG and TC levels

Plasma triglyceride (TG) levels of rats fed with ***Rubus coreanus*** Miquel bamboo salt and purple bamboo salt samples showed the similar results but ***Rubus coreanus*** Miquel bamboo salt and purple bamboo salt showed the signigicantly lower TG levels than control group (Fig. 41). The plasma levels of TC in CCl_4-treated rats markedly increased when compared with control rats, the ***Rubus coreanus*** Miquel bamboo salt showed the highest total cholesterol (TC) levels of sample groups (Fig. 42). These results suggested that ***Rubus coreanus*** Miquel bamboo salt was a good functional food for preventing hepatitis, TC levels is a standard to test hepatitis as AST and ALT levels, but were not best standard (194). TG levels were not related with hepatitis in other studies as same as this study.

(4) Expressions of inflammation related genes of NOS, COX-2, TNF-α and IL-1β in liver

To investigate whether the hepatitis preventive effect of bamboo salt was associated with decreased inflammation related genes of iNOS, COX-2, TNF-α and IL-1β expressions. The levels of COX-2, iNOS mRNA and protein expressions in rat

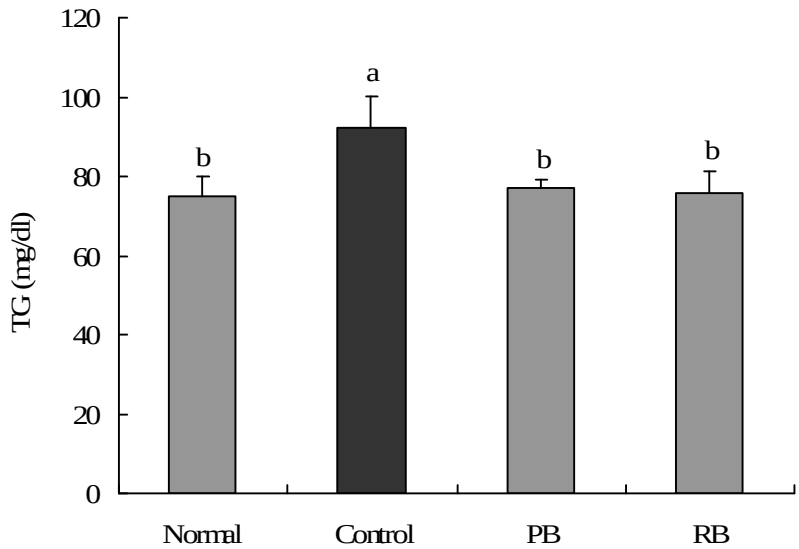


Fig. 41. Effect of ***Rubus coreanus*** Miquel bamboo salt (400 mg/kg) on TG (Plasma triglyceride) levels in rats treated with CCl_4

PB: Purple bamboo salt

RB: ***Rubus coreanus*** Miquel bamboo salt

[a~b] Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

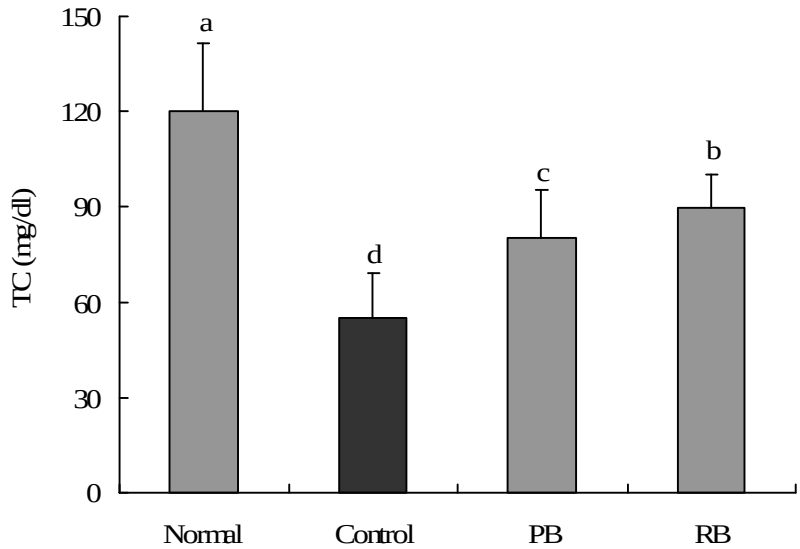


Fig. 42. Effect of ***Rubus coreanus*** Miquel bamboo salt (400 mg/kg) on TC (Total cholesterol) levels in rats treated with CCl_4

PB: Purple bamboo salt

RB: ***Rubus coreanus*** Miquel bamboo salt

$^{a\sim d}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

liver were shown in Fig. 43 and 44. *Rubus coreanus* Miquel bamboo salt decreased mRNA and protein expressions of COX-2 and iNOS compared to purple bamboo salt.

Purple bamboo salt significantly decreased mRNA and protein expressions of TNF-α and IL-1β. The more decreased mRNA and protein expressions of *Rubus coreanus* Miquel bamboo salt showed the better antiinflammtion effects than purple bamboo salt. Accordingly, *Rubus coreanus* Miquel can be expected to contribute to the prevention of hepatitis. TNF-α and IL-1β inflammatory cytokines took an important role in the occurrence and development of severe hepatitis (195). The inflammation genes analysis could determine the hepatitis preventive effects of salts. From the results, it suggested that *Rubus coreanus* Miquel showed a strong hepatitis preventive effect.

2) Gastritis preventive effects

(1) Gastric injury levels

Rubus coreanus Miquel bamboo salt and bamboo salt administration on gastritis in rats led to reduce the gastric injury. Purified salt and solar salt showed the gastric injury inhibition rate of 68.8% and 75.4, 1 time and 9 times baked bamboo salt showed a better effect to prevent gastritis (96.8 and 98.8%),

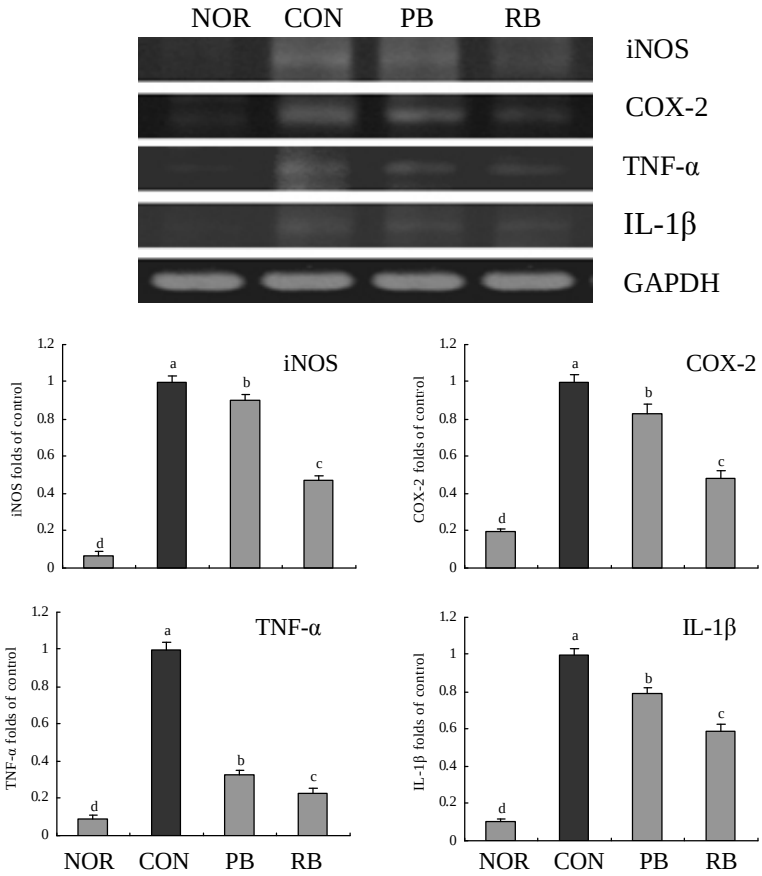


Fig. 43. Effects of *Rubus coreanus* Miquel bamboo salt (400 mg/kg) on mRNA expressions of iNOS, COX-2, TNF-α and IL-1β in liver of rats treated with CCl_4

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / GAPDH × control numerical value (control fold ration: 1)

$^{a\sim d}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

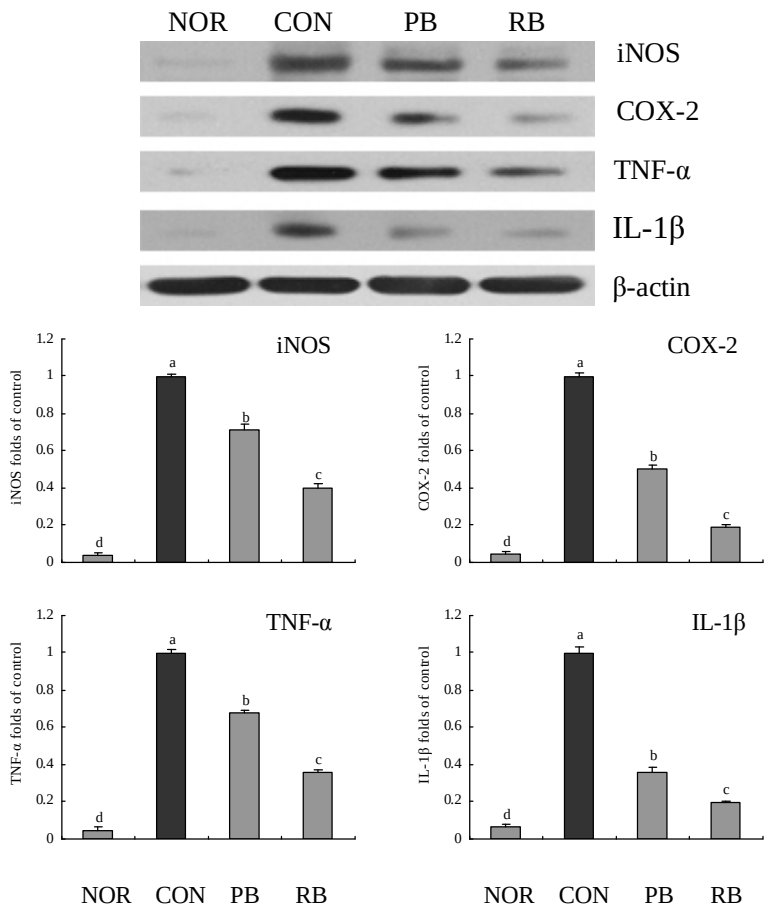


Fig. 44. Effects of ***Rubus coreanus*** Miquel bamboo salt (400 mg/kg) on protein expressions of iNOS, COX-2, TNF-α and IL-1β in liver of rats treated with CCl_4

The intensity of the band was measured with densitometer and expressed as fold-rate of control. Fold ration: Gene expression / β -actin × control numerical value (control fold ration: 1)

$^{a\sim d}$ Means with the different letters are significantly different ($p<0.05$) by Duncan's multiple range test.

Rubus coreanus Miquel bamboo salt (99.3%) showed the best gastritis preventive effect as that of omeprazole (100%) (gastritis drug) (Table 13, Fig. 45). It was reported that bamboo salt expressed anti-gastric ulcer activity and anti-gastritis activity (196, 197) as this study. The results suggested that *Rubus coreanus* Miquel bamboo salt showed a strong gastritis preventive effect as the medicine of omeprazole.

(2) Gastric secretion volume and pH of gastric juice

Gastritis-caused material causes increased gastric secretion volume and acid output, with significant decreased gastric pH (198). The gastric secretion volume of the normal, control, purified salt, solar salt, bamboo salt (1×), bamboo salt (9×), *Rubus coreanus* Miquel bamboo salt and omeprazole were 1.4 ml, 3.4 ml, 3.0 ml, 2.8 ml, 2.0 ml, 1.9 ml, 1.7 ml and 1.6ml, respectively (Fig. 46). The pH of them were 3.8, 1.6, 1.8, 1.9, 2.6, 2.9, 3.5 and 3.6, respectively (Fig. 47). The results suggested that *Rubus coreanus* Miquel bamboo salt has the least gastric secretion volume and showed the highest pH of the salt samples. The gastritis preventive effect of *Rubus coreanus* Miquel bamboo salt and bamboo salt were better than purified salt and solar salt.

Table 13. Gastritis preventive effects of salts treatment in rats treated with HCl/ethanol

Group (400 mg/kg)	Inhibition rate of gastric injury	
	Gastric injury (mm^2)	Inhibition rate (%)
Normal	0.0±0.0 [c]	100 [1)]
Control	15.0±3.7 [a]	0.0
Purified salt	4.7±4.7 [b]	68.8
Solar salt	3.7±2.7 [b]	75.4
Bamboo salt (1×)	0.5±1.0 [c]	96.8
Bamboo salt (9×)	0.2±0.4 [c]	98.8
Rubus coreanus Miquel bamboo salt	0.1±0.2 [c]	99.3
Omeprazole	0.0±0.0 [c]	100

[1)] inhibition rates (%).

[a~c] Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

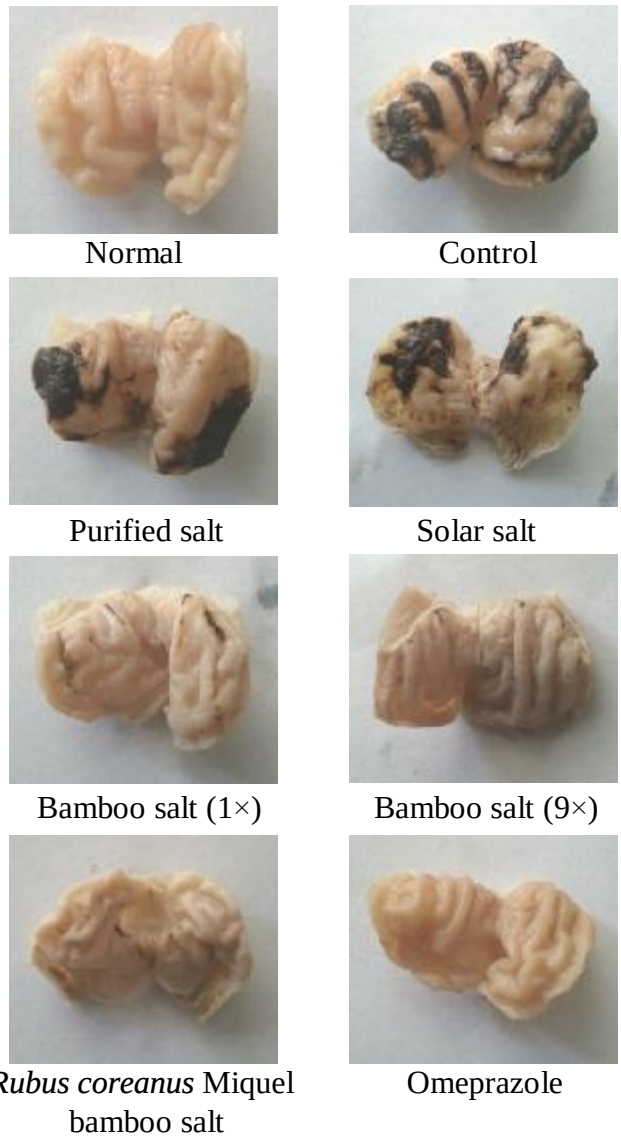


Fig. 45. Stomach appearance of salts treatment in rats treated with HCl/ethanol

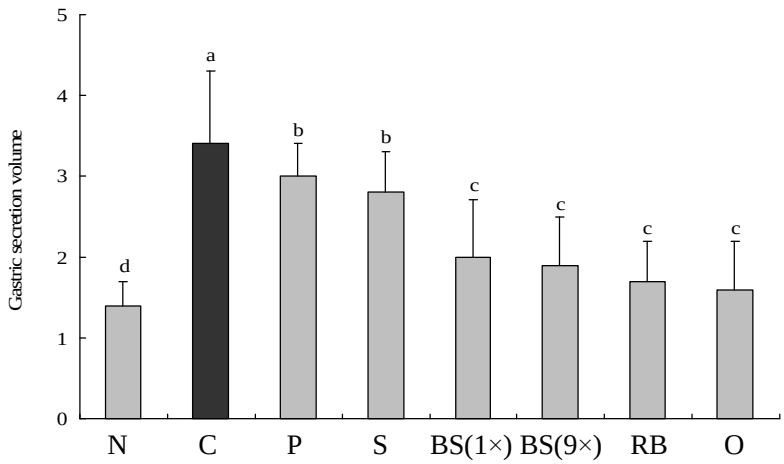


Fig. 46. Gastric secretion volume of salts treatment in rats treated with HCl/ethanol

N: Normal

C: Control

P: Purified salt

S: Solar salt

BS: Bamboo salt

RB: ***Rubus coreanus*** Miquel bamboo salt

O: Omeprazole

[a~d] Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

Treated with 1% salt (400 mg/kg) or omeprazole (20 mg/kg)

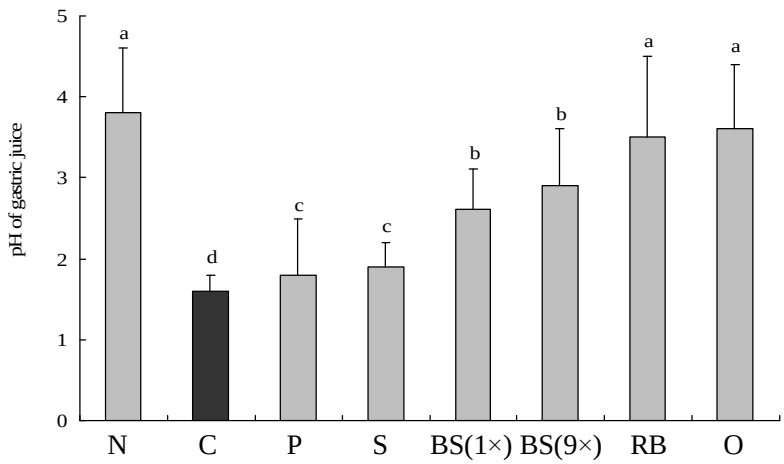


Fig. 47. pH of gastric juice of salts treatment in rats treated with HCl/ethanol

N: Normal

C: Control

P: Purified salt

S: Solar salt

BS: Bamboo salt

RB: ***Rubus coreanus*** Miquel bamboo salt

O: Omeprazole

[a~d] Means with the different letters in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

Treated with 1% salt (400 mg/kg) or omeprazole (20 mg/kg)

Ⅴ. SUMMARY AND CONCLUSIONS

Bamboo salt contained more minerals, such as potassium, calcium, magnesium, and manganese compared to purified salt and solar salt. Bamboo salt (BS) showed higher pH, and higher reduction potential and contained more OH group compared to purified salt and solar salt. BS exhibited antioxidative activity determined by DPPH assay. It showed antimutagenicity against MNNG though the other salts showed comutagenicity. BS also showed anticancer activities on HCT-116, HT-29 and AGS human cancer cells. It induced apoptosis and showed antiinflammatory effect, regulating mRNA and protein expressions of Bax, Bcl-2, iNOS and COX-2. BS significantly reduced *in vivo* metastasis using colon 26-M3.1 carcinoma cells in Balb/c mice compared to the other salts though purified salt increased the metastasis by the colon cells. BS revealed to prevent hepatitis and gastritis in SD rats induced by CCl_4 and ethanol, respectively. The *Rubus coreanus* Miquel bamboo salt (RBS) showed even higher functionalities than those of the BS. The health benefits of BS were significantly increased by the addition of *Rubus coreanus* Miquel (20%) to the BS.

Thus it can be concluded by the followings from these studies:

1) SEM pictures indicate that when the number of baking times of the bamboo salt (BS) increased, the particle size decreases. There are many mineral elements in BS, such as Na, Ca, Mg, Fe, Mn, P, S and K. BS (9×) contains the highest contents of Fe, Mn, P and K.
2) BS is a NaCl-KCl salt. From the XPS analysis, BS showed a complex structure materials which include NaCl, KCl and $MgCl_2$.
3) BS contained more OH^- than purified salt and solar salt, and seemed to contain other antioxidation substances.
4) BS showed much better radical scavenging activity than solar salt and purified salt. However, RBS showed much better radical scavenging activity than 9 times baked BS.
5) BS (9×) showed a good antimutagenic effects, and RBS showed the better antimutagenic effects than 9 times baked bamboo salt. However, solar salt and purified salt showed the comutagenic effects.
6) BS exhibited much better *in vitro* anticancer activity than solar salt and purified salt by MTT assay. However, RBS showed the better anticancer activity than the 9 times baked BS.

7) The anticancer mechanisms of BS and RBS in the human cancer cells were inducing apoptosis by increasing apoptotic bodies, and regulating mRNA and protein levels of apoptosis related genes of Bax and Bcl-2, and showing antiinflammatory effects by regulating iNOS and COX-2 genes.
8) BS and RBS showed much better *in vivo* anticancer activity than solar salt and purified salt on tumor metastasis, however, purified salt increased metastasis in the mouse.
9) The hepatitis and gastritis induced in SD rats were effectively prevented by BS and RBS.

In this study, from the results of physicochemical property tests using various experimental methods, BS was a new salt substance different from the common salts. Because of more mineral compositions and new chemical construction, it increased antioxidation, antimutagenicity, anticancer and antiinflammation effects *in vitro* and *in vivo*. When *Rubus coreanus* Miquel added to the BS, the above functionalities were even more increased. Thus BS and RBS can be used as functional foods from this study. However, it is still needed more detailed research for preparing better products.

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복분자 죽염과 죽염의

항암 및 항염증 기능성

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국문요약

소금은 체내의 필수적인 무기물로서 신경이나 근육흥분성을 유지하며 신진대사 및 세포의 삼투압을 조절하는 등 생체조절물질로서 중요한 역할을 한다.

고대에는 생식(生食)으로 음식을 섭취하여 별도의 염분섭취가 필요없었으나 음식을 조리해 먹으면서 소금을 얻기 위한 노력이 시작되었다고 한다.

소금은 KS 규격에 따라 천일염과 정제염으로 나누어지고, 정제염은 다시 기계염과 가공염으로 분류되며, 기계염은 이온교환을 통하여 대량 생산되며, 가공염은 가열공정을 거친것으로 구운소금, 죽염등이 있다. 죽염은 일반 소금과는 달리 인체에 필요한 무기질을 풍부하게 함유하고 있으며 여러 가지 질환에 효과가 있는 것으로 알려져 있는데 이와 관련하여 위염, 위궤양, 소화기계통의 질환에 대한 치료 효과와 외상치료, 해독작용, 풍치, 치통에 대한 완화 효과가 보고 되었다. 또한 죽염을 혼합한 치약이 구강 내 연쇄상구균의 수를 감소시키고, 치은염의 증상을 완화 시킨다는 실험결과도 발표되었다. 본 연구에서는 죽염의 이화학적인 특성과 항산화, 항암 기능성을 연구하였다.

죽염은 입자가 불규칙하여 뚜렷한 형태를 보이지 않는 반면 천일염은 규칙적이여 형태가 명확히 관찰된다. 죽염결정 알맹이 크기는 천일염 결정 알맹이 크기보다 대략 10 배 작다. 또한 죽염 결정체에서는 결정격자기변이 발생하였으나 천일염 구조에서는 결정격자의 기변을 불 수 없었다. 따라서, 죽염의 결정, 격자기변은 죽염의 구조가 준안정상태 에너지가 높은 상태에 있다는 것을

의미해서 죽염은 천일염과 정제염보다 높은 에너지 상태에 있는 새로운 물질이고 일반소금과 완전히 다른 물질이라 생각된다.

죽염의 DPPH 항산화 결과는 천일염과 정제염보다 좋았고 복분자 죽염의 항산화 효과는 9 회 자죽염보다 더 좋았다. 죽염은 직접돌연변이원인 자염 유발 및 MNNG 을 이용한 Ames test 를 통하여 복분자 죽염과 9 회 자죽염은 항돌연변이 결과는 나타났고 다른 소금은 보돌연변이 효과를 보였다. 죽염의 *in Vitro* 항암실험은 MTT 방법으로 HCT-116 인체 대장암세포, HT-29 인체 결장암세포와 AGS 인체 위암세포를 이용하여 실험한 결과 1% 농도에서는 천일염과 정제염보다 약 15% 더 항암효과가 우수하였다. 복분자 죽염의 *in Vitro* 항암 효과는 9 회 자죽염보다 더 좋았습니다. 죽염에 의한 항암적인 Bax, Bcl-2, iNOS, COX-2 는 mRNA 및 단백질 발현 억제 효과는 복분자 죽염과 죽염이 가장 우수했다.

in vivo 전이 실험을 통하여 복분자 죽염과 죽염은 항암 기능성이 나타났고 천일염과 정제염은 암 유발 효과를 보였다. 사염화탄소를 투여하여 유발된 간장해에 대한 복분자 죽염의 간 보호 효과는 AST, ALT 및 LDH 의 감소효과에 대한 방어적 작용도 관여하는 것으로 보인다. 또한 iNOS, COX-2, TNF-α, IL-1β 와 같은 염증성 억제 효과도 나타났다. HCl-Ethanol 처치로 유발된 위장해에 대한 복분자 죽염, 9 회죽염과 3 회죽염의 위 보호 효과가 정제염과 천일염보다 높았으여 복분자 죽염은 약(Omeprazole)과 유사한 효과를 나타냈다.

그러나 죽염과 복분자 죽염은 항산화, 항암, 항염증 기능성을 천일염과 정제염보다 좋고 계속적인 확인 연구가 필요하다.

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