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The Effect of Vegetable Extracts on the Activity of Alcohol Dehydrogenase from *Saccharomyces cerevisiae*

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Abstract

We investigated the effects of bean sprouts (*Glycine max*), dropwort (*Oenanthe javanica*), and radish (*Raphanus sativus* var. *hortensis for. acanthiformis*) extracts on alcohol dehydrogenase (ADH). The extracts from three kinds of vegetables were prepared by extracting with boiling water, distilling water, and ethyl alcohol. Among extracts, boiling water extract showed the highest activating effect on ADH, respectively and distilled water extract had a greater effect on ADH activation than that of alcohol extract. The ADH facilitating effect of bean sprout extract by distilled water was significantly higher than dropwort or radish, but the effect of the bean sprout extract by ethyl alcohol was lower than others. The facilitating effect on ADH of mixture extracts of bean sprout and dropwort were mixed at 1 : 1 mixture of boiled-water extract showed the highest effectiveness. And bean sprout extract separated below 3000 molecular weight (MW) range of extract fraction had greater ADH activity than large MW parts.

Key words: alcohol dehydrogenase (ADH), facilitating effects, bean sprouts, radish

INTRODUCTION

When liquor is ingested, the alcohol is absorbed in the intestines and then dispersed to each part of the body through the blood. Thereafter the alcohol is transported to the liver where it is oxidized by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), and becomes acetic acid. Then it is discharged after being converted to CO_2 and urine with passing acetyl CoA. The digestive quantity of alcohol is controled by the quantity of ADH, ALDH and alcohol induced cytochrome P450 (CYP2E1) mainly existing in the liver of each person. The digestive rate of alcohol is thus controled by factors affecting ADH and ALDH activity (1-3). Therefore, to control the digestive rate of alcohol after drinking liquor, the quantity of ADH and ALDH existing in the body is important and substances that affecting the activity of these enzymes and foods generating these substances need to be investigated. ADH participating in alcohol digestion is an enzyme that reversibly oxidizes first and second grade alcohol to aldehyde, ketone, and NADH, and widely exists in animals, plants and microorganisms (4). It has been found that ADH activity is inhibited by pyrazole (5), rice seedlings (6) and daidzein between components of *Puerariae thunbergiana* radix (7), reactivated by Zn^{2+} and Co^{2+}, and facilitated by EDTA and 2-mercaptoethanol (8). After liquor is absorbed inside the body, it causes mental and behavioral problems due to an altered mental status (9-11), plus secondary problems due to biochemical reactions inside of the body. As such, there is great interest in accelerating the digestive rate of alcohol so as to alleviate the problems related to a hangover. In Korea, there are many popular traditional remedies including bean sprouts, dropwort, radish, dried persimmon, larva, *Rhus chinensis* gall, flower of *Pueraria lobata*, and *Allium tuberosum* scallion, all of which are easy to get from nature. These traditional remedies have been used in foods and beverages, yet so far there has been no proven scientific and medical evidence. Most previous researchers have focused on the metabolism (12-14) of these vegetables, and recently, there have been various related commercial products developed as remedies for hangovers. Water, sugar, and alcohol dehydrogenase are all needed to alleviate a hangover. Vegetables containing these components are known to help in treating a hangover, and have been used for generations. It has been reported that bean sprouts decrease the activity of the causal factor or antinutrient, trypsin inhibitor (15) and are effective in treating a hangover because they contain lots of aspartic acid, asparagine, Zn^{2+}, and Ca^{2+} thereby aiding in alcohol degradation. Also, dropwort and radish have been used to treat a hangover and restore the liver function because they contain asparagine, glucose, sucrose, and Ca^{2+}. Accordingly, the current study investigated the effects of these vegetable extracts on ADH activity *in vitro* to provide scientific evidence. Also, the effects *in vivo* are estimated as well as possible industrial uses.

MATERIALS AND METHODS

Enzyme and chemicals

ADH extracted from *Saccharomyces cerevisiae* accord-

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ing to Ganzhorn et al's method (16). ADH was concentrated to 36 Units/mL, and plus 13 mM NAD (Sigma), and a 0.05 M Tris-buffer (pH = 8.8) made with Trizma-base (Sigma) (17). These chemicals were all refrigerated until use. ADH and NAD solution were kept in -20°C, and Tris-buffer was kept in 4°C.

Preparation of vegetable extracts

Visibly fresh bean sprout, dropwort, and radish were extracted by distilled water and ethyl alcohol (Daejung, 95.8% ethanol). The extraction by distilled water was prepared according to the following method. Each type of vegetable was homogenized (Omni Mixer Homogenizer, OMNI-17106) in units of 50 g, and filtered using an Asparator (EYELA, A-3S) and filter paper (Whatman, 110 mm), then diluted with 50 mL distilled water. Next the extracts were centrifuged (Kontron Instruments, T-324) at 16,000 rpm, for 20 min at 4°C, and then were kept in -20°C until use. In addition, each extract was made with boiling water and ethyl alcohol, prepared using the same methods as the distilled water extracts. Alcohol extraction of each vegetable was digested in units of 50 g in 95.8% ethanol for 24 hours. The homogenizing and filtering process was similar to the method of extraction by distilled water. Next the extracts were concentrated using a Rotary Vacuum Evaporator (EYELA, N-N Series), diluted, centrifuged and kept in -20°C as previously described.

Separation of extracts for approximate molecular weight range

All extracts were separated by use of an Ultrafiltration Cell (Amicon, 8400). The filters used in this study were 3000 and 10000 pore size. The approximate molecular weight range (MW) was below 3000 MW, above 3000 MW and below 10000 MW, and above 10000 MW. The extracts were kept in the same method as the extraction by distilled water.

Determination of ADH activity

The ADH activity was determined by monitoring the formation of NADH at 340 nm with a Diode Array Spectrometer (Hewlett Packard, 8452A) (17,18). After the optimal conditions for ADH activity were identified, the ADH activity was estimated at an enzyme concentration of half the maximum value between the reaction time and the enzyme concentration where the ADH activity was represented by a straight line. For investigation of activation effect of fresh bean sprout, dropwort, radish, and vegetable mixtures on ADH activity, 0.2 mL of alcohol, 0.4 mL of NAD and 0.03, 0.05, 0.07, 0.09 and 0.11 mL of each vegetable extract and vegetable mixtures was added to each test tube, then the total volume was made 5 mL by adding a buffer. The vegetable mixtures were blended at 1 : 1 ratio of boiled-water extract from bean sprout, dropwort and radish each other. Thereafter the solution was incubated at 25°C for 10 min in a water bath (Vision, KMC-1205 SW), then the reaction was started by the addition of 0.04 mL of ADH and allowed to react for 35 min. After the reaction was stopped, the ADH activity was determined at 340 nm using the above spectrometer.

RESULTS AND DISCUSSION

Effects of boiled and non-boiled extracts on ADH activity

The vegetable extracts used in this investigation were made using alcohol, distilled and boiling water. When compared with the control, the effects were represented by an increase in the ratio (%) of ADH activity relative to all the other extracts when activation ratio of the control was zero. All extracts facilitated ADH activity *in vitro*. In particular, when the extracts made with boiling water were added, they strongly facilitated the ADH activity and the increase in the rate for the bean sprout, dropwort, and radish was 236.07%, 223.56% and 202.72%, respectively as shown Fig. 1. In the kinds of solvent for extraction, the ADH activity of boiled vegetable extracts by distilled water and ethyl alcohol increased from a minimum of 152.03% to a maximum of 196.31% than non-boiled extracts made using distilled water and ethyl alcohol (Fig. 2). And the ADH activity of bean sprout extract by distilled water was significantly higher than dropwort or radish, but the sprout extract by ethyl alcohol was lower than others (Fig. 2A-2B). It has been previously reported that various components, including protein, vitamins and inorganic substances, are degenerated or destroyed when vegetables are boiled (19). Accordingly it is thought that the reason for the results in the current study may be quantitative changes in these components, however, further investigation through a component analysis using analytical chemistry and food chemistry is needed. The ADH activity may be facilitated because proteins preventing ADH activity are destroyed or denatured by boiling. Also there are possibilities increasingly facilitate ADH activity by quantitative increasing of facilitating factor because the cells are destroyed by boiling.

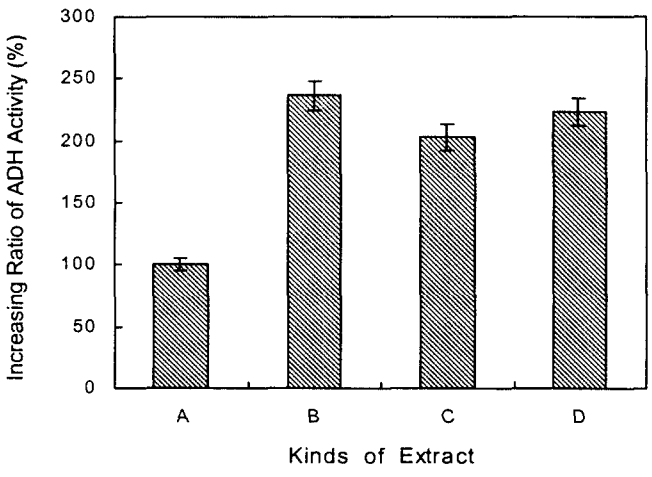

Fig. 1. Effects of boiled extracts on ADH activity of *Saccharomyces cerevisiae*. A, Control (ADH activity of *Saccharomyces cerevisiae*); B, Bean Sprout; C, Dropwort; D, Radish.

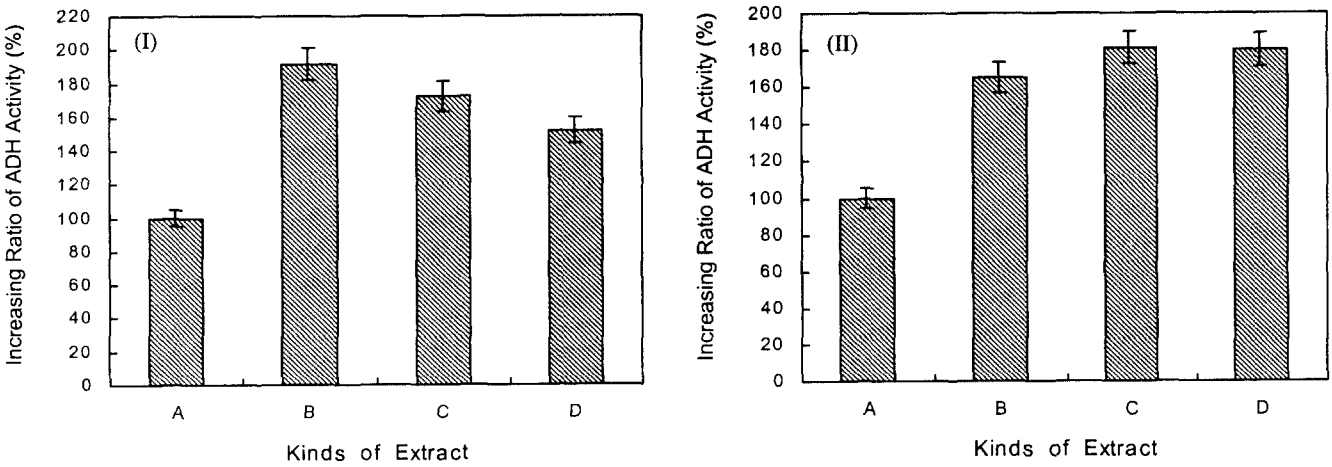

Fig. 2. Effects of boiled extracts by distilled water (I) and alcohol (II) on ADH activity of *Saccharomyces cerevisiae*. A, Control (ADH activity of *Saccharomyces cerevisiae*); B, Bean Sprout; C, Dropwort; D, Radish.

In case of extracts by non-boiled and distilled water, it is thought these components may facilitate ADH activity more than other extracts because the components facilitating ADH activity such as aspartic acid, Ca^{2+}, and Zn^{2+} are well dissolved in water. These results are in accord with reports (8,20) that the factors such as aspartic acid, Ca^{2+}, and Zn^{2+} facilitate ADH activity by regenerating NAD^{+}. Also it is thought that there are possibilities that the facilitating rate is lower because of the destruction or denaturing of apoenzymes by boiling because ADH need apoenzymes in boiled extracts. But it is thought that the reasons for the above results need to be investigated more closely through components analysis by analytical chemistry and food chemistry.

Effects of mixtures of each vegetable extract on ADH activity

The activation effects on ADH activity of two types of extracts of homogenized vegetable extract and boiled extract after homogenizing are shown in Fig. 1, and the boiled extracts facilitated higher ADH activity than the homogenized extracts. In addition, for the boiled mixed extract and non-boiled mixed extract made with two vegetable extracts at a 1 : 1 ratio of bean sprout, dropwort and radish, the boiled bean sprout and dropwort mixture facilitated more ADH activity than any other mixture as shown Fig. 3 (A). The strongest facilitating effect was in the following order, bean sprout and dropwort, bean sprout and radish, and dropwort and radish. As in the effects of the boiled and non-boiled extracts on the ADH activity, it would seem that the differences in the facilitating effects of the extracts were due to the material components. Also it was found that there was no advantage in using a mixture. But these rising effects were shown only in mixtures adding bean sprout and dropwort to radish except the mixture of bean sprout and dropwort (Fig. 3). Judging from these results, it is indicated that there is no need to use mixtures of these vegetables in the case of using bean sprouts for treating the aftereffects of the night's drink. Also it was found that mixtures of these vegetables such as dropwort and radish were more

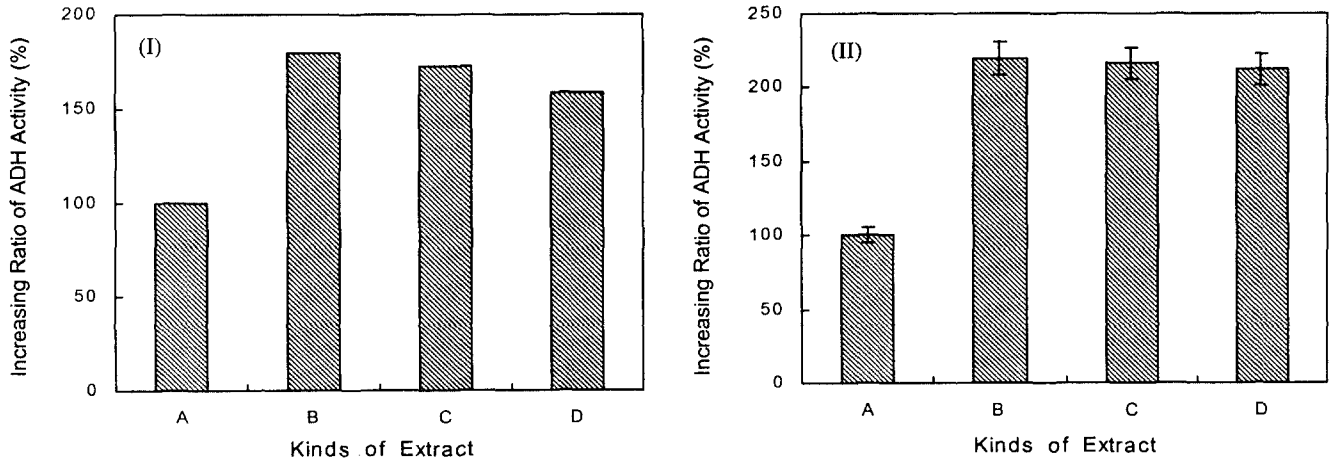

Fig. 3. Effects of mixtures by boiled extract (I), and nonboiled extract (II) of two vegetables at 1 : 1 ratio on ADH activity. A, Control (ADH activity of *Saccharomyces cerevisiae*); B, Bean Sprout + Dropwort; C, Bean Sprout + Radish; D, Dropwort + Radish.

efficacious.

Effects of approximate molecular weight range of extracts on ADH activity

In the investigation on the range of approximate molecular weight (MW) of vegetable extracts, there were differences in the ratio of increase and decrease from 5% to 40% between the extracts below 3000 MW and the nonseparated extracts, whereas the extract above 3000 MW showed a lower increase than a large molecular weight as shown Fig. 4~6. The extracts below 3000 MW increased the ADH activity the most, while the extracts above 3000 MW were similar (Fig. 4~6). In the investigation based on the approximate molecular weight range, the extracts below 3000 MW facilitated more ADH activity than the other extracts yet similar to the extracts above 3000 MW (Fig. 4~6). This indicates that the concentration of the components in the extracts below 3000 MW was higher than in the other extracts because the components in the extracts below 3000 MW passed through the pore size of the filter used. As discussed above, it would appear that components with a relatively small molecular weight may have the main effect on ADH activity.

Effects relative to kind of extract fraction on ADH activity

In the case of extracts made using distilled water and alcohol, ADH activity was most facilitated in the following order, bean sprouts, dropwort, and radish as shown Fig. 2~6. With the below 3000 MW, the rate increase with bean sprouts and radish was similar at 172.82% and 175.32% yet with dropwort it was somewhat lower at 169.57% (Fig. 4A). The strength of the effects of the extracts on facilitating ADH activity were in the following order, bean sprouts, radish, and dropwort. But the ADH activity of the extract fraction by ethyl alcohol was different. The radish extract was highest a-

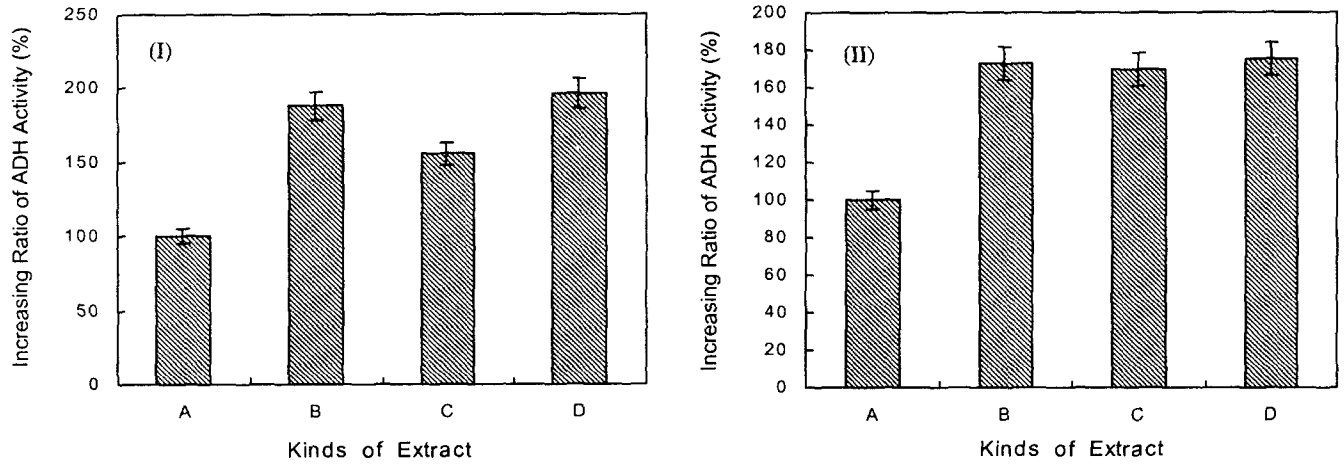

Fig. 4. Effects of extracts (below 3000 MW) by water (I) and alcohol (II) on ADH activity of *Saccharomyces cerevisiae*. A, Control; B, Bean Sprout; C, Dropwort; D, Radish.

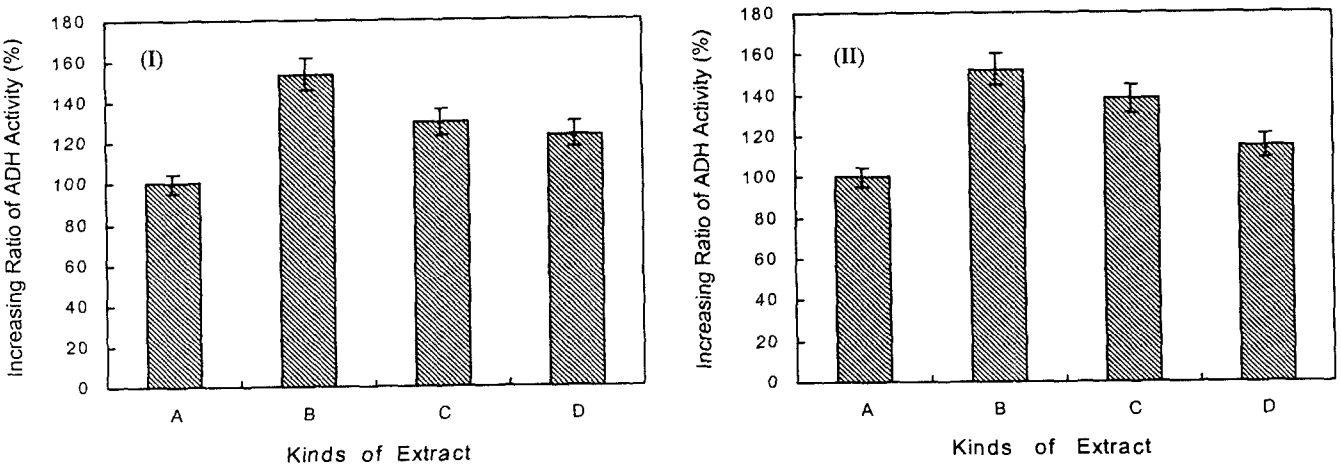

Fig. 5. Effects of extracts (between above 3000 MW and below 10000 MW) by water (I) and alcohol (II) on ADH activity of *Saccharomyces cerevisiae*. A, Control; B, Bean Sprout; C, Dropwort; D, Radish.

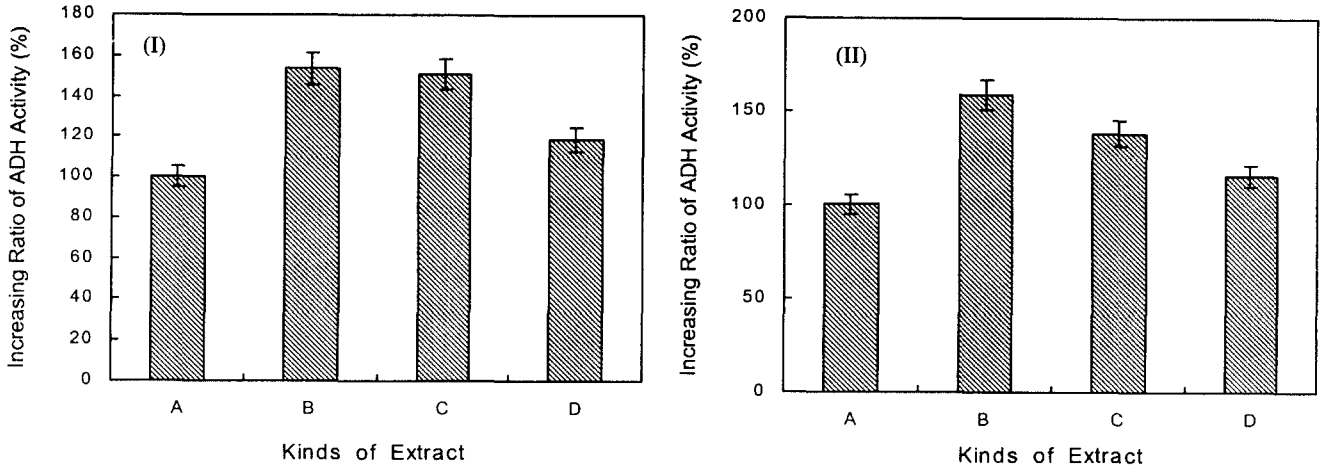

Fig. 6. Effects of extracts (above 10000 MW) by water (I) and alcohol (II) on ADH activity of *Saccharomyces cerevisiae*. A, Control; B, Bean Sprout; C, Dropwort; D, Radish.

mong them and dropwort extract was significantly low as shown in Fig. 4B. It has been previously reported that ADH activity is directly and indirectly facilitated by amino acids including aspartic acid, asparagine and inorganic substances of Ca^{2+} and Zn^{2+} (8,20). It has also been reported that the contents of the components helping to dissolve the aftereffects of liquer are aspartic acid 1200 mg%, Ca^{2+} 50 mg%, and Zn^{2+} 1 mg% in raw bean sprouts, and asparagine 250 mg%, Ca^{2+} 71 mg%, Zn^{2+} 0 mg% in raw dropwort, and aspartic acid 450 mg%, and a very small amount of Ca^{2+} in raw radish (19,21). As indicated above, if the contents of these components are compared, the contents in bean sprouts are higher than the others. Also the component content in raw cabbage are very small or not detected, thus it was not investigated in the current study. The results of the present investigated showed that the bean sprout extracts facilitated ADH activity more any other of the extracts. This indicates that the component content is very important factor on affecting ADH activity.

Effects of extracting solvents on ADH activity

Extracts made using distilled water facilitated ADH activity more than the extracts made using alcohol yet below a dropwort extract of 3000 MW. The facilitating rate of those made using alcohol was about 14% higher than that of the extracts made using distilled water (Fig. 2~6). When investigating the effect of the extracting solvent, extracts made using distilled water facilitated more ADH activity than the extracts made using alcohol. But in case of effects by molecular range, there were not great differences between distilled water and alcohol extracts above 3000 MW. This shows that the concentration of amino acids and inorganic substances helping to dissolve the aftereffects of liquer in the extracts made using distilled water was much higher than in the extracts made using alcohol. This was because the important components were well dissolved in the distilled water yet not in the alcohol. Also, as discussed before, it is thought that there are not great differences between by distilled water and by alcohol in above 3000 MW because there are not these components in above 3000 MW.

Consequently, although there are differences between *in vitro* and *in vivo* environmental conditions, the key components are still absorbed inside the body if people eat or drink such extracts and may directly facilitate ADH activity regardless of chemical reactions or other pathways under suitable conditions. As such it would seem that these extracts can directly facilitate ADH activity under the appropriate condition both *in vitro* and *in vivo*. It was found that the extracts made using boiled water were better than the others. This type of extract can easily be produced on a large scale for commercial use.

In conclusion, it is more efficacious to use bean sprouts, especially boiled bean sprouts, to dissolve the after effects of liquor. Therefore, bean sprouts would appear to have great commercial potential. For example, beverages can be produced at a low cost including a water-boiled bean sprout solution with the addition of an aromatic components with a mouth cleaning function to eliminate any alcohol aroma.

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