The Total Biotin Level in Dried Soybeans Treated with Hydrochloric Acid Quantified Using an Agar Plate Bioassay

J Rehabil Health Sci 2005; 3:25-26

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The Total Biotin Level in Dried Soybeans Treated with Hydrochloric Acid Quantified Using an Agar Plate Bioassay

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Received November 28, 2005; accepted December 8, 2005

Key words: biotin; acidic hydrolysis; soybean

1 Introduction

Biotin is a water-soluble vitamin that serves as a coenzyme for acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylaze, and 3-methyl-crotonyl-CoA carboxylase in humans.[1] These enzymes are involved with lipogenesis, glucogenesis, and branched-chain amino acid metabolism.[1] A biotin deficiency retards growth, causes neurological impairments and other disorders, but the morbidity rate of the deficiency has been low, because the humans can utilize biotin that is contained by various foods and produced by intestinal bacteria.[1-4]

Some of the biotin in various foods bonds with proteins and peptides, while the remaining is free biotin.[5] Therefore, the linkage between biotin and proteins or peptides must are cleaved, in order to measure total biotin amounts in the foods. Biotinylated proteins and peptides can be hydrolyzed under acidic conditions, since almost biotins are joined to ε-amino groups of lysine residues and the N-terminus of proteins or peptides.[4] Sulfuric acid has been generally used as an acid reagent for hydrolysis and the total biotin quantities obtained using sulfuric acid in various foods have been reported.[4-9]

In this study we used hydrochloric acid as a hydrolyzing reagent for determining the total biotin quantities in dried soybeans. The biotin concentration was measured using an agar plate bioassay.

2 Materials and Methods

2.1 Materials

Dried soybeans (*Glycine max*) were supplied by the Federation of Japan 'NATTO' Manufacture's Cooperative Society, Tokyo, Japan. *Lactobacillus plantarum* ATCC8014 was a gift from T. Fukui, Byotai-Seiri Laboratory, Tokyo, Japan. The following materials were obtained from the sources indicated: sterilized plates (230 × 80 × 14.5 mm) from Eikenkizai Co.,Ltd., Tokyo, Japan; Lactobacili Inoculum Broth® and Biotin Assay Medium® from Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; agar (noble) from Difco Laboratories, Detroit, U.S.A.; biotin from Sigma Chemical Co., St. Louis, U.S.A. All other chemicals were of reagent grade or better. The water used was 17-Mohm grade.

2.2 Quantitation of biotin using an agar plate bioassay

Fukui et al.[10] previously developed an agar plate biotin bioassay method using *L. plantarum* ATCC8014, which is a biotin-dependent microorganism. Briefly, the bacteria were pre-cultivated in Lactobacili Inoculum Broth®. A small aliquot of samples was added to each well on the agar plate containing bacteria in Biotin Assay Medium® and the agar plate was incubated at 37℃ for about 16 h. The diameter of the growth zone was measured and compared with the standard curve.

2.3 Acid treatment of dried soybeans

One gram of dried soybeans was homogenized in 10 mL of purified water. Ten milliliters of 12 mol L^{-1} (12 N) hydrochloric acid or 2.25 mol L^{-1} (4.5 N) sulfuric acid were added to the homogenate and the mixture was autoclaved at 121℃ for 1 h. The acid-treated mixture was neutralized with 10 mol L^{-1} sodium hydroxide and the volume of the mixture

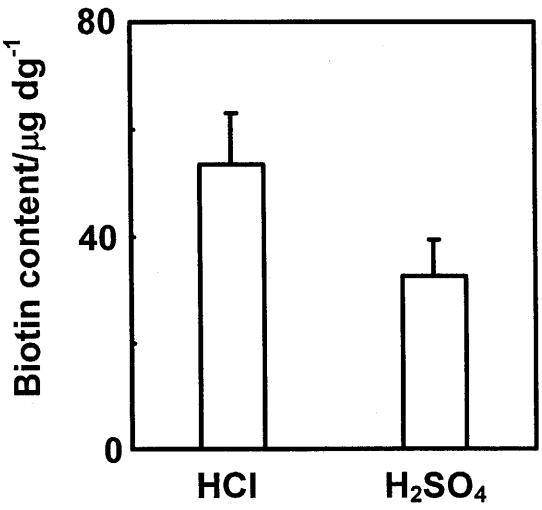

Fig. 1 The total biotin levels in dried soybeans measured using the agar plate bioassay after hydrochloric acid- or sulfuric acid-treatment.
The concentrations of hydrochloric acid and sulfuric acid were 12 and 2.5 mol L^{-1}, respectively. The vertical bars indicate the standard deviation for the mean of the experiments (n = 8). $P < 0.001$.

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neutralized was finally measured. The biotin level in the mixture was measured using the agar plate bioassay.

2.4 Statistics

Simple comparisons of the means of data were performed using unpaired Student's *t*-test.

3 Results and Discussion

For quantitations of the biotin level in various foods, 2.25 mol L^{-1} sulfuric acid has frequently been used. Therefore, we also used the same concentration of sulfuric acid to treat the dried soybeans, resulting in the finding that the biotin level in dried soybeans was 32.6 ± 6.60 μg dg^{-1}. However, using 12 mol L^{-1} hydrochloric acid led to a finding of 53.5 ± 9.41 μg dg^{-1}. The total biotin level obtained using hydrochloric acid in dried soybeans was approximately 1.6 times that of those obtained by sulfuric acid treatment and the former value was significantly higher than the latter ($P < 0.001$). The principle underlying the agar plate bioassay used in this study is that the growth of *L. plantarum* depends on the concentration of biotin, that is, the diameter of the growth zone increases as the biotin concentration of samples increases.[10] Treating the samples with hydrochloric acid then neutralizing the samples with sodium hydroxide adds an abundance of ions, Cl^- and Na^+, to the samples. The increase of those ions may interact with the growth of bacteria. In order to confirm the influence, we added the same amounts of Cl^- and Na^+ to the biotin standards as found in the samples after acid treatment and neutralization were performed. When the standards with excess amounts of the ions were applied to agar plate bioassay, it was demonstrated that the addition of excess ions did not affect the growth of the bacteria (data not shown), indicating the hydrochloric acid treatment was more effective than sulfuric acid for measuring the total biotin level in dried soybeans. Most biotins are probably joined to primary amine groups in the side chain of lysine residues and the N-terminus of proteins or peptides in the dry soybeans and acids cleave the linkage, liberating biotins from the proteins or peptides.[4] Hydrochloric acid might destroy the structure of dried soybeans more effectively than sulfuric acid does, but the effects of hydrochloric acid and sulfuric acid on hydrolysis can be considered the same. Why hydrochloric acid processing was more effective than sulfuric acid is not clear at present.

Hydrochloric acid (12 mol L^{-1}) was a more effective hydrolyzing reagent than sulfuric acid (2.25 mol L^{-1}) for measuring the total biotin levels in dried soybeans.

Acknowledgements

This study was supported in part by grants from the Federation of Japan 'NATTO' Manufacture's Cooperative Society and from Osaka Prefecture College of Health Sciences.

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