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## Identification of Angiotensin I-converting enzyme Inhibitory Activities from traditional Mongolian fermented milk products

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**Abstract:** Several angiotensin-converting enzyme (ACE) inhibitory peptides have been detected in milk products. There are many traditional milk products in Mongolia. For this study, some Mongolian milk products were collected, and the ACE inhibitory activities of these samples were tested; an active fraction was found in aaruul made from mare's milk. After purification by dialysis and HPLC, the active fractions were isolated. The molecular weight of the active component was 362.05 M, as determined by mass spectrometry. An authentic standard was used to determine the IC<sub>50</sub> value of the inhibitory activity. From 5'-GMP is not much higher than that of the active peptide in sour milk and some flavonoids. However, this is the first report that shows that 5'-GMP inhibits ACE activity. These results will provide useful information for the development of hypertension therapy agents.

**Keywords:** traditional Mongolian milk product, angiotensin-converting enzyme, 5'-guanosine monophosphate

### Introduction

Angiotensin I-converting enzyme (ACE) is one of the vasopressor principle. ACE convert angiotensin I to angiotensin II, that has a vasopressor action, in the rennin-angiotensin system and also inactivates bradykinin that is in an antihypertensive peptide. Recently, modification of the conventional ACE inhibition assay procedure has been requested because of the use of harmful organic solvent such as ethyl acetate for the extraction of hippuric acid cleaved from Hippuryl-His-Leu by ACE and its complicated procedure. Fermented milk products, in addition to providing both energy and nutrients, are an excellent source of bioactive peptides. Numerous peptides with bioactive properties

have been isolated from fermented dairy products. These include antibacterial, anticancer, immunomodulatory, mineral-binding, opioid and antihypertensive peptides. The dairy product making tradition, which has a 15,000 years of history in the region was a known skill for its ancient dwellers. Milk is sacred in Mongolia where milk and milk products are staple foods and produced in great abundance from over 30 million head. Though more than 100 regional varieties are produced, traditional products are broadly classified as fat or protein-based or fermented. The Protein-based products aaruul (dried curd) is made that thick aarts is put on the wooden panel and pressed by a weight and cut it by threads, knife, and scraper or crumpled up and dried up finally. It is a

strong tasty, sour like acid and light brown colored.



Mare's milk shows some structural and functional peculiarities that make it more suitable for human nourishment than cow's milk. Mare's milk is rich in vitamins and combines rare elements of zinc, calcium, copper and cobalts. It contains hormone of 'acetylcholine' that restores cells of nervous system, improves blood circulation by enhancing the coronary activity and reduces high blood pressure. Cow's milk is suitable making dairy products and also for daily consumption.

### Experimental

Aaruul (Dried curd), eezgii, bayslag samples were collected from in the Arhangai, Bayankhongor region of Mongolia.

The ACE inhibitory activity was assayed using commercially available ACE kit WST<sup>®</sup> assay kit (Dojindo Co. LTD.) Briefly, diluted samples were put into the well of 96well microplate, and enzyme-working mixture was added to each well. After incubation for 60 min, indicator-working solution was added to each well. After 10 min, the plates were read the absorbance at 450 nm with microplate reader. The IC<sub>50</sub> value was defined as the concentration of ACE inhibitory sample required to inhibit 50% of the ACE activity under the above assay conditions and was determined by a regression analysis of ACE inhibition (%) versus sample concentration. Ethanol content was quantified by the chemical oxidation method after microdistillation of the aaruul samples, which was performed by Ethanol kit.

Calculate the ACE inhibit activity (inhibition rate %) using the following equation;  
 ACE inhibitory activity (inhibition rate %)= $[(A_{\text{blank1}}-A_{\text{sample}})/(A_{\text{blank1}}-A_{\text{blank2}})] \times 100$

Blank 1: positive control (without ACE inhibition),  
 blank 2: reagent blank

Solution	Samples	Blank-1	Blank-2
Sample solution	20μL	-	-
Deionized water	-	20μL	40μL
Substrate buffer	20μL	20μL	20μL
Enzyme working Solution	20μL	20μL	-
37 <sup>o</sup> C 60 min			
Indicator Working Solution	200μL	200μL	200μL
Room temperature. 10 min			
450 nm with a microplate reader			

If the color of the sample solution is strong, subtract the absorbance of the sample blank, that is prepared by sample solution (10μl) and deionized water (100μl), from that of the sample (A<sub>sample</sub>) per each sample.

The concentration (mg/mL or μM) that inhibited 50% of ACE activity (IC<sub>50</sub>) was determined by regression analysis of ACE inhibition (%) versus peptide concentration in duplicate.

### Results and Discussion

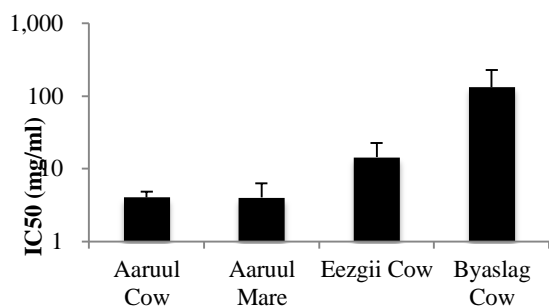
The ACE inhibitory activity of aaruul, eezgii was not affected by ACE preincubation. The ACE inhibitory activity of aaruul makes it commercially attractive in the future as a health-enhancing ingredient in the production of functional foods. The results of IC<sub>50</sub> values are shown in Table 1. Aaruul is a type of coagulated milk, eezgii is a kind of cottage cheese, while byaslag is a mild cheese, and all are produced I Mongolia. The IC<sub>50</sub> values of aaruul samples ranged between 2.59 and 5.30 mg/ml, while the IC<sub>50</sub> of eezgii was between 4.82 and 28.04 mg/ml, and the IC<sub>50</sub> values of the byaslag samples ranged from 123.37 to 141.47 mg/mL. It has been previously reported that many peptides in sour milk have strong ACE inhibitory activity.

**Table 1.** Angiotensin- converting enzyme inhibitory activity of Mongolian traditional milk products Extracts.

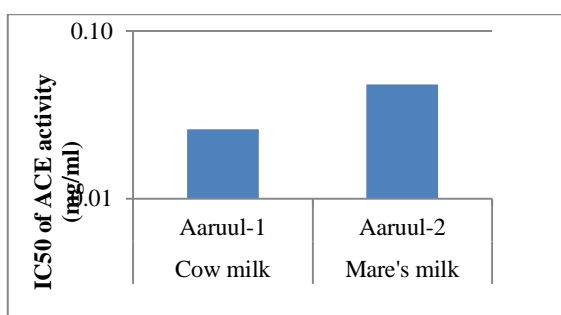
Sample	Origin	IC <sub>50</sub> (mg/mL)*
Aaruul-1	Cow	2.59
Aaruul-2	Cow	5.30
Aaruul-3	Cow	4.27
Aaruul-4	Mare	4.83
Aaruul-5	Mare	4.02
Aaruul-6	Mare	3.21
Eezgii-1	Cow	28.04
Eezgii-2	Cow	4.82
Eezgii-3	Cow	10.23
Byaslag-1	Cow	123.37
Byaslag-2	Cow	141.47

\*: Inhibitory concentration of 50%

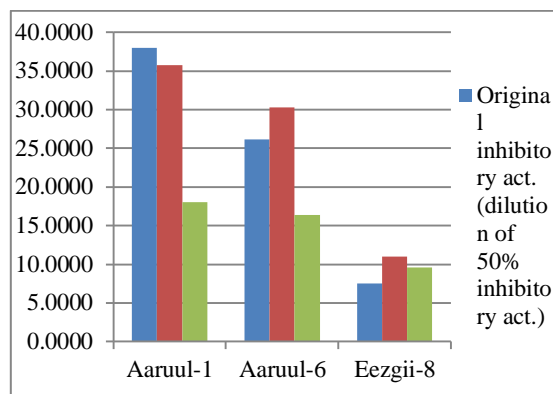
Our present results demonstrated that aaruul has high ACE inhibitory activity. Figure 1 shows the results of the collective IC<sub>50</sub> value between the different types of milk products were compared.



**Fig.1.** ACE inhibitory activities of various types in Mongolian traditional milk products.



**Fig. 2.** Stability of angitensin I-converting enzyme (ACE) activity from aaruul (cow's milk, mare's milk)



**Fig.3.** The ACE inhibitory activity between the original and the treatments (relative ACE inhibitory index=50% inhibitory concentration)

There are many types of fermented milk products in Mongolia. Therefore, differences in the IC<sub>50</sub> value between the different types of milk products were compared. The relatively higher IC<sub>50</sub> values (4.02±0.81 [mean ±standard deviation] mg/mL) were found in the aaruul (mare) group. Aaruul (cow) showed almost a similar inhibitory activity, at 4.05±1.37 mg/mL, whereas that of eezgii (cow) was 14.36±12.15 mg/mL. The lowest IC<sub>50</sub> (132.42±12.80 mg/mL) was found in byaslag (cow).

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