熱帯植物ギンネム中のミモシンの簡易除去法(農芸化学科)

A Simple Reduction Method of Mimosine in the Tropical Plant Leucaena*

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Summary

Because of the presence of mimosine (β- 〔N-(3-hydroxypyridone-4)-α-amino-propionic acid) in leucaena (*Leucaena leucocephala de Wit*), the ingestion of the plant by animals causes alopecia, growth retardation, cataract and infertility. The mimosine degradation product 3-hydroxy-4(1H)-pyridone (DHP) is also reported to be goitrogenic in animals. Inactivation or reduction of mimosine and DHP in the plant before or during the preparation of the plant as feed would enhance the use of the legume as a source of protein for livestock feed. Although purified mimosine is not soluble in distilled water, it become very soluble in the ionic solutions. When the leaves of leucaena were leached in 0～100% sea water or in various concentrations of acids and bases for 24 hrs at 25°C, about 60～95% of the mimosine was extracted. As the rate of mimosine extraction from the leaf of leucaena depended on the concentration of reagents, it was found that 0.05N CH_3COONa was the most effective at extracting 95% of the mimosine. There were no loss of the nutrients, important for use as a livestock feed, such as crude fat, crude fiber and crude protein. The method is relatively simple and removes simultaneously both mimosine and DHP, therefore, it is beleaved to be a method practically applicable for both the small scale treatment of a local farmer and the large scale treatment of a commercial enterprise.

Introduciton

It is known that one of the mimosine degradation products, 3-hydroxy-4(1H)-pyridone (DHP),

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causes goitrogen in mouse and livestock[1]. In present methods of use of untreated leucaena, it is reported that feed with less than 30% leucaena for cattle and less than 10% for swine and less than 5% for poultry do not affect the growth of the livestock at all. In addition to adjustments of the amount of leucaena fed to individual animals, various treatments of leucaena have been reported, including the reduction of mimosine in leucaena leaves by heating[9], by the utilization of endogenous mimosine degradation enzyme[6], and by the addition of iron salts to make a mimosine complex for the purpose of inhibition of absorption in the body[7]. However, each of these methods is considered to entail problems such as the loss of nutrients from leucaena, the formation of DHP, and the surplus absorption of the iron in the body.

The present study was carried out to determine an effective method of reduction of mimosine and DHP without any loss of protein in leucaena for the ultimate purpose of utilizing leucaena as a safe feed.

Methods

Quantitative analysis of mimosine

50 ml of 0.1N HCl was added to 2g of freeze-dried leucaena leaves which were made into impalpable powder by Wiley's mill, and the mixture was then homogenized for 10 min by polytron with ice cooling. The obtained mixture of leucaena was centrifuged at 12.000 rpm by Hitachi 20 PR, and the supernatant was filtered by sucking through Toyo filterpaper No. 52. The filtrate was brought to a volume of 100 ml by the addition of 0.1N HCl, and then the solution was passed through the diskpaper for high performance liquid chromatography (HPLC). 2μl of the solution was injected to HPLC (Shimadzu LC-6A) for analysis. The column used was Shim-pack CLC-ODS (15cm x 6mm i.d.), and the column temperature was 50 °C. Mobile phase employed was the mixture solution of 10 mM potassium-di-hydrogen phosphate : 10 mM phosphoric acid : acetonitrile (45 : 45 : 10), and finally 0.1% sodium 1-octanesulfonate was added to the mixture as the surface active agent. They were detected at 250 nm with flow rate 1.5 ml/min.

Reduction of mimosine in leucaena by a leaching method

100g of leucaena leaves in a nylon bag were leached for 24 hrs at room temperature in 1 l each of 0.1N solution of salts and acids. The eluted mimosine and DHP in the medium, and the remaining mimosine and DHP in the leucaena leaves were simultaneously determined by HPLC. The changes of mimosine content after and before the leaching were compared. Additionally, the general compositions were compared before and after the leaching and also the level of protein loss was examined.

Results and Disucussion

Degradation or removal of mimosine is an indispensable condition for feeding leucaena to livestock. At present, it is known that there are some methods for detoxifying of leucaena, as by the addition of iron salts or the heating of leucaena leaves, but both these methods introduce a further problem by the formation of DHP which is a mimosine degradation product. Table 1 shows the results of HPLC analysis of the residual mimosine content in leucaena which was leached for

Table.1 Mimosine reduction by the leaching method with each 0.1N solution.

Untreated leucaena	100 〔%〕
H_2O	7.2
HCl	31.2
NaOH	3.6
NaCl	12.0
KCl	13.2
$CaCl_2$	13.2
CH_3COONa	8.7
$CuCl_2$	31.5
$ZnCl_2$	39.4
$MgCl_2$	10.7
$FeCl_3$	36.1
$FeSO_4$	27.4
formic acid	11.6
Acetic acid	8.8
Lactic acid	7.8
Fumaric acid	10.2
Succinic acid	8.2
Malic acid	10.2
Tartaric acid	11.2

24 hrs at 25°C in water and in various 0.1N solutions of acids and salts. It was confirmed that water was the most effective for the reduction of mimosine: as much 92.8% of the mimosine was removed, as shown in the Table. Metal salts such as $CaCl_2$, $ZnCl_2$ and $FeSO_4$ instead inhibited the elution of mimosine. Organic acids and CH_3COONa were more effective on elution than were NaCl, KCl and $CaCl_2$. Since NaOH discolors leucaena, and has to be washed out with a large amount of water after treatment, we decided to investigate primarily with CH_3COONa or with sea water, because it is readily available in great quantities. Taking the mimosine content in untreated leucaena to be 100%, Fig 1 shows the mimosine contents which were leached for 24 hrs at 25°C in 0～100% sea water or in 0.01～1.0N CH_3COONa. In this case, the most effective reagent was 0.05N CH_3COONa in that it removed 94.4% of the mimosine. In the case of 60% sea water, 90.1% of the mimosine was removed; therefore, the utilization of sea water seemed to be an effective method for saving water.

A typical example of HPLC analysis of mimosine and DHP is shown in Fig 2. The left spectrum in the Fig shows the HPLC analysis of the medium of the leucaena which was leached by 0.05N CH_3-COONa for 24 hrs at 25°C. The middle spectrum shows the untreated leucaena and exibits an absence of DHP. A large amount of mimosine seemed to be degraded to form DHP during the

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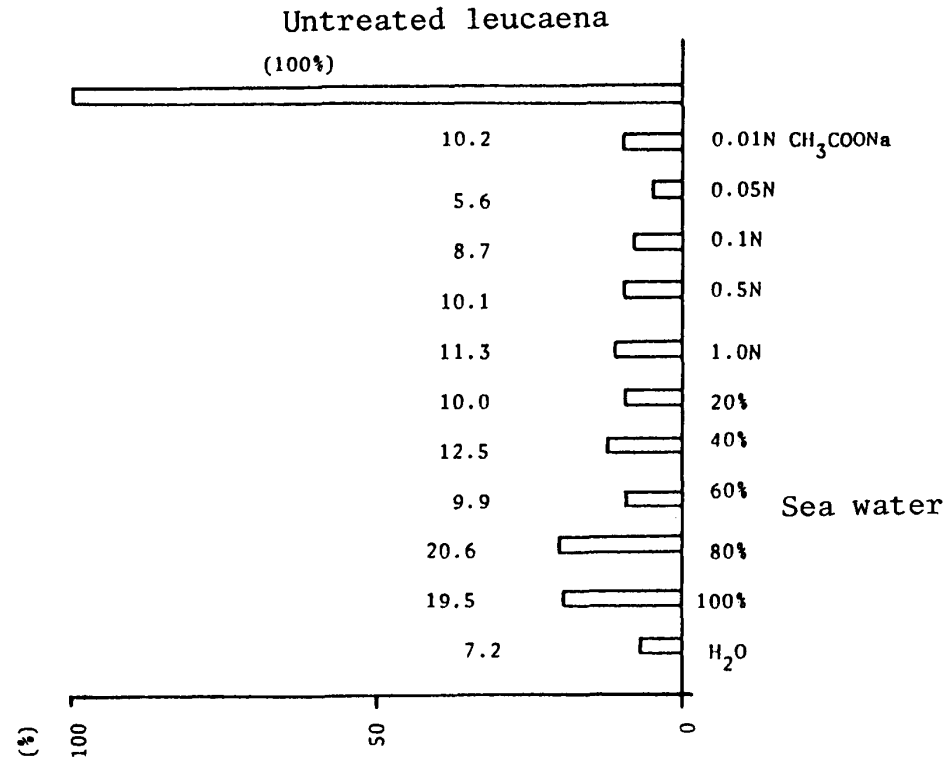

Fig.1 Mimosine reduction by the method with sea water and CH_3COONa.

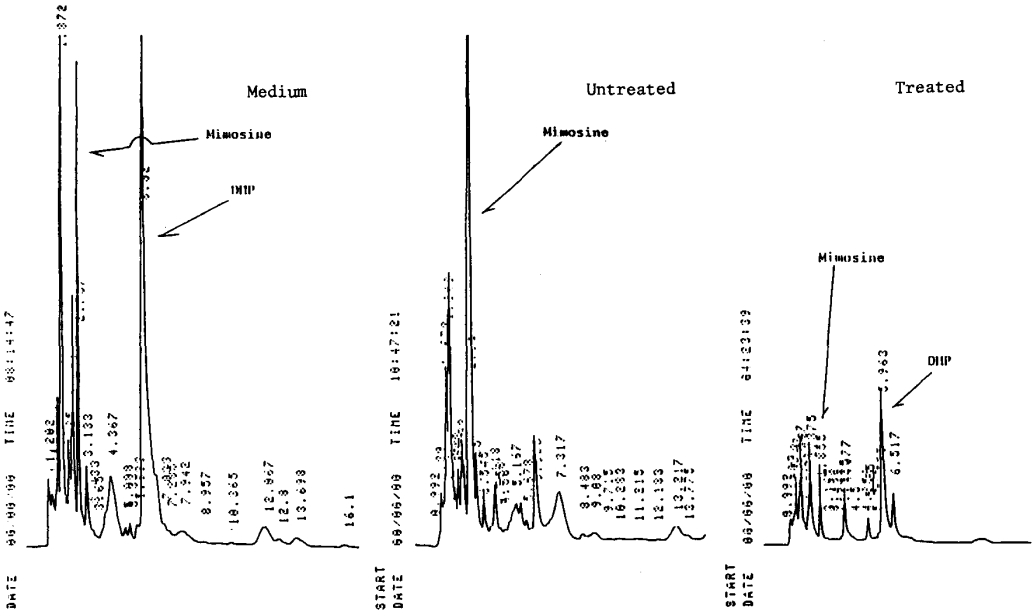

Fig. 2 Mimosine contents in the medium, untreated and treated leucaena leaves.

leaching. The degradation seemed to be due to the presence of the endogenous enzyme in leucaena. The right spectrum in the Fig shows that small amounts of both mimosine and DHP are present in leucaena after the leaching. The residue rate of mimosine was 4.5%, and it appeared that most of the DHP was also transfered in the solution. Fig 3 shows the results of the residual mimosine and

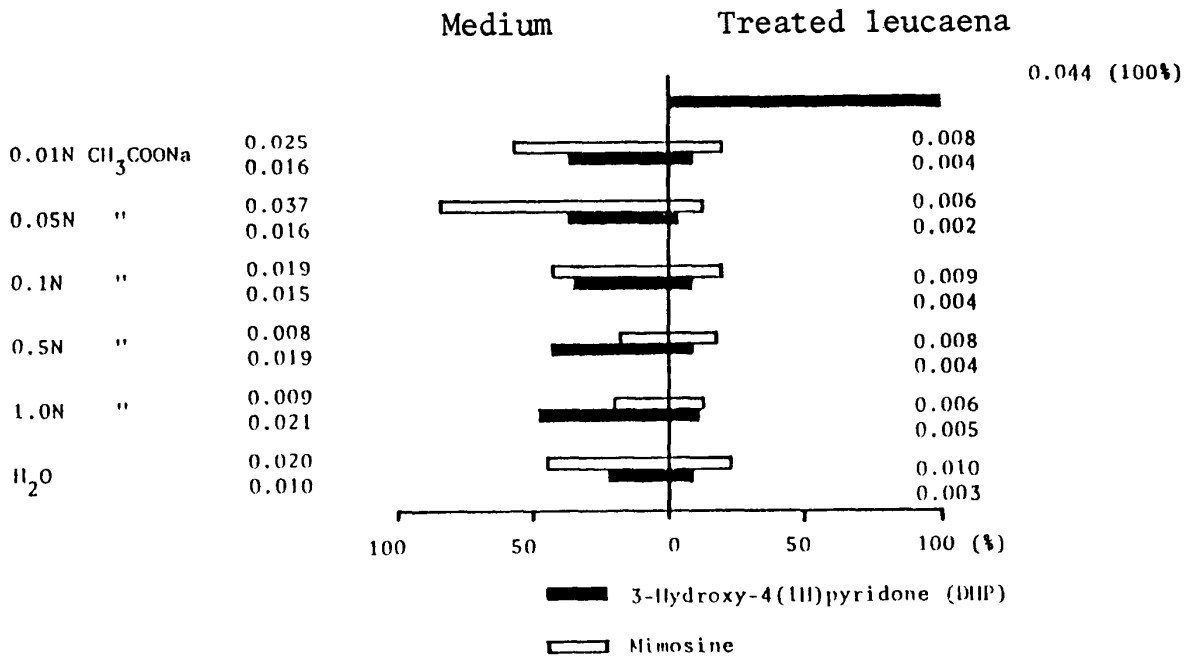

Fig. 3 Analyses of mimosine and DHP in the medium and treated leucaena leaves.

DHP in the treated leucaena, and the eluted mimosine and DHP in the medium which was leached in 0.01~1.0N CH_3COONa soltions and water. In the treatment by 0.05N CH_3COONa, the formation rate of DHP in the leucaena was about half that in the water treatment. If the toxicity of DHP is considered, the most effective leaching reagent might be 0.05N CH_3COONa. Accordingly, it was confirmed that a safer feed of leucaena could be prepared by this method. A comparison of the general composition of 0.05N CH_3COONa treated leucaena and untreated leucaena is shown in Table 2. The total weight of leucaena after the leaching decreased about 3%. In indivedual composition,

Table. 2 General composition of untreated and treated leucaena.

	Crude ash	Crude fiber	crude fat	crude protein	Water soluble carbohydrate	Momosine (%)
Treated leucaena	5.4	17.8	8.3	20.8	47.7	5.6
Untreated leucaena	9.5	13.1	6.2	21.7	49.5	100

Leucaena: *Leucaena leucocephala de Wit*
Untreated: Dry basis leucaena leaves
Treated: Leucaena leaves was leached in 0.05N CH_3COONa for 24 hrs at 25°C

crude ash 4.1%, water soluble carbohydrate 1.8%, and crude protein 0.9% were decreased, respectively. By contrast, crude fiber and crude fat, which are the sources of nutrients in feed for livestock, were increased by 4.7% and 2.1%, respectively. A small amout of various free amino acids and about 1% mimosine are present in untreated leucaena, and these are considered to be eluted in the medium, together with other inorganic compounds such as K, Ca, and P, and others. Therefore it is considered that polymer compounds such as proteins, which are present in the cells, did not elute at all.

From the studies mentioned above, it can be seen that from leucaena, which is presently an unused resource that grows wild everywhere in Okinawa, it is possible to remove about 95% of mimosine by leaching without any loss of the nutrients, important for use as a livestock feed, such as crude fiber, crude fat and crude protein; therefore, it is considered that the absolute unilization of leucaena could be possible in the near future. The leaching method seemed to have been investigated by Szyszka et al [8], though they did not report it in detail. As the method is relatively simple and removes simultaneously both mimosine and DHP, it is considered to be the most superior method. It is beleaved to be a method practically applicable for both the small scale treatment of a local farmer and the large scale treatment of a commercial enterprise.

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熱帯植物ギンネム中のミモシンの簡易除去法

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摘　　要

ギンネム（*Leucaena leucocephala de Wit*）中には、ミモシン（β-〔N-(3-hydroxy pyridone-4)-α-aminopropionic acid）が存在するため，動物がそれを摂取すると脱毛症，成長障害，白内障，不妊症などを引き起こす。ミモシンの分解産物3-hydroxy-4(IH)-pyridone (DHP) もまた動物に甲状腺腫誘発性があると報告されている。飼料として調製中あるいは調製前にギンネム中のミモシンおよびDHPを不活性化あるいは除去できるのならば、家畜飼料のタンパク源としてギンネムの利用が大きく高揚するだろう。精製ミモシンは蒸留水には難溶性であるが，イオン性溶液には易溶性を示した。ミモシン茎葉部を0～100％海水あるいは酸類，塩類の各種濃度液に25°Cで24時間浸漬すると，およそ60～95％のミモシンが溶脱された。ギンネムからのミモシンの溶脱率は試薬の濃度に左右され，最も効果的なものは0.05N CH_3-COONaで95％ものミモシンが抽出される事がわかった。家畜飼料として利用するのに重要な栄養源である粗脂肪，粗組織，粗タンパク質の損失はほとんどなかった。この方法は比較的簡単であり，ミモシンもDHPも同時に除去する事から，農家における小規模な処理法から，企業による大規模生産まで応用できる実用的な方法であると思われた。

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