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TOXIC SUBSTANCE PRESENT IN THE OIL FRACTION OF THE SAGABEAN

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ABSTRACT

Penelitian sebelumnya menunjukkan adanya suatu zat toksis pada biji saga yang tidak tergolong faktor "anti-nutritive" seperti "tripsin inhibitors", "phyto-agglutinins" dan "saponins".

Ternyata bahwa zat toksis tersebut yang belum dapat diekstraksi dan diidentifikasi, berada di dalam fraksi minyak biji saga.

Di samping adanya zat toksis, ditemukan pula bahwa kualitas protein biji saga lebih rendah daripada kacang kedele. Hasil analisa asam amino menunjukkan bahwa kadar methionine dan threonine adalah terbatas (limiting), apabila ditambah dengan kedua asam amino ini dalam jumlah yang cukup, kualitas proteinnya menjadi sama tingginya dengan kacang kedele rebus tanpa tambahan.

INTRODUCTION

From the result of previous investigations which has been reported (Oey et al. 1981, 1983), it was concluded that the sagabean *(Adenanthera pavonina*–Linn.) must contain a toxic substance (s) leading to a very decreased food intake resulting in loss of weight or very depressed growth of the experimental rats. This toxic substance must be different from the anti-nutritive factors such as trypsin-inhibitors, haemagglutinins and saponins, commonly found in many edible legume-seeds and which can be inactivated by ordinary moist-heat-treatment such as boiling.

Experiments with defatted saga were therefore undertaken to obtain more information on the postulated or contended toxic substance. Saga cotyledons were defatted using n-hexane and incorporated as the single source of protein in various experimental rat-diets for further testing.

MATERIALS

Preparation of Defatted Samples.

Sample A : Decortication, Soaking, Boiling, Defatting (Sample prepared in Nutrition Unit Diponegoro Jakarta).
Crushed, decorticated raw sagabeans were soaked in water for 36 hours with repeated washings and boiled in sufficient water for at least one hour, as described in our previous article (Oey et al. 1983). For defatting the dried grits were extracted using n-hexane (boiling point 69°C), which was carried out at the National Institute for Chemistry in Bandung.

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Sample B : Decortication, Defatting and Steaming. (Sample prepared in LKN–LIPI Bandung).

- Another defatted sample of saga was prepared at the National Institute for Chemistry in Bandung as follows:
- Whole sagabeans were dried in the oven at 50°C. for 60 hours, followed by crushing in a mill and decorticated manually. The split cotyledones were dried again at 55°C. for 24 hours and ground into grits using a sieve of 9 mesh.
- The grits were then subjected to the same procedure of defatting using n-hexane during 76 hours (19 circulations) (Soxhlet).
- Half of the n-hexane defatted saga sample was further extracted with alcohol, with the aim of reducing the saponine content.
- Before incorporating into experimental rat diets, both defatted samples were separately steamed in small batches in a common household rice cooker for one hour. Before steaming the batch of dry defatted saga flour was sprinkled with sufficient water for moistening (wetting) to ensure appropriate heat-treatment.

METHODS AND PROCEDURES

- The methods for the chemical analysis and the rat-feeding experiment have been described in our previous publications (Oey et al. 1981, 1983).
- The composition of the experimental rat diets is given in table 4.

In all these experimental saga diets, the saga was the only source of protein fed at approximately 10% protein level.

RESULTS

a) Chemical analysis:

The results of the chemical analysis are needed for the preparation of the experimental rat diet, and the result is presented in Table 1. No data are available on the amino acid composition of the decorticated, defatted, steamed sagabeans due to technical reasons (amino-acid analyzer out of order). However, it might be expected that the amino acid composition will be in close resemblance to the amino acid composition of the saga, boiled whole in the skin and decorticated as reported in our first report. (Oey et. al. 1981).

Table 1. Proximate principles of various Saga meal

Average per 100 gram of dry saga meal	Decorticated Soaked Boiled		Decorticated Defatted Steamed	
	not defatted	Defatted n-hexane	n-Hexane extraction	n-Hexane and then Alcohol extraction
Moisture %	4.2	4.4	5.2	7.1
Nitrogen %	4.68	6.64	6.58	6.87
Protein (N x 5.71) %	26.7	37.9	37.6	39.2
Fat %	36.1	11.2	0.8	0.8
Carbohydrates by diff. %	30.1	41.7	50.5	47.1
Minerals %	2.9	4.8	5.9	5.8

b) Rat feeding experiments:

The results of the rat feeding experiment (PER or growth study) is given in Table 2 and Table 3.
More details can be seen in Table 5.

DISCUSSION

Toxic substance (s)

a) Not defatted Saga diets.

As can be seen from table 2, the average total food-intake of the rats fed on non-defat-

ted saga diets was very low, both for the non-supplemented as well as supplemented diets with methionine and threonine (viz. 67 g and 85 g respectively). As can be seen from the growth-curves, (Graph 1) the rats on these two diets did not grow, on the contrary they lost weight.

b) Defatted Saga diets.

When the rats were fed on defatted saga, the average total food-intake of the rats on the non-supplemented defatted saga diet was slightly higher than that of the non-defatted diets, viz. 99 g. But when supplemented with Me & Th the defatted saga diet was well eaten, viz. 224 g, a total intake even higher than for soy, boiled, supplemented (Table 2).

The growth curve of the rats on the non supplemented although defatted saga diet was negative, there was no growth at all. But the defatted supplemented saga diet gave a very good growth, almost the same as for the supplemented boiled soy diet, although somewhat lower.

The process of defatting and amino acid supplementation must have a very beneficial effect on the palatibility and the food intake of the experimental rats, resulting in better growth.

One may therefore conclude that through defatting the saga has become detoxicated (non-toxic) and that the toxic substance (s) must therefore be present in the oil fraction of the saga bean.

Amino Acid Pattern Of Prepared Saga Sample
(Biological value of the Saga protein)

The non supplemented but defatted, detoxicated saga diet was not well eaten by the experimental rats. It also did not give a good growth as compared to the defatted saga diet supplemented with Me and Th. As the saga was detoxicated already this refusal means that the protein in the saga as prepared must be very li-

Table 2. Summary of Results of Protein Efficiency Ratio Determinations

Sample A. Nutr. Unit Diponegoro – Jakarta (4 weeks)

	Experimental Diet (10% protein level)	No. of Rats n	Protein Content of of XP–diet g %	Avg. Wght. at Start g	Total Wght Gain at End g	Total Wght. Gain at End %	Total Food Intake at End g	Total Protein Intake at End g	PER ± SD
1.	Protein-free	10	0	49.95	–18.4	–36.8	66.5	–	–
2.	Skim milk	10	10.12 (N×6.38)	50.00	58.3	116.6	207.6	21.0	2.79 ± 0.25
3.	Soy, boiled	6	9.52 (N×5.71)	47 9	45.5	95.0	194.0	18.47	2.46 ± 0.24
4.	Soy, boiled + Me + Th	10	9.23 (N×5.71)	48.0	63.1	131.5	203.3	18.76	3.35 ± 0.32
5.	Saga, Dc, S. B Not defatted	6	10.29 (N×5.71)	47.9	–11.9	–24.8	67.0	6.89	(–1.87 ± 0.69)
6.	Saga, Dc. S. B. Not defatted + Me + Th	10	10.22 (N×5.71)	48.0	+ 2.2	+ 4.6	84.8	8.67	(+0.24 ± 0.34)
7.	Saga, Dc. S.B. Defatted	10	10.53 (N×5.71)	49.95	– 0.7	– 1.4	98.9	10.4	(–0.15 ± 0.75)
8.	Saga, Dc. S.B. Defatted + Me + Th	10	10.15 (N×5.71)	49.95	54.3	108.7	223.5	22.7	2.38 ± 0.36

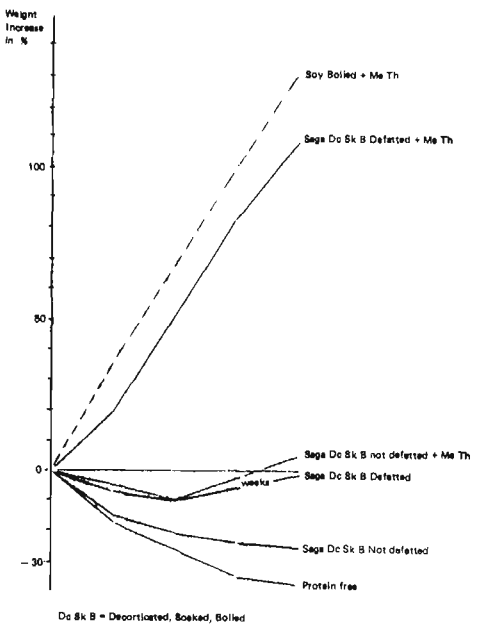
miting in one or more amino acids. And as indicated by the results of the amino acid analysis from Mahidol University Bangkok reported by Oey et al, (1983) and confirmed by the results of these rats experiments, the limiting amino acids in the saga protein prepared must be indeed methionine and threonine.

Anti-Nutritive Factors

The results of feeding experiments with defatted detoxicated saga bean, both raw as well as steamed are very useful for the evaluation whether the anti-nutritive factors are eliminated by moist heat-treatment viz. steaming.

The results of the feeding experiments with sample B (prepared at the LKN–LIPI Bandung) was intentionally selected for discussion on the anti-nutritive factors, as this sample prepared was not soaked and boiled but still in the raw state, defatted and then afterwards steamed. No leaching out of the saponine from the sample is therefore expected.

Graph 1. Sample A:
Growth Curves of young weanling albino rats fed Defatted and non-defatted Sagabeans with and without supplementation with Me and Th.



When the detoxicated saga still in the raw state was fed to the experimental rats, even supplemented with Me and Th, these raw saga diets were totally refused by the rats (Table 3 and Graph 2).

The experimental rats on the raw saga diets started already to die in the third week of the experiments. When steamed however, the defatted (detoxicated) non supplemented saga diet started to give a positive growth after 2 weeks, none dying, as shown in graph 2.

Supplemented with the limiting amino acids Me and Th, there was a very good growth almost as good as soy (Graph 2).

As can be seen from the growth curves, the growth of the experimental rats on the supplemented diets both n-hexane extracted as well as n-hexane and alcohol extracted do not show any difference.

One may conclude that it will not be necessary to inactivate the saponin content by alcohol extraction as the steaming process will already be adequate.

Graph 2. Sample B :
Growth Curves of young weanling albino rats fed Raw and Steamed *but* Defatted Saga (LKN) with and without Supplementation with Me and Th

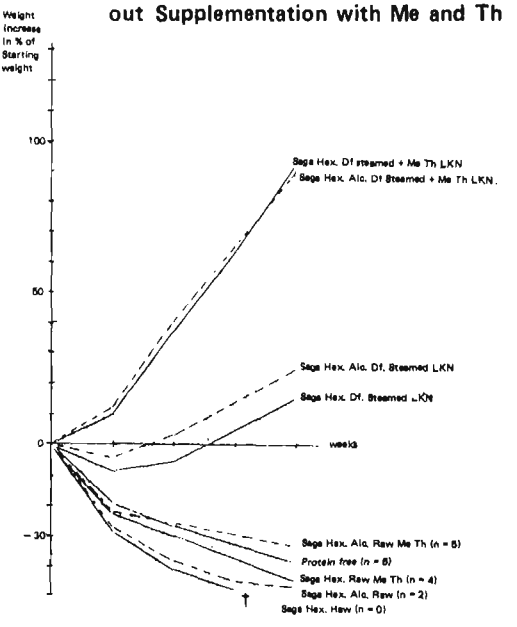


Table 3. Summary of Results of Protein Efficiency Ratio Determinations (4 weeks)
Sample B. LKN – LIPI

Basic Ingredients in grams (approx. 10% protein level)	Protein free	Skim milk	Sample A: Saga soaked boiled	Saga soaked boiled + Me&Th	Saga soaked boiled defatted	Saga soaked boiled defatted + Me&Th	Sample B: Saga Hexane (raw) or steamed	Saga Hexane (raw) or steamed + Me&Th	Saga Hexane Alcohol (raw) or steamed	Saga Hexane Alcohol (raw) or steamed + Me&Th
Fat added	100	98	–	–	70	70	97	97	97	97
Starch	720	526	555	555	596	592	577	577	578	578
Glucose	150	50	50	50	50	50	50	50	50	50
Salt mixture	20	20	20	20	20	20	20	20	20	20
Vitamin mixture	+	+	+	+	+	+	+	+	+	+
Cellu flour	10	20	–	–	–	–	–	–	–	–
DL–Methionine	–	–	–	1.954	–	1.954	–	1.953	–	1.953
L–Threonine	–	–	–	2.145	–	2.145	–	2.146	–	2.145
Skim milk	–	286	–	–	–	–	–	–	–	–
Saga soaked, boiled not defatted	–	–	375	375	–	–	–	–	–	–
Saga soaked, boiled defatted	–	–	–	–	264	264	–	–	–	–
Saga Hexane (raw) or (steamed)	–	–	–	–	–	–	256	256	–	–
Saga Hexane Alcohol (raw) or (steamed)	–	–	–	–	–	–	–	–	255	255
Total in grams	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Table 4. Composition of Experimental diets for Rat feeding Experiments.

Experimental Diet (10% protein level)	No. of Rats n	Protein Content of of XP–diet g %	Avg. Wght. at Start g	Total Wght. Gain at End g	Total Wght. Gain at End %	Total Food Intake at End g	Total Protein Intake at End g	PER ±SD
1. Protein free	6	0	51.5	–19.8	–38.7	75.2	0	–
2. Skim milk	6	10.2	51.5	62.3	121.2	220.2	22.38	2.77 ± 0.35
Raw Defatted Saga								
3. Saga Hex. extr. raw	6	10.0	47.9	– n = 0	–	–	–	–
4. Saga Hex. extr. raw + Me Th.	6	10.0	48.0	–19.3 n = 4	–40.5	51.4	5.14	(–3.91 ±0.46)
5. Saga Hex. Alc. extr. raw	6	10.2	48.0	–22.0 n = 2	–46.4	50.5	5.15	(–4.32 ±0.39)
6. Saga Hex. Alc.extr. raw + Me Th	6	9.8	48.0	–16.0 n = 5	–33.6	57.3	5.62	(–2.95 ±0.90)
Steamed Defatted Saga								
7. Saga Hex.extr. steamed	6	10.22	51.5	7.8	15.1	116.2	11.87	0.65 ± 0.23
8. Saga Hex. steamed Me + Th	6	10.27	51.5	47.0	91.3	190.6	19.58	2.41 ± 0.22
9. Saga Hex. then alc. extr. steamed	6	10.55	51.5	12.5	24.3	134.0	14.13	0.88 ± 0.17
10. Saga Hex. then alc. extr. Steamed + Me + Th	6	10.59	51.5	46.3	89.9	188.6	19.97	2.32 ± 0.09

Table 5. Summary of Results of Protein Efficiency Ratio Determination per rat by weeks of: Decorticated, Soaked, Boiled Saga Non-defatted and Defatted (Sample A from Nutrition Research Unit Diponegoro Jakarta)

Experimental Diet at approx. 10 % protein level	No. of rats	Protein content of XP-diet	Avg. Wght. at Start	Avg. Cumulative Wght. Increase in % of Starting Weight at End of week				Total weight Gain	Avg. Total Food-Intake per rat by weeks during week no.				Total Food-Intake	Total Protein Intake	PER ± SD
				I	II	III	IV		I	II	III	IV			
	n	g %	g	%	%	%	%	g	g	g	g	g	g	g	
Protein-free	10	0	49.95	–17.4	–26.3	–36.7	–36.8	–18.4	20.11	14.7	13.9	17.8	66.5	–	–
Skim milk	10	10.12 (Nx6.38)	50.00	25.0	57.2	86.6	117.0	58.3	41.4	52.5	54.7	59.1	207.6	21.0	2.79 ± 0.26
Soy, boiled	6	9.52 (Nx5.71)	47.9	23.7	47.7	71.3	94.9	45.5	41.7	48.9	54.4	48.9	194.0	18.47	2.46 ± 0.24
Soy, boiled + Me + Th	10	9.23 (Nx5.71)	48.0	36.5	69.3	96.9	130.8	63.1	45.6	53.0	53.7	51.1	203.3	18.76	3.35 ± 0.32
Saga, Dc. Sk. B.	6	10.29 (Nx5.71)	47.9	–14.8	–20.8	–23.7	–25.0	–11.9	15.6	16.5	18.1	16.8	67.0	6.89	(–1.87 ± 0.69)
Saga, Dc. Sk. B. + Me + Th	10	10.22 (Nx5.71)	48.0	– 3.8	–10.2	– 3.2	+ 4.8	+ 2.2	20.0	17.0	25.8	24.0	84.8	8.67	(+0.24 ± 0.34)
Saga, Dc. Sk. B. Defatted	10	10.53 (Nx6.71)	49.95	– 7.2	– 9.4	– 6.0	– 1.3	– 0.7	19.4	24.9	26.5	28.2	98.9	10.4	(–0.15 ± 0.76)
Saga, Dc. Sk. B. Defatted + Me + Th	10	10.15 (Nx5.71)	49.95	20.2	52.6	82.6	108.6	54.3	39.1	58.0	63.0	63.4	223.5	22.7	2.38 ± 0.36

Table 6. Summary of Results of Protein Efficiency Ratio Determination per rat by weeks of: Saga defatted using n-hexane and alcohol after decortication. (Sample B prepared by the National Inst. for Chemistry LKN–LIPI, Bandung)

Experimental Diet approx. 10 % protein level	No. of rats	Protein content of XP-diet as analysed	Avg. Wght. at Start	Avg. Cumulative Wght. Increase in % of Starting Weight at End of week no.				Total Wght. Gain	Avg. Total Food–Intake per rat by weeks during week no.				Total Food-Intake	Total Protein Intake	PER ± SD
				I	II	III	IV		I	II	III	IV			
	n	g %	g	%	%	%	%	g	g	g	g	g	g	g	
Protein-free	6	0	51.5	–19.4	–27.0	–33.5	–38.7	–19.8	20.5	19.1	17.6	18.1	75.2	0	–
Skim milk	6	10.16 (Nx6.38)	51.5	24.0	57.9	90.1	121.2	62.3	40.9	55.5	60.6	63.3	220.2	22.38	2.77 ± 0.35
Saga, Hex.extr.	6	10.00 (Nx5.71)	47.9	–28.3	–40.7	–47.3	– * (n = 0)	–	9.4	11.4	12.5	–	–	–	–
Saga, Hex. + Me + Th	6	10.00 (Nx5.71)	48.0	–22.3	–30.3	–17.8	–40.5* (n = 4)	–19.8	10.5	13.6	12.9	12.4 (n = 4)	51.4	1.4	(–3.91 ± 0.46)
Saga, Hex. Alc. extr.	6	10.21 (Nx5.71)	48.0	–27.1	–38.5	–44.8	–46.4* (n = 2)	–22.0	10.0	12.0	10.8	9.6 (n=2)	50.5	5.15	(–4.32 ± 0.39)
Saga, Hex.Alc. extr. + Me + Th	6	9.80 (Nx5.71)	48.0	–21.5	–26.5	–30.1 (n=5)	–33.6* (n=5)	–16.0	12.4	15.5	14.7 (n=5)	14.7 (n=5)	57.3	5.62	(–2.95 ± 0.90)
Saga Hex.	6	10.22 (Nx5.71)	51.5	– 8.6	– 5.7	3.4	15.3	7.8	22.1	27.5	32.5	34.2	116.2	11.87	0.65 ± 0.23
Saga Hex. + Me + Th	6	10.27 (Nx5.71)	51.5	9.8	38.9	62.8	91.5	47.0	30.2	50.3	52.2	58.0	190.6	19.58	2.41 ± 0.22
Saga Hex. Alc.	6	10.55 (Nx5.71)	51.5	– 3.9	2.92	12.7	24.6	2.5	27.0	33.0	35.5	38.5	134.0	14.13	0.88 ± 0.17
Saga Hex. Alc. + Me + Th	6	10.59 (Nx5.71)	51.5	11.7	40.8	63.9	90.0	46.3	32.8	51.1	50.1	54.6	188.6	19.97	2.32 ± 0.09

*) The number (n) within brackets indicates the number of surviving rats, as the XP–rats started to die.

From the result of this experiment with detoxicated saga, one may therefore conclude that ordinary moist-heat treatment such as boiling or steaming, must be sufficient to inactivate the tryprin inhibitor, haemagglutinin and saponines present in the raw sagabeans.

CONCLUSION

The still unidentified toxic substance (s) in the sagabean is present in the oil fraction. Defatting with n-hexane will eliminate the toxicity of the saga meal.

Ordinary household cooking procedures such as moist heat treatment (boiling or steaming) proves to be sufficient or adequate to inactivate the anti-nutritive factors, but these methods alone will not be capable to detoxicate the sagabean.

Soaking the cotyledons after crushing and decortication of the beans with repeated washings will result in considerable loss of soluble matters.

The amino-acid pattern of the saga protein proves to be not as good as soy protein as was mentioned previously. The sulfurcontaining amino-acids (a.o. methionine), threonine and tryptophan are limiting.

CONCLUDING REMARKS

- Subacute toxicity feeding test with young rats being carried out in our laboratory to obtain more information on the toxic nature of the saga oil.
- Acute toxicity tests with youg rats will be carried out with the extracted saga oil in cooperation with the National Institute for Chemistry (LKN–LIPI) Bandung.
- Roasted sagabeans, which are consumed as snacks by certain segments of the population in the northern part of Central-Java, are also being investigated.

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REFERENCES

1. Oey Kam Nio, Lie Goan Hong, J. Herlinda, G. Nainggolan–Sihombing, Risnawati Aminah and Sumardi. (1981). An Unknown Toxic (or anti-nutritive) Substance in the sagabean. Health Studies in Indonesia. Vol. 9. no. 1 : 37 – 45. 1981.

2. Oey Kam Nio, J. Herlinda, G. Nainggolan–Sihombing, Lie Goan Hong. (1983). More Evidence on the Presence of an Unknown Toxic Substance (s) in the Sagabean. Paper presented at the Fourth Asian Congress of Nutrition, held in Bangkok, Thailand.