EFFECT OF THE BOILING AND DECANTING METHOD OF KHESARI *(Lathyrus sativus)* DETOXIFICATION, ON CHANGES IN SELECTED NUTRIENTS

Krishna Jha[1]

College of Basic Sciences and Humanities
Rajendra Agricultural University
Pusa (Samastipur), India

SUMMARY

It is a well-known fact that the legume Khesari *(Lathyrus sativus)* causes lathyrism, a disease characterized by paralysis of the lower limbs in human beings. The toxic constituent is an amino acid identified as B-Oxalyl-Amino L-Alanine (BOAA).

It has been reported that if the legume is boiled for two hours and the water is then decanted, almost 85°/o of the toxic amino acid is eliminated. Therefore, this investigation constitutes an effort to prevent the loss of other nutrients, simultaneously to the elimination of toxicity.

As has been observed, as much as half the protein content, as well as 80.36°/o total sugars, 63.13°/o reducing sugars, 86.05°/o amino acids, and all thiamine, riboflavin and niacin are lost from dahl (dehulled, separated cotyledons), while the respective losses from the whole seeds are 47.25°/o, 45.73°/o, 74.69°/o and 80.00°/o, and all vitamins, in just a one-hour treatment. The losses of the toxic amino acid from dahl and whole seeds are 71.46°/o and 68.74°/o, respectively. The data for losses occurring in the two-hour and three-hour treatment are also described.

INTRODUCTION

Khesari *(Lathyrus sativus)* is a legume, well known to cause lathyrism in humans when consumed continuously. Nevertheless, it constitutes the staple food of a large number of people, mostly the poor masses in Eastern and Central India. This legume crop requires little cultivation practices and is highly resistant to drought conditions (1). The States of Madhya Pradesh and Bihar of India, alone, are known to produce seven to eight million tons of legume seeds, aside from the leaves which are consumed as

Manuscrito modificado recibido: 18-4-86.

1 Assistant Professor cum Junior Scientists, Department of Microbiology, College of Basic Sciences and Humanities, Rajendra Agricultural University, Pusa (Samastipur) 848 125, India.

vegetable, and used as fodder. The toxic constituent in this legume has been identified as B-Oxalyl Amino Alanine (BOAA) (2-4).

It has been reported that the above-mentioned toxin is not eliminated solely by boiling or roasting for a two-hour period, but if the excess water is decanted after the boiling treatment has been applied, the toxin level decreases by approximately 80-85% (5). Nevertheless, almost nothing is known as to what happens to the nutritive value of the food submitted to this treatment. The present investigation was therefore undertaken to study the effect that the boling and decanting treatment exerted on the proteins, carbohydrates, amino acids, thiamine, riboflavin, niacin and toxic constituents of Khesari, respectively.

MATERIAL AND METHODS

Boiling and Decanting Treatment

The whole seeds and dahl (dehulled and separated cotyledons) were washed apart in running tap water, so as to remove dust and dirt particles, etc. Then, weighed and transferred to 20 Erlenmeyer flasks of 500 ml capacity. Two hundred ml of tap water were added to each flask. One flask each of whole seeds and dahl were kept as control while the others were placed on a hot plate for boiling. All were covered with watch glass to avoid excess evaporation. After one, two and three hours boiling started, three flasks were removed from each group and the water immediately decanted. These materials were used for analysis.

Moisture Determination

A 10 g content from all flasks, including the controls, were transferred to pre-weighed moisture boxes and moisture determined by oven drying at 100°C (6).

Estimation of Protein, Carbohydrates and Amino Acids

Extraction – Two grams of material from each flask were thoroughly mashed three times in 20 ml of 80% aqueous ethanol, using a pestle mortar. After each extraction supernatants were collected by centrifugation at 3,000 rpm for 15 min, using a table centrifuge (Model-Remi, India). All supernatants were then pooled and flash-evaporated at 60°C to reduce final volume to 4 ml. Volumes were then adjusted with sterile distilled water when required, and the extracts were stored in a refrigerator at 4°C for future analysis.

Protein was determined by Lowry's method (7), and total and reducing sugar were estimated according to Dreywood (8) and Nelson-Somogyi (9), respectively.

Thiamine, riboflavin and niacin were microbiologically assayed using *Lactobacillus fermentum* (NRRL B-585), *L. plantarum* (NRRL B-531) and *L. casei* (NRRL B-1445), respectively. The stock cultures as well as samples and standard solutions were prepared as per Difco (10). The methodology of the Association of Vitamin Chemists (11) and Sarrett

and Cheldih (12), was followed in detail.

The BOAA contents were estimated by preparative paper chromatography. A 500 ml sample from each treatment was applied to a 46 X 57 cm Whatman No. 1 sheet throughout along the width, and 8 cm above the edge. The chromatograms were then run for 48 hr by the descending method using butanol:acetic acid:water (4:1:5) as solvent. The papers were dried at room temperature, and a strip of 1 cm width was cut along the length where the standard was also loaded. This strip was then developed with ninhydrin spray. By overlapping the developed strip on the full chromatogram, the BOAA band was demarcated and cut along the width. This strip containing BOAA was then eluted with an excess of 80°/o aqueous ethanol. After adjusting the volume to 1.0 ml by evaporation, the BOAA concentration was estimated colorimetrically according to the method of Jayaraman (13).

RESULTS AND DISCUSSION

Prior to presenting the findings of our study, it was deemed convenient to include, herein, the proximal composition of *Lathyrus sativus* seeds, considering that, as stated previously, it constitutes the staple food of large sectors of the poor masses in Eastern and Central India. Therefore, for information purposes, the reader is referred to Table 1. The main results of this research are now discussed.

Loss of BOAA

The toxic constituent BOAA was eliminated within one hour to the extent of 71.77°/o and 68.74°/o from dahl and whole seeds, respectively. The corresponding losses after two hours were 79.35°/o and 78.66°/o, which are slightly less than 80°/o, as reported by Liener (5). The lesser loss in whole seeds appears to be due to the hard-seed coat. Nevertheless, as boiling was increased to three hours, the loss in seed also became equivalent to that from dahl, probably on account of softening (Table 2).

Although such a loss appears to be quite appreciable, it cannot be considered altogether safe. According to some authors, the *L. sativus* toxin shows selective retention in brain tissues of monkey. Thus, foods with a low toxin content will produce symptoms of lathyrism after prolonged consumption, i.e. there will be slow poisoning-like effects. Moreover, the study herein presented also indicated complete loss of three important vitamins, thiamine, riboflavin and niacin, apart from proteins, carbohydrates and amino acid losses. Therefore, such a drastic treatment does not appear to be of much significance.

Loss of Protein

As much as 52.97°/o and 47.25°/o of soluble proteins were lost from dahl and whole seeds with one hour boiling, respectively. In the recommended 2 hr-treatment, the respective losses were 53.41°/o and 58.48°/o. The increase of loss in the whole seed can also be explained on the basis

TABLE 1

PROXIMAL COMPOSITION OF KHESARI *(Lathyrus sativus)* SEEDS

Constituents	Contents per 100 g (dry weight)
Proteins (N x 6.25)	28.5 g
Total sugars	56.6 g
Reducing sugars	17.8 g
Total amino acids	0.569 g
Thiamine	0.44 mg
Riboflavin	0.21 mg
Niacin	0.02 mg

of seed-coat softening, because after three hours the loss values have levelled off.

Loss of Carbohydrates

In the case of dahl, the loss was 80°/o of total sugars and 63.13°/o reducing sugars within one hour, while from whole seeds only 45°/o of total and 74.69°/o of reducing sugar were lost, probably due to the same basic reason of hard-seed coat. The higher loss of reducing sugars may be due to the higher content in the seed coat, and higher solubility.

Loss of Amino Acids

Once again, the loss of amino acids also was higher in dahl during the one-hr and two-hr treatments, but after three hours, the values in both dahl and whole seeds became almost equal (Table 2). A considerable variability in loss of total amino acids and BOAA -which is an amino acid- may be implicated. This could occur due to the fact that loss of nutrients during cooking largely depends on their solubility in water and on the substrate itself, as reported by Pushpama, Geervani and Krishnakumari (14). These authors have also observed such variation in case of loss of lysine from green gram *(Vigna aureus)* and bengal gram *(Cicer arietinum).* In the latter case, the respective losses were 20.9°/o and 51.3°/o during fat-frying for a minute and-a-half only.

Loss of Vitamins

It is well known that vitamins are heat-labile; therefore, it is not altogether unusual for such a drastic loss to occur during the one-hour cooking treatment. According to Vijaylaxmi (15), merely frying for one and a half minutes, leads to a complete loss of vitamins, irrespective of the substrate.

ACKNOWLEDGEMENTS

The author is highly thankful to the authorities of Rajendra Agricul-

TABLE 2

LOSS OF NUTRIENTS FROM Lathyrus sativus BY THE BOILING AND DECANTING DETOXIFICATION METHOD

Material	Treatment	Per cent* loss (dry weight basis) of							
		BOAA	Proteins	Total sugars	Reducing sugars	Total amino acids	Thiamine	Riboflavin	Niacin
Dahl	1 hour	71.47	52.97	80.36	63.13	86.05	100	100	100
	2 hours	79.35	53.41	82.43	66.62	86.81	—	—	—
	3 hours	87.98	53.75	83.82	80.23	86.89	—	—	—
Whole seed	1 hour	68.74	47.25	45.73	74.69	80.00	100	100	100
	2 hours	78.66	58.48	63.88	82.44	85.17	—	—	—
	3 hours	88.98	59.15	82.68	89.98	91.37	—	—	—

* Averages of three replications.

tural University for providing the necessary facilities to perform this research. He also extends his sincere appreciation to Dr. K.D.N. Singh and Professor M. Mishra, for supplying the BOAA standard compound.

RESUMEN

EFECTO DEL METODO DE DETOXIFICACION, POR EBULLICION Y DECANTACION DEL KHESARI *(Lathyrus sativus)* SOBRE CAMBIOS EN NUTRIENTES SELECCIONADOS

Es un hecho más que establecido que la leguminosa conocida como Khesari *(Lathyrus sativus)* causa latirismo, enfermedad que en los humanos se caracteriza por parálisis de los miembros inferiores. El constituyente tóxico es un aminoácido identificado como "B-Oxalyl-amino L-alanina" (BOAA).

Se ha informado que si la leguminosa se hierve durante dos horas, y luego se decanta el agua, se logra eliminar casi el 85°/o del aminoácido. Esta investigación, por lo tanto, constituye un esfuerzo para prevenir que —en forma simultánea a la eliminación de la toxicidad— ocurran pérdidas de otros nutrientes.

En el caso del dahl (descascarados y separados los cotiledones), se ha observado que se pierde casi la mitad de proteínas, 80.36°/o de azúcares totales, 63.13°/o de azúcares reductoras, 86.05°/o de aminoácidos, y toda la tiamina, riboflavina y niacina, mientras que con tan sólo una hora de tratamiento, las pérdidas respectivas de las semillas enteras son de 47.25°/o, 45.73°/o, 74.69°/o y 80.00°/o, así como todas las vitaminas. Las pérdidas del aminoácido tóxico del dahl y de las semillas enteras son de 71.46°/o y 68.74°/o respectivamente. Se describen, asimismo, los datos en cuanto a las pérdidas que ocurren con el tratamiento de dos y de tres horas.

BIBLIOGRAPHY

1. Ganapathy, K.T. & M. Dwivedi. **Studies on Clinical Epidemiology of Lathyrism.** New Delhi, Indian Council of Medical Research, 1961.
2. Roy, D.N., V. Nagarajan & C. Gopalan. Production of neuro-lathyrism in chicks by the injection of *Lathyrus sativus* concentrates. **Current Sci., 32**:116, 1963.
3. Rao, S.L.N., P. Adiga & P.S. Sarma. Isolation and characteristics of B-Oxalyl L-a, B-diamino propionic acid. A neurotoxin from the seeds of *Lathyrus sativus*. **Biochemistry, 3:432, 1964.**
4. Murti, V.V.S., T.R. Sheshadri & S. Venkitsubraminian. Neurotoxic compound of the *Lathyrus sativus*. **Phytochemistry, 3**:73, 1964.
5. Liener, I.E. Cited in: **Chemistry and Biochemistry of Legumes.** S.K. Arora (Ed.). New Delhi, Oxford and IBH Publishing Co., 1982.
6. Association of Official Analytical Chemists. **Official Methods of Analysis of the AOAC.** 12th ed. Washington, D.C., The Associacion, 1975.
7. Lowry, O.H., N.J. Rosebrough, A.L. Farr & R.J. Randall. Protein measurement with Folin-phenol reagent. **J. Biol. Chem., 163**:265-275. 1954.
8. Dreywood, R. Cited in: **Methods in Enzymology,** Vol. III. Sidney P. Colowick and Nathan O. Kaplan (Eds.). New York, N.Y., Academic Press, Inc., 1957, p. 84, ftnote 32.
9. Nelson, N. A photometric adaptation of the Somogyii's method for determination of glucose. **J. Biol. Chem., 195**:19, 1944.

10. **Difco Manual of Dehydrated Media and Reagents for Microbiological and Clinical Procedures.** Detroit, Michigan, Difco Laboratories, Inc., 1977.
11. Association of Vitamin Chemists. **Methods of Vitamin Assays.** New York, N.Y., Interscience Publishers, 1966.
12. Sarrett, M.P. & H. Dheldlin. The use of *Lactobacillus fermentum* 36 for thiamine assay. **J. Biol. Chem., 155**:153, 1944.
13. Jayaraman, J. **Laboratory Manual of Biochemistry.** New Delhi, Wiley Eastern Ltd., 1982, p. 64-65.
14. Pushpamma, P., P. Geervani & K. Krishnakumari. Losses of some nutrients in cereals, millets and legumes; recipes commonly used in Andhra Pradesh. **Indian J. Nutr. Dietet., 19**:342, 1982.
15. Vijayalaxmi, C. Modified by Haanumantha, K. Rao & M. Subramanian. Essential amino acid composition of commonly used Indian pulses by paper chromatography. **J. Food Sci. Technol., 7**(1):31, 1981.