



Research Article

THE ROLE OF FOOD PROCESSING TECHNIQUES IN THE DETOXIFICATION OF ODAP IN *LATHYRUS SATIVUS*

Sujata Yerra, Swathi Putta and *Eswar Kumar Kilari

A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh

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ABSTRACT

Lathyrus sativus (Leguminaceae) is commonly known as grass pea. *Lathyrus sativus*, well known as grass pea or commonly as Kesari dal in India. The relation between the consumption of grass pea and lathyrism is well known where the cause is β -N-oxalyl-L- α , β -diaminopropionic acid (ODAP). Lathyrism is noticed in the cases of ODAP toxicity due to the consumption of the grass pea seeds, if more than 75% of the diet intake for a period of 3 months. The production and consumption of this nutrition rich crop and other bright prospects of grass pea are handicapped by the stigma of its toxicity. Since there is no data available on the detoxification of ODAP in *Lathyrus sativus* grown in India by employing different food processing techniques and that the pulse has excellent nutritional qualities, especially, protein and minerals. The present study aims to evaluate the detoxification of ODAP in grass pea seed samples collected from different States of India- West Bengal, Chattisgarh and Bihar by various food processing techniques of roasting, soaking prior to roasting, soaking prior to boiling, treatment with tamarind water, germination, autoclaving and frying in oil with varying processing time of 15, 25, 45 minutes.

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INTRODUCTION

The rich potential of grass pea is undermined though it is a good source of protein of about 28-32% (Urga et al., 2005). It has been serving as a staple food for the poor farmers and tribal folk in countries like India, Bangladesh, China and Ethiopia mostly during droughts and famines as it sustains the harshest of agroclimatic conditions. An epidemiological association between grass pea and neurolathyrism (crippling and paralysis of lower limbs) (Tacon, 1995) is well known and the main causative factor is a neurotoxic non-protein amino acid, commonly known as ODAP (2-N-Oxalyl-2, 3, diaminopropionic acid) or BOAA (Beta-Oxalyl-aminoalanine) (Spencer et al. 1983, 1986; Campbell et al., 1994; Hanbury et al., 2000; Smulikowska et al., 2008). Neurolathyrism is noticed in adults who consume large quantities of it, about 300g/day for prolonged period of 2-3 months. (Dwivedi et al., 1964; Getahun et al., 1999; Hanbury et al., 2000; Smulikowska et al., 2008). Some researchers independently isolated the neurotoxic non protein amino acid, ODAP (Murti et al., 1964; Rao et al., 1964) which is suspected culprit of neurolathyrism (Hanbury et al., 2000).

*Corresponding author : Dr. Eswar Kumar, K.,
Assistant Professor (Senior), A.U. College of Pharmaceutical
Sciences, Andhra University, Visakhapatnam-530003,
Andhra Pradesh, India.

In India, grass pea is well known as kesari dal (Central Government Act, 1955). The consumption and sale of kesari dal is banned in India (PFA, 1954). Though its consumption and sale is restricted by the Indian Government Agencies yet the continued cultivation and consumption is noticed in almost all States of the Indian subcontinent. This clearly is an indicator for its good acceptability in Indian human diets. In contrary, no outbreaks of lathyrism are noticed since three decades despite the regular consumption of kesari dal, especially by the poor peasants. It might be due to the fact that kesari dal is no more used as a staple food in human population and that the detoxification of ODAP present in it is practised by employing various food processing techniques at domestic levels. The high variability of the ODAP biosynthesis is the major problem in this endeavor. The ODAP levels in *Lathyrus sativus* are highly variable and depend on variety, growth location, soil, fertilisation, plant part and age (Gurung et al., 2011; Jiao et al., 2006; Jiao et al., 2011). Various environmental factors of drought, zinc deficiency, abundance of iron and the presence of heavy metals in the soil can considerably increase the level of ODAP in the seeds of *Lathyrus sativus* grown in farmers' fields as compared to more optimal experimental fields. The presence of cadmium in the soil can increase the ODAP level up to six-fold (Lambein et al., 2007).

Food processing plays an important role in the elimination of ODAP in *Lathyrus sativus*. The toxic amino acids get readily soluble in water and can be easily leached. Some of the moist heat food processing methods like boiling and steaming denature protease inhibitors depleting the protective sulphur amino acids and enhancing the toxic effect of raw grass pea. Steeping and boiling decreased ODAP levels by 90% (Padmaja prasad *et al.*, 1997), while extrusion reduced ODAP levels by 46% (Ramachandran *et al.*, 2004). It is a well known practise that the toxin can be removed from the seeds of *Lathyrus sativus* by simply soaking them in hot water and then discarding the latter or by consuming it after the removal of its coat.

Thus, about 65-85% detoxification of ODAP can be seen by other techniques of roasting, soaking prior to roasting, treatment with tamarind water, germination, autoclaving and frying in oil with varying processing time of 15, 25, 45 minutes. Hence, in the present study, the detoxification of the neurotoxic, non protein amino acid- ODAP in *Lathyrus sativus* seed samples collected from different States of India- West Bengal, Chattisgarh and Bihar, is carried out to unveil the prospects of the pulse and bring about public awareness regarding its safe consumption.

MATERIALS AND METHODS

Chemicals

Reagents used for analysis were purchased from Sigma Aldrich Company. All chemicals and reagents used were analytical reagent grade.

Sample Collection

Lathyrus sativus(LS) seed samples were collected from West Bengal, Chattisgarh and Bihar.

Sample Preparations

The seeds were cleaned manually to remove foreign matters, immature and damaged seeds.

Raw

The cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water and immediately dried in drying oven at 55 °C for 12 h, under air circulation, and then grind by grinder to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until analysis.

Food Processing Techniques

Roasting

The seeds of *Lathyrus sativus* were roasted at 180° C for 15, 30 and 45 minutes in open pan, over a gas burner.

Roasting After Soaking Seeds in Water

The seeds were soaked overnight in water and the next day the water was removed. The seeds were then roasted under the same conditions as mentioned earlier.

Boiling In Fresh Water

The seeds were boiled in fresh water after soaking overnight at 80-90° C until soft to press.

Soaking In Alkaline Water and Then Boiling

Seeds were soaked in 1% Calcium hydroxide solution in a ratio of 1:5 (W/V) for 6 hours and then wrapped in muslin cloth and cooked in boiling water for 15, 25, 45 minutes. The seeds were drained, dried and powdered.

Soaking In Tamarind Water and Then Boiling

The seeds were washed, soaked in tamarind water (1:3 W/V) for 6 hours. The tamarind slurry was prepared by stirring the tamarind pulp in water (10g pulp to 300ml water). After draining off the tamarind water, the seeds were rinsed with drinking water, boiled in water for 15, 25, 45 minutes and then drained. The seeds were drained, dried and powdered.

Germination

The soaked seeds were germinated on glass plates over a damp muslin cloth and then incubated for 30-36 hours for sprouting.

Autoclaving under Pressure

The seeds were soaked overnight, water removed, and then the seeds were pressure- cooked at 15 psi for 15, 30 and 45 minutes.

Frying In Oil

The overnight soaked, drained seeds were slightly dried and then deep fried in cooking oil (refined groundnut oil).

Chemical Analysis

ODAP Estimation

The DAP (diaminopropionic acid) and ODAP were estimated by the OPT method of Rao, 1978. This is a very sensitive, rapid and specific chemical method resulting in a colored product on the reaction of *O*-phthadehyde (OPT) with DAP.

Procedure

Grinding samples to BSS (British Standard Sieve) 100-mesh sized fine powder. 10-20g of the sample was extracted for 6 hours using 70% neutral alcohol. The clear supernatant was used for the estimation of the said toxin. Extraction under this condition is 99%. 100ml of the samples were heated in a boiling water bath in an open tube with 0.2ml. Of OPT reagent (0.1% OPT in 0.5 M Potassium tetraborate containing 0.2ml. of mercaptoethanol). A suitable blank without alkali hydrolysis was included and free DAP content of the sample was determined using OPT reagent.

RESULTS AND DISCUSSION

The food processing methods including roasting, soaking prior to boiling, germination, and autoclaving greatly influence the nutritive values of legumes.

Table 1. Effects of domestic cooking practices on detoxification of *Lathyrus sativus* seeds collected from West Bengal

Processing conditions	Temperature (°C)	Time (min.)	ODAP (mg/100g) (after processing)	% ODAP (destroyed in processing)
Control dry seeds	--	--	812	--
Roasting	180	15	493.8	39.2
	180	45	309.6	61.9
Roasting after soaking in water	180	15	257.2	68.3
	180	25	488.7	39.8
Boiling after overnight soaking	180	25	329.3	59.4
	180	45	281.2	65.4
	90	15	329.1	59.5
Tamarind water treatment	90	25	262.9	67.6
	90	45	243.7	69.9
	80	15	351.3	56.7
Germination		25	332.4	59.1
		45	299.4	63.1
	36	36 hrs.	444.3	45.3
Autoclaving	212	15	312.9	61.5
	212	25	298.2	63.3
Frying in oil	212	45	278.5	65.7
	180	3	229.2	71.8
		5	134.7	83.4

Table 2. Effects of domestic cooking practices on detoxification of *Lathyrus sativus* seeds collected from Chattisgarh

Processing conditions	Temperature (°C)	Time (min.)	ODAP (mg/100g) (after processing)	% ODAP (destroyed in processing)
Control dry seeds	--	--	823	--
Roasting	180	15	499.1	39.4
	180	25	322.2	60.9
Roasting after soaking in water	180	45	278.4	66.2
	180	15	490.2	40.4
Boiling after overnight soaking	180	25	345.8	57.9
	180	45	297.3	63.9
	90	15	342.9	58.3
Tamarind water treatment	90	25	289.3	64.8
	90	45	265.2	67.8
	80	15	376.3	54.3
Germination		25	356.8	56.6
		45	319.0	61.2
	36	36 hrs.	463.8	43.5
Autoclaving	212	15	335.2	59.3
	212	25	309.8	62.4
Frying in oil	212	45	292.1	4.5
	180	3	246.7	70.0
		5	160.3	80.5

Table 3. Effects of domestic cooking practices on detoxification of *Lathyrus sativus* seeds collected from Bihar

Processing conditions	Temperature (°C)	Time (min.)	ODAP (mg/100g) (after processing)	% ODAP (destroyed in processing)
Control dry seeds	--	--	838	--
Roasting	180	15	514.5	38.6
	180	25	367.5	56.1
Roasting after soaking in water	180	45	297.4	64.5
	180	15	509.3	39.2
Boiling after overnight soaking	180	25	370.2	55.8
	180	45	301.9	63.9
	90	15	363.2	56.7
Tamarind water treatment	90	25	302.5	63.9
	90	45	287.5	65.7
	80	15	394.3	53.1
Germination		25	378.4	54.8
		45	324.3	61.3
	36	36 hrs.	491.8	41.3
Autoclaving	212	15	358.3	57.2
	212	25	322.5	61.5
Frying in oil	212	45	301.4	64.0
	180	3	266.3	68.2
		5	197.7	76.4

Of these, soaking prior to boiling and germination plays an important role as it influences the bioavailability and utilization of nutrients and improves palatability, which incidentally may result in enhancing the digestibility and nutritive value (Ramakrishna *et al.*, 2006).

Previously, the effect of different processing techniques (roasting, germination and autoclaving) on the nutritive value of grass pea had also studied by Ramachandran and Ray, 2008. Different traditional processing methods including roasting, boiling food samples were collected and assayed for β -ODAP

levels by Teklehaimanot *et al.*, 1993. The effect of soaking time and soaking solution on the nutritional quality of grass pea seeds were investigated by Urga and Gebretsadik 1993. The effect of cooking, roasting, autoclaving and germination on the content of β -ODAP in the whole seeds and flour of grass pea were determined at different levels of temperature, time, pH, degree of soaking and moisture content by Akalu *et al.*, 1998. It was found that soaking of the seed in water could lower β -ODAP content but not sufficiently for continuous safe human consumption. Physical and chemical treatments have also been used in the detoxification. Further efforts would be a necessary for further improvements (Institute of Tropical Medicine, 2008). In the present investigation, the content of ODAP (mg /100gm) of *Lathyrus sativus* raw seeds was found to be 812 mg/100gm in West Bengal, 823 mg/100gm in Chattisgarh and 838 mg/100gm in Bihar. Boiling in water or repeated steeping in hot water and discarding the extracts can detoxify the seeds. Roasting of seeds, at 140°C for 15 to 20 minutes, result in 80 to 90 % destruction of the neurotoxins. Some people soak the seeds overnight and decant the water before cooking. This eliminates about 90% of the toxin. Toxic amino acids are readily soluble in water and can be leached. Germination is useful to reduce β -ODAP content. Moist heat (boiling, steaming) denatures protein inhibitors, which other wise add to the toxic effect of raw grass pea through depletion of protective sulfur amino acid (Rao SLN, 2001). When grass pea is processed, the protein inhibitor and other anti-nutritional factors, which inhibit the protein digestibility and chelate the mono, di and trivalent metal ions and form insoluble complexes will be degraded to a smaller molecular form and release the protein and the essential elements (Urga *et al.*, 2005).

Compound β 1 is a water- soluble amino acid present in the ODAP that can be leached from seed by soaking in water (Mohan *et al.*, 1966; Tekele-Haimanot *et al.*, 1993). Steeping grass pea in a large volume of cold water for 3 minutes, leached out approximately 30% of 1, with greater losses when hot water was employed (Tekele-Haimanot *et al.*, 1993). Similarly, steeping dehusked seed in hot water for several hours and boiling the seed in water removed 70–80% of the neurotoxin. Moslehuddin and colleagues, 1987 also found that washing seed partially removed the toxin to a greater level. Padmaja Prasad and associates, 1997 reported that boiling grain and discarding the water reduced 1 level up to 90%. Boiling has widely been used in the preparation of *Lathyrus sativus* seed as dahl, in bread-making and in vegetable preparations (Kay, 1979). In this study, during each food processing technique, a varying time of 15, 25 and 45 minutes was carried out, except for germination which was carried out for 36 hours and frying in oil was done for 3 and 5 minutes. It must be also noted that the processing time was a vital factor wherein we found that as the processing time is increased, the content of ODAP after processing is decreased manifold. It is also noticed that frying in oil was the best method as 61-83% of ODAP was found to be eliminated. This was followed by autoclaving, soaking prior to both boiling and roasting food processing techniques for the detoxification of ODAP in *Lathyrus sativus* seed samples collected from the States of West Bengal, Chattisgarh and Bihar. Thus, about 65-85% detoxification of the toxin- ODAP can be seen by the above-mentioned methods. This might be due to the very high temperatures employed during the food processing techniques.

Preliminary soaking of the samples prior to roasting as well as boiling is noticed to immensely detoxify ODAP rather than the food processing techniques of roasting without any preliminary soaking. Almost all recent research publications emphasize that there is no neurolathyrism when the pulse is consumed as part of a normal diet and this is very true in the Indian context. No legume other than *Lathyrus sativus* has in fact ever served as a staple food. (Surya S. Singh and S.L.N. Rao, 2013). Finally, it should be stressed that excessive consumption of grass pea is the single most predisposing factor of neurolathyrism. Moreover, it was found that causation of the disease is poorly understood both by the population and by health workers working in neurolathyrism-prone areas (Getahun *et al.*, 2002).

Conclusion

Hence, the use of various food processing techniques alongwith the efficient manipulation of the processing time on the grass pea seeds collected from different States of India, can invariably improve the optimal use and benefits of the pulse-grass pea, rendering it safe for human consumption. Therefore, this investigation constitutes an effective effort to prevent the outbreaks of neurolathyrism in human folklore simultaneously by the detoxification of ODAP present in *Lathyrus sativus*.

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