

Kinetics of degradation of ODAP in *Lathyrus sativus* L. flour during food processing

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Abstract

A nonprotein neurotoxic amino acid, β -*N*-oxalyl-L-2,3-diaminopropionic acid (ODAP), found in *Lathyrus sativus* (grass pea or chickling vetch) seeds is known to be relatively heat stable. The present study aims at development of a kinetic model for degradation of ODAP in *Lathyrus sativus* subjected to a defined set of processing conditions. This study was carried out at pH 4.0 and 9.2. Isothermal condition experiments were carried out over a temperature range of 60–120 °C. For nonisothermal conditions, three different cooking methods viz., – open pan, pressure cooking and cooking in recently developed and patented fuel efficient ‘EcoCooker’ were used. The degradation of ODAP was adequately modeled by Arrhenius type of equation. A mathematical model based on the time temperature data of the nonisothermal heat process and isothermal kinetic rate parameters has been developed to predict the degradation of ODAP in any nonisothermal heating process of known time temperature profiles.

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1. Introduction

Grass pea (*Lathyrus sativus* L.), an annual pulse crop belonging to the tribe *Vicieae* in the family *Fabaceae* (Biswas & Biswas, 1997) is known by a wide range of common names, include chickling vetch, Indian vetch, *khesari* or *batura* (India) and dhal (Muehlbauer & Tullu, 1997). The genus *Lathyrus* is believed to have originated in southwest and central Asia, with a significant subsequent spread to the east of the Mediterranean (Smartt, 1984). All grass pea lines appear to divide into two geographical origins – one group derives from the Indian subcontinent, and another from the Mediterranean/European region. The genus *Lathyrus* comprises over 150 species (Allkin, Macfarlane, White, & Adey, 1983). Among these, four species, viz., *L. sativus*, *Lathyrus odoratus*, *Lathyrus ochrus* and *Lathyrus aphaca* are found in India. *L. sativus* is cultivated for grain and *L. odoratus* is grown as ornamental. The

seeds of *L. sativus* contain 31% protein, 41% carbohydrate, 17% total dietary fiber (2% soluble and 15% insoluble), 2% fat and 2% ash, on a dry matter basis (Akalu, Johansson, & Nair, 1998; Aletor, Abd El Moneim, & Goodchild, 1994). It can be utilized as thickening agents in food pastes and sauces and for incorporation into foods designed for improved satiety and delayed glycaemic responses (Akalu et al., 1998). Grass pea grows well under adverse environmental conditions, and its many cultivars possess different attributes including the ability to resist drought and flooding, high climatic adaptability and the ability to grow in cool climates and at high altitudes (Tiwari & Campbell, 1996a, 1996b). One of the major drawbacks of grass pea is the presence of a major antinutritional compound β -*N*-oxalyl-L- α , β -diaminopropionic acid (ODAP) (Padmanaban, 1980; Wang, Chen, Chen, Qin, & Li, 2000; Zhao et al., 1999). Overconsumption of grass pea for an extended period of time can cause spastic paraparesis of the legs in up to 6% of the population, affecting mainly the young males. Since grass pea is deficient in cysteine and methionine, and consumption of cereals richer in these amino

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acids and condiments rich in antioxidants seem to be protective factors (Getahun, Lambein, Vanhoorne, & Van der Stuyft, 2003, 2005), malnutrition and oxidative stress have to be considered as contributing factors in the aetiology of neurolethyrism, together with the ingestion of the neurotoxin. The paralytic disease known as 'lathyrism' or 'neurolethyrism', manifests as paralysis of the leg muscles, muscular rigidity and weakness (Mehta, Ali, & Barna, 1994; Rao, Adiga, & Sarma, 1964). The action of β -ODAP is subtle; it hyper-excites the neurons causing spastic movements of the legs and leads to paralytic neurolethyrism. Children fed grass pea showed poor growth with underdeveloped nervous system (Grela, Studzinski, & Matras, 2001).

Naturally occurring ODAP exists in two isomeric forms, viz., α and β (Bell & O'Donovan, 1966), with α -isomer being less toxic (De Bruyn et al., 1994; Harrison, Nunn, & Hill, 1977). The concentration of the β -form is about 95% of the total ODAP. Isomerization of β -ODAP to α -ODAP is known to depend on time/temperature conditions (Padmajaprasad, Kaladhar, & Bhat, 1997). Thermal isomerization has been used for detoxification of ODAP. The equilibrium concentration of α - and β -ODAP has been reported to be in the ratio of 40:60 at 55–60 °C, and 30:70 at room temperature (Zhao, Li, Li, Chen, & Hu, 1999). The conversion of β -form to the α -form is also known to follow zero order rate law (Belay, Moges, Solomon, & Johansson, 1997). Long, Ye, and Xing (1996) observed that higher temperature and pH affects the isomerization of β -ODAP.

Different processing methods have been proposed for the reduction of overall ODAP content (Tekle-Haimont et al., 1993), but none could completely destroy β -ODAP. Cooking of seeds for 1 h at pH 8.0 in boiling water reduced β -ODAP content by 57%; presoaking in water prior to cooking reduces β -ODAP levels up to 67%. Roasting of seeds at 150 °C for 1 h reduced β -ODAP content by 82%, while dry autoclaving of seeds for 30 min reduced β -ODAP content by 39% (Akalu et al., 1998). Cooking and processing of grass pea only resulted in \approx 40% loss of β -ODAP (Padmajaprasad et al., 1997). Highest losses of 65–70% were observed in freshly boiled water, alkaline and tamarind solutions as compared to soaking in drinking water (Srivastava & Khokhar, 1996). Fermentation reduced β -ODAP by 80–90% (Kuo, Bau, Quemener, Khan, & Lambein, 1995).

The most widely used method for ODAP detection is the colorimetric method (Rao, 1978). It involves an alkaline hydrolysis and a colour reaction between *o*-phthaldehyde (OPT) with α,β -diaminopropionic acid (DAP). The yellow color developed was measured at 420 nm on a spectrophotometer. Other methods are flow injection analysis (Moges & Johansson, 1994), paper chromatography, liquid chromatography with precolumn derivatization with 1-fluoro-2,4-dinitrobenzene (Wang et al., 2000, Wang, Chen, Chen, Qin, & Li, 2003) and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) (Chen, Wang, Chen, Qin, & Li, 2000), capillary zone electrophoresis (Arentoft &

Greirson, 1995) and HPTLC (Paradkar, Singhal, & Kulkarni, 2003). Of these, the TLC method was found to be simple, rapid, reliable and inexpensive.

Kinetic studies helps engineers and scientists to optimize processing systems and design processes, to improve or optimize existing processes and develop control systems for processing operations by finding rates of reaction that occur during heat processing operations. Knowledge of the rates of various reactions helps to predict how quick the reaction mixture is able to move to its equilibrium condition and its mechanism (Atkins, 1990). The destruction of antinutritional factors (ANF) is a function of temperature, heating protocol, moisture content or water activity, pH, pressure, amount of reactants and many other experimental conditions (Fennema & Tannenbaum, 1985). Knowledge of kinetic modeling aids predicting the intentional or unintentional food quality losses or improvement like by destruction of toxins as a function of processing conditions.

Different processing methods are being used in usual household cooking. Some of them are, normal open pan cooking, pressure cooking, and slow cooking using slow cookers. A fuel-efficient cooker has been recently developed in our institute (Joshi & Patel, 2003). The principle of this cooker is based on multiple effect evaporation; slow heating proportional to pick up rate of heat by the cooking vessel, and insulation; and on the logic of combining these principles in one unit. Literature survey done over the past 50 years did not give any information on the degradation kinetics of ODAP in foods cooked differently.

This study was undertaken with the following objectives: (1) to determine the kinetic parameters for ODAP degradation in *L. sativus* over a temperature range of 60–120 °C (isothermal conditions); (2) to study the degradation kinetics of ODAP under different cooking methods (nonisothermal conditions); (3) to develop a mathematical model relating the calculated kinetic rate data from the isothermal conditions and extend the same by taking into account the time temperature profiles under different cooking methods (nonisothermal conditions), and (4) to apply this model to predict the degradation of ODAP for nonisothermal process from its time temperature data, and comparing it with the observed degradation. This could then be used to assess the nutritional values of the cooked item as a function of the method of cooking.

2. Materials and methods

2.1. Materials

Standard β -*N*-oxalyl-L-2,3-diaminopropionic acid (β -ODAP) was supplied by Dr. S.L.N. Rao of Lathyrus Technologies, Hyderabad, India, TLC plates of silica gel GF₂₅₄ (0.25 mm) were purchased from E. Merck, Germany. Grass pea samples of four varieties namely Ratan, Pusa, Prateek and local market samples were generously provided by Indira Gandhi Krishi Vidyapeeth, Raipur, India.

All other reagents used were of analytical grade. Oil and water baths were used with an accuracy of $\pm 1^\circ\text{C}$.

2.2. Sample preparation

Seeds of *L. sativus* was dehusked and spilt. They were ground to pass through 200-mesh screen in cyclotech 1093 sample mill (Tecator). Samples were kept in an air-tight container at $+20^\circ\text{C}$.

2.3. Heat treatment

Heat treatments were carried out at different temperatures (60, 80, 90, 100, and 120°C) for 0–60 min. A water bath was used as a heating device for temperatures up to 100°C , while for the studies at 120°C an oil bath was used. 1 g of ground *L. sativus* was suspended in 10 ml buffer of pH 4.0 and pH 9.2 and heated at various time/temperature conditions including 120°C on an oil bath, after which the ODAP contents of the entire mass were analyzed as reported in Section 2.6 below.

2.4. Cooking methods

For cooking studies normal open pan cooking (60 min at a gas flow rate of 1283 Kcal/h), pressure-cooking (20 min at a gas flow rate of 1283 Kcal/h) and the newly developed slow cooker named 'EcoCooker' (60 min at 'sim', i.e., at a gas flow rate of 513 Kcal/h and 30 min holding period without any further heating) were selected as different cooking method for the grass pea flour. The time of heating at the desired temperature and gas flow rates for open pan and pressure-cooking were selected as per the protocol used in the household practices. The heating time and gas flow rates for 'EcoCooker' was selected as per the instructions given for its usage (Joshi & Patel, 2003). The samples were withdrawn periodically, and analyzed for ODAP content.

2.5. Time–temperature data

Time–temperature data for each cooking methods was monitored using a thermocouple with a measuring accuracy of $\pm 1^\circ\text{C}$.

2.6. Determination of ODAP by HPTLC

2.6.1. Standard curve for ODAP

Standard solutions were prepared by dissolving standard ODAP in dilute sodium bicarbonate (0.01 mg/ml). 10 μl of aliquots (containing 0.02–0.70 μg) was applied by Camag Linomat IV applicator on silica gel GF₂₅₄ TLC plate, and run twice up to 9 cm using *n*-butanol–pyridine–water (10:10:5) as the mobile solvent system. The plates were visualized by 0.2% ninhydrin in acetone and heating for 15 min at 120°C . The plates so developed gave spot corresponding to standard ODAP at $R_f \approx 0.21$ which were densitometrically determined at 500 nm on Camag II

densitometer. The peak area corresponding to the spot was plotted against concentration of ODAP, μg (x). The standard curve so obtained gave a regression equation, $y = 1426.8x$ ($R^2 = 0.97$).

2.6.2. Statistical parameters

Suitability of the method developed was found by studying the statistical parameters. The reproducibility of the method was investigated by performing replicate analysis. Less than 1% standard deviation was observed, indicating the method to be precise. Recovery studies were carried out by standard addition technique (at three different concentrations in triplicate) using low toxin variety of grass pea. This analytical technique was reproducible and gave 96.50 percent recovery. Further the values of confidence intervals at 95% and 99% confidence level were calculated using the formula:

$$X \pm \frac{Z\sigma}{\sqrt{N}},$$

where X is the experimental mean, σ is the standard deviation and $Z\sigma$ is the confidence level.

The confidence interval for the method found was 4.5 ± 0.47 at 95% confidence interval and 4.5 ± 0.61 at 99%. Limit of detection found for the visual and signal to noise ratio method was 0.07 μg .

2.7. ODAP content in grass pea flour

One gram grass pea flour was transferred into 50 ml test tubes containing 10 ml of buffer of pH 4.0 and 9.2, and subjected to the desired time/temperature conditions. After the specified time of heat treatment(s), samples were extracted with water 10 ml distilled water and 0.1 ml 1 N HCl for 18 h at room temperature on mechanical orbital shaker. The ODAP containing extract was then centrifuged. Then total volume was made to 25 ml. A deproteinisation procedure was used to separate the nonprotein amino acid, ODAP. A 0.5 ml aliquot of the extract was mixed with 1 ml acetonitrile in an Eppendorf tube, and left standing overnight at 20°C . The proteinaceous precipitate so formed was removed by centrifugation (10000 rpm, 10 min). A 10 μl of aliquots was applied by Camag Linomat IV applicator on silica gel GF₂₅₄ TLC plate. The solvent system used was *n*-butanol–pyridine–water (10:10:5). The plates were visualized by 0.2% ninhydrin in acetone and heating for 15 min at 120°C . The α -isomer showed a peak at $R_f \approx 0.18$ and the β -isomer showed a peak at $R_f \approx 0.21$. Quantification of the β -isomer was densitometrically determined at 500 nm on Camag II densitometer.

2.8. Kinetic calculations

A general reaction rate expression for the degradation kinetics can be written as follows (Labuza & Riboh, 1982; Ramaswami, Van De Voort, & Ghazala, 1989; Van Boekel, 1996).

$$-d[C]/dt = k[C]^m, \quad (1)$$

where 'C' is the quantitative value of the concentration of the target molecule under consideration, 'k' is the reaction rate constant (min^{-1}), and 'm' is the order of the reaction. The equation for first order kinetics ($m = 1$) after integration of Eq. (1) can be written as

$$\text{Ln}([C]_t/[C]_0) = -kt, \quad (2)$$

where '[C]₀' is the concentration of the reactants under consideration at time 0, and '[C]_t' is the value after reaction (heating) time 't'.

The relationship of the reaction rate to temperature is generally quantified by the Arrhenius relationship as indicated below:

$$k = A_0 \exp(-E_a/RT), \quad (3)$$

where 'E_a' (cal/mole) is the activation energy of the reaction, 'R' is the gas constant, 'T' is absolute temperature (°C), and 'A₀' is a pre-exponential constant.

Each experiment was done in triplicates and the average values were taken for the kinetic parameters estimation. Kinetic data were analyzed by regression analysis using MS Excel.

3. Results and discussion

3.1. Amount of ODAP in *L. sativus* flour at isothermal conditions

Varietal analysis of the grass pea samples showed the local variety to contain the highest amount of ODAP (4.5 ± 0.63 mg/g) of flour, while those in Pusa, Prateek and Ratan had 2.42 ± 0.20 , 1.12 ± 0.07 and 1.22 ± 0.01 mg/g ODAP, respectively. Hence, the local variety was used to carry out all further studies. The grass pea seed coat contains very little ODAP (0.1 ± 0.01 mg/g). [Deshpande and Campbell \(1992\)](#) have reported ODAP content to vary from 0.2 to 7.2 mg/g depending on the type and location of the variety. [Table 1](#) shows the effect of heat treatments at different temperatures 60–20 °C over different time periods on the resultant concentration of ODAP at pH 4.0 and 9.2, respectively. The initial concentration of ODAP in flour found in the study was 4.5 mg/g. During isothermal heat treatment, maximum of about 54% loss

of ODAP was observed at 120 °C at pH 9.2. ODAP is reported to be a relatively heat stable compound. In alkaline medium (pH 9.2), greater loss of ODAP was observed more than in acidic medium (pH 4.0). Similar observations have been reported by [Akalu, Tufvesson, Jonsson, and Nair \(1998\)](#) and [Gupta \(1980\)](#). ODAP is water-soluble. Its reduction could be due to subsequent isomerization of ODAP during heating.

3.2. Kinetic data for degradation of ODAP in grass pea flour

In order to arrive at the reaction rate constants, a first order degradation was presumed. Accordingly, ' $\text{Ln}([C]_t/[C]_0)$ ' was plotted versus 't', from which rate constant, 'k' was calculated as the slope of the line. A correlation coefficient >0.9 in all the cases confirmed the assumption of degradation to follow the first order kinetics. Similar data were obtained at all the other temperatures.

$T_{1/2}$, the time required for ODAP to degrade to 50% of its original value was calculated from the rate constant as ' $0.693/k$ '. [Table 2](#) shows the rate constants and ' $t_{1/2}$ ' (min) for ODAP in grass pea flour at pH 4.0 and pH 9.2. It is evident that the rate of ODAP degradation increased with an increase in the temperature. At pH 4.0, it increased from 0.0041 min^{-1} at 60 °C to 0.0132 min^{-1} at 100 °C. At pH 9.2, it increased from 0.0055 min^{-1} at 60 °C to 0.0128 min^{-1} at 100 °C. Activation energies E_a (cal M^{-1}) was calculated as a product of gas constant, R ($1.987 \text{ cal M}^{-1} \text{ K}^{-1}$) and the slope of the graph obtained by plotting ' $\text{Ln}k$ ' versus ' $1/T$ '. [Fig. 1](#) shows the Arrhenius plots for ODAP degradation in grass pea flour under acidic and alkaline conditions. The linear nature of the plot obtained gave activation energy of ODAP degradation in *L. sativus* flour as 7.29 kcal/mole at pH 4.0, and 5.48 kcal/mole at pH 9.2.

3.3. Time–temperature data of the three modes of cooking

To extend the results obtained from isothermal heat treatment experiments to the nonisothermal condition encountered in the three modes of cooking, viz., open pan cooking, pressure cooking and cooking in 'Eco-Cooker', time–temperature data during the processing of each was recorded ([Fig. 2](#)).

Table 1
Effect of heating on ODAP concentration^a (mg/g) in *Lathyrus sativus* flour^b at various temperatures at pH 4.0 (A) and 9.2 (B)

Time (min)	60 °C	80 °C	90 °C	100 °C	120 °C
15 (A)	4.39 ± 0.13	4.38 ± 0.49	4.34 ± 0.24	4.33 ± 0.34	3.66 ± 0.25
15 (B)	4.41 ± 0.10	4.33 ± 0.20	4.29 ± 0.18	4.14 ± 0.10	3.79 ± 0.24
30 (A)	3.95 ± 0.02	3.84 ± 0.54	3.83 ± 0.01	3.69 ± 0.21	3.13 ± 0.14
30 (B)	4.04 ± 0.25	3.96 ± 0.04	3.85 ± 0.35	3.56 ± 0.19	3.46 ± 0.08
45 (A)	3.75 ± 0.11	3.52 ± 0.14	3.26 ± 0.04	3.17 ± 0.04	2.31 ± 0.25
45 (B)	3.79 ± 0.02	3.23 ± 0.03	3.17 ± 0.52	3.11 ± 0.30	2.60 ± 0.03
60 (A)	3.64 ± 0.01	2.94 ± 0.27	2.65 ± 0.11	2.38 ± 0.29	2.10 ± 0.09
60 (B)	3.42 ± 0.10	2.97 ± 0.02	2.47 ± 0.29	2.28 ± 0.53	2.24 ± 0.14

^a Values are mean \pm SD of three or more individual determinations.

^b The ODAP content of the *L. sativus* flour chosen in the study was 4.5 ± 0.63 mg/g.

Table 2
Rate constant and half-life of ODAP degradation in *Lathyrus sativus* flour at pH 4.0 and pH 9.2

Temperature (°C)	pH 4.0		pH 9.2	
	Regression equation for degradation (R^2)	$T_{1/2}$ (min)	Regression equation for degradation (R^2)	$T_{1/2}$ (min)
60	$Y = -0.0041x + 0.0161$ (0.93)	169.02	$Y = -0.0055x + 0.0631$ (0.99)	126.00
80	$Y = -0.0086x + 0.1065$ (0.98)	80.58	$Y = -0.0094x + 0.119$ (0.98)	73.72
90	$Y = -0.0109x + 0.1479$ (0.99)	63.58	$Y = -0.0123x + 0.1741$ (0.97)	56.34
100	$Y = -0.0132x + 0.1889$ (0.98)	52.50	$Y = -0.0123x + 0.1394$ (0.96)	54.14
120	$Y = -0.0132x + 0.0071$ (0.96)	52.90	$Y = -0.0124x + 0.0457$ (0.96)	55.88

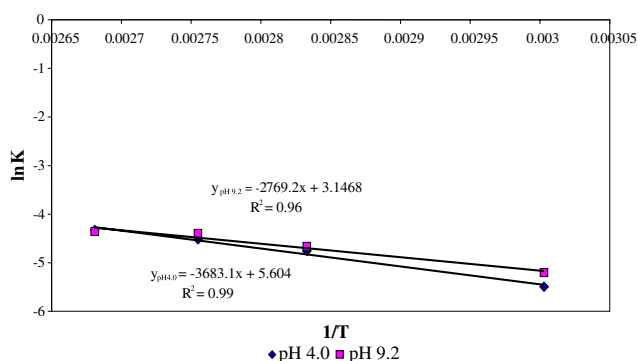


Fig. 1. Arrhenius plot for ODAP degradation in *Lathyrus sativus* flour at pH 4.0 and pH 9.2.

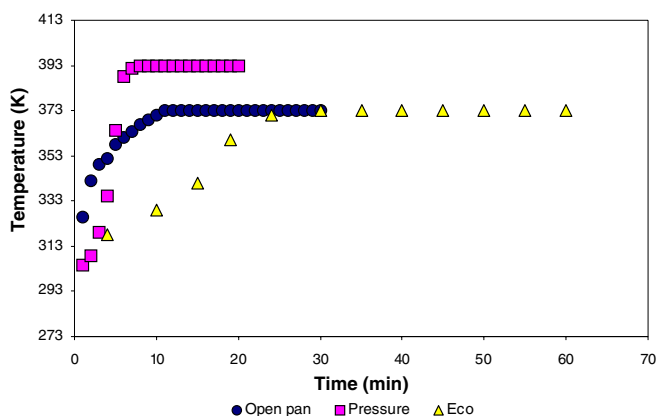


Fig. 2. Time-temperature profile of the different cooking methods used.

3.4. Degradation profile and half-life values of ODAP in *L. sativus* flour under the three modes of cooking

ODAP degradation was followed in each of these modes of cooking similarly as for *L. sativus* flour heat treatment under nonisothermal conditions. The results are documented in Table 3. The loss of ODAP was higher in alkaline condition than in acidic condition. Long et al. (1996) and Chavan et al. (2003) have also observed greater loss of ODAP in basic medium.

3.5. Prediction of ODAP loss during unsteady state heating/processing

To predict the amount of ODAP degradation occurring in the *L. sativus* flour during a given nonisothermal cook-

ing process, the following equation was derived from the integrated first order rate law.

$$k_i = A_0 \exp(-E_a/RT_i), \quad (4)$$

where ' k_i ' is the rate constant at time ' t_i ' which depends on the temperature T (K) at that time. ' E_a ' is the activation energy of the reaction, ' R ' is the gas constant and A_0 is a pre-exponential constant, which are already calculated from the isothermal experiments. The rate constant ' k_i ' at each temperature was calculated using Eq. (4) substituting for ' T ' from the time temperature data under nonisothermal cooking operations. Knowing the rate constant ' k ' at that temperature from the earlier isothermal experiments, the rate dC/dt_i , the amount degraded during the time interval zero to t_i , and the final concentration ' C ' can be calculated as follows.

Rate = Rate constant $k_i \times$ Initial concentration C .

Amount degraded during $t_i (\Delta C) = \text{Rate} \times t_i$.

Concentration after time $t_i = C - \Delta C$.

These calculations were continued for the entire time period (heating and constant temperatures) for which each of the cooking process was carried out. An MS excel based computer program was used to calculate the above parameters.

The total amount degraded after complete cooking = $\sum \Delta C$. The final concentration thus will be = $C_0 - \sum \Delta C$, where C_0 is the initial concentration of ODAP present in the *L. sativus* flour. The resulting predictions and the actual degradation obtained experimentally are given in Table 4. As seen, is a reasonably good agreement between the actual and the predicted degradation/retention of ODAP has been obtained. Using this method, the degradation of ODAP can be predicted for any heat processing method, if the time-temperature profile of that processing operation is known.

4. Conclusions

The pressure-cooking showed marginally higher extent of degradation of ODAP than open pan cooking and Eco-Cooker at acidic and basic conditions. 'EcoCooker', although, fuel-efficient showed similar degradation compared to normal open pan. The work accentuates the need to study the degradation of other antinutrients during food processing.

Table 3
Degradation profile and kinetics of ODAP in *Lathyrus sativus* flour at different cooking methods at pH 4.0 and 9.2

Method of cooking	Time (min)	ODAP concentration ^a (mg/g)		Rate constant, k^b (min^{-1}) (R^2)		$t_{1/2}$ (min)	
		pH 4.0	pH 9.2	pH 4.0	pH 9.0	pH 4.0	pH 9.2
Pressure cooking	15	2.29 ± 0.34	1.92 ± 0.05	0.0170 (0.99)	0.013 (0.96)	40.76	53.30
	30	1.85 ± 0.36	1.54 ± 0.09				
	45	1.45 ± 0.45	1.37 ± 0.08				
	60	1.11 ± 0.12	1.03 ± 0.02				
Open pan cooking	15	3.17 ± 0.15	3.00 ± 0.05	0.0121 (0.94)	0.0112 (0.96)	57.27	61.87
	30	2.50 ± 0.48	2.68 ± 0.17				
	45	2.38 ± 0.23	2.06 ± 0.04				
	60	1.83 ± 0.23	1.87 ± 0.01				
EcoCooking ^c	15	3.45 ± 0.50	3.62 ± 0.07	0.0098 (0.89)	0.0096 (0.96)	70.71	72.18
	30	2.58 ± 0.10	2.94 ± 0.09				
	45	2.36 ± 0.07	2.76 ± 0.02				
	60	2.18 ± 0.11	2.29 ± 0.30				

^a Values are mean ± SD of three or more individual determinations.

^b The content of the ODAP chosen in the study was 4.5 ± 0.63 mg/g.

^c The readings were taken after 30 min holding period as per the protocol for EcoCooker.

Table 4
The actual and predicted retention of ODAP in the cooking methods

Cooking methods	ODAP content (mg/g) ^a at pH 4.0		ODAP content (mg/g) ^a at pH 9.2	
	Predicted retention	Actual retention	Predicted retention	Actual retention
Open pan	2.08	1.83	2.07	1.86
Pressure	1.37	1.11	1.56	1.01
EcoCooking	2.27	2.18	2.22	2.29

^a The ODAP content of the *Lathyrus sativus* flour chosen in the study was 4.5 ± 0.63 mg/g.

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References

- Akalu, G., Johansson, G., & Nair, B. M. (1998). Effect of processing on the content of b-N-oxalyl- α,β -diaminopropionic acid (β -ODAP) in grass pea (*Lathyrus sativus*) seeds and flour as determined by flow injection analysis. *Food Chemistry*, 62, 233–237.
- Akalu, G., Tufvesson, F., Jonsson, C., & Nair, B. M. (1998). Physico-chemical characteristics and functional properties of starch and dietary fiber in grasspea seeds. *Starch/Stärke*, 50, 374–382.
- Aletor, V. A., Abd El Moneim, A., & Goodchild, A. V. (1994). Evaluation of the seeds of selected lines of three *Lathyrus* spp. for β -N-oxalylamino-L-alanine (BOAA), tannins, trypsin inhibitor activity and certain in vitro characteristics. *Journal of the Science of Food and Agriculture*, 65, 143–151.
- Allkin, R., Macfarlane, T.D., White, R.J., & Adey, M.E. (1983). Name and synonyms of species and subspecies in the viciae. Viciae database project publication, Issue No. 2 Southampton.
- Arentoft, A. M. K., & Greirson, B. N. (1995). Analysis of 3-(N-oxalyl)-L-2,3-diaminopropanoic acid and its alpha-isomer in grasspea (*Lathyrus sativus*) by capillary zone electrophoresis. *Journal of Agricultural and Food Chemistry*, 43, 942–945.
- Atkins, P. W. (1990). The rates of chemical reaction. In P. W. Atkins (Ed.), *Physical chemistry* (4th ed., pp. 778–811). Oxford: Oxford University Press.
- Belay, A., Moges, G., Solomon, T., & Johansson, G. (1997). Thermal isomerisation of the neurotoxin b-N-oxalyl-L- α,β -diaminopropionic acid. *Phytochemistry*, 45, 219–223.
- Bell, E. A., & O'Donovan, P. (1966). The isolation of a and c-oxalyl derivatives of a, c-diaminobutyric acid from seeds of *Lathyrus latifolius*, and the detection of the α -oxalyl isomer of the neurotoxin α -amino- β -oxalylaminopropionic acid which occurs together with the neurotoxin in this and other species. *Phytochemistry*, 5, 1211–1219.
- Biswas, S. C., & Biswas, A. K. (1997). Induced translocation heterozygosity and sterility in *Lathyrus sativus* L. *Bangladesh Journal of Botany*, 26, 131–136.
- Chavan, U. D., Shahidi, F., Chavan, J. K., & Amarowicz, R. (2003). Methanol-ammonia-water extraction of beachpea and grasspea. *Journal of Food Science and Technology*, 40, 209–211.
- Chen, X., Wang, F., Chen, Q., Qin, X. C., & Li, Z. X. (2000). Analysis of neurotoxin 3-N-oxalyl-L-2,3-diaminopropionic acid and its α -isomer in *Lathyrus sativus* by high-performance liquid chromatography with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatisation. *Journal of Agricultural and Food Chemistry*, 48, 3383–3386.
- De Bruyn, A., Becu, C., Lambein, F., Kebede, N., Abegaz, B., & Nunn, P. B. (1994). The mechanism of the rearrangement of the neurotoxin b-ODAP to a-ODAP. *Phytochemistry*, 36, 85–89.
- Deshpande, S. S., & Campbell, C. G. (1992). Genotype variation in BOAA, condensed tannins, phenolics and enzyme inhibitors of grass pea (*Lathyrus sativus* L.). *Canadian Journal of Plant Science*, 72, 1037–1047.

- Fennema, O. R., & Tannenbaum, S. R. (1985). Introduction to food chemistry. In O. R. Fennema (Ed.), *Food chemistry* (pp. 1–20). New York: Marcel Decker.
- Getahun, H., Lambein, F., Vanhoorne, M., & Van der Stuyft, P. (2003). Food-aid cereals to reduce neurotoxicity related to grass-pea preparations during famine. *Lancet*, *362*, 1808–1810.
- Getahun, H., Lambein, F., Vanhoorne, M., & Van der Stuyft, P. (2005). Neurotoxicity risk depends on type of grass pea preparation and on mixing with cereals and antioxidants. *Tropical Medicine and International Health*, *10*, 169–178.
- Grela, E. R., Studzinski, T., & Matras, J. (2001). Antinutritional factors in seeds of *Lathyrus sativus* cultivated in Poland. *Lathyrus Lathyrism Newsletter*, *2*, 101–104.
- Gupta, Y. P. (1980). Khesari dal: consumption, a health hazard. *Indian Farming*, *30*, 7–9.
- Harrison, F. L., Nunn, P. B., & Hill, R. R. (1977). Synthesis of α and β -ODAP and their isolation from seeds of *Lathyrus sativus*. *Phytochemistry*, *16*, 1211–1215.
- Joshi, J.B., & Patel, S.B. (2003). Fuel-efficient steam cooking device. US Patent 6,668,707 B2.
- Kuo, Y. H., Bau, H. M., Quemener, B., Khan, J. K., & Lambein, F. (1995). Solid-state fermentation of *Lathyrus sativus* seeds using *Aspergillus oryzae* and *Rhizopus oligosporus* sp. T-3 to eliminate the neurotoxin β -ODAP without loss of nutritional value. *Journal of the Science of Food and Agriculture*, *69*(1), 81–89.
- Labuza, T. P., & Riboh, D. (1982). Theory and application of Arrhenius kinetics to the prediction of nutrient losses in foods. *Food Technology*, *36*, 66–74.
- Long, Y. C., Ye, Y. H., & Xing, Q. Y. (1996). Studies on the neuroexcitotoxicity of β -N-oxalyl-L- α , β -diaminopropionic acid and its isomer α -N-oxalyl-L- α , β -diaminopropionic acid from the root of *Panax* species. *International Journal of Protein and Peptide Research*, *47*, 42–46.
- Mehta, S. L., Ali, K., & Barna, K. S. (1994). Somaclonal variation in a food legume – *Lathyrus sativus*. *Journal of Plant Biochemistry and Biotechnology*, *3*, 73–77.
- Moges, G., & Johansson, G. (1994). Flow injection assay for neurotoxin β -ODAP using an immobilized glutamate oxidase reactor with prereactors to eliminate glutamate interferences. *Analytical Chemistry*, *66*, 3834–3839.
- Muehlbauer, F.J., & Tullu, A. (1997). New crop Fact sheet —*Lathyrus sativus* L. internet publication. Updated 24 February 1998. <http://www.hort.purdue.edu/newcrop>.
- Padmajaprasad, V., Kaladhar, M., & Bhat, R. V. (1997). Thermal isomerisation of β -N-oxalyl-L- α , β -diaminopropionic acid, the neurotoxin in *Lathyrus sativus* during cooking. *Food Chemistry*, *59*, 77–80.
- Padmanaban, G. (1980). Lathyragens. In I. E. Liener (Ed.), *Toxic constituents of plant foodstuffs* (2nd ed., pp. 244–250). New York: Academic Press.
- Paradkar, M. M., Singhal, R. S., & Kulkarni, P. R. (2003). Detection of *Lathyrus sativus* in processed chickpea and red gram based products by thin layer chromatography. *Journal of the Science of Food and Agriculture*, *83*, 727–730.
- Ramaswami, H. S., Van De Voort, F. R., & Ghazala, S. (1989). An analysis of TDT and Arrhenius methods for handling process and kinetic data. *Journal of Food Science*, *54*, 1322–1326.
- Rao, S. L. N., Adiga, P. R., & Sarma, P. S. (1964). Isolation and characterization of β -N-oxalyl-L- α , β -diamino propionic acid, a neurotoxin from seeds of *Lathyrus sativus*. *Biochemistry*, *3*, 432–436.
- Rao, S. L. N. (1978). A sensitive and specific colorimetric method for the determination of α , β -diaminopropionic acid and the *Lathyrus Sativus* neurotoxin. *Analytical Biochemistry*, *86*, 386–395.
- Smartt, J. (1984). Evolution of grain legumes. I. Mediterranean pulses. *Experimental Agriculture*, *20*, 275–296.
- Srivastava, S., & Khokhar, S. (1996). Effects of processing on the reduction of β -ODAP (β -N-oxalyl-L-2,3-diaminopropionic acid) and anti-nutrients of khesari dhal, *Lathyrus sativus*. *Journal of the Science of Food and Agriculture*, *71*, 50–58.
- Tekle-Haimont, R., Abegaz, B. M., Wuhib, E., Kassina, A., Kidane, Y., Kebede, N., et al. (1993). Pattern of *Lathyrus sativus* consumption and β -N-oxalyl-L- α , β -diaminopropionic acid (β -ODAP) content of food samples in the lathyrism endemic region of northwest Ethiopia. *Nutrition Research*, *13*, 1113–1126.
- Tiwari, K. R., & Campbell, C. G. (1996a). Inheritance of seed weight in grasspea (*Lathyrus sativus* L). *FABIS Newsletter*(38/39), 30–33.
- Tiwari, K. R., & Campbell, C. G. (1996b). Inheritance of neurotoxin (ODAP) content, flower and seed coat colour in grass pea (*Lathyrus sativus* L). *Euphytica*, *91*, 195–203.
- Van Boekel, M. A. J. S. (1996). Statistical aspects of kinetic modeling for food science problems. *Journal of Food Science*, *61*, 477–485.
- Wang, F., Chen, X., Chen, Q., Qin, X., & Li, Z. (2000). Determination of neurotoxin 3-N-oxalyl-2,3-diaminopropionic acid and non-protein amino acids in *Lathyrus sativus* by precolumn derivatization with 1-fluoro-2,4-dinitrobenzene. *Journal of Chromatography A*, *883*, 113–118.
- Wang, F., Chen, X., Chen, Q., Qin, X. C., & Li, Z. (2003). Determination of neurotoxin 3-N-oxalyl-2,3-diaminopropionic acid and non-protein amino acid. *Lathyrus Lathyrism Newsletter*, *3*, 58–62.
- Zhao, L., Chen, X. G., Hu, Z. D., Li, Q. F., Chen, Q., & Li, Z. X. (1999). Analysis of β -N-oxalyl-L- α , β -diaminopropionic acid and homoarginine in *Lathyrus sativus* by capillary zone electrophoresis. *Journal of Chromatography*, *857*, 295–302.
- Zhao, L., Li, Z., Li, G., Chen, X., & Hu, Z. (1999). Kinetics studies on thermal isomerization of β -N-oxalyl-L- α , β -diaminopropionic acid by capillary zone electrophoresis. *Physical Chemistry Chemical Physics*, *1*, 3771–3773.