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A study on degradation kinetics of riboflavin in green gram whole (*Vigna radiata* L.)

P. Nisha [a], Rekha S. Singhal [a,b,*], Aniruddha B. Pandit [b]

[a] *Food and Fermentation Technology Department, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai – 400 019, India*
[b] *Chemical Engineering Department, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai – 400 019, India*

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Abstract

The kinetics of riboflavin degradation in green gram whole (*Vigna radiata* L.), as well as in pure riboflavin solutions, at initial concentrations present in green gram were studied over a temperature range of 50–120 °C (steady state temperature process). Riboflavin degradation followed first order kinetics, where the rate constant increased with an increase in the temperature. The temperature-dependence of degradation was adequately modelled by the Arrhenius equation. The degradation kinetics of riboflavin, in normal open pan cooking, pressure-cooking and a newly developed and patented fuel-efficient 'Eco-cooker', were also studied (unsteady state heating process). A mathematical model, to predict the losses of riboflavin from the time–temperature data of the unsteady state heating/cooking process, has been developed using the steady state kinetic parameters obtained. The results indicate riboflavin degradation of a similar magnitude in all three modes of cooking used in the study.

Keywords: Riboflavin degradation; Kinetics; Green gram; Cookers

1. Introduction

Kinetic parameters, such as reaction order, rate constant and activation energy, are essential for predicting food quality loss during storage as well as thermal processing. Thermal processing of food gives microbial safety, texture and flavour, but causes losses of vitamins and minerals. Water-soluble vitamins are the most sensitive to heat treatments. There are conflicting reports with respect to thermal stability of riboflavin in various foods. Riboflavin, or vitamin B_2, is stable toward temperature, oxygen and acid, but very unstable to alkali and light (Belitz & Grosch, 1999; Steaven, 1988; Steaven, Veron, & Michael, 1985). The losses occur mainly due to leaching during processing.

Riboflavin retention, in peas and lima beans subjected to blanching and processing was reported to be greater than 70% (Guerrant & O'Hara, 1953). A study of the effects of cooking methods on thiamin and riboflavin contents of chicken meat showed riboflavin to be fairly well retained (46–94%) (Al Khalifa & Dawood, 1993). Alkali treatments of common bean (*Phaseolus vulgaris*), followed by cooking, retain more of the riboflavin and niacin than other vitamins (Jyothi & Sumathi, 1995). Studies conducted by Aryanci and Kaya (1993) on kinetic analysis of the loss of thiamin, niacinamide and riboflavin during the cooking of macaroni, at 50, 75, 80, 85 and 90 °C, demonstrated the losses of all three vitamins, exhibiting first order behaviour, and losses were in the order thiamin > niacin > riboflavin. It was concluded that the leaching of these vitamins into cooking water was mainly responsible for their loss during macaroni preparation.

Data on the kinetics of riboflavin degradation during cooking, and particularly with respect to food commodities used in the Indian subcontinent, such as cereals, pulses and legumes, are almost non-existent. Different processing methods, such as open pan cooking, pressure-cooking and slow cooking are used in household cooking. A fuel-efficient cooker has recently been developed in our institute (Joshi & Patel, 2000).

* Corresponding author. Fax: +91-022-2414-5614.
E-mail address: rekha@udct.org (R.S. Singhal).

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The principle of this cooker is based on multiple effect evaporation, slow heating proportional to pick up rate of the supplied heat by the cooking vessel and insulation, and on the logic of combining these principles in one. Green gram (*Vigna radiata* L.) is one of the most consumed legumes in India. It grows throughout southeast Asia, central Africa and the warmer part of China and the USA. It is an excellent source of protein with high digestibility, vitamins, especially thiamin, riboflavin and niacin, and minerals (Adsule, Kadam, & Salunke, 1989; Gopalan, Sastri, & Balasubramanian, 1996). It is consumed in many forms, such as boiled dry bean, dhal curry (green gram splits boiled to porridge-like consistency), sprouts, noodles and fried dhal (green gram splits) (Adsule et al., 1989). Among legumes, riboflavin content is highest in green gram (Gopalan et al., 1996) and hence it was selected for the study.

The main objectives of the present study were:

(1) to determine the kinetic parameters of riboflavin degradation in green gram whole (*V. radiata* L.), a major Indian food ingredient, as well as in a pure solution of riboflavin at the concentration found in the green gram, over a temperature range of 50–120 °C (steady state temperature, typically used in cooking). The temperature rise study was one minute,

(2) to study the degradation kinetics of riboflavin for different cooking methods (unsteady state process),

(3) to develop a mathematical model relating the calculated kinetic data from the steady state temperature and time–temperature profiles of different cooking methods (unsteady state process),

(4) to apply this model to predict the riboflavin degradation for the unsteady state heating process, from the time–temperature data of the unsteady state heating process and comparing this with the actual degradation values, which could then be used to asses the nutritional values of the green gram as a function of method of cooking.

2. Materials and methods

2.1. Material

Green gram was procured from a local market of Mumbai city. Pure riboflavin was obtained from SD. Fine Chemicals, Mumbai, India. All the chemicals used were of AR grade.

2.2. Heat treatment

Heat treatments were carried out at different temperatures (50, 60, 70, 80, 90, 100, 110 and 120 °C) for 0–60 min. The temperatures were measured using a thermocouple with ±0.1 °C accuracy and the come up time was less than a minute. A water bath was used as a heating device for temperatures up to 100 °C while, for 110 and 120 °C, an autoclave was used. To study the effect of other constituents of green gram on degradation of riboflavin, degradation kinetics of pure riboflavin at concentration found in the green gram (0.286 mg/100 ml) was also undertaken. Ten grammes of green gram was transferred to a 100 ml beaker containing 30 ml distilled water, pre-heated to the desired temperature. Samples were withdrawn periodically and immediately analyzed for riboflavin using a fluorimetric method (AOAC, 1984). For pure riboflavin solutions, samples were taken directly for the determination of riboflavin after the heat treatments.

2.3. Cooking methods

For cooking studies, normal open pan cooking (30 min at a gas flow rate of 15 ml/s), pressure-cooking (15 min at 15 ml/s) and the newly developed slow cooker named 'Eco-cooker' (30 min at a gas flow rate of 4.5 ml/s and 30 min holding period) were selected as different cooking methods. The time and gas flow rates for open pan and pressure-cooking were selected according to the protocol used in household practices that ensures complete cooking of the green gram. The time and flow rates for the 'Eco-cooker' were selected according to the instructions given for its usage (Joshi & Patel, 2000). A ratio of 1:3 (w/v) of green gram to water is required for the complete cooking of green gram. Therefore, in the present study, the same ratio of green gram to water was taken and cooked as above in the three modes of cooking. The samples were withdrawn periodically, and analyzed for riboflavin.

2.4. Time–temperature data

Time–temperature data for each cooking method were monitored using a thermocouple.

2.5. Determination of riboflavin

Riboflavin was analyzed using the procedure described by AOAC (1984). For determination of riboflavin, 10 g of green gram were transferred into a 100 ml beaker containing 30 ml distilled water, pre-heated to the desired test time–temperature conditions. After the heat treatment(s), samples of the mash were taken out immediately and 50 ml of 0.2 N HCl added to each. They were was then heated on a boiling water bath for 30 min. pH was adjusted to 6 using 5 N NaOH, and then to pH 4.5 using 1 N HCl so as to precipitate the protein. The solution was made up to 250 ml and centrifuged. The procedure was repeated until precipitate formation ceased. To 10 ml of the supernatant, 1 ml glacial acetic acid and 0.5 ml 0.5% $KMnO_4$ solution were added, held for 2 min, and then 0.5 ml 3% H_2O_2 was added. The

solution was shaken well in order to expel the bubbles. The fluorescent intensity was measured at an excitation wavelength of 470 nm and an emission wavelength of 575 nm. Riboflavin was estimated from a standard curve, prepared by using standard riboflavin in the range of 0.0–1.0 μg.

For pure riboflavin solutions, heat treatments were carried out as above. The amount of riboflavin was determined as described above.

The reproducibility of the analytical method was confirmed by conducting the following experiment. One hundred grammes of green gram was added to 300 ml distilled water at 100 °C. It was held for 60 min at 100 °C. After 60 min, it was mashed in a mixer-grinder to achieve uniform consistency. It was then divided in to nine samples of 40 g each. The amount of riboflavin was estimated using the above described method. The concentration of riboflavin was found to be 0.2061 ± 0.0032 mg/100 g and the variation in the measured value was ±1.6%. Thus, the riboflavin concentration over the range studied in this work could be estimated within ±1.6% certainty.

2.6. Kinetic calculations

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

$$-\mathrm{d}[C]/\mathrm{d}t = k[C]^m, \quad (1)$$

where '[C]' is the quantitative value of the component under consideration, 'k' is the reaction rate constant, and 'm' is the order of the reaction. The equation for first order kinetics after integration of Eq. (1) can be written as:

$$\ln([C]_t/[C]_0) = -kt. \quad (2)$$

The dependence of the degradation rate constant (k_T) on temperature was quantified by the Arrhenius equation

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

where $[C]_0$ and $[C]_t$ = Concentration of riboflavin at time zero and time 't' (min), respectively, E_a = activation energy of the reaction (kJ mol^{-1}), R = universal gas constant (8.3145 J mol^{-1} K^{-1}), T = absolute temperature (K), A_0 = frequency factor (min^{-1}) is a pre-exponential constant.

Each experiment was done in triplicate, and average values were taken for the analysis. Kinetic data were analyzed by regression analysis using MS Excel.

3. Results and discussion

3.1. Concentration of riboflavin in green gram after the time–temperature treatments as indicated

After the heat treatment, the entire mass (green gram as well as water) was mashed and analyzed for riboflavin. Tables 1 and 2 show the concentrations of riboflavin in green gram as well as in riboflavin solution containing riboflavin at 0.286 mg/100 ml (the concentration found in green gram in the present study) after heat treatments at 50–120 °C. The pH of the green gram cooked in water was 6.7 ± 0.02. From the tables, it is evident that riboflavin is more stable in green gram than in the pure solutions. This is speculated to be due the protective effects of the phytochemicals present in green gram on the riboflavin. There are no literature reports to substantiate this observation. It would be interesting to investigate the identity of such riboflavin-protecting phytochemicals.

3.2. Kinetic data for degradation of riboflavin in green gram and pure riboflavin

Using linear regression, the degradation data were analyzed using the standard integrated rate equation to determine the overall order and rate constant for the

Table 1
Effect of heating on riboflavin concentration (mg/100 g[a,b]) in green gram at various temperatures

Time (min)	Temperature (°C)							
	50	60	70	80	90	100	110	120
5	–	–	–	–	–	–	0.277 ± 0.0081	0.280 ± 0.0078
10	0.281 ± 0.0026	0.281 ± 0.0008	0.285 ± 0.0010	0.282 ± 0.0011	0.281 ± 0.0005	0.280 ± 0.0004	0.272 ± 0.0059	0.258 ± 0.0053
15	–	–	–	–	–	–	0.255 ± 0.0037	0.240 ± 0.0076
20	0.276 ± 0.0015	0.276 ± 0.0029	0.281 ± 0.0019	0.280 ± 0.0021	0.279 ± 0.0050	0.273 ± 0.0058	0.243 ± 0.0032	0.222 ± 0.0012
30	0.272 ± .0006	0.268 ± .0004	0.267 ± .0008	0.260 ± 0.0025	0.260 ± 0.0023	0.255 ± 0.0017	–	–
40	0.269 ± 0.0005	0.259 ± 0.0008	0.251 ± 0.0038	0.252 ± 0.0021	0.247 ± 0.0024	0.240 ± 0.0022	–	–
50	0.263 ± .0007	0.256 ± .0013	0.247 ± 0.0005	0.242 ± 0.0014	0.231 ± 0.0016	0.216 ± 0.0009	–	–
60	0.259 ± 0.0011	0.252 ± 0.0028	0.238 ± 0.0026	0.223 ± 0.0040	0.214 ± 0.0023	0.202 ± 0.0018	–	–

[a] Values are means ± SD of three or more individual determinations.
[b] The riboflavin content of the green gram chosen in the study was 0.286 ± 0.0018 mg/100 g.

Table 2
Effect of heating on riboflavin concentration (mg/100 ml[a,b]) in pure solution at various temperatures

Time (min)	Temperature (°C)							
	50	60	70	80	90	100	110	120
5	–	–	–	–	–	–	0.242 ± 0.0014	0.279 ± 0.0027
10	0.289 ± 0.0023	0.282 ± 0.0015	0.284 ± 0.0020	0.281 ± 0.0024	0.281 ± 0.0018	0.28 ± 0.0024	0.271 ± 0.0028	0.235 ± 0.0018
15	–	–	–	–	–	–	0.240 ± 0.0018	0.193 ± 0.0017
20	0.284 ± 0.0036	0.274 ± 0.0031	0.272 ± 0.0030	0.271 ± 0.0043	0.272 ± 0.0037	0.274 ± 0.0016	0.226 ± 0.0036	0.162 ± 0.0016
30	0.280 ± 0.0018	0.267 ± 0.0027	0.258 ± 0.0027	0.25 ± 0.0016	0.252 ± 0.0035	0.241 ± 0.0022	–	–
40	0.277 ± 0.0033	0.260 ± 0.0051	0.244 ± 0.0018	0.24 ± 0.0023	0.234 ± 0.0029	0.217 ± 0.0017	–	–
50	0.271 ± 0.0027	0.246 ± 0.0024	0.235 ± 0.0014	0.223 ± 0.0012	0.218 ± 0.0041	0.202 ± 0.0007	–	–
60	0.265 ± 0.0018	0.239 ± 0.0013	0.226 ± 0.0031	0.208 ± 0.0015	0.198 ± 0.0071	0.177 ± 0.0025	–	–

[a]Values are means ± SD of three or more individual determinations.
[b]The riboflavin content of the pure vitamin solution in the study was 0.286 ± 0.0008 mg/100 ml.

degradation reaction. A correlation coefficient >0.9 in all the cases confirmed that the degradation of riboflavin in green gram, pure solution and in cooking methods follows first order reaction at all temperatures. Since, the entire mass (green gram as well as water) was mashed and analyzed for riboflavin after the heat treatment, adsorption and diffusion phenomena do not play any roles. Thus we can safely conclude that the degradation is indeed a kinetic phenomenon and can be modelled as first order reaction.

Figs. 1–3 show the representative plots for green gram and pure riboflavin solution at 50, 80 and 120 °C, respectively. Previous studies also indicate that the riboflavin degradation follows first order kinetics (Aryanci & Kaya, 1993; Okmen & Bayindirli, 1999). $T_{1/2}$, the

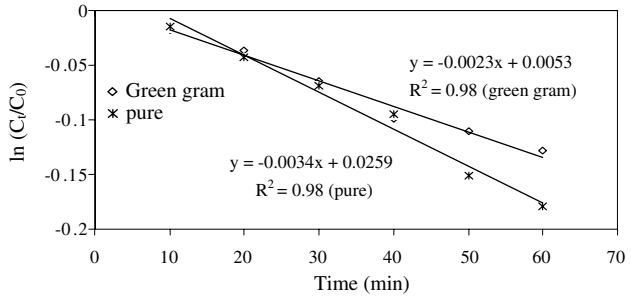

Fig. 1. First order plot of riboflavin degradation in green gram and in pure solution at 60 °C.

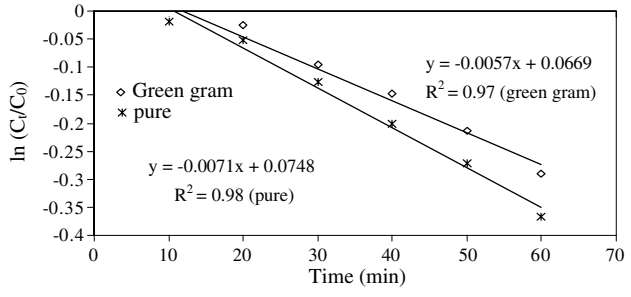

Fig. 2. First order plot of riboflavin degradation in green gram and in pure solution at 90 °C.

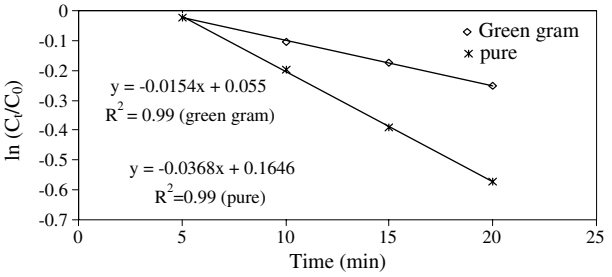

Fig. 3. First order plot of riboflavin degradation in green gram and in pure solution at 120 °C.

time required for riboflavin to degrade to 50% of its original value was calculated from the rate constant as '0.693/k'.

Table 3 documents the rate constants and '$t_{1/2}$' (min) for riboflavin in green gram and in pure solutions. The rate constants for riboflavin degradation in green gram increased from 0.0016/min at 50 °C to 0.0154/min at 120 °C with a corresponding decrease in half-life from 433 to 45 min. A similar trend was observed with pure vitamin solutions. It can be seen that the degradation is faster in pure vitamin solution than green gram at all temperatures. This may be due the protective effect of some constituents, present in green gram, on the degradation reaction.

Fig. 4 shows the Arrhenius plot for riboflavin degradation in green gram. The linear natures of the plots obtained indicate that thermal destruction of riboflavin conforms to the Arrhenius equation. Activation energies, E_a (kJ mol^{-1}) were calculated as a product of the gas constant, R (8.3145 J mol^{-1} K^{-1}) and the slope of the graph obtained by plotting '$\ln k$' vs. '$1/T$'. The activation energy of riboflavin in green gram was found to be 29.8 kJ mol^{-1}.

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

To extend the results obtained from steady state experiments to the unsteady state encountered in the three

Table 3
Rate constant and half-life of riboflavin degradation in green gram and in pure solution

Temperature (°C)	Green gram			Pure vitamin solution		
	Rate constant k (min^{-1})	R^2	$t_{1\backslash 2}$ (min)	Rate constant k, (min^{-1})	R^2	$t_{1/2}$ (min)
50	0.0016	0.99	433	0.0017	0.99	408
60	0.0023	0.98	301	0.0034	0.98	204
70	0.0039	0.97	177	0.0047	0.99	147
80	0.0047	0.97	147	0.0061	0.99	114
90	0.0057	0.97	122	0.0071	0.99	98
100	0.0069	0.98	100	0.0095	0.98	73
110	0.0092	0.97	75	0.0157	0.97	44
120	0.0154	0.99	45	0.0368	0.99	19

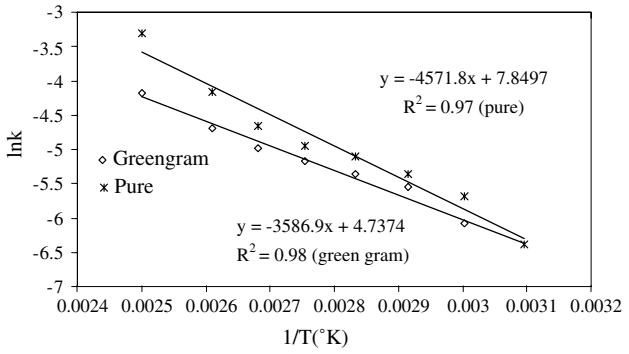

Fig. 4. Arrhenius plot for riboflavin degradation in green gram and in pure solutions.

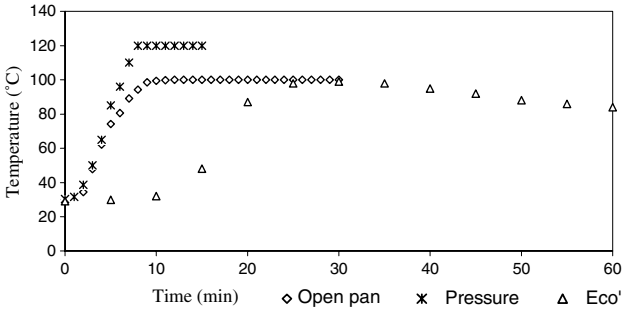

Fig. 5. Time–temperature profiles of the different cooking methods used.

modes of cooking, namely, open pan cooking, pressure-cooking and cooking in 'Eco-cooker', time–temperature data during the processing of each were recorded (Fig. 5).

3.4. Degradation profile and half-life values of riboflavin in red gram splits in the three modes of cooking

Riboflavin degradation was followed in each of these modes of cooking as for green gram under steady state conditions. The results, documented in Table 4, indicate that degradation was of a similar order of magnitude in all the modes of cooking.

3.5. Prediction of riboflavin loss during unsteady state heating processing

To predict the degradation of riboflavin occurring during a given unsteady state heating process, the Arrhenius equation, $k_T = A_0 \exp(-E_a/RT)$ (Eq. (3)) was used, where 'k_T' is the rate constant at any absolute temperature 'T' and time't'. 'E_a' is the activation energy of the reaction, 'R' is the gas constant, and A_0 is a pre-exponential constant, already calculated for the steady state heating process. The rate constant 'k_T' at each temperature was calculated using Eq. (3) substituting for

Table 4
Degradation profile and kinetics of riboflavin in green gram at different cooking methods

Method of cooking	Time (min)	Riboflavin concentration (mg/100 g[a,b])	Rate constant k[b] (min^{-1}) (R^2)	$t_{1\backslash 2}$ (0.693/k, min)
Open pan cooking	5	0.281	0.0071 (0.98)	98
	10	0.257		
	20	0.244		
Pressure-cooking	5	0.283	0.0066 (0.99)	105
	10	0.274		
	15	0.265		
Bachat cooking[c]	10	0.284	0.0070 (0.90)	99
	20	0.276		
	30	0.247		

[a] Values are means ± SD of three or more individual determinations.
[b] The riboflavin content of the green gram chosen in the study was 0.286 mg/100 g.
[c] The readings were taken after 30 min holding period.

Table 5
The actual and predicted retention of riboflavin in the cooking methods

Cooking method	Green gram (mg/100 g)		Pure solution (mg/100 ml)	
	Actual retention	Predicted retention	Actual retention	Predicted retention
Open pan	0.244	0.230	0.223	0.210
Pressure	0.265	0.250	0.235	0.227
Bachat	0.247	0.210	0.243	0.207

'T' from the time–temperature data of the unsteady state heating process. Knowing the rate constant k_T, the rate (dC/dt), amount degraded (ΔC) during the short time interval, zero to t ($=\Delta t_T$), and the final concentration, '$C_{t+\Delta t}$', can be calculated as follows:

Rate = Rate constant $k_T \times$ Initial concentration C_t,

Amount degraded during $\Delta t_T(\Delta C)$ = Rate $\times \Delta t_T(k_T \cdot C \cdot \Delta t_T)$,

Concentration after time $\Delta t_T = C_t - \Delta C$.

These calculations were continued for the entire time period (heating and constant temperatures) during which each cooking process was carried out. An MS excel-based computer programme 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 riboflavin. The resulting predictions and the actual degradation obtained experimentally are given in Table 5. As seen, a good agreement between the actual and the predicted degradation/retention of riboflavin was obtained. Using this method, the degradation of riboflavin can be predicted for any processing method, if the time–temperature profile of that processing operation is known.

4. Conclusions

The degradation of riboflavin in green gram whole and in pure solutions of riboflavin followed first order reaction kinetics. A mathematical model was successfully developed, using the activation energy of riboflavin degradation and the time–temperature profile of the processing method, to predict the loss riboflavin, if the initial concentration of riboflavin in the product is known. The order of magnitude of retention of riboflavin was found to be the same in all the cooking methods used, namely, open pan, pressure-cooking and cooking using 'Eco-cooker'. Based on the nutrient retention, in this particular case riboflavin, and fuel savings, an overall judgment in favour of the slow 'Eco-cooker' is suggested.

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