Kinetics of Trypsin Inhibitor Reduction in Two Accessions of Soaked and Unsoaked Velvet Beans Treated by UV Radiation

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Abstract

The influence of UV radiation on the kinetics of trypsin inhibitor degradation in two accessions (black colored seed coated seed and white colored seed coated seed) of velvet bean (soaked and unsoaked) was examined. The resulting trypsin inhibitor activity declining rates data were well fitted by simple zero-order kinetics with correlation value \( r^2 \) ranging from 0.95-0.97. A zero-ordered correlation suggests that the initial high activity of trypsin inhibitor will not increase the rate of trypsin inhibitor degradation after being exposed to UV radiation. In both velvet beans accessions, trypsin inhibitor degradation rate of soaked seed was always higher than that of unsoaked seeds. Based on literature findings and the outcomes of regression analysis in this study, it can be concluded that the minimum exposure time to UV radiation required to ensure minimal negative influence on broiler performance should not be less than 46 minutes and 36 minutes for the unsoaked and soaked velvet beans, respectively.

Keywords

Soaking, Trypsin Inhibitor Activity, Velvet Beans, UV Radiation, Zero-Order Kinetic

1. Introduction

Trypsin inhibitors are considered as a unique class of proteins found in some feed ingredients that inhibit protease enzymes in the digestive tract by forming indigestible complexes with dietary protein [2]. Protease inhibitors have been reported to reduce trypsin activity and to a lesser extent chymotrypsin; therefore reduce protein digestion by monogastric animals and some young ruminant and animals [8]. Velvet bean have been reported to have substantial level of trypsin inhibitor which restrict its inclusion level in animal feed [6]. Several approaches have been reported to reduce activity of trypsin inhibitor in animal feed such as fermentation [3], toasting [2], extrusion [10], microwave treatment [4] and exposure to UV radiation [6]. Reduction in trypsin inhibitor activity in velvet bean by UV radiation has been reported to be dependent on exposure time [6]. However, the correlation magnitude and the kinetics of trypsin inhibitor degradation in velvet bean after being exposed to UV irradiation were not quantified. The main objective of this study was to identify the correlation magnitude and the kinetics (i.e. rate) of trypsin inhibitor degradation after exposing two accessions of velvet bean (for soaked and unsoaked seeds) to UV irradiation. This has practical application in estimating trypsin inhibitor content in velvet bean after exposure to UV radiation which is considered to be an important antinutritonal factor in animal nutrition.

2. Treatments and Data Fitting

2.1. Treatments

Velvet bean seed (Mucuna pruriens (L.) DC var. utilis (Wall.ex Wight) Bak. ex Burck) origin, processing description and analysis of trypsin inhibitor activity (TIA) of both seed accessions (black and white colored seed coat) were described and obtained from recently published work described by Kala and Mohan [6]. Seeds of both velvet bean accessions were placed in petridishes and exposed under UV-B light (15 W Philips lamp, Tempo Instruments and Equipment Pvt. Ltd, Bombay) for different exposure time intervals: 0, 10, 20, 30, 45, 60 and 90 minutes. Same treatments were also given to seeds of both velvet bean accessions that were soaked (overnight) in distilled water. The soaked seeds then were dried at 55°C. Both the raw and treated seeds were ground by using Willey Mill (60 mesh size). The effect of UV radiation on trypsin inhibitor activity of the two velvet bean accessions was obtained from previous work of Kala and Mohan [6] (Table 1) and after acquiring the publisher approval (Tropical and Subtropical Agroecosystems).
Table 1. Effect of different UV radiation exposure period on TIA (TIU / mg protein)* of two accessions of VB (White colored seed coat and Black colored seed coat) (adapted from Kala and Mohan [6])

<table>
<thead>
<tr>
<th>Accessions</th>
<th>Time duration (min)</th>
<th>No soaking</th>
<th>With soaking**</th>
<th>No soaking</th>
<th>With soaking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White colored seed coat</td>
<td>0</td>
<td>46.4±0.56</td>
<td>46.4±0.56</td>
<td>43.7±0.68</td>
<td>43.7±0.68</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>32.1±0.19</td>
<td>30.3±0.17</td>
<td>31.2±0.24</td>
<td>29.1±0.19</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>26.4±0.12</td>
<td>24.1±0.15</td>
<td>28.1±0.15</td>
<td>22.4±0.20</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>18.3±0.08</td>
<td>10.4±0.17</td>
<td>14.3±0.08</td>
<td>10.3±0.13</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>7.4±0.01</td>
<td>4.4±0.04</td>
<td>7.2±0.06</td>
<td>2.3±0.01</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.3±0.04</td>
<td>null</td>
<td>null</td>
<td>null</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>null</td>
<td>null</td>
<td>null</td>
<td>null</td>
</tr>
</tbody>
</table>

| Black colored seed coat  |                     |            |                |            |              |
|                          | 0                   | 43.7±0.68  | 43.7±0.68      | 43.7±0.68  | 43.7±0.68    |
|                          | 10                  | 31.2±0.24  | 29.1±0.19      | 29.1±0.19  | 29.1±0.19    |
|                          | 20                  | 28.1±0.15  | 22.4±0.20      | 22.4±0.20  | 22.4±0.20    |
|                          | 30                  | 14.3±0.08  | 10.3±0.13      | 10.3±0.13  | 10.3±0.13    |
|                          | 45                  | 7.2±0.06   | 2.3±0.01       | 2.3±0.01   | 2.3±0.01     |
|                          | 60                  | null       | null           | null       | null         |
|                          | 90                  | null       | null           | null       | null         |

*One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein (Kala and Mohan (2011))

** Means±SE (n=3). Low SE value suggests that measurements are reproducible

2.2. Data Fitting

For simplicity, simple linear regression was implemented to evaluate the rate of TIA reduction for both velvet bean accessions after being exposed to UV radiation at different time-duration range from 0 – 90 minutes (0 (raw)), 10, 20, 30, 45, 60 and 90 minutes). Simple linear regression is given by the following equation (1)

\[
Y = AX + B
\]

where \( Y \) is the TIA at time \( X \); \( A \) is the slope (rate of decline in TIA per unit time), \( X \) is the exposure duration to UV Radiation.

For the purpose of data fitting, the value of \( A \) was obtained by linear-least-squares fit of the solution of eq. (1) using excel sheet (Microsoft, 2007). The linearity of such a plot (indicated by correlation value) will provide a measure of the applicability of zero-order kinetics. Zero–order kinetics indicate the rate that is independent of the initial TIA activity.

3. Results

![Graph](image.png)

**Figure 1.** Time dependence of TIA of soaked white colored seed coat velvet bean after different exposure durations to UV radiation
Simple regression analysis showed that the TIA after exposure of both accessions of velvet bean (with or without soaking) to UV irradiation is highly correlated and time dependent ($r^2$ range from 0.95 to 0.97). In all treatments, the slope of regression line indicate decline in TIA (i.e changing the activity of trypsin inhibitor per unit time). Although no statistical analysis were performed to compare activity rate of trypsin inhibitor after certain treatment, soaking white colored coated seed before being treated by UV radiation showed a higher reduction in activity rate of trypsin inhibitor (slope=0.92) (figure 1) compared to unsoaked seeds (slope=0.71) (figure 2). Regression analysis showed that soaking of white colored coated seed over night reduced activity rate of trypsin inhibitor by 30%. However, activity rate of trypsin inhibitor of black colored seed coat after soaking is less pronounced. Soaking black colored coated seed before being treated by UV radiation showed slightly a higher reduction in activity rate of trypsin inhibitor (slope=0.91) (figure 3) compared to unsoaked seeds (slope=0.81) (figure 4). The results of regression analysis showed that soaking of black colored coated seed over night reduced activity rate of trypsin colored inhibitor by only 13%.

Regression analysis of unsoaked seeds between the two accessions showed that the decline in activity rate of Trypsin inhibitor in black colored coated seed was higher than in white colored coated seeds (0.808 vs. 0.717, respectively) (figure 2 vs. 4). However, soaking seeds before being exposed to UV irradiation eliminate any differences in trypsin inhibitor activity rate between the black colored coated and white colored coated seed (0.910 vs 0.929, respectively).

Figure 2. Time dependence of TIA of unsoaked white colored seed coat velvet bean after different exposure durations to UV radiation

Figure 3. Time dependence of TIA of soaked black colored seed coat velvet bean after different exposure durations to UV radiation
4. Discussion

The regression analysis was conducted in order to quantify the correlation and the declining rate of trypsin inhibitor activity in the two accessions of velvet bean after being treated by UV radiation with or without pre-soaking treatment. Excellent fitting of the data indicate that the initial high concentration of trypsin inhibitor will not accelerate the declining rate of trypsin inhibitor activity after being exposed to UV radiation. In other words, the reduction in trypsin inhibitor activity is proportional to the time being exposed to UV radiation treatment and independent to initial activity of trypsin inhibitor. Exposure time of the two accessions of velvet bean to UV radiation is critical in eliminating negative effect of trypsin inhibitor in overall poultry performance. Trypsin inhibitor has been reported to inhibit the proteolysis of dietary protein within the animal body by forming trypsin inhibitor-dietary protein complexes which are resistant to digestive enzymes [7]. Presence of trypsin inhibitor in poultry diets have been reported to increase pancreases weight (double) [1] to overcome the reduction in protein digestibility by increasing the synthesis and secretion of proteolysis enzymes. Consequently, presence of trypsin inhibitor can increase the maintenance of energy requirement in poultry. In addition, trypsin inhibitor can severely depress poultry feed intake [5, 12] and reduce protein retention within poultry body and thus reduce overall poultry growth performance [9]. From health prospective, Palliyegurua et al. [11] reported that there was a linear increase in sub-clinical necrotic enteritis lesions in the duodenum, jejunum, mid small intestine and ileum with increasing the level of trypsin inhibitor in poultry diets (trypsin inhibitor level in the diets ranged from 1.90 - 8.46 mg/g protein). From processing prospective, Dominguez [2] reported that feed ingredients that contain trypsin inhibitor activity above 18 TIU/mg are considered not well processed. From nutritional prospective, feed ingredients should be less than 8.8 TIU/mg in order to avoid negative influences on overall growth and health performance in poultry [9]. Based on the previous literature findings and the outcomes of regression analysis in this study, it can be concluded that the minimum exposure time to UV radiation that is required to ensure minimal negative influence on broiler performance should not be less than 46 minutes and 36 minutes for the unsoaked and soaked velvet beans, respectively.

REFERENCES


