Thermal degradation kinetics of hemaglutinin in African yam bean (*Sphenostylis stenocarpa*) seeds using partial cooking method

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Abstract

In this study, the thermal degradation kinetics of hemagglutinins in African yam bean (*Sphenostylis stenocarpa*) seeds during partial wet cooking was investigated. Heat treatments were carried out at different temperatures of 80, 85, 90, and 95˚C over a period of 10 to 40 minutes at isothermal conditions. The degradation of hemagglutinin was adequately modelled by the fractional conversion model and Arrhenius equation. There was a progressive decrease in hemagglutinin activity for each thermal treatment as the cooking time increased. High values of Coefficient of linear regression; R^2 ranging from 0.795–0.931 confirmed the degradation to follow the first order thermal kinetics. Thermal treatment of 95˚C for 40 minutes was sufficient to reduce 82% of hemagglutinin activity in African yam bean seeds.

KEY WORDS: African yam beans, Hemagglutinin, Thermal degradation.

INTRODUCTION

Kinetic studies help food process engineers and scientists to optimize processing systems and design processes, to improve and optimize existing processes and develop control systems for processing operations in terms of reaction rates. These studies help to predict how quick the reaction mixture will attain equilibrium condition, and also in predicting the reaction mechanism (Kavita et al., 2006).

African Yam Bean (AYB) is an herbaceous leguminous plant occurring throughout tropical Africa. It is grown as a minor crop in association with yam and cassava. AYB serves as security crop; it has the potential to meet year round protein requirements if grown on a large scale (Aburime, 2012). African Yam Bean (AYB) is highly nutritious with high protein, mineral and fibre content, its protein content is reported to be similar to that of some major and commonly consumed legumes (Nwosu, 2013). Its amino acid profile is comparable if not better than those of cowpea, soy bean and pigeon pea (Aburime, 2012). Aside the nutritional benefits of AYB, the seeds are encumbered by the presence of anti- nutrients (Udeogu and Awuchi, 2016). Habtamu and Negussie (2014) defined anti-nutrients as chemicals which have been evolved by the plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in the food and decreasing the nutritive value. Lectin, also called hemagglutinin is one of the anti-nutrients found in AYB seeds (Udeogu and Awuchi, 2016). Lectins may be inactivated, destroyed or drastically reduced by some processing operation, such as cooking (El-Adawy, 2002) and malting (El-Adawy, 2002).

Importantly, hemagglutinin can be detoxified by several processing method such as soaking, cooking, boiling, germination, autoclaving, fermentation, sprouting, genetic manipulation and other processing methods, which however may interfere with the level of protein and fibre contents used as indicator of high nutritive value as reported by Apata and Ologhobo (1994). However, Betch et al. (2005) reported that while fermentation can substantially improve the nutritional quality of AYB and reduce loses due to thermal influences of most food values, roasting was found to have greater efficiency in the elimination or reduction of the levels of phytate available in AYB and boiling seemed to eliminate hemagglutinin more efficiently when compared with roasting and others. Information on heat processing and kinetics of degrading the toxicity of hemagglutinin in AYB is insufficient (Beckley et al., 2012), hence the aim of this study.

MATERIALS AND METHODS

The brown (dark coloured) African yam bean (*Shpenostylis stenocarpa*) used for the work was obtained from a local market (Wurukum market) in Makurdi, Benue state. Heat treatments were carried out at different temperatures of 80, 85, 90, and 95˚C over a period of 10 to 40 minutes in a water bath (DK420) using standard methods, further -more, determination of hemagglutinin level was done using standard methods (AOAC, 2012).

Kinetic Calculation

A general reaction rate expression for the degradation kinetics can be written as follows: (Tola and Ramaswamy, 2015).

$$-\frac{d[C]}{dt} = k[C]^n \quad (1)$$

Where C is the quantitative value of the concentration of the degraded molecule under consideration, *k* is the reaction rate constant, and *n* is the order of the reaction. The equation for first order rate kinetics can be obtained by the integration of Eq. (1) as

$$\ln\left(\frac{[C]_0}{[C]_t}\right) = -kt \quad (2)$$

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.

According to Tola and Ramaswamy (2015), the intensity of heat on degradation of most nutrients in foods can be determined using the Arrhenius kinetic model. The effect of temperature in absolute form on the reaction rate constant *k* is explained by Arrhenius, in Eq. (3 and 4).

$$k = A_o \exp\left(-\frac{E_a}{RT}\right) \quad (3)$$

$$\ln k = \ln k_{Tref} + \left[\frac{E_a}{R}\left\{\frac{1}{T_{ref}} - \frac{1}{T}\right\}\right] \quad (4)$$

Where, k_{Tref} is reaction rate constant at reference temperature, E_a is the activation energy (kJ/mole), and R the molar gas constant (8.314 J mole^{-1} K^{-1}), T temperature (K) at time t.

The graph *lnk* versus $\frac{1}{T}$ will give us a linear line from which the inverse slope is used to calculate the E_a required for the reaction.

$$Slope = \frac{Ea}{2.303R} \quad (5)$$

RESULTS AND DISCUSSION

Quantity of Hemagglutinins in African Yam Bean Flour at Isothermal Conditions

Table 1 shows the effect of wet-heat treatment at different temperatures (80-95 ˚C) for different time periods on the resultant concentration of Hemagglutinins. The initial concentration of Hemagglutinins in African Yam Bean flour found in this study was 1.205 mg/100 g, whereas the Hemagglutinin activity of African Yam Bean flour reported by El-Adawy (2002) was 6.22 mg/100 g. The variation in the content may be due to varietal difference as reported by Abioye et al., (2015), these include different cultivars of AYB planted on soils with different soil nutrient makeup.

Furthermore, there was a progressive decrease in Hemagglutinin activity for each thermal treatment as the cooking time was increased. This clearly indicates that Hemagglutinins are relatively heat labile constituents (Qayyum et al., 2012). This trend in degradation obviously indicates the degradation to follow a first order kinetics.

Similar experiment by Kavita et al., (2006) showed the degradation of Saponins in Soya bean flour to follow the first order kinetics.

The reduction of Hemagglutinin during heating suggest that they would be a great reduction in the ability of the sugar binding proteins to bind and agglutinate (Nwosu, 2013). While heating at 95˚C for 40 min reduced hemagglutinin levels by 82% (Table 2). Nwosu (2013) reported that soaking and malting for 48 h reduced Hemagglutinin levels by 49% and 38% respectively. Acceptable level of Hemagglutinin in food and food products as approved by the U.S department of Agriculture (2007) ranges from 0.1 to 0.33 mg/100 g, hence reduction at 95˚C for 40 min gives the best level of Hemagglutinin with minimal negative effect.

Table 1. Effect of Heating on Hemagglutinin (mg/100 g) in AYB Flour at Various Temperatures

Time (min)	Temperature (˚C)			
	80	85	90	95
10	1.074^a ± 0.02	0.993^a ± 0.02	0.724^a ± 0.02	0.520^a ± 0.02
20	1.052^a ± 0.01	0.990^a ± 0.02	0.522^b ± 0.02	0.498^a ± 0.02
30	0.926^b ± 0.02	0.715^b ± 0.01	0.400^c ± 0.02	0.336^b ± 0.03
40	0.613^b ± 0.02	0.414^c ± 0.02	0.382^c ± 0.03	0.215^c ± 0.02
$LSD_{0.05}$	0.03	0.09	0.05	0.07

-Values are mean ± Standard deviation of triplicate determination. Mean Values with same superscript letters on the same column are not significantly different (p<0.05)

-The Hemagglutinin content of the raw AYB flour was found to be 1.205 ± 0.02 mg/100 g.

LSD=Least Significant Difference

Thermal Degradation Kinetics of African Yam Bean (AYB) Hemagglutinin after Cooking Treatments.

In order to find the kinetic model that better fits the obtained experimental data points, the two-step method was used as a first approach (Goncalves et al., 2010).

Considering each Hemagglutinin parameter, a regression analysis was applied, in a first step, for each isothermal experiment to calculate the corresponding reaction rate

Table 2. Percentage Reduction of Hemagglutinin (mg/100 g) in AYB at Various Temperature and Time

Temp. (°C)	Percentage Reduction			
	10 min	20 min	30 min	40 min
80	11	13	23	49
85	18	18	41	66
90	40	57	67	68
95	57	59	72	82

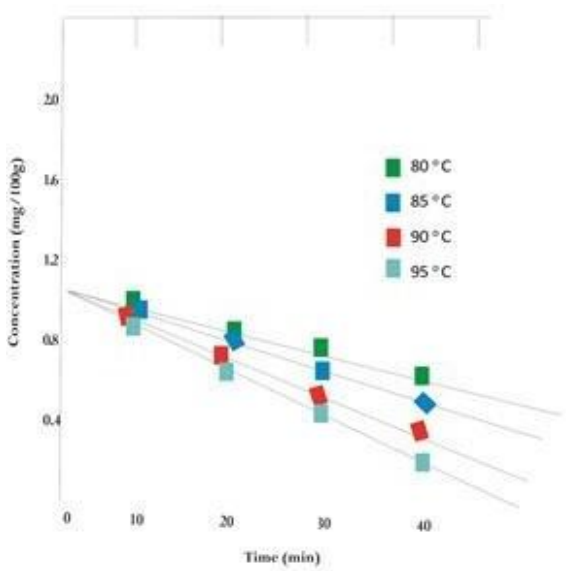

Figure 1. First Order Plot of Hemagglutinin degradation in AYB seed flour at 80, 85, 90 and 95^{0}C.

constant.

Accordingly, $ln(^{[C]_0}/_{[C]_t})$ was plotted vs. t, from which rate constant, k was calculated as the slope of the linear plot. Figure 1 shows the first order reaction model (Eq. (1)) applied to the hemagglutinin values, respectively.

Table 3 shows the Rate Constants (*k*) and regression coefficient (R^2) of Hemagglutinin degradation in AYB Flour. The rate constants increased with a corresponding increase in temperature. This is because temperature increase the rate of reaction. Similar findings have been reported by Kavita et al., (2006). High values of correlation coefficient ranging from 0.795 to 0.931 confirmed the degradation to follow the first order kinetics.

In order to make a better estimate of the kinetic parameters, a one-step non-linear regression was applied to all data (Table 4). Based on the examination of the residuals, this proved to be the most adequate model, hence the distribution of residuals has no visual tendency (were randomly distributed around zero).

Estimated activation energies and rate constants at the reference temperature of 88°C and corresponding 95% ($p<0.05$) confidence intervals are reported in Table 3. An inversely proportional relationship was found to exist between the activation energies and rate constant at 88˚C (reference temperature). Similar results were obtained by Goncalves et al., (2010) who studied peroxidase inactivation of Pumpkin during blanching and found that increase in Activation energy led to decrease in the reaction rate constant at 88˚C.

Table 3. Rate Constants (*k*) and Regression Coefficient (R^2) of Hemagglutinin Degradation in AYB Seed Flour

Temperature (^{0}C)	Rate Constant, *k* (min^{-1})	Regression coefficient(R^2)	Standard error
80	0.891	0.795	0.116
85	0.943	0.88	0.102
90	0.948	0.888	0.064
95	0.965	0.931	0.046

Table 4. Kinetic Parameters for Hemagglutinin degradation of AYB using one-step Non-linear Regression Method

Samples	E_a (KJ/mol)	$_{0C}$ (min_{-1})	C_0	C_r	R^2
A	102 ± 3.4	0.946 ± 0.01	1.052 ± 0.02	0.615 ± 0.02	0.795
B	119 ± 2.1	0.906 ± 0.02	0.993 ± 0.09	0.414 ± 0.04	0.88
C	123 ± 3.6	0.821 ± 0.01	0.724 ± 0.01	0.383 ± 0.01	0.888
D	139 ± 1.8	0.796 ± 0.11	0.523 ± 0.01	0.214 ± 0.01	0.931

Activation Energies and Rate constants at Reference Temperature of 88˚C and Corresponding 95% Confidence Interval

CONCLUSION

The study reveals that thermal treatment of 95°C for 40 minutes was sufficient to reduce 82% of hemagglutinin activity with minimal negative effect in African yam bean seeds. This can also be achieved with dissimilar optimal temperatures for other hemagglutinin rich flour sources. The values of the regression coefficient, R^2 (ranged between 0.795-0.931) showed the reliability of the suggested model. The Arrhenius model described the temperature dependence of the reaction rate constant of all the considered parameters

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