Kinetics of Thermal Inactivation of Peroxidase and Color Degradation of African Cowpea (*Vigna unguiculata*) Leaves

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Abstract: Cowpea leaves form an important part of the diet for many Kenyans, and they are normally consumed after a lengthy cooking process leading to the inactivation of peroxidase (POD) that could be used as an indicator for the potential shelf life of the vegetables. However, color degradation can simultaneously occur, leading to poor consumer acceptance of the product. The kinetics of POD *in situ* thermal (for thermal treatments in the range of 75 to 100 °C/120 min) inactivation showed a biphasic first-order model, with Arrhenius temperature dependence of the rate constant. The kinetic parameters using a reference temperature (T_{ref}) of 80 °C were determined for both the heat-labile phase ($k_{ref} = 11.52 \pm 0.95 \times 10^{-2}$ min^{-1} and E_a of 109.67 ± 6.20 kJ/mol) and the heat-stable isoenzyme fraction ($k_{ref} = 0.29 \pm 0.07 \times 10^{-2}$ min^{-1} and E_a of 256.93 ± 15.27 kJ/mol). Color degradation (L*, a*, and b* value) during thermal treatment was investigated, in particular as the "a*" value (the value of green color). Thermal degradation (thermal treatments between 55 and 80 °C per 90 min) of the green color of the leaves followed a fractional conversion model and the temperature dependence of the inactivation rate constant can be described using the Arrhenius law. The kinetic parameters using a reference temperature ($T_{refC} = 70$ °C) were determined as $k_{refC} = 13.53 \pm 0.01 \times 10^{-2}$ min^{-1} and $E_{aC} = 88.78 \pm 3.21$ kJ/mol. The results indicate that severe inactivation of POD (as an indicator for improved shelf life of the cooked vegetables) is accompanied by severe color degradation and that conventional cooking methods (typically 10 min/100 °C) lead to a high residual POD activity suggesting a limited shelf life of the cooked vegetables.

Keywords: cowpea leaves, green color, inactivation, kinetics, POD

Practical Application: The thermal inactivation of peroxidase (POD) and thermal degradation of green color are important for evaluating the adequacy of the traditional cooking process of cowpea leaves. Proper inactivation of POD can lead to an improved shelf life of the cooked product. The results of the study can be used to optimize the cooking process of cowpea leaves in terms of green color retention while improving shelf life based on POD inactivation as an indicator enzyme.

Introduction

Cowpea (*Vigna unguiculata*) leaves are an important source of essential nutrients in the diet (Wawire and others 2011, 2012) of many in Kenya. Like many African leafy vegetables in Kenya, these vegetables are normally cooked by boiling for about 10 min (Ajayi and others 1980; Imungi and Potter 1983; Sreeramulu and others 1983; Mathooko and Imungi 1994). The benefits of heat treatment have been widely documented (Kleinschmidt 1971; Shams and Thompson 1987; Canet 1989; Préstamo and others 1998; Reyes De Corcuera and others 2004). However, thermal treatment has some adverse effects, especially on the quality attributes of the vegetables. On the other hand, an improper heat treatment can lead to incomplete enzyme inactivation which may result in quality changes, such as texture, color, flavor, and nutritional losses, during storage.

Peroxidase (POD, E.C.1.11.1.7) is one of the most widely distributed enzymes in vegetables and like many other vegetable enzymes, it is located in a soluble form in the cell cytoplasm, and in an insoluble form being ionically or covalently bound to the cell wall (Gkinis and Fennema 1978; McLellan and Robinson 1981; Vamós-Vigyázó 1981; Morales-Blancas and others 2002).

Complete inactivation of POD can lead to increased shelf life of vegetable products, as it is a reasonable assumption that other quality-related enzymes will also have been inactivated (Gonçalves and others 2007). However, because POD is more thermostable than many enzymes that lead to food deterioration, its complete inactivation by thermal processing may lead to an excessive heat treatment of the product and cause other quality problems, such as color changes, tissue softening, and nutritional losses (Howard and others 1999; Murcia and others 1999; Oboh 2005; Gonçalves and others 2007). Thus, an optimized thermal process, in terms of the balance between thermal inactivation of enzymes and degradation of quality attributes of the vegetables is important. This can be achieved by monitoring changes of some quality parameters, such as color in the case of cowpea leaves, of the vegetables during thermal processing.

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Color is a primary consumer-perceived product characteristic and plays an important role in food. Furthermore, the degree of greenness, attributed to chlorophyll pigments, is important in determining the final quality of thermally processed green vegetables and is often expected to be close to the raw ones (MacDougall 2002). Thermally processed green vegetables exhibit poor green-color quality as compared to the fresh ones, due to chlorophyll degradation. Chlorophyll degradation can be due to the action of chlorophyllase or tissue damage (Heaton and Marangoni 1996; McFeeters 2002). The chlorophyll degradation pathway entails the replacement of magnesium in the centre of the chlorophyllic group by hydrogen ions to form pheophytin and further formation of the degradation products such as pheophorbides (White and others 1963; Schwartz and Von Elbe 1983; Canjura and others 1991; Schwartz and Lorenzo 1991; Heaton and Marangoni 1996; Van Boekel 1999, 2000). Pigment degradation can be related to physical color measurements (Muftugil 1986; Sims and others 1993; Bao and Chang 1994; Martins and Silva 2002; McFeeters 2002).

The study of the kinetics of the thermal inactivation of POD (to represent the effectiveness of the cooking process) and the kinetics of the thermal degradation of the green color (to represent the consumer perception of the degree of adverseness of the cooking process) forms a framework for optimizing the cooking process of cowpea leaves. The commonly used kinetic models and parameter estimates, for POD inactivation and color degradation for a number of fruits and vegetables have been extensively reviewed (Gonçalves and others 2007). In summary, the models are described by zero or first-order kinetics. However, when there are different heat-resistant fractions available (heat-labile and heat-stable fractions), a biphasic first-order model is used to describe their thermal inactivation kinetics (Ling and Lund 1978).

The objective of this study was to evaluate the kinetics of cowpea leaves POD inactivation and color changes during cooking to create a starting point for the evaluation of the adequacy of cooking processes in terms of POD residual activity (a potential indicator for improving the shelf-life of the cooked vegetables) and its impact on the green color of the vegetables. To the best of our knowledge, this information is not available in open literature.

Materials and Methods

Growing of the cowpea leaves and sample preparation after harvest

Cowpea (*Vigna unguiculata*) seeds were grown in a greenhouse (Katholieke Univ. Leuven greenhouse, Heverlee, Belgium) where the temperature and relative humidity were automatically regulated and averaging 25.5 °C and 80%, respectively. The plants had an adequate supply of water through a flood irrigation system whereby the troughs which contained the perforated pots (used to grow the cowpeas) were flooded once a day. There was no application of commercial fertilizer and/or pesticides and there was no visible indication of stress in the plants (that is, leaves drying up, wrinkling/curling up). The leaves were harvested at 6 wk after planting (WAP). This harvest stage was determined based on the time when the vegetables began to pod, which is when they are normally consumed in Kenya. The vegetables were harvested by uprooting the plants from the soil, plucking out the leaves. The moisture content of the leaves averaged 81.8% ± 0.4% (fresh weight). The color of the leaves was also monitored throughout the study (L^*, a^*, and b^* values are 10.55 ± 1.5, –16.21 ± 0.54, and 12.72 ± 4.49, respectively). Defective and yellowing leaves were not used in this study.

After harvesting, the leaves were immediately packed in a bulk of 5 kg in cooling boxes to aid in slowing down the respiration and transpiration during transport from the greenhouse to the research centre. On arrival at the laboratory, the leaves were rinsed under flowing tap water, and the excess water on the leaves was removed by dabbing them in pieces of dry cotton cloth. Then they were chopped into pieces and afterwards packed (approximately 10 g/pouch) in plastic pouches (140 × 200 × 40 mm; Dakla-Pack United Kingdom, Chiswick, London) followed by vacuum-sealing at 34 millibars (Multivac C200 Vacuum Chamber, Sepp Haggenmueller GmbH and Co, Wolfertschwenden, Germany) to minimize oxidative reactions.

The packed fresh leaves were randomly divided into 2 batches of samples, whereby one batch was frozen in liquid nitrogen and stored at –80 °C for later thermal treatment, extraction and measurement of POD activity while another batch was subjected to the study on color degradation after thermal treatment.

In situ thermal stability of POD in cowpea leaves

The thermal stability of POD in cowpea leaves was repeated twice using independent samples. The measurement and quantification of POD activity was conducted in duplicate on each of the samples.

Screening the POD thermal stability. The frozen samples were thawed at 25 °C in a temperature controlled water bath (Memmert water bath WB 22, Memmert GmbH + Co. KG, Schwabach, Germany). After thawing, the plastic pouches containing the sample were heated for 10 min (simulating the average cooking time of the vegetables for households in Kenya) at a predefined temperature from 30 up to 100 °C in a temperature controlled water bath (Memmert Water Bath WBU 45, Memmert GmbH + Co. KG). Pouches containing leaves that were not subjected to any thermal treatment after thawing were used as blanks to assess the initial enzyme activity (A_0) for the temperature screening of the thermal stability of POD. After heat treatment, the pouches containing the samples were cooled in an ice water bath for 20 min, to a temperature of just about 0 °C, and used for extraction and residual POD activity measurement.

Kinetics of thermal inactivation of POD. Samples of 10 g of frozen leaves were thawed at 25 °C in a temperature controlled water bath (Memmert water bath WB 22, Memmert GmbH + Co. KG). The plastic pouches containing the samples were heated at predefined temperatures between 75 and 85 °C for different time intervals in a temperature controlled water bath and from 90 to 100 °C for different time intervals in a temperature controlled oil bath (Memmert oilbath model 1, Memmert GmbH + Co. KG). The temperature in the samples was measured by inserting a thermocouple (T thermocouples; Ellab, Hillerød, Denmark) into the pouches. The longest come up time in this study was 2.10 min which occurred at the lowest temperature used in this study.

Leaves that were not subjected to any thermal treatments after thawing were used as blank (initial enzyme activity, A_0) for the estimation of kinetic parameters for the thermal inactivation of POD. After heat treatment, the plastic pouches containing the samples were cooled in an ice water bath (it took approximately 10 min to reach the ice water bath temperature of just above 0 °C) for 20 min and then subjected to POD extraction.

Extraction of POD. The procedure of POD extraction (Gonçalves and others 2007) was optimized (in terms of the dilution ratio of enzyme extract to buffer and the mode of extraction that is, magic bullet blender, J-26, (Ningbo Vanguard Import and

Export Co., Ningbo, China), and a grinder (Grindomix, GM 200, Germany).

After the thermal treatment ("Kinetics of thermal inactivation of POD" section above), the thermally treated samples (10 g) and the blank samples were opened and the contents emptied in a blender (magic bullet blender, J-26, Ningbo Vanguard Import and Export Co.) and then mixed with sodium phosphate buffer (0.1 M, pH 6) containing 1 M NaCl and 1% polyvinylpyrrolidone (PVPP). This mixture was mixed for 20 s and then the extract was filtered through a 1 mm² sieve with the aid of a vacuum pump. The filtrate was then centrifuged at 17700 × *g* at 4 °C for 30 min. The filtrate was discarded and the supernatant diluted 5 times in sodium phosphate (0.1 M, pH 6.5). This crude extract solution was afterwards frozen in liquid nitrogen and stored at –80 °C until POD assay.

POD activity. The supernatant obtained from the extraction procedure above was thawed in a water bath at 25 °C (Memmert water bath WB 22, Memmert GmbH + Co. KG) and subjected to a POD assay. The measurement of POD activity was done using a spectrophotometric assay in duplicate as described by Bergmeyer (1974) and Gonçalves and others (2007), with slight modifications, with 1% (v/v) guaiacol as a substrate and 0.1% (v/v) H_2O_2 as a cosubstrate. The rate of change in absorbance, A_{470} nm, was monitored using a spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences, Uppsala, Sweden) connected to portable PC running the SWIFT II software (Amersham Biosciences).

The reaction mixture contained 20 μL of enzyme extract, 125 μL H_2O_2 (0.1%), and 1075 μL of 1% guaiacol. The increase in absorbance was followed for 5 min. The blank sample included 100 μL sodium phosphate buffer (0.1 M, pH 6.5), 125 μL H_2O_2 (0.1%) and 1075 μL guaiacol (1%). The assay was carried out at 25 °C implemented by using a 3 L recirculating bath, Haake C10-B, (Karlsruhe, Germany).

One unit of POD activity is defined as the amount of enzyme which oxidizes 1 μmol guaiacol per minute at pH 7 and 25 °C. The specific activity was expressed as units of activity per gram of dry matter of the cowpea leaves. The initial enzyme activity was determined from 4 samples of fresh product (Bergmeyer 1974; Gonçalves and others 2007).

Data analysis to calculate kinetic parameters for POD thermal inactivation

Due to the presence of POD isoenzymes with different thermostability (most often 2 fractions, that is, heat-labile and heat-stable fraction; Ling and Lund 1978; Gonçalves and others 2007) whereby, each fraction of the isoenzyme was inactivated simultaneously according to a first-order kinetic model (Ling and Lund 1978; Gonçalves and others 2007). The k_L and k_S value at each temperature was estimated based on Eq. (1), k_{ref} values and the activation energies (E_a), A_{L0} and A_{S0} (= 1 – A_{L0}) for both fractions were estimated using a 1-step nonlinear regression approach (Eq. (2)). (Ling and Lund 1978; Lund 1983; Arabshahi and Lund 1985; Gonçalves and others 2007; Ludikhuyze 2003).

$$A_L + A_S = A_{L0} \exp(-k_L t) + A_{S0} \exp(-k_S t) \quad (1)$$

$$A_L + A_S = A_{L0} \exp\left(-(k_{refL} \exp\left[\frac{E_{aL}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right])t\right) + A_{S0} \exp\left(-(k_{refS} \exp\left[\frac{E_{aS}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right])t\right) \quad (2)$$

Alternatively, D and z-values can be calculated. The D-value represents the time required to reduce the POD activity by 90%, and z values, the temperature required for a 90% decrease in D value (Ariahu and Ogunsua 2000). These were estimated as follows:

$$A_L + A_S = A_{L0} \times 10^{(-t/D_L)} + A_{S0} \times 10^{(-t/D_S)} \quad (3)$$

$$A_L + A_S = A_{L0} \times 10^{\left(\frac{-t}{D_{refL} \times 10^{((T_{ref}-T)/Z_L)}}\right)} + A_{S0} \times 10^{\left(\frac{-t}{D_{refS} \times 10^{((T_{ref}-T)/Z_S)}}\right)} \quad (4)$$

where A is the total residual, experimentally measured, POD activity (in units), A_L is the residual enzyme activity (in units) of the thermolabile fraction while A_S is the residual enzyme activity (in units) of the thermostable fraction after heat treatment for time (t) in minutes at a given temperature, A_{L0} and A_{S0} is the initial enzyme activity at $t = 0$ for the thermolabile and thermostable fractions of the enzyme, respectively, k is the inactivation rate constant (min^{-1}) whereby for thermolabile phase $k = k_L$ and for the thermostable phase $k = k_S$, k_{ref} the inactivation rate constant

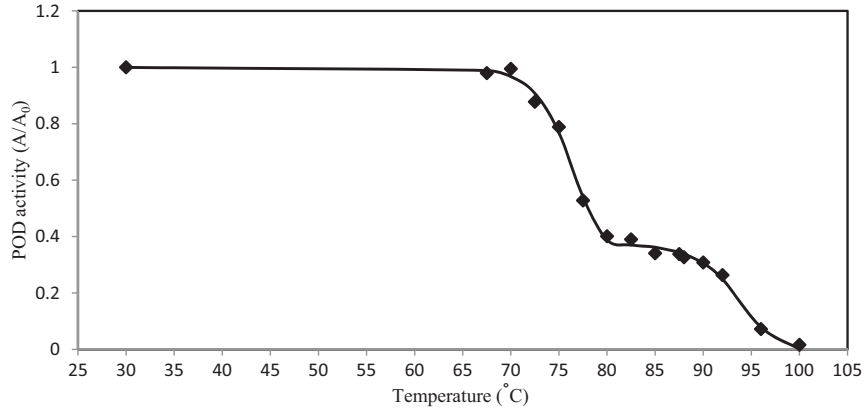

Figure 1–Residual activity of peroxidase (POD) as a function of temperature for cowpea leaves after a thermal treatment of 10 min and subsequent extraction of POD using a blender (magic bullet).

(in min^{-1}) at reference temperature, E_a is activation energy (in kJ/mol), R is the ideal gas constant (8.314 J/mol.K), T is the actual treatment temperature (in K), and T_{ref} was the average value of the range considered (that is, T_{ref} = 80 °C), aiming at improving parameter estimation. Variables D_{refL} and D_{refS} represent the D values at reference temperature T_{ref} (80 °C) for the thermolabile and the thermostable enzyme fractions respectively. The D values (D_L and D_S for the thermolabile and the thermostable enzyme fractions respectively) were estimated based on nonlinear regression analysis (Eq. (3)) while the Z values (Z_L and Z_S for the thermolabile and the thermostable enzyme fractions respectively) were estimated based on 1 step nonlinear regression analysis (Eq. (4)).

Parameter precision was evaluated based on confidence intervals at 95%, and the quality of the regression was assessed by the coefficient of determination (r^2), and randomness and normality of residuals (Hill and Grieger-Block 1980). SAS 9.1 software (SAS Institute Inc., Cary, N.C., U.S.A.) was used for all regression analysis procedures (using least squares estimation and Levenverg–Marquart method, for minimizing the sum of squares of the deviations between experimental values and the ones predicted by the mathematical model).

Moisture content

The moisture content of the leaves was determined by the A.O.A.C method (2002).

Thermal stability of the green color

The thermal stability of the green color in cowpea leaves was performed twice using independent samples. The green color measurement was conducted by taking 6 color measurements on each side of the sample in the pouch.

Screening the thermal color degradation. The thermal treatment (30 to 90 °C) for temperature screening of green color stability was carried out for 10 min (simulating the average cooking time of the vegetables for households in Kenya) as indicated in "Kinetics of thermal inactivation of POD" section above. Samples that were not subjected to any thermal treatment were used to assess the initial green color (C_0).

Kinetics of thermal degradation of green color. To obtain full kinetic data, the thermal treatments (55 to 80 °C) were done as indicated in "Kinetics of thermal inactivation of POD" section above, for different time intervals in a thermostated water bath. Samples that were not subjected to any thermal treatment were used to assess the initial green color (C_0).

Color properties

The color of the leave samples was measured using a handheld tristimulus colorimeter (Minolta Chroma Meter CR-300, Osaka, Japan) and a CIE standard illuminant C to determine CIE color space co-ordinates, L*, a*, and b* values (Francis and Clydesdale 1975). The L* value (lightness value) indicates how dark/light the sample is (varying from 0 for black to 100 for white), a* value is a measure of greenness/redness (varying from –60 to +60), and b* value is a measure of blueness/yellowness (also varying from –60 to +60). The colorimeter was calibrated against a standard white reference tile. The color measurements were performed on the samples in the plastic pouches in which they had been previously packed. The color measurements were done by taking measurements from 6 different spots on each side of the pouch containing the leaves.

Data analysis to calculate kinetic parameters for color thermal degradation

A fractional conversion model was used to describe the thermal degradation of green color of the leaves. This model represents a first-order process reaching a final stable color value after prolonged heating.

$$C = C_\infty + (C_0 - C_\infty)\exp(-k_t \times t) \quad (5)$$

The temperature dependence of the rate constant (at a reference temperature, T_{refC} = 70 °C) is described by an Arrhenius behavior (Eq. 6),

$$k_t = k_{refC}\exp\left[\frac{E_{aC}}{R}\left(\frac{1}{T_{refC}} - \frac{1}{T}\right)\right] \quad (6)$$

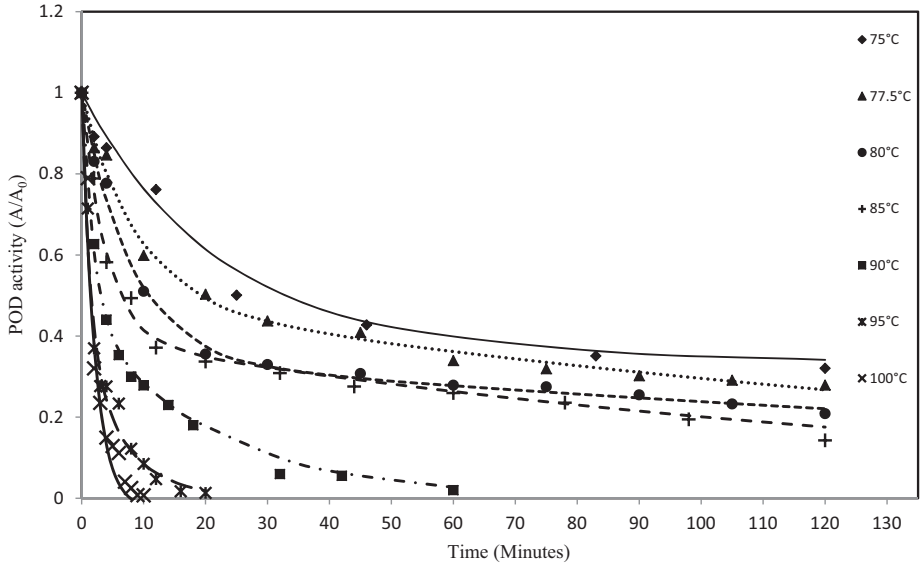

Figure 2–Residual activity of peroxidase (POD) in cowpea leaves as a function of time at different temperatures. Symbols represent experimental values, and lines correspond to individual fittings of the first-order kinetics model to each temperature with an Arrhenius temperature dependence using nonlinear regression analysis (Eq. (2)).

where C is the measured color (in units), after heat treatment of time (t) in minutes at a given temperature, C_0 refers to the initial measured color (before heat treatment, at $t = 0$) and C_∞ is the estimated color after reaching a color loss (degradation) plateau. While k_t is the degradation rate constant (min^{-1}), k_{refC} is the inactivation rate constant (in min^{-1}) at reference temperature. E_{aC} is activation energy (in kJ/mol), R is the ideal gas constant (8.314 J/mol.K), T is the actual treatment temperature (in K), and T_{refC} is the reference temperature (343 K). The k and C_∞ value at each temperature was estimated based on a nonlinear regression analysis (Eq. (5)). The E_{aC} and the K_{refC} values were estimated by integrating the kinetic models considered (Eq. (5)) with Arrhenius equation (Eq. (6)) (Lund 1983; Arabshahi and Lund 1985). A 1-step nonlinear regression approach was used to simultaneously estimate E_{aC}, k_{refC}, and C_∞. The reference temperature used was the average value of the range considered (that is, $T_{refC} =$ 70 °C), aiming at improving parameter estimation. The precision of the estimated parameters was evaluated as earlier indicated in "Data analysis to calculate kinetic parameters for POD thermal inactivation" section.

Results and Discussion

Extraction of POD

The total POD activity was recorded at 2793.7 ± 332.6 (units/g dry weight) and the moisture content (% wet basis) was 81.8 ± 0.4. This total POD activity represents the sum of the free and bound enzyme since sodium chloride was used in the extraction medium to release the POD fraction ionically bound to the cell wall.

In situ thermal stability of POD

Thermal stability screening of POD. The results on the thermal stability screening of POD after 10 min of thermal treatments are indicated in Figure 1. The residual enzyme activities, presented as ratio of measured enzyme activity to initial enzyme activity, as a function of heating temperature were plotted. The thermal stability screening study indicates that POD inactivation in the leaves exhibited a biphasic behavior. In general, the plot in Figure 1 showed that POD is thermally stable (temperature-dependent thermal inactivation) up to around 65 °C. However, POD was inactivated at temperatures above 65 °C where the first fraction was inactivated between 65 and 80 °C and the second fraction was inactivated between 85 and 100 °C. The first fraction is characterized by a steeper decline indicating higher temperature sensitivity compared with the second fraction. These results were important in designing the kinetic experiment to study the thermal inactivation of POD in cowpea leaves, that is, setting of experiments that allow the simultaneous estimation of kinetic parameters of the 2 separate POD fractions.

Thermal inactivation kinetics of POD. Starting from the POD thermal stability screening results, the determination of the thermal inactivation kinetics of POD in the leaves was carried out in a temperature range of 75 to 100 °C for a time of up to 120 min depending on the experimental temperature. Experimental data are presented in Figure 2.

The figure shows 2 sets of distinct curves; those above 85 °C and those below 85 °C. The curves also showed an initial steep straight line, an intermediate curved portion and a final straight line with a shallow slope. These results confirm those obtained for the thermal stability of POD, above. At a given temperature, the inactivation results can be described with the biphasic first-order model proposed by Ling and Lund (1978) based on the presence of 2 isoenzyme groups with distinct thermal stabilities, a heat-labile fraction (initial steep straight line, (Figure 2), that inactivates rapidly and a heat-stable fraction which cannot be inactivated completely (a final straight line with a shallow slope, (Figure 2). The biphasic pattern is also known as the 2-fraction model (Weng and others 1991; Saraiva and others 1996; Rodrigo and others 1997).

The thermal inactivation kinetic parameters (k and D values) were estimated from Eq. (1) and (3) respectively while the z/D_{ref} and E_a/k_{ref} values were estimated from Eq. (2) and (4) respectively and presented in Table 1.

In general, k values increase with increasing temperature (Table 1) and those for heat-labile fractions (k_L) are several times (from 5 to 51) higher than those for heat-stable (k_S) fraction (Table 2). This explains the higher decrease in enzyme activity during the steep section of the graphic presentation of the data (Figure 2) of the thermal treatments.

The temperature-dependence of the inactivation rate constant (k) is explained using the concept of activation energy (E_a). Estimated E_a values for cowpea leaves POD are shown in Table 1. The activation energy of the heat-stable fraction was almost double that of the heat-labile fraction. This can be attributed to the fact the 2 different isoenzymes (proteins) posses different thermal

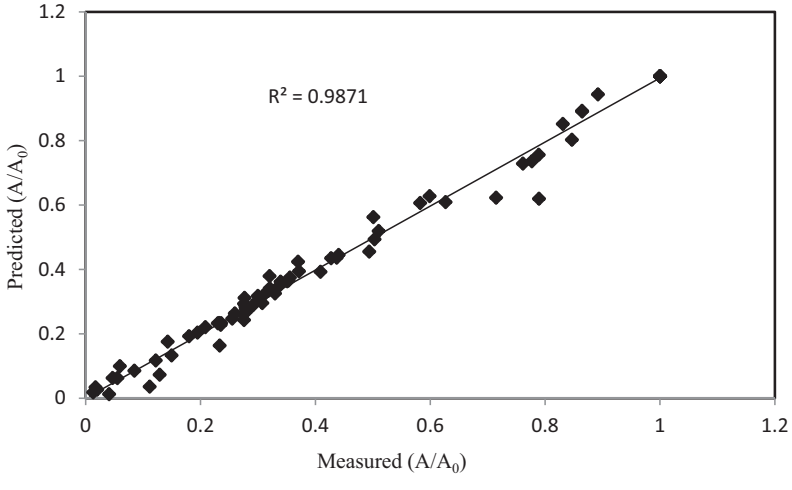

Figure 3–Linearized scatter plot of predicted compared with measured residual activity of peroxidase (POD) activity in cowpea leaves.

Table 1–Estimated kinetic parameters for thermal inactivation of peroxidase (POD) in cowpea leaves.

Parameter estimate						
Temperature	Heat-labile fraction			Heat-stable fraction		
(°C)	k ($\times 10^{-2}$ min^{-1})	D (min)	Fraction of enzyme	k ($\times 10^{-2}$ min^{-1})	D (min)	Fraction of enzyme
75	4.67 ± 1.24	49.33 ± 13.13	0.64	0.09 ± 0.003	2610.90 ± 89.97	0.36
77.5	11.41 ± 1.27	20.19 ± 2.24	0.51	0.50 ± 0.07	459.20 ± 61.96	0.49
80	12.64 ± 0.92	18.21 ± 1.33	0.65	0.38 ± 0.07	605.70 ± 11.20	0.35
85	25.09 ± 2.47	9.18 ± 0.90	0.60	0.68 ± 0.09	340.80 ± 45.18	0.40
90	50.53 ± 6.60	4.56 ± 0.59	0.55	4.72 ± 0.52	48.74 ± 5.40	0.45
95	75.38 ± 23.9	3.06 ± 0.97	0.80	15.30 ± 0.05	15.05 ± 4.92	0.20
100	ND	ND	0.99	45.84 ± 10.45	5.69 ± 0.81	0.04
T_{ref} = 80 °C	k_{ref} ($\times 10^{-2}$ min^{-1})	D_{ref} (min)		k_{ref} ($\times 10^{-2}$ min^{-1})	D_{ref} (min)	
	11.52 ± 0.95	19.63 ± 1.71		0.29 ± 0.07	687.4 ± 148.2	
	E_a (kJ/mol)	Z value (°C)		E_a (kJ/mol)	Z value (°C)	
	109.67 ± 6.20	22.75 ± 1.34		256.93 ± 15.27	10.03 ± 0.59	

ND, not detected.
Note: The standard errors are defined at 95% confidence level.

Table 2–Ratio of the estimated kinetic parameters (k_L/k_S) for thermal inactivation of POD in cowpea leaves.

Temperature (°C)	Ratio of k_L/k_S
75	51.9
77.5	22.8
80	33.3
85	36.9
90	10.7
95	4.9
100	ND

ND, not detected.

stability with the most common mechanism of inactivation being denaturing of proteins and to some extend loss of haem POD (Lu and Whitaker 1974; Tamura and Morita 1975; Anthon and Barrett 2002). A higher activation energy for the heat-stable fraction compared to the heat-labile fraction was also observed by Günes and Bayindirh (1993) whereby POD in peas (4.1×10^4 J/mol against 7.5×10^4 J/mol), green beans (5.7×10^4 J/mol against 7.7×10^4 J/mol), and carrots (5.2×10^4 J/mol against 5.7 × 104 J/mol) was lower for the heat-labile fraction compared with the heat-stable fraction.

The estimated E_a values for POD in the leaves were higher than those reported for often vegetables such as broccoli (florets), green asparagus (tip), green asparagus (stem), carrot (cortex), and carrot (core) by Morales-Blancas and others (2002) where the values ranged from 61 to 97 kJ/mol for the heat-labile fraction and 43 to 83 kJ/mol for the heat-stable fraction respectively. However, the cowpea leaves POD values compared well with those recorded by Gonçalves and others 2009 (159 kJ/mol) for experiments that were performed *in situ* on broccoli samples. The experimental results obtained for POD in cowpea leaves are satisfactorily described by a first-order kinetic model (biphasic), for all temperatures tested (Figure 2). The quality of the model fit was assessed by analyses of a scatter plot between measured and predicted values of A/A_0 and their corresponding values of R^2 (Figure 3). Having established that cowpea leaves POD consists of both a heat-labile and heat-stable isoenzyme fraction and keeping in mind the fact that the residual activity could play a role in the vegetable shelf life (or be used as an indicator), it is important to determine the amount of heat-labile compared with heat-stable fraction. The results are shown in Table 1, where approximately 64% of the enzyme activity represents the heat-labile fraction, while 36% is heat-stable. These results are similar to those obtained by Morales-Blancas and others (2002) for the carrot core section (35.1% ± 6.1% for the heat stable fraction). Residual enzyme activity is dependent on the initial enzyme load since a low percentage of residual enzyme activity could still represent a significant enzyme activity if the initial total activity was high enough.

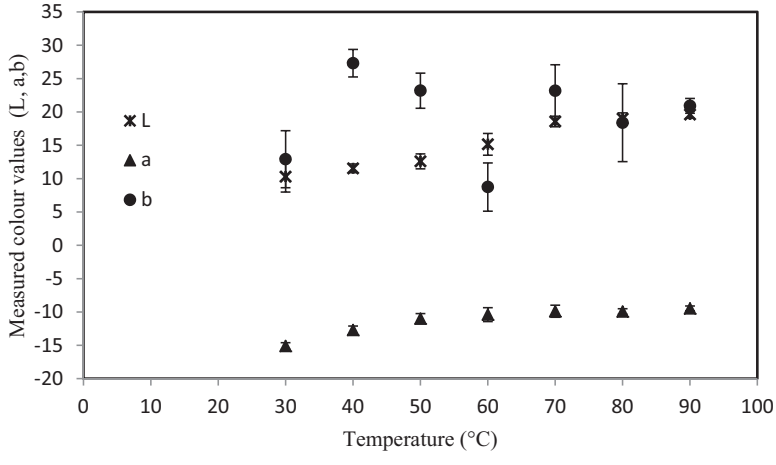

Figure 4–Changes in the color of cowpea leaves, measured as color values L∗, a∗, and b∗ as a function of temperature after a thermal treatment of 10 min. Symbols represent experimental values. The error bars are standard errors at 95% confidence interval. *Initial L*, a*, and b* values are 10.55 ± 1.5, –16.21 ± 0.54, and 12.72 ± 4.49, respectively.

Green color

Effect of temperature/time on green color of cowpea leaves. Figure 4 shows the effect of temperature/time treatment on the green color of the leaves expressed as Hunter L*, a*, and b* values. There is a consistent decrease in L* and "-a*" values with an increase in the treatment time and temperature. However, there was no consistent change in b* value. This phenomenon was also observed by Nisha and others (2004) in thermal treatments on spinach. The change in L* and b* values may be due to pheophytin conversion or due to degradation/reaction of other components present in the vegetables (Weemas and others 1999). Thus, since the greenness is indicated by "-a*," and due to the fact that the green is the dominant color for quality attributes of vegetables, the kinetic study were carried out only with respect to "- a*" values.

Figure 4 also indicates a rapid loss of green color between 30 and 75 °C, and from 75 °C, the "-a*" values reach a plateau phase. This indicates a biphasic model of the fractional conversion type as opposed to POD which indicated a further POD inactivation after the plateau (or interphase or flattening out), a characteristic of the biphasic model of the consecutive step type.

Degradation kinetics of green color. Using nonlinear regression, the green color degradation data were analyzed using Eq. (5) and (6) to determine the rate constant for the degradation reaction. The degradation rate constants are shown in Table 3, indicating a general increasing trend of the k values with increasing temperature, as corroborated by Figure 5.

Table 3–Estimated kinetic parameters for thermal degradation of green color (measured as "-a*" values) in cowpea leaves.

Temperature (°C)	Parameter estimate k ($\times 10^{-2}$ min^{-1})	C_∞
55	1.76 ± 0.01	0.49 ± 0.08
60	6.00 ± 0.01	0.33 ± 0.02
65	7.83 ± 0.02	0.36 ± 0.05
70	11.4 ± 0.02	0.41 ± 0.05
75	19.4 ± 0.03	0.42 ± 0.03
80	29.67 ± 0.05	0.43 ± 0.03
T_{refC} = 70 °C	k_{refC} ($\times 10^{-2}$ min^{-1}) 13.53 ± 0.01	
	E_{aC} (kJ/mol) 88.78 ± 3.21	

Note: The standard errors are defined at 95% confidence level.

Table 3 also shows that a little more than 50% of the color degraded leading to a final plateau value of around 46.4%. This means that the green color of the vegetables will rapidly deteriorate in the first few minutes of cooking and afterwards the color will remain stable for much of the entire cooking time. The results in Table 3 and Figure 5, show that the thermal degradation of the green color in vegetables is of first-order kind with Arrhenius temperature dependence. The activation energy, E_a, for green color in the leaves (88.78 ± 3.21 kJ/mol) was higher than that in spinach puree, approximately 37.57 kJ/mol (Nisha and others (2004), and E_a of 28.55, 41.15, and 34.01 kJ/mol for spinach

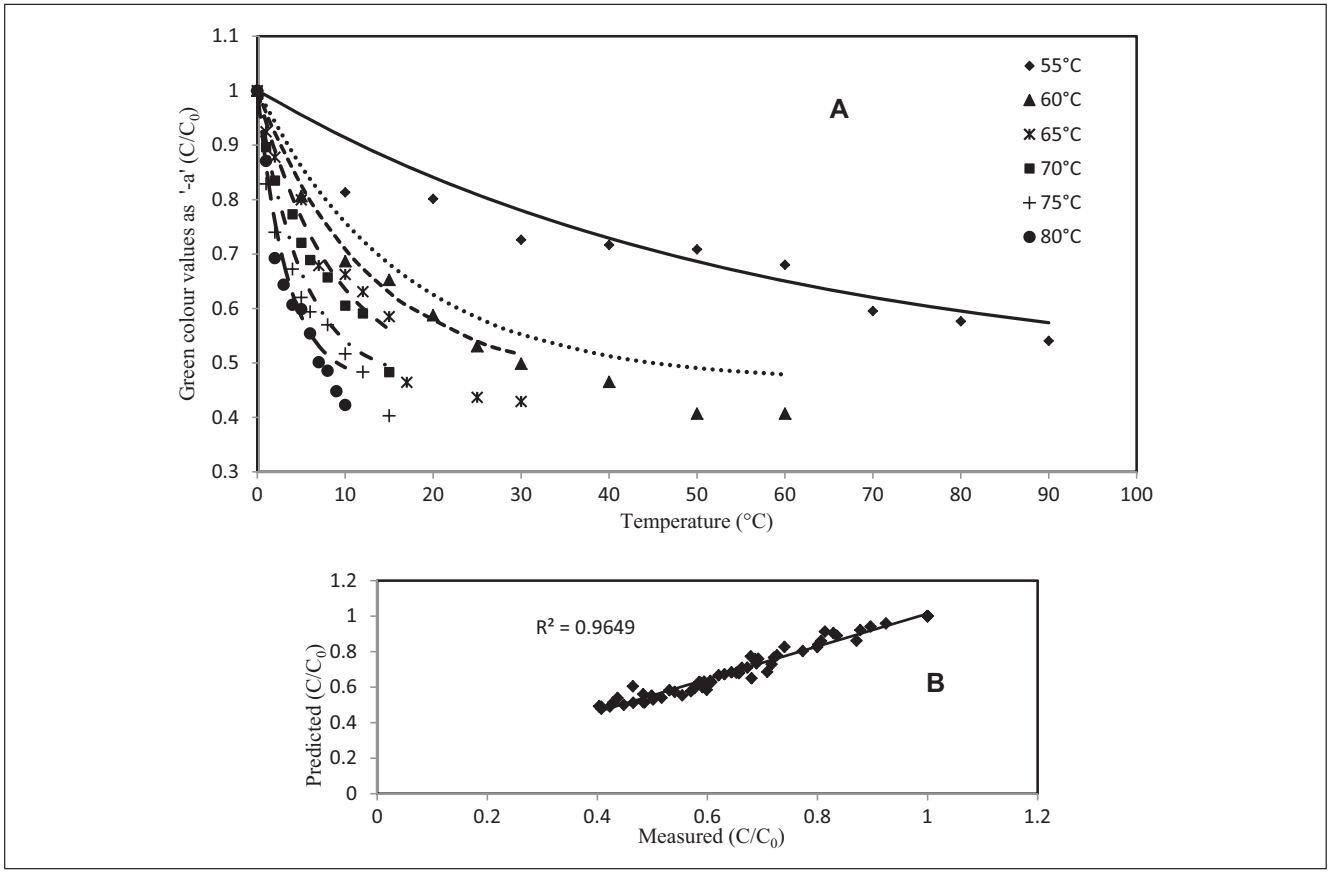

Figure 5–(A) Residual green color, (measured as '-a*' values) in cowpea leaves, as a function of time at different temperatures. Symbols represent experimental values, and lines correspond to individual fittings of the first-order kinetic model to each temperature with an Arrhenius temperature dependence using nonlinear regression analysis. (B) Linearized scatter plot of predicted compared with residual green color (measured as '-a*' values) in cowpea leaves.

puree, mustard leaves, and a mixed puree, respectively (Ahmed and others 2002).

Ahmed and others (2002) reported an E_a of 11.34 to 15.98 kJ/mol for color degradation in green chilli puree while Weemas and others (1999) reported an E_a of 72.01 kJ/mol for heated broccoli juice. These variations may be attributed to the differences in the raw material due to variations in levels of pH, salt, minerals, and the general matrix structure that can affect the green color stability.

Conclusion

This study shows that the kinetics of POD inactivation and color degradation are quite different. Color degradation of the leaves occurred much faster than the POD inactivation and, therefore, other methods than direct thermal processing to stabilize the green color (pH, use of salts) need to be considered while inactivating POD. The impact of a blanching process on quality attributes of the vegetables can be gauged by monitoring a parameter that represents the "cooked quality" of the processed food product, in this case represented by the green color. In order to optimize product quality, it is desirable to keep this cook value as low as possible, while assuring the required time needed to inactivate the undesirable microorganisms and enzymes as indicated by the inactivation of POD.

Based on the kinetic data obtained in this study, it is suggested that color degradation during thermal processing can be used to describe the cook value of the processing of cowpea leaves, however this process value will result in partial POD inactivation. As a consequence, shelf life studies, should be undertaken in future research.

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Author Contributions

Michael Wawire designed the study, collected test data, interpreted the results and drafted the manuscript, reviewed the manuscript. Indrawati Oey designed the study, collected test data, interpreted the results, reviewed the manuscript. Francis M. Mathooko designed the study, reviewed the manuscript. Charles K. Njoroge collected test data, reviewed the manuscript. Douglas Shitanda interpreted the results, reviewed the manuscript. Marc Hendrickx designed the study, interpreted the results, reviewed the manuscript.

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