Elicitation kinetics of phenolics in common bean (Phaseolus vulgaris) sprouts by thermal treatments

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Abstract
Phenolics are plant secondary metabolites with numerous health benefits, produced via the phenylpropanoid pathway in plants in response to environmental conditions. In this study, the mathematical relationship between thermal elicitation (25°C, 30°C, 35°C, and 40°C) of phenolic compounds through the accumulation of oxidative stress markers (hydrogen peroxide [H₂O₂], malondialdehyde [MDA], catalase [CAT], and guaiacol peroxidase [GPX]) and activation of phenylpropanoid triggering enzymes (phenylalanine ammonia-lyase [PAL] and tyrosine ammonia-lyase [TAL]) was kinetically modeled at different sprouting stages of common bean. The rate of H₂O₂ and MDA formation showed an increasing trend with an increase in sprouting temperature. However, activation rates of CAT, GPX, PAL, and TAL were highest at 30°C, after which there were significant reductions. Also, activation rate of PAL was lower as compared with TAL, which was further established with its low activation energy Eₐ value of 150 kJ/mol compared with TAL (221 kJ/mol). Also, activation energy values for total phenolic acids (30.4 kJ/mol) and flavonoids (64.0 kJ/mol) showed that they required less energy for formation during sprouting, compared with anthocyanins (209 kJ/mol), with the activation energy results obtained from their estimated kinetic rate constants and production percentages. Thus, manipulation of sprouting temperature can increase the potential use of common beans as natural functional foods with improved health benefits.

KEYWORDS
common bean, elicitation, kinetics, sprout, temperature

1 | INTRODUCTION

The phenylpropanoid pathway (PPP) has been identified as one of the main defense mechanisms triggered under environmental stress for production of plant defense secondary metabolites such as phenolics. Numerous enzymatic steps are associated with the PPP (Singh, Singh, Kaur, & Singh, 2017). The initial stage of PPP requires the deamination of phenylalanine into trans-cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL). The cinnamic acids are subsequently used as intermediates by different enzymes for production of downstream phenolic compounds (Deng & Lu, 2017). Phenolics are benzene-ringed secondary metabolites with one or more hydroxyl groups present in their structure. They are classified into phenolic acids, flavonoids, stilbenes, and tannins based on structural differences (Aquino-Bolaños et al., 2016). In vivo and in vitro studies have connected the consumption of phenolic-rich foods to health benefits...
such as antioxidative, anti-inflammatory, antidiabetic, anticancer, and antimutagenic effects (Curran, 2012). Thus, demand exists for minimally processed plant foods that have high phenolic contents.

Common beans (Phaseolus vulgaris) are pulse foods that have become dietary components of human populations since ancient times (Gan et al., 2016). The wide consumption of common bean is not only due to its protein, fiber, resistant starch, and B-vitamin content but also due to the presence of bioactive secondary metabolites such as phenolics (Gan et al., 2016). However, depending on cultivar, growing conditions, geographical location, and growth stage, the concentration of phenolics differs among common bean varieties (Sutivisedsak et al., 2010). In order to increase phenolic yield, technologies including genetic engineering, tissue culture, and sprouting have been applied in plant foods. Genetic engineering is limited by its high cost and low consumer acceptance, whereas tissue culture has been shown to cause cell damage as well as resulting in lower yield (Owolabi et al., 2018). However, although sprouting is a cheap, simple, and consumer acceptable household process, it is a slow process and has low yield compared with genetic engineering and tissue culture (Swieca & Baraniak, 2014), thus the need to develop technologies that can increase the rate of PPP during sprouting for enhanced phenolic accumulation.

One such approach is the process of elicitation. Elicitation is the induction of physiological processes in plants, in the presence of an elicitor/stressor. During elicitation, applied elicitors/stressors (e.g., temperature, chemicals, light, and ultrasound) induce wounds, osmotic disruptions, and membrane weakening, leading to increased production of oxidative stress markers (e.g., reactive oxygen species such as hydrogen peroxide [H₂O₂] and OH radicals) and subsequent stimulation of the PPP for biosynthesis of phenolics as antioxidative protectants (Liu et al., 2019). For instance, a previous study by Swiec and Baraniak (2014) reported on 40⁰C-elicited lentil sprouts with total phenolic acid and flavonoid contents of 23.70 and 2.50 mg/g, respectively, compared with control sprouts (25⁰C) which possessed 19.8 and 1.84 mg/g contents, respectively. However, no comparative investigation on triggering of PPP, phenolics accumulation, and antioxidative properties of common bean at the sprouting developmental phase has been reported. A good understanding of the optimum elicitation conditions during sprouting will be very significant in designing processing systems for optimizing phenolic synthesis and producing phenolic-enriched sprouts. Thus, the objectives of this study were to establish the kinetic relation between thermal elicitation and stress markers, catalysis of PPP stimulating enzymes, and phenolic concentrations of common bean at different sprouting stages by kinetic modeling.

2 | MATERIALS AND METHODS

2.1 | Common bean seeds

Common bean cultivar “Kabulengeti” was provided by the Council for Scientific and Industrial Research Center of Zambia. This cultivar has high yield stability, high tolerance to diseases and pests, and possesses protein and lipid contents of 24.38% and 1.38%, respectively. However, it has low levels of total phenolic acids (3.76 mg CE/100 g) and flavonoids (2.24 mg RE/100 g). Therefore, it was chosen with this study as a model for common bean cultivars with low concentrations of phenolic compounds.

2.2 | Elicitation processes

Common bean seeds (100 g) were disinfected (1% sodium hypochlorite in 500 ml) for 15 min and rinsed afterwards to obtain neutral pH. For thermal elicitation, disinfected seeds were soaked in distilled water (1:3 w/v, 25°C, 24 h; Mubarak, 2005). Afterwards, hydrated seeds were sprouted in darkness at 25°C, 30°C, 35°C, and 40°C for 24, 48, 72, and 96 h in a temperature regulated incubator (Model-12-140E, Quincy Laboratory, INC. Chicago, USA). During the sprouting process, sprouts were sprayed with distilled water at intervals of 12 h. After each treatment, the treated sprouts were frozen and lyophilized for analysis. For each treatment, spraying was performed in triplicates.

2.3 | Determination of oxidative stress markers

2.3.1 | H₂O₂ and MDA determination

H₂O₂ content was evaluated as described by Alexieva, Sergiev, Mapelli, and Karanov (2001). Approximately 0.1 g of sample flour was homogenized with 1 ml of mixed solution (0.25 ml of 0.1% trichloroacetic acid [TCA], 0.5 ml of 1 M potassium iodide [KI], and 0.25 ml of 10 mM potassium phosphate buffer, pH 7.0) at 4°C for 10 min. Afterwards, the obtained mixture was centrifuged (10,000 g, 15 min, 4°C); the supernatant was recovered and kept in the dark for 30 min and its absorbance measured at 390 nm with a microplate reader. A control was prepared with H₂O instead of KI.

Lipid peroxidation was determined by measuring accumulation of malondialdehyde (MDA) as described by Dhindsa, Plumb-Dhindsa, and Thorpe (1981). Approximately, 0.2 g of sample was homogenized in 2 ml of 5% TCA for 10 min and centrifuged at 13,500 g for 15 min at 25°C. Afterwards, 1 ml of supernatant was mixed with 1 ml of 0.5% (v/v) thio-barbituric acid (in 20% (v/v) TCA). The obtained mixture was heated at 96°C for 30 min, cooled in ice bath and centrifuged at 9,500 g for 10 min. After centrifugation, absorbance of the recovered supernatant was measured at 532 nm, and the value for nonspecific absorption at 600 nm was measured and subtracted. MDA concentration was expressed as nmol MDA per g of dry weight.

2.3.2 | Determination of antioxidant enzymes

Extract preparations and protein content determination

Total protein assay. Protein contents of enzyme extracts were measured according to the method of Bradford (1976). Bovine
serum albumin was used as the reference protein to calibrate the assay.

**Enzyme extraction.** Briefly, 200 mg of sample was homogenized at 4°C for 20 min in 4 ml of sodium phosphate buffer (100 mM, pH 6.4) containing 0.2 g of polyvinylpyrrolidone. Next, homogenized mixture was centrifuged (12,000 g, 4°C, 30 min), and supernatant collected as enzyme extract (Swieca, Seczyk, & Gawlik-Dziki, 2014). Catalase (CAT) and guaiacol peroxidase (GPX) assays were performed on the same day of extraction. Enzyme extraction was performed in triplicate.

**CAT and GPX assays.** For CAT activity, the reaction mixture (0.05 ml of enzyme extract, 0.95 ml of 10 mM H$_2$O$_2$ in 100 mM sodium phosphate buffer, pH 7) was incubated at 30°C for 1 min, and decomposition of H$_2$O$_2$ was measured at 240 nm. One unit (1 U) of CAT activity was defined as the amount of CAT capable of decomposing 1.0 μmol H$_2$O$_2$ per min under the conditions of the assay (Swieca et al., 2014).

GPX assay was performed as described by Burguieres, McCue, Kwon, and Shetty (2007). The reaction mixture (0.1 ml of enzyme extract, 2 ml of 8 mM guaiacol in 100 mM sodium phosphate buffer-pH 6.4) was incubated at 30°C for 1 min. Afterwards, 1 ml of 24 mM H$_2$O$_2$ was added, and the change in absorbance was measured at 460 nm.

### 2.4 | Activation of phenylpropanoid triggering enzymes

**2.4.1 | Enzyme extraction**

Two hundred (200) milligrams of sample flour were homogenized with 2 ml extracting buffer (0.2 M boric acid buffer containing, 1 mM ethylenediaminetetraacetic acid [EDTA] and 50 mM β-mercaptoethanol, pH 8.8) for 20 min at 4°C. Afterwards, the homogenized mixture was centrifuged (12,000 g, 4°C, 30 min) and supernatant collected as enzyme extract (Swieca et al., 2014). Enzymatic extractions were performed in triplicate.

**2.4.2 | Determination of PAL and TAL activities**

For PAL activity, the reaction mixture (300 μl of enzyme extract, 1.2 ml of 0.02 M L-phenylalanine and 2 ml of PAL extracting buffer) was incubated at 30°C for 60 min. Afterwards, the reaction was terminated by adding 0.5 ml of 10% TCA, centrifuged (15,000 g, 10 min, 4°C) and absorbance of supernatant measured at 290 nm; 1 U was defined as the amount of PAL that produced 1.0 μmol trans-cinnamic acid per min under conditions of the assay (Assis, Maldonado, Munoz, Escribano, & Merodio, 2001).

Activity of tyrosine ammonia-lyase (TAL) was determined by incubating reaction mixture (100 μl of enzyme extract and 0.9 ml of 0.02 M L-tyrosine) for 60 min at 30°C. Next, the reaction was stopped by adding 0.5 ml of 10% TCA, centrifuged (15,000 g, 10 min, 4°C) and absorbance of supernatant read at 310 nm. One unit (1 U) was defined as the amount of TAL that produced 1.0 μmol p-coumaric acid per min under conditions of the assay (Assis et al., 2001).

### 2.5 | Assessment of phenolic compounds and antioxidant capacities

#### 2.5.1 | Extraction of phenolic compounds

Methanolic extraction of phenolics was carried out as described by Marathe, Rajalakshmi, Jamdar, and Sharma (2011). Approximately 1 g of sample flour was extracted for 20 min with 15 ml of 80% aqueous methanol. Pooled triplicate extractions were centrifuged (5,000 rpm, 10 min), and supernatant organic solvents were removed at 35 ± 3°C by using a rotary vacuum evaporator. The obtained extract was freeze-dried and dissolved in 80% aqueous methanol prior to ultraviolet (UV)-vis quantification.

#### 2.5.2 | Determination of total phenolic acids and anthocyanins

Total phenolic acids and anthocyanin contents of methanolic extract were measured according to the method of Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999), with their total contents calculated as catechin and cyanidin-3-glucoside equivalents in mg/100 g of sample flour. Standard curves were prepared with (+)-catechin (0–200 μg ml$^{-1}$) and cyanidin-3-glucoside (0–20 μg ml$^{-1}$) for quantitative measurements of total phenolic acids and anthocyanins, respectively.

#### 2.5.3 | Determination of total flavonoids content

Total flavonoids were analyzed as described by Hairi, Sallé, and Andary (1991), and expressed as rutin equivalent in mg/100 g of sample flour. A standard curve for quantitative measurement was prepared with rutin (0–50 μg ml$^{-1}$).

### 2.6 | Kinetic analysis

Kinetics of the experimental data were determined by the dimensionless normalized concentration $\frac{A}{A_0}$ at the different sprouting temperatures and time; where $A_0$ and $A$ are the concentrations of phenolic compounds in elicited and untreated samples, respectively. According to Chen and Ramaswamy (2002), the first-order kinetic model is suitable for studying activities of enzymes and accumulation of biochemical components. Thus, kinetics of all investigated biomolecules, enzymes, and antioxidant capacities were calculated according to the following first-order equation:
\[ \ln(A) = -Kt + \ln A_0. \]  

(1)

Furthermore, the Arrhenius equation was used to evaluate the temperature dependence of the reaction rate \( k \):

\[ k = k_0 \exp \left( \frac{-E_a}{RT} \right), \]

(2)

where \( k_0 \) is the pre-exponential factor \( h^{-1} \), \( E_a \) is the activation energy of the enzymatic reaction, \( R \) is the gas constant, and \( T \) is the absolute temperature.

2.7 | Statistical analysis

Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey test at a probability (\( p \)) of 0.05 using SAS statistical software 2019 (version 9.4, SAS Institute Inc. Cary, North Carolina, USA). Results were presented as mean ± standard deviation. Pearson correlation test was used to evaluate correlation among variables.

3 | RESULTS AND DISCUSSION

3.1 | Accumulation kinetics of oxidative stress markers

The kinetic parameters for accumulation of oxidative stress markers H\(_2\)O\(_2\), MDA, CAT, and GPX are presented in Table 1. The accumulation rate \( k \) (\( h^{-1} \)) of H\(_2\)O\(_2\) increased with elicitation temperatures, with treatment of 40°C eliciting the highest H\(_2\)O\(_2\) rate constant of 0.95 \times 10^{-2}. Thus, there was a 1.83-fold increase in the rate of H\(_2\)O\(_2\) production with the increase in elicitation temperatures (25°C–40°C) applied to sprouts.

According to Kumar et al. (2013), exposure of plant cells to temperatures above threshold levels increases cellular damage and respiratory activities, leading to an increased accumulation of free radicals (e.g., H\(_2\)O\(_2\)) as metabolic end-products. Also, the determination coefficients (\( R^2 \)) were very high at 0.99 (except 0.58 at 25°C), implying that the experimental data for H\(_2\)O\(_2\) fit well the first-order kinetic model under the temperatures tested.

Similar to H\(_2\)O\(_2\) accumulation, an increase in elicitation temperatures of sprouts increased the rate of MDA production. As shown in Table 1, the estimated rate constant, \( k \) (\( h^{-1} \)) for production of MDA at 25°C was 0.417 \times 10^{-2}. This rate increased significantly by 46.9% over a 15°C temperature rise. Exposure of plant cells to elevated temperatures induces oxidative stress through peroxidation of polyunsaturated fatty acids into harmful aldehydes such as MDA (Swieca et al., 2014). Furthermore, determination coefficients (\( R^2 \)) estimated for MDA production ranged from 0.92 to 0.98, suggesting that obtained experimental data fitted well the first-order kinetic model under investigated temperatures.

Activities of endogenous antioxidant enzymes such as CAT and GPX are significant during stress to protect plant cells. CAT is the enzyme responsible for maintaining threshold levels of H\(_2\)O\(_2\), by breaking it down into water and oxygen. In this study, increasing thermal elicitation to 30°C resulted in an estimated rate constant \( k \) (\( h^{-1} \)) of 0.883 \times 10^{-2} for CAT activation (Table 1). However, this rate was

| Oxidative stress marker | Thermal elicitation (°C) | 25 | 30 | 35 | 40 |
|-------------------------|--------------------------|----|----|----|----|
| H\(_2\)O\(_2\)          |                          |    |    |    |    |
| \( k \) \( [k \times 10^{-2}(h^{-1})] \) | 0.523 ± 0.000            | 0.820 ± 0.000 | 0.927 ± 0.000 | 0.957 ± 0.001 |
| \( A_0 \)              | 0.074 ± 0.001            | 0.112 ± 0.008 | 0.136 ± 0.003 | 0.176 ± 0.005 |
| \( R^2 \)              | 0.576                    | 0.998 | 0.994 | 0.996 |
| MDA                    |                          |    |    |    |    |
| \( k \) \( [k \times 10^{-2}(h^{-1})] \) | 0.417 ± 0.001            | 0.510 ± 0.000 | 0.550 ± 0.001 | 0.785 ± 0.001 |
| \( A_0 \)              | 10.689 ± 1.403           | 38.913 ± 2.273 | 44.535 ± 2.886 | 58.087 ± 3.986 |
| \( R^2 \)              | 0.979                    | 0.921 | 0.922 | 0.971 |
| Catalase               |                          |    |    |    |    |
| \( k \) \( [k \times 10^{-2}(h^{-1})] \) | 0.350 ± 0.000            | 0.883 ± 0.002 | 0.517 ± 0.001 | 0.290 ± 0.000 |
| \( A_0 \)              | 6.370 ± 0.004            | 42.909 ± 1.335 | 35.296 ± 0.586 | 6.127 ± 1.122 |
| \( R^2 \)              | 0.960                    | 0.978 | 0.994 | 0.998 |
| Guaiacol peroxidase    |                          |    |    |    |    |
| \( k \) \( [k \times 10^{-2}(h^{-1})] \) | 0.030 ± 0.000            | 1.643 ± 0.001 | 0.913 ± 0.001 | 0.860 ± 0.002 |
| \( A_0 \)              | 0.654 ± 0.006            | 8.752 ± 0.286 | 5.254 ± 0.356 | 3.999 ± 0.042 |
| \( R^2 \)              | 0.801                    | 0.988 | 0.978 | 0.997 |

Note: Data are presented as mean ± SD of three independent experiments. 
Abbreviations: H\(_2\)O\(_2\), hydrogen peroxide; MDA, malondialdehyde; \( k \), rate constant; \( R^2 \), coefficient of determination.
significantly reduced by threefold when the temperature was increased by 10°C. GPX activation showed the same trend. Elicitation at 35°C estimated a rate constant \( k (\text{h}^{-1}) \) of 1.643 \( \times 10^{-2} \) for activation of GPX, with this value decreasing by 47.67% when temperature was further increased to 40°C. Reduced activation rates observed for CAT and GPX above 30°C can be attributed to elevated levels of \( \text{H}_2\text{O}_2 \) and MDA at higher temperatures. Above threshold levels, \( \text{H}_2\text{O}_2 \) and MDA have been reported to induce unfolding of enzyme tertiary structure, reduce catalytic activity, and subsequent inactivation (Djanaguiraman & Prasad, 2010). Overall, results on oxidative stress markers demonstrated that, although increasing sprouting temperatures up to 45°C can elevate the production rate of \( \text{H}_2\text{O}_2 \) and MDA, the recommended temperature for optimum activation rate of antioxidant enzymes during sprouting is 35°C. This is because production of high \( \text{H}_2\text{O}_2 \) and MDA is not the object of phenolics elicitation, but rather undesirable outcomes.

### 3.2 | Activation of phenylpropanoid triggering enzymes

#### 3.2.1 | Activation kinetics of PAL and TAL activities

Accumulation of \( \text{H}_2\text{O}_2 \) and MDA during elicitation has been linked to the activation of PAL and TAL, the enzymes responsible for triggering PPP (i.e., the main phenolic biosynthetic pathway). Kinetic parameters for activation of PAL and TAL during thermal elicitation of common bean sprouts are shown in Table 2. Within investigated temperatures, the rate of PAL activation varied from 0.4900 \( \times 10^{-2} \) to 1.40 \( \times 10^{-2} \). The first-order rate constant \( k (\text{h}^{-1}) \) for PAL activation was statistically higher in sprouts incubated at low temperatures (30°C) compared with high thermal treatments (35°C and 40°C).

As observed for PAL activation, high elicitation temperatures reduced the activation rate of TAL. The highest TAL activation rate of 2.190 \( \times 10^{-2} \) was observed at 30°C, with this trend decreasing by about 91.8% when sprout elicitation temperature reached 40°C. Overall, the estimated activation rates of PAL and TAL from this study suggest that elicitation temperatures more than 30°C induce lower expression of enzymes responsible for stimulating the PPP, as further confirmed with estimated determination coefficients \( (R^2) \) within ranges of 0.92–0.99 (PAL) and 0.70–0.99 (TAL). This trend can be linked to elevated production rates of \( \text{H}_2\text{O}_2 \) and MDA with increasing temperatures. Also, Goldson-Barnaby and Scaman (2013) reported 30°C as the optimum temperature for maximum activation of PAL and TAL, compared with 40°C and 50°C, attributing their observation to heat denaturation of functional tertiary structures.

### 3.3 | Phenolics accumulation during thermal elicitation of common bean sprouts

Figure 1 shows the heat-induced formation of total phenolic acids, flavonoids, and anthocyanins as common bean sprouting temperature and time increased. With all evaluated phenolic compounds, there were continuous increments in their concentrations with increasing sprouting time, relative to their initial concentration. For total phenolic acids, the highest percentage formation of 3,720% was observed with 30°C treatment at 96 h of sprouting time. This trend was 72.98%, 85.49%, and 89.43%, significantly higher compared with the percentage formation of total flavonoids with 35°C, 40°C, and 25°C treatments, respectively at the same sprouting time. The formation of total flavonoids was lower at higher temperatures, compared with 30°C treatment. In increasing order, the percentage formation of total flavonoids was 5,334 (30°C) > 3,557 (35°C) > 2,843 (40°C) > 1,567 (25°C) at 96 h of sprouting, compared with the initial flavonoid concentration (2.24 mg/100 g).

Similarly, treatment at 30°C for 96 h of sprouting yielded the highest percentage formation of total anthocyanins by 637%, compared with its initial concentration of 1.64 mg/100 g. This observation at 30°C was 1.18, 1.47, and 2.87 fold higher than total anthocyanin

### TABLE 2  
First-order kinetic parameters for activation of phenylpropanoid triggering enzymes during thermal elicitation of common bean sprouts

| PPP enzyme | Thermal elicitation (°C) |   |   |   |
|------------|-------------------------|---|---|---|
|            | 25                      | 30 | 35 | 40 |
| PAL        |                         |   |   |   |
| \( k (\times 10^{-2}[\text{h}^{-1}]) \) | 0.490 ± 0.000 | 1.400 ± 0.002 | 1.310 ± 0.001 | 1.210 ± 0.003 |
| \( A_0 \)  | 1.074 ± 0.008           | 5.088 ± 0.144 | 1.728 ± 0.006 | 1.175 ± 0.025 |
| \( R^2 \)  | 0.922                   | 0.975         | 0.979         | 0.997         |
| TAL        |                         |   |   |   |
| \( k (\times 10^{-2}[\text{h}^{-1}]) \) | 1.140 ± 0.001 | 2.190 ± 0.008 | 1.480 ± 0.002 | 0.180 ± 0.001 |
| \( A_0 \)  | 0.531 ± 0.030           | 3.571 ± 0.414 | 1.829 ± 0.149 | 0.099 ± 0.042 |
| \( R^2 \)  | 0.701                   | 0.995         | 0.961         | 0.943         |

Note: Data are presented as mean ± SD of three independent experiments. 
Abbreviations: \( k \), rate constant; PAL, phenylalanine ammonia-lyase; PPP, phenylpropanoid pathway; TAL, tyrosine ammonia-lyase; \( R^2 \), coefficient of determination.
percentages observed with 35°C, 40°C, and 25°C treatments, respectively, at the same sprouting time. The enhanced percentage production of phenolics at 30°C is in synchrony with optimum activation rates of PAL and TAL at the same temperature, compared with other evaluated temperatures. Thus, increased activation rates of phenylpropanoid triggering enzymes with a 30°C elicitation resulted in the production of minimally processed phenolic-enriched common bean sprouts.

### 3.4 Accumulation kinetics of total phenolic acids, flavonoids, and anthocyanins

Also, as shown in Table 3, the rate of formation \( k (h^{-1}) \) of total phenolic acids, flavonoids, and anthocyanins was dependent on elicitation temperatures. The highest rate formation for phenolic acids, flavonoids, and anthocyanins was estimated as \( 6.2200 \times 10^{-2} \), \( 6.4400 \times 10^{-2} \), and \( 3.3800 \times 10^{-2} \), respectively. At temperatures more than 30°C, there were reductions in \( k (h^{-1}) \) formations for all evaluated phenolic compounds.

These kinetic parameters are in synchrony with phenolic percentage formation results discussed earlier, where 30°C elicited maximum concentration of all tested phenolics. Also, the results are in synchrony with kinetic parameters of phenylpropanoid triggering enzymes (PAL and TAL), where their estimated activation rates were highest with 30°C treatment, thus, accounting for the observed maximum rate and percentage accumulations of phenolic compounds at sprout elicitation temperature of 30°C.

From this study, it can be hypothesized that the rate of formation of \( \text{H}_2\text{O}_2 \) and MDA at temperatures more than 30°C which were above threshold levels, lowered activation rates of antioxidant enzymes (CAT and GPX), phenylpropanoid triggering enzymes (PAL and TAL), and final expression of phenolics as defense compounds against accumulated stress markers. Thus, 30°C can be estimated as the elicitation temperature for optimum rate formation of phenolics in common bean sprouts. Furthermore, the high determination coefficients \( R^2 \), which ranged from 0.88 to 0.99 (total phenolic acids), 0.71 to 0.99 (total flavonoids), and 0.80 to 0.97 (total anthocyanins).

### TABLE 3 First-order kinetic parameters for formation of phenolic compounds during thermal elicitation of common bean

| Phenolic compound | Thermal elicitation (°C) | 25 | 30 | 35 | 40 |
|-------------------|---------------------------|----|----|----|----|
| **TPA**           |                           |    |    |    |    |
| \( k [k \times 10^{-2}(h^{-1})] \) | 3.740 ± 0.001 | 6.220 ± 0.001 | 5.480 ± 0.042 | 3.540 ± 0.001 |
| \( A_0 \)         | 1.429 ± 0.135             | 23.929 ± 1.592 | 2.067 ± 0.283 | 0.285 ± 0.012 |
| \( R^2 \)         | 0.993                     | 0.879             | 0.867            | 0.978          |
| **TFC**           |                           |    |    |    |    |
| \( k [k \times 10^{-2}(h^{-1})] \) | 0.870 ± 0.001 | 6.440 ± 0.002 | 1.480 ± 0.004 | 1.050 ± 0.003 |
| \( A_0 \)         | 1.387 ± 0.067             | 92.877 ± 0.413   | 78.521 ± 6.849  | 16.420 ± 0.401 |
| \( R^2 \)         | 0.815                     | 0.949             | 0.998            | 0.706          |
| **ANTH**          |                           |    |    |    |    |
| \( k [k \times 10^{-2}(h^{-1})] \) | 0.050 ± 0.001 | 3.380 ± 0.008 | 0.730 ± 0.002 | 0.460 ± 0.003 |
| \( A_0 \)         | 8.011 ± 0.052             | 13.120 ± 0.518   | 9.096 ± 0.359   | 0.812 ± 0.006  |
| \( R^2 \)         | 0.808                     | 0.965             | 0.891            | 0.730          |

Note: Data are presented as mean ± SD of three independent experiments.

Abbreviations: ANTH, total anthocyanins; \( k \), rate constant; \( R^2 \), coefficient of determination; TFC, total flavonoids contents; TPA, total phenolic acids.
flavonoids), and 0.73 to 0.96 (total anthocyanins), show that the experimental data obtained fit well the first-order kinetic model used to assess phenolic formations under the tested temperatures.

3.5 | Activation energy for stress markers, phenolic triggering enzymes, and phenolic accumulations during thermal elicitation of sprouts

Temperature dependence for elicitation of phenolic compounds in common bean sprouts was estimated by the Arrhenius equation in Equation 2. Among oxidative stress markers, the activation energies $E_a$ (kJ/mol) for $H_2O_2$ and MDA were reported to be 15.7 and 41.6 kJ/mol, respectively, whereas $E_a$ for antioxidant enzymes, that is, CAT and GPX, were estimated as 55 and 174 kJ/mol, respectively, (Table 4). This result further explains why the rate of $H_2O_2$ and MDA formation was increasing with increased temperature, as well as why the estimated activation rate of CAT was higher compared with GPX. It signifies that during thermal elicitation of common bean sprouts, less energy was required for the formation of free radicals and lipid peroxidation compared with activation rates of antioxidant enzymes. Therefore, the demand for phenolic signaling as an alternative defense mechanism against accumulated stress markers ($H_2O_2$ and MDA).

In response to accumulation of $H_2O_2$, MDA, CAT, and GPX, the activation energies for activities of phenolic triggering enzymes were calculated as 150 and 221 kJ/mol for PAL and TAL, respectively. Low $E_a$ (kJ/mol) observed for PAL explains its estimated low activation rate and high end-product formations, compared with TAL. Thus, less energy was required by PAL to catalyze the deamination of phenylalanine into trans-cinnamic acids. Also, the activation energies calculated for total phenolic acids, flavonoids, and anthocyanins were 30.4, 64.0, and 209 kJ/mol, respectively. This confirms the higher formation rates of phenolic acids and flavonoids, compared with anthocyanins, as also seen in Figure 1 and Table 3.

4 | CONCLUSION

This study showed that biochemical processes leading to the accumulation of phenolics along the sprouting phase of common bean are temperature dependent. Increasing sprouting time increased the kinetic rates of all investigated biochemical and enzyme mechanisms. From this study, it was established that, although the production rates of $H_2O_2$ and MDA were increased at high sprouting temperatures, this trend was not reflected by activation kinetic rates of antioxidant enzymes, phenylpropanoid triggering enzymes, and final accumulation rates of phenolic compounds. Thermal elicitation of common bean sprouts at 30°C resulted in a strong correlation between oxidative stress markers, activation of phenylpropanoid triggering enzymes, and final accumulation rates of phenolic compounds. Thus, optimization of sprouting conditions is a potential alternative for the functional food industry to produce phenolic-enriched common bean sprouts with improved health benefits.

TABLE 4 | Activation energy of stress markers, phenylpropanoid triggering enzymes, and phenolic accumulation during thermal elicitation

| Evaluated component | $E_a$ (kJ/mol) | $R^2$ |
|---------------------|---------------|-------|
| Stress marker       |               |       |
| $H_2O_2$            | 15.74 ± 8.18  | 0.94  |
| MDA                 | 41.62 ± 3.15  | 0.88  |
| CAT                 | 55.41 ± 1.79  | 0.61  |
| GPX                 | 174.54 ± 11.58| 0.69  |
| PPP triggering enzyme|             |       |
| PAL                 | 221.12 ± 18.41| 0.70  |
| TAL                 | 149.80 ± 6.95 | 0.82  |
| Phenolic compound    |               |       |
| TPA                 | 30.40 ± 1.76  | 0.84  |
| TFC                 | 63.98 ± 1.76  | 0.93  |
| ANTH                | 208.63 ± 12.59| 0.94  |

Note: Data are presented as mean ± SD of three independent experiments. Abbreviations: ANTH, total anthocyanins; CAT, catalase; $E_a$, activation energy; GPX, guaiacol peroxidase; $H_2O_2$, hydrogen peroxide; MDA, malondialdehyde; PAL, phenylalanine ammonia-lyase; PPP, phenylpropanoid pathway; $R^2$, coefficient of determination; TAL, tyrosine ammonia-lyase; TFC, total flavonoids contents; TPA, total phenolic acids.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Josephine Ampofo design the study, performed the experiments, analyzed the data, and wrote the manuscript. Hosahalli Ramaswamy provided supervision, reviewed, and edited the manuscript. Michael Ngadi did the supervision, funding, concept testing, and reviewing of the manuscript. All authors contributed to the final review and editing of the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human and animal subjects.

DATA AVAILABILITY STATEMENT

Due to technical limitations, the full dataset is unable to be published at this time. However, it is available upon request from the first author.

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