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# Investigating the Kinetics of Tannin Removal in Sorghum *[Sorghum Bicolor (L.) Moench]* Grains through a Soaking Process

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**Abstract.** Sorghum (*Sorghum bicolor (L.) Moench*) grains contain tannins that significantly affect their nutritional value and potential utilizations in food product development. This study aims to investigate the kinetics of tannin removal during the soaking process and to optimize the operating condition. To achieve the goals, sorghum grains were subjected to various soaking durations, temperatures, and soaking solution concentrations. The soaking process employed potassium hydroxide and calcium hydroxide solutions at concentrations ranging from 0.05% to 0.15%; soaking temperatures were varied at 30°C, 40°C, and 50°C. Soaking was performed for 8 hours, with samples withdrawn every hour. The dissolved tannin was analyzed for its concentration, and the data were fitted to first-order rate equation kinetic models to determine the rate constants associated with tannin reduction. This study provides valuable insights into the soaking kinetics of sorghum grains, highlighting the potential for improving sorghum-based products' nutritional quality through optimized soaking treatments. From the investigation results, the type and concentration of the solvent, as well as the soaking temperature, affected the tannins removal rate from sorghum grains. The highest dissolution rate and constant ($k$) were obtained during soaking in 0.15% calcium hydroxide solution at 50°C, with values of 0.436 ppm. min$^{-1}$ and 0.00616 min$^{-1}$, respectively. The average percentage error of the first-order reaction kinetics model was below 0.2%, which suggested its high suitability for application in the soaking process of sorghum grains using an alkaline solvent. The findings can guide food processors in developing more efficient methods for tannin reduction, enhancing the utilization of sorghum as both a staple food and food ingredient.

**Keywords:** Kinetics, Model, Soaking, Sorghum, Tannin.

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## INTRODUCTION

Food crisis is often exacerbated by rapid population growth and climate change (Yanto *et al*., 2019). Rapid population growth increases the demand for safe and high-quality foods while changing climate patterns and erratic rainfall, as well as extreme temperature changes, disrupt food production by damaging crops, reducing their yields, and affecting the quality of agricultural land (Myers *et al*., 2022). So, the need to perform research on the search for alternative food ingredients is very urgent (Bahlawan *et al.,* 2020). Sorghum grain is one of the alternative food ingredients yet to be widely used. Sorghum (*Sorghum bicolor (L.) Moench*) is a multipurpose plant that belongs to the grass family (*Poaceae*) that has been used as an alternative food crop in various parts of the world, especially in areas with dry and semi-arid climates (Bahlawan *et al*., 2022). Depending on the cultivar, this plant has strong and upright stems, with a height ranging between 1 and 4 meters. The leaves are long, narrow, and green, closely similar to corn leaves, and grow alternately along the stem. In addition, sorghum plants can grow on marginal soil and lack a continuous water supply (Zarei *et al.,* 2022). Currently, sorghum leaves are widely used as animal feed, and more recently, the stems are used as raw material for bioethanol production (Stamekovic *et al*., 2020). Sorghum grains have quite good nutritional content, which comprises 70-80% carbohydrates, 8.00-18.00% protein, 1.00-5.00% fat, and 3.00% fiber (Curti *et al*., 2023). Furthermore, sorghum grains are excellent food ingredients due to their low gluten content. Excess consumption of gluten by some individuals can damage the lining of the small intestine, interfere with nutrient absorption, and cause symptoms such as diarrhea, stomach ache, bloating, and fatigue (Posner *et al*., 2024). However, sorghum is rarely used as a food ingredient because it has a reasonably high tannin content (Cecilia *et al*., 2022).

Tannins are polyphenolic compounds that occurring naturally in various plant types, including fruit, bark, leaves, and nuts (Fragra *et al*., 2021). Tannins are known for their bitter and astringent taste and their role in protecting plants from herbivores and pathogens (Iqbal *et al.,* 2024). Thus, plant-based food ingredients with high tannin concentration usually possess low economic value. Moreover, high tannin content in food can trigger unfavorable binding, precipitation, and reduction of protein absorption in human and animal digestion systems (Rinaldi *et al.,* 2020). However, at low concentrations, tannins have numerous benefits in the human body because they can function as antioxidants (Agarwal *et al.,* 2023), anti-cancer (Smeriglio *et al*., 2017), anti-inflammatory (Maheswari *et al.,* 2022), immunomodulatory (Safriani *et al*., 2021), and antithrombotic (Marcinczyk *et al*., 2022). The maximum tannin content in sorghum in food products is at most 0.5% on dry matter. Therefore, sorghum grains must be appropriately processed to reduce their tannin content, making them safer as an alternative food ingredient.

Because generally, processing raw food materials can potentially reduce their nutritional quality, appropriate treatment must be done to reduce tannin content without significantly reducing the nutritional quality (Das *et al*., 2020). Both biological and chemical treatments are the common methods used for this purpose. In physical treatment, dehulling has been proven to effectively reduce tannin content in sorghum grains because their outer shell contains relatively high tannin content (Zahra *et al*., 2020). In biological treatment, fermentation reduces tannin content by up to 30% (Cardoso *et al*., 2021). Meanwhile, chemical treatment involves soaking the grains in a base, acid, or salt solution to reduce tannin content to a safe level (Nautiyal *et al*., 2023; Fraga-Coral *et al*., 2020).

This study provides a comprehensive kinetic analysis of tannin removal from sorghum grains through alkaline soaking, addressing a critical bottleneck in utilizing sorghum as a wheat flour alternative by systematically investigating solvent type. This work advances a fundamental understanding of tannin-sorghum interactions. It delivers practical parameters for developing sorghum-based food products, contributing to global efforts in diversifying staple crops for food security. The validated kinetic model is a valuable tool for scaling up the production of nutritionally enhanced sorghum flour suitable for gluten-free applications.

## MATERIALS AND METHODS

### Materials

Sorghum grains used in this work were white sorghum cultivars obtained from local farmers in the Gunung Kidul district of Yogyakarta, Indonesia. The raw sorghum consisted of grains that had been pre-milled and cleaned from impurities, such as leaves, gravel, and other debris. Only whole and unbroken sorghum grains were selected as raw material for tannin removal experiments.

### Soaking Methods

The soaking experiments involved randomly selecting 100 g sorghum grains, which were then soaked in 200 mL of alkaline solutions. The solutions included Potassium Hydroxide (Merck, Germany), Calcium Hydroxide (Merck, Germany), and Sodium Hydroxide (Merck, Germany), with concentrations ranging from 0.05% to 0.15% w/v, and the soaking process was conducted at temperatures of 30°C, 40°C, and 50°C. The concentrations used were chosen to remain below the safe limits for food-grade chemicals. In addition, the soaking temperatures were controlled to stay below the gelatinization point of the sorghum grains at 61.6 to 114.4 °C (Xouyan *et al*., 2022). The soaking process lasted 8 hours, with hourly sampling. Distilled water was used as the control soaking medium. At each one-hour time interval, measurement was performed for water absorption and tannin content. After soaking, the grains were rinsed with distilled water, dried in an oven at 55°C for 2 h, and ground for further investigations, which focused on the kinetics of tannins removal during soaking and nutrient content.

### Tannin Content Analysis

Tannin content analysis on sorghum grains was conducted using the method described by (Adamczyk *et al*., 2017). $Na_2WO_4 \cdot 2H_2O$ (20.66 g), dodeca-molybdo-phosphoric acid (4.13 g), and 85% phosphoric acid (10 mL) were dissolved in 150 mL of distilled water, with gentle stirring for 1 h to prepare the Folin-Denis reagent. The mixture was stirred at 100 rpm for 2 h, then diluted to 500 mL with distilled water in a volumetric flask. A sodium carbonate solution was prepared by dissolving 106 g of sodium carbonate in 1000 mL of water. A tannic acid solution with 6% tannin content was also prepared by dissolving 250 mg of tannin in 500 mL of double-distilled water. 2-3 drops of a 0.1% sodium azide solution were added to prevent fungal and bacterial contamination.

This solution was then diluted to 1:100 with double-distilled water before use. All analytical solutions were stored at 4°C before their use for analysis.

The extracted solution from the soaking process was transferred to sterile tubes using a pipette. Then, 7.9 mL of distilled water and 0.5 mL of Folin-Denis reagent were added, and the mixture was stirred for 3 minutes. This step added 1.5 mL of a 20% sodium carbonate solution. The solution was vortexed and incubated in a dark cabinet at room temperature for 2 h. Absorbance was then measured using a UV-VIS spectrophotometer (GENESYS™ 20 Thermo Fisher Scientific, Karlsruhe, Germany) at a wavelength of 725 nm. The tannin concentration was determined using a calibration curve derived from tannic acid standards, which were diluted to concentrations ranging from 0 to 140 ppm. Absorbance values from these standards were used to create the calibration curve and regression equation.

### Kinetics of Tannins Removal Study

Experimental data on soaking sorghum grain on tannin concentration was obtained from (Bahlawan *et al.*, 2024) by fitting the model data with a first-order kinetics model. The rate of change in the concentration of tannin can be described using the first-order rate equation:

$$\frac{dC(t)}{dt} = -k.C(t) \tag{1}$$

Integrating this equation with the initial condition

$$C_{(0)} = C_0 : C_{(t)} = C_0.e^{-kt} \tag{2}$$

Temperature and alkaline concentration dependence of the rate constant (*k*) was assumed to obey the Arrhenius equation, which describes the effect of temperature and concentration on reaction rates:

$$k = k_0.e^{-\frac{Ea}{R(T+273,15)}}.An \tag{3}$$

Combining the above equations, the final model for tannin concentration over time is:

$$C_t = C_0.e^{-\left(\frac{Ea}{R(T+273,15)}.An\right).t} \tag{4}$$

The data measured during the soaking process was the concentration of tannin dissolved in the solution at various soaking durations, type of solvent, concentration of alkaline solution, and soaking temperature. The % error was calculated by comparing the experiment data with the selected kinetics model. The formula used was:

$$\%error=\sum_{i=1}^{n}\left(\frac{experiment\ data-model\ data}{experiment\ data}\right)\times 100\% \tag{5}$$

## RESULTS AND DISCUSSION

### Effect Type and Concentration of Alkaline Solution on Tannins Removal Kinetics at 30° C

The study investigates the kinetics of tannin removal from sorghum grains during soaking in alkaline solutions (KOH and $Ca(OH)_2$) at concentrations of 0.05%, 0.10%, and 0.15% at a constant temperature of 30°C. The primary objective is to investigate how different concentrations of alkaline solutions would affect the tannin removal rate. Figure 1 demonstrates a clear trend that increased alkaline solution concentration increases tannin removal rate from sorghum grains. This phenomenon can be attributed to the increased availability of hydroxide ions ($OH^-$) in solution, which cleaves tannin-protein and tannin-starch complexes, easing tannin solubilization (Adamczyk *et al.*, 2017). Table 1 shows that at 0.05% KOH, the tannin removal rate was relatively slow (0,082 ppm/ minute), indicating that the low concentration of $OH^-$

ions was insufficient to break the bonds in tannin-protein and tannin-starch complexes. As KOH solution concentration increased to 0.10%, a significant increase in the tannin removal rate was observed, suggesting that the threshold concentration for effective tannin removal had already been achieved. At 0.15% KOH, the tannins removal rate reached its peak, showing that further increases in concentration may result in diminishing returns as the tannins removal rate becomes limited by factors other than the availability of $OH^{-}$ ions, such as external and internal diffusion limitations or the inherent solubility of tannins (Fraga-Coral *et al*. 2020).

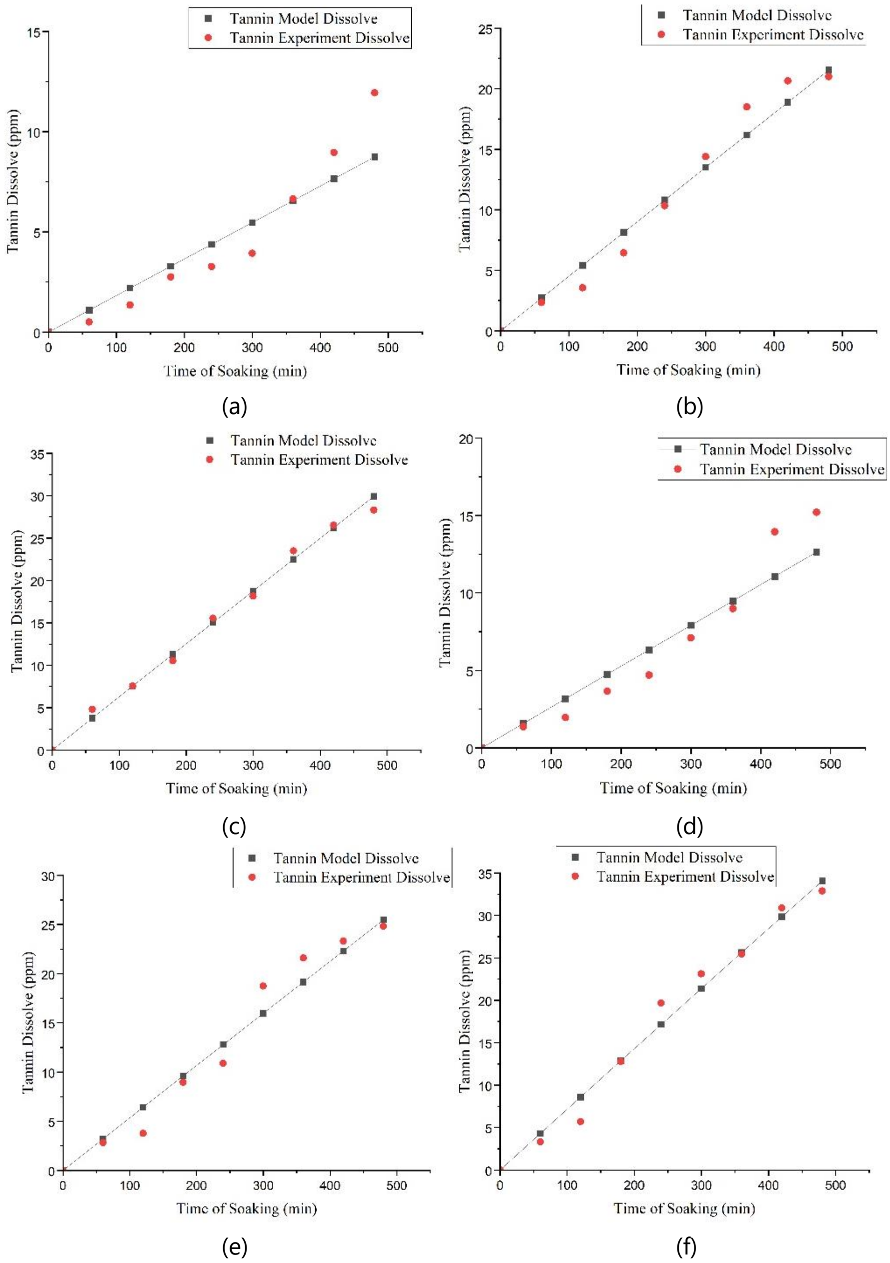


**Fig. 1**: Tannin dissolution kinetics during sorghum grain soaking at 30°C in different alkaline solutions: (a) 0.05% KOH, (b) 0.10% KOH, (c) 0.15% KOH, (d) 0.05% $Ca(OH)_2$, (e) 0.10% $Ca(OH)_2$, and (f) 0.15% $Ca(OH)_2$. Data points represent experimental measurements, while solid lines show first-order kinetic model fits.

**Table 1.** First-order kinetic parameters for tannin removal from sorghum grains during alkaline soaking: dissolution rates (*R*), rate constants (*k*), and model errors at varying solvent concentrations and temperatures.

| No | Solutions | Rate of dissolution ($ppm.min^{-1}$) | k ($min^{-1}$) | Error (%) |
|---|---|---|---|---|
| Soaking Temperature of 30° C | | | | |
| 1 | KOH 0,05% | 0,082 | 0,00143 | 0,012 |
| 2 | KOH 0,10% | 0,202 | 0,00354 | 0,048 |
| 3 | KOH 0,15% | 0,281 | 0,00493 | 0,075 |
| 4 | $Ca(OH)_2$ 0,05% | 0,119 | 0,00207 | 0,037 |
| 5 | $Ca(OH)_2$ 0,10% | 0,239 | 0,00419 | 0,028 |
| 6 | $Ca(OH)_2$ 0,15% | 0,321 | 0,00562 | 0,030 |
| Soaking Temperature of 40° C | | | | |
| | KOH 0,05% | 0,112 | 0,00166 | 0,307 |
| 2 | KOH 0,10% | 0,280 | 0,00413 | 0,042 |
| 3 | KOH 0,15% | 0,324 | 0,00479 | 0,031 |
| 4 | $Ca(OH)_2$ 0,05% | 0,145 | 0,00214 | 0,155 |
| 5 | $Ca(OH)_2$ 0,10% | 0,292 | 0,00431 | 0,028 |
| 6 | $Ca(OH)_2$ 0,15% | 0,367 | 0,00543 | 0,042 |
| Soaking Temperature of 50° C | | | | |
| 1 | KOH 0,05% | 0,140 | 0,00197 | 0,031 |
| 2 | KOH 0,10% | 0,318 | 0,00448 | 0,052 |
| 3 | KOH 0,15% | 0,357 | 0,00504 | 0,014 |
| 4 | $Ca(OH)_2$ 0,05% | 0,195 | 0,00275 | 0,014 |
| 5 | $Ca(OH)_2$ 0,10% | 0,330 | 0,00466 | 0,061 |
| 6 | $Ca(OH)_2$ 0,15% | 0,436 | 0,00616 | 0,030 |

Similar patterns were observed with soaking of sorghum using $Ca(OH)_2$ solutions. However, the tannin removal rates were generally higher than those observed for soaking sorghum using KOH solution at the same concentrations. The highest tannins removal rate occurred at a $Ca(OH)_2$ concentration of 0.15%, reaching 0.321 ppm. $min^{-1}$. Table 1 shows that $Ca(OH)_2$ can be a more effective soaking medium than KOH for extracting tannins from sorghum. This may be because calcium ions ($Ca^{2+}$) in the $Ca(OH)_2$ solution can interact with tannin more intensively and produce a more stable and insoluble calcium-tannin complex (Koopmann *et al.,* 2020). The formation of this complex helps remove tannins from sorghum grains microstructure more effectively. On the other hand, $Ca(OH)_2$ may also interact with other molecules in sorghum grains, such as proteins or polysaccharides, possibly affecting overall solvent stability (Xue *et al.*, 2024).

This study fitted the tannins removal rate from sorghum grains during soaking in KOH and $Ca(OH)_2$ solutions with the first-order kinetics model. This model was chosen based on the assumption that the tannins dissolution rate from sorghum grains depends directly on the tannins concentration in the solvent. At a low solvent concentration (0.05%), the tannin dissolution rate is slower, which is indicated by the small value of the rate constant (*k*). As the solvent concentration increased to 0.10% and 0.15%, the *k* value increased significantly, indicating an acceleration of the tannin dissolution rate (Wang *et al.*, 2020). This phenomenon happens due to increased $OH^-$ ions in the solution, accelerating the breakdown of tannin bonds in the sorghum grain matrix (Choudhary *et al.*, 2024). Table 1 displays that the highest *k*-constant value in the KOH solvent occurs at a concentration of 0.15%, reaching 0.00493 $min^{-1}$, and $Ca(OH)_2$ at a concentration of 0.15%, reaching 0.00562 min-1. From Table 1, the evaluation of the % error in the first-order reaction kinetic model using KOH and $Ca(OH)_2$ as the soaking media shows ideal results with values of % error were all below 10%. These results prove that the kinetic model used matches the experimental data. This shows that the model can accurately describe the reaction dynamics of tannin dissolution in sorghum grains with KOH and $Ca(OH)_2$ solutions under these conditions.

**Effect Type and Concentration of Alkaline Solution on Tannins Removal Kinetics at 40° C**

From Figure 2, it can be seen that

increasing the temperature during the soaking process can increase the speed of the soaking reaction. At a KOH concentration of 0.15%, the rate of dissolution value reached 0.324 ppm. $min^{-1}$. Meanwhile, at a $Ca(OH)_2$ concentration of 0.15%, the rate of dissolution value reached 0.367 ppm.min-1. This is also directly proportional to the resulting constant k value. As the dissolution temperature increases, the constant k value also increases. The constant value at a temperature of 40ºC produces a maximum k value reaching 0.00479 $min^{-1}$ at 0.15% KOH and 0.00543 $min^{-1}$ at 0.15% $Ca(OH)_2$, respectively. Increasing temperature not only accelerates the tannin dissolution process by increasing the kinetic energy of molecules but also affects the reaction rate constant ($k$) (Lu *et al*., 2024). According to the Arrhenius equation, constant $k$ increases with increasing temperature because the activation energy becomes more accessible to solvent and tannin molecules (Carvallo-Silva *et al*., 2019). With constants $k$ a more significant, the tannin dissolution rate increases, making the process more efficient and faster at higher temperatures (Das *et al*., 2020). Table 1 shows that higher temperatures strengthen the influence of the constant $k$ in accelerating the dissolution of tannin. Evaluating the % error in the first-order reaction kinetic model for tannins removal using KOH and $Ca(OH)_2$ solutions demonstrates excellent accuracy, with values falling below 10%. This indicates that the kinetic model aligns well with the experimental data, effectively capturing the reaction dynamics of tannin dissolution in sorghum grains using KOH and $Ca(OH)_2$ at a temperature of 40ºC.

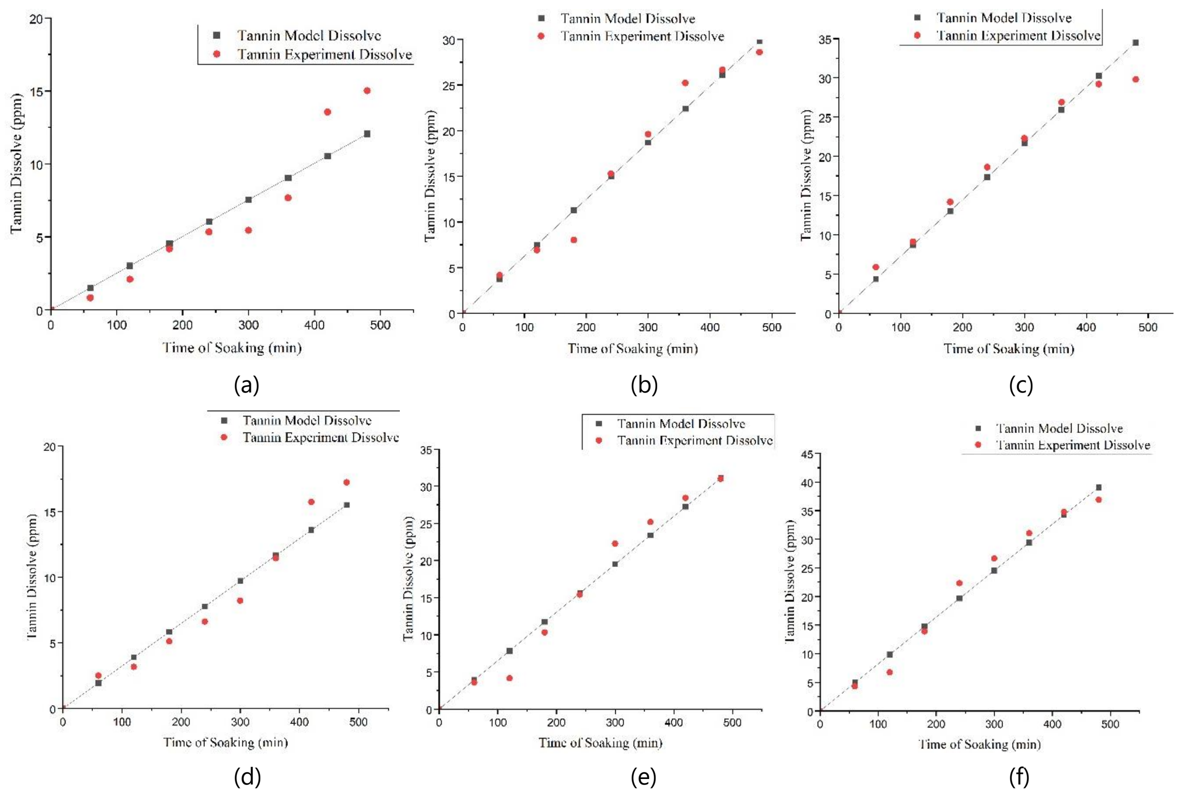


**Fig. 2**: Tannin dissolution kinetics during sorghum grain soaking at 40°C in different alkaline solutions: (a) 0.05% KOH, (b) 0.10% KOH, (c) 0.15% KOH, (d) 0.05% $Ca(OH)_2$, (e) 0.10% $Ca(OH)_2$, and (f) 0.15% $Ca(OH)_2$. Data points represent experimental measurements, while solid lines show first-order kinetic model fits.

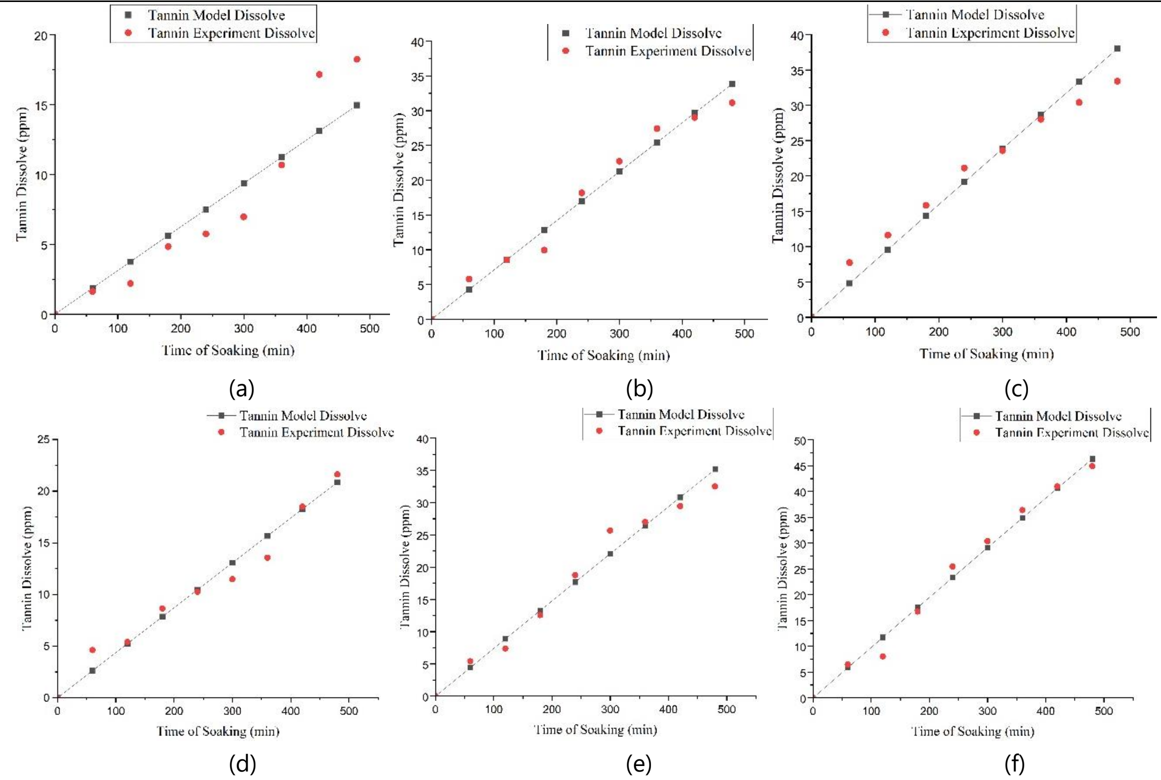


**Fig. 3**: Tannin dissolution kinetics during sorghum grain soaking at 50°C in different alkaline solutions: (a) 0.05% KOH, (b) 0.10% KOH, (c) 0.15% KOH, (d) 0.05% $Ca(OH)_2$, (e) 0.10% $Ca(OH)_2$, and (f) 0.15% $Ca(OH)_2$. Data points represent experimental measurements, while solid lines show first-order kinetic model fits.

**Effect Type and Concentration of Alkaline Solution on Tannins Removal Kinetics at 50° C**

From Figure 3, it can be seen that there was a significant increase in the removal of tannins with the increase in $Ca(OH)_2$ and KOH concentration. The highest tannin removal rate occurred when KOH and $Ca(OH)_2$ solution were used at a concentration of 0.15%, reaching 0.357 ppm. $min^{-1}$ and 0.436 ppm, respectively. Apart from that, increasing temperature also impacts increasing *k* values. The highest *k* values were also obtained in 0.15% KOH and $Ca(OH)_2$ solutions, which were 0.00504 $min^{-1}$ and 0.00616 $min^{-1}$, respectively. At a temperature of 50°C, tannin in sorghum grains experiences a significant acceleration of the dissolution process due to synergistic factors. This increase in temperature increases the kinetic energy of solvent and tannin molecules, which causes an increase in the frequency and energy of collisions between molecules (Das *et al.*, 2020). These more frequent and energetic collisions allow the solvent molecules to overcome the energy barrier required to break the bonds in the tannin molecules, thereby speeding up the dissolution process (Gil-Martin *et al.*, 2022).

In addition, the activation energy ($E\alpha$) required to dissolve tannin becomes more easily achieved at 50°C, allowing more molecules to participate in the dissolution reaction in a shorter time. The reaction rate constant ($k$), which increases exponentially with increasing temperature as described by the Arrhenius equation, also shows a significant increase at this temperature, which means that the tannin dissolution reaction rate is faster and more efficient (Lim *et al.*, 2021). Furthermore, higher temperatures can cause changes in the molecular structure of tannin, such as softening or structural modification, which makes it more accessible to solvent molecules, thereby increasing the rate and efficiency of dissolution (Liu *et al.*, 2021). As a result, at a temperature of 50°C, the tannin extraction process from sorghum seeds becomes faster and more efficient, making this temperature an optimal condition. From Table 1, a similar phenomenon can be seen in dissolution at temperatures of 30°C and 40°C, where the model used is proven to be suitable and able to describe the dissolution process well with error values below 10%.

## CONCLUSIONS

This study systematically examined the kinetics of tannin removal from sorghum grains through alkaline soaking, particularly optimizing process parameters for enhanced efficiency. The experimental results demonstrated that tannin extraction followed first-order reaction kinetics, with the highest removal rate (0.436 ppm·min$^{-1}$) and rate constant (0.00616 min$^{-1}$) achieved using 0.15% calcium hydroxide solution at 50°C. The excellent agreement between experimental data and the kinetic model, evidenced by an average percentage error below 0.2%, validates the model's robustness for process optimization. Temperature emerged as a critical factor, with elevated temperatures significantly accelerating tannin dissolution through increased molecular collision frequency and reduced activation energy barriers. Comparative analysis revealed calcium hydroxide's superior performance over potassium hydroxide, likely due to the formation of stable calcium-tannin complexes. These findings provide valuable insights for food processing industries seeking to improve sorghum's nutritional quality while maintaining process efficiency. The optimized conditions established in this study offer practical solutions for reducing antinutritional factors in sorghum-based food products, thereby enhancing their potential as gluten-free alternatives.

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## NOMENCLATURE

| | | |
|---|---|---|
| $C_{(t)}$ | : | Concentration of dissolved tannin at time t [mg/L] |
| $C_{(0)}$ | : | Initial concentration of tannin in the sorghum grains [mg/L] |
| $K$ | : | Rate constant for tannin dissolution [1/h] |
| $T$ | : | Temperature [°C] |
| $A$ | : | Concentration of the alkaline solution [%] |
| $t$ | : | Soaking time [h] |
| $K_{(0)}$ | : | Pre-exponential factor (frequency of collisions with proper orientation) |
| $E_a$ | : | Activation energy [J/mol] |
| $R$ | : | Universal gas constant [8.314 J/[mol·K]] |

| | | |
|---|---|---|
| *n* | : | Reaction order with respect to the alkaline concentration |

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