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Purification and characterization of urease from three sprouted melon varieties

Joseph Peter S.[1&3*], Amusan Temiloluwa O.[2], Ogunbiyi Oluwagbenga J.[1], Musa Dickson A.[3], Egwim Evans C.[4]

[1]Biology Unit, Air Force Institute of Technology, Kaduna, Kaduna State, Nigeria.
[2]Department of Chemistry, Air Force Institute of Technology, Kaduna, Kaduna State, Nigeria.
[3]Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria.
[4]Department Biochemistry, Federal University of Technology Minna, Niger State, Nigeria.

[*]**Correspondence**
Joseph Peter S.
jpshabz@gmail.com

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Background: Urease is a nickel-dependent enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide, facilitating nitrogen transformation and enhancing its bioavailability for plant uptake. Beyond its agronomic relevance, urease also serves as a defense protein in certain plants and is produced by various microorganisms. This study focused on the purification and characterization of urease enzymes isolated from three sprouted melon varieties: Watermelon, Whitemelon, and Blackmelon sourced from the Lapai market in Niger State, Nigeria.

Methods: The extraction process involved sequential steps: ammonium sulfate precipitation, dialysis, gel filtration chromatography, and SDS-PAGE analysis. Enzyme activity was assessed under varying pH levels and temperatures and in the presence of selected metal ions. The molecular weights of the purified enzymes were determined using SDS-PAGE.

Results: Purification folds were 4.0, 2.5, and 1.6, with corresponding yields of 19.5%, 18.4%, and 19.2% for Watermelon, Whitemelon, and Blackmelon, respectively. Optimal pH values were 6.0 for White and Blackmelon and 7.5 for Watermelon. The temperature optima were recorded at 30°C (Watermelon), 40°C (Blackmelon), and 50°C (Whitemelon). SDS-PAGE revealed distinct protein bands at 55, 38, and 35 kDa for Watermelon and White melon, whereas no defined band was observed for Black melon. Nickel ions significantly enhanced enzyme activity, while Mg^{2+}, Hg^{2+}, EDTA, Ba^{2+}, Ca^{2+}, and K^{+} exhibited inhibitory effects in decreasing order. Kinetic parameters revealed V_{max} values of 0.000611, 0.000410, and 0.000163 U/sec for watermelon, blackmelon, and whitemelon, respectively, with corresponding K_m values of 0.0341, 0.0469, and 0.0021 mg/mL.

Conclusion: These findings suggest that urease derived from these sprouted melon varieties holds potential for immobilization and utilization in urea biosensing and other urease-based biotechnological applications.

Keywords: *urease, sprouted, melon, purification, characterization*

Introduction

Urease (urea amidohydrolase, EC 3.5.1.5) is a nickel-dependent metalloenzyme produced by plants, fungi, and bacteria, but not synthesized by animals. It is currently recognized as one of the most efficient enzymes known, with widespread distribution across various biological systems (Joseph et al., 2022). Urease catalyzes the hydrolysis of urea into ammonia

and carbamate, the latter of which spontaneously decomposes into an additional molecule of ammonia and carbon dioxide, as depicted in Equations (1) and (2):

Equation (1): $NH_2\text{-}CO\text{-}NH_2 + H_2O \rightarrow NH_3 + NH_2\text{-}COOH$

Equation (2): $NH_2\text{-}COOH + H_2O \rightarrow NH_3 + CO_2 + H_2O$

Compared to the uncatalyzed decomposition of urea, urease accelerates the reaction rate by a factor of at least 10^{14} (Real-Guerra et al., 2013; Mobley & Hausinger, 1989). In plants, urea serves as a significant nitrogen source, requiring conversion to ammonia for assimilation (El-Hefnawy et al., 2017). Beyond its role in nitrogen metabolism, plant urease also exhibits defensive functions against pathogenic fungi and herbivorous insects (El-Shora, 2001). Urease has been isolated from a broad range of organisms, including bacteria (El-Hefnawy et al., 2017; Singh et al., 2017), fungi and yeasts (Smith et al., 1993; Mirbod et al., 2002; Amin et al., 2010), and various plant species (Bello et al., 2024; Krishna et al., 2011; Hamzah, 2014). Structurally, plant and fungal ureases are typically homo-oligomeric, consisting of repeating identical subunits, whereas bacterial ureases are hetero-oligomeric, composed of two or three distinct subunits of varying molecular weights (Krishna et al., 2011). The enzyme's activity is strongly influenced by its tertiary structure and environmental parameters such as temperature, pH, and substrate concentration (Pervin et al., 2013).

In the context of human health, urease is implicated in several pathophysiological conditions, particularly gastroduodenal and urinary tract infections, and even certain cancers. Clinically, it is employed for diagnostic purposes to quantify urea levels in biological fluids such as blood and urine (Liu et al., 2012). Industrially, urease holds significant relevance due to its role in degradation of urea, making it valuable in producing fertilizers, hair conditioners, adhesives, plastics, animal feeds, and even food processing (e.g., pretzel manufacturing). Additionally, it serves as a biocatalyst in detecting environmental contaminants such as pesticides and heavy metals and has been extensively used in mercury (II)-inhibition assays as a model enzyme (Tsai & Doong, 2005). Urease-producing bacteria have also been applied in bio-cementation processes to enhance concrete strength (Varalakshmi & Devi, 2014).

The present study was inspired by the need to identify and utilize local plant sources for urease extraction, building upon previous findings that reported high urease activity in some members of the Cucurbitaceae family (Fahmy et al., 1993). Despite the vast biodiversity available for enzyme sourcing, most industrial applications still rely on commercially procured urease, which can be economically limiting. This study focuses on optimizing urease extraction and partial purification from three sprouted melon varieties watermelon, blackmelon, and whitemelon. Key enzymatic properties characterized include optimal pH and temperature, kinetic parameters (Km & Vmax), metal ion effects, and electrophoretic banding patterns, all aimed at assessing their potential for industrial and biotechnological applications.

Materials and methods

Sample collection and authentication

Three varieties of melon: Watermelon (*Citrullus lanatus*), Blackmelon, and Whitemelon (*Colocynthis citrullus*) were purchased from the local market in Lapai, Niger State, Nigeria (Latitude: 9.0444°N; Longitude: 6.5709°E). The plant materials were authenticated by a botanist in the Biology Unit, Air Force Institute of Technology, Kaduna, with authentication reference numbers: AFITBIO/WM-002-0004 (watermelon), AFITBIO/BM-002-0003 (blackmelon), and AFITBIO/SM-001-0005 (whitemelon). Specimens were deposited in the departmental herbarium. All chemicals and reagents used, including Nessler's reagent, Tris buffer, HCl, dialysis tubing cellulose (Sigma-Aldrich), and Sephadex G-100, were of analytical grade.

Sprouting of melon varieties

The seeds of the three melon varieties (Figure 1) were evenly distributed on moistened woolen material and covered with the same. Regular watering was carried out to maintain humidity. Under warm and moist conditions, the seeds swelled and subsequently sprouted. Urease was extracted daily, and its activity was monitored to determine the stage of optimal enzyme activity. Sprouting was discontinued once activity began to decline.

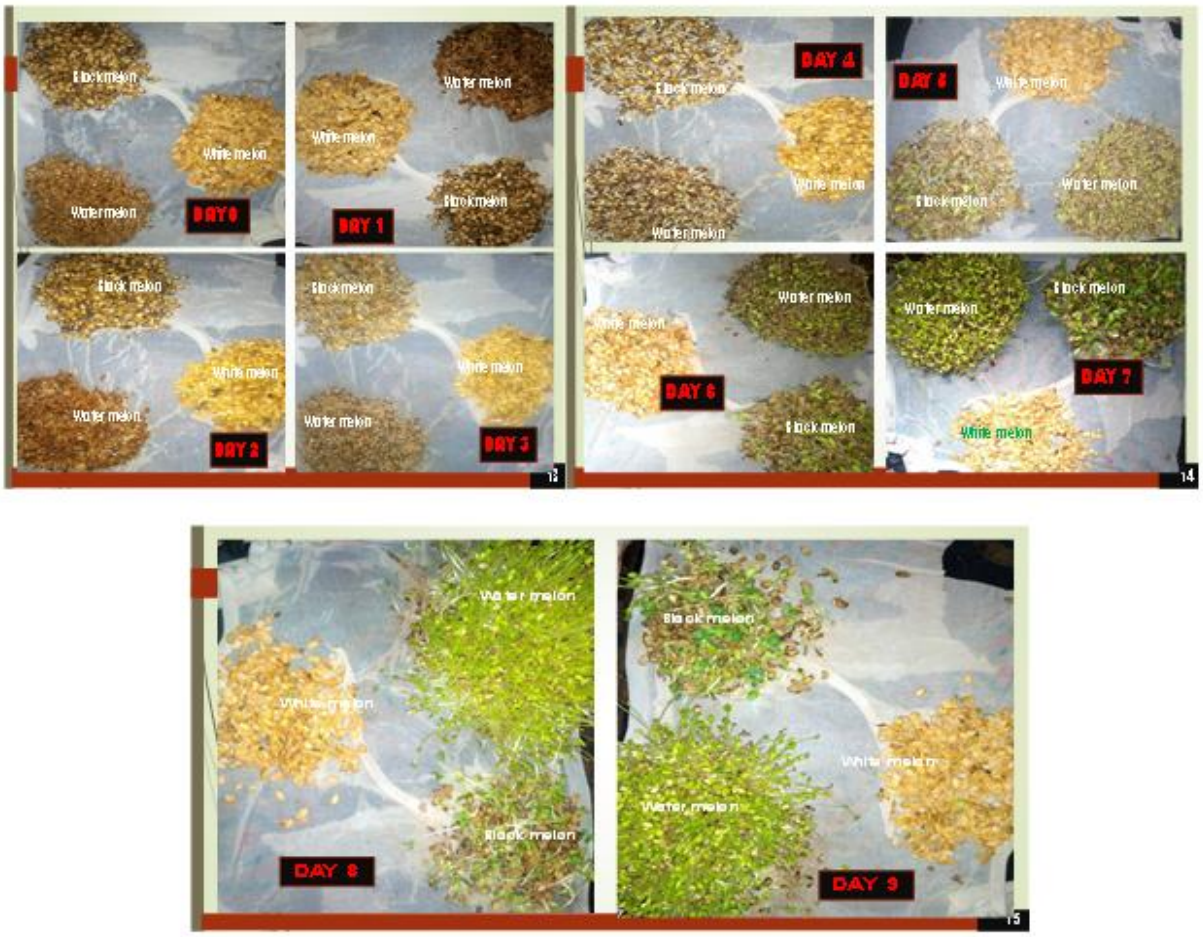

Figure 1. Sprouted melon varieties

Extraction of crude urease

Fifty grams of each sprouted melon seed variety were coarsely ground using a mortar and pestle, and soaked overnight in 100 mL of extraction buffer (0.025 M Tris-acetate, pH 6.5) at 4°C. Although the typical pH range for Tris buffer is 7.5–9.5, a lower pH was chosen to enhance enzyme extraction and improve yield (Kayastha & Nilanjana, 1999). After 24 hours, the homogenate was agitated for 2 minutes, filtered through four layers of pre-washed muslin cloth, and centrifuged at 15,000 rpm for 15 minutes at 4°C. The supernatant was collected as a crude enzyme extract and stored at 4°C for further analysis.

Protein determination

Total protein concentration was determined via UV spectrophotometry following cold acetone precipitation, as described by (Whitaker & Granum., 1980)1. Briefly, 0.1 mL of crude extract was diluted with 9.9 mL of 0.05 M Tris-acetate buffer (pH 6.5). One milliliter of the diluted sample was further made up to 10 mL, and absorbance readings were taken at 260 nm and 280 nm. Protein concentration was calculated using the following empirical formula:

$$\mathbf{X = 1.45 \times A_{80} - 0.74 \times A_{260}}$$

Where **X** is the protein concentration (g/L), and $\mathbf{A_{260}}$ and $\mathbf{A_{280}}$ represent absorbance values at 260 nm and 280 nm, respectively.

Urease activity assay

Urease activity was determined by quantifying the ammonia released from urea using Nessler's reagent (Kulshrestha & Husain, 2006). The reaction mixture consisted of 0.9 mL of assay buffer (0.05 M Tris-acetate, pH 7.0), 0.1 mL of diluted enzyme (20-fold in assay buffer), and 1 mL of 0.2 M urea. The mixture was incubated at 60°C for 30 minutes. The reaction was terminated by cooling to 10°C. One milliliter of the reaction mixture was transferred to a beaker, and 1 mL of Nessler's reagent was added. After 1 minute of reaction, the total volume was made up with distilled water. Absorbance was measured at 405 nm using a UV/VIS spectrophotometer (Biochrom UV 2800). A blank lacking both urea and enzyme was used as a control. One unit of urease activity (U) was defined as the amount of enzyme that releases 1 µmol of ammonia per minute under the assay conditions. All assays were performed in triplicate.

Protein precipitation with acetone

Cold acetone precipitation was used to concentrate the protein (Wolfgang, 2007). One-third volume of ice-cold acetone was added to the crude enzyme extract and maintained at 0°C with constant swirling for 15 minutes. The mixture was centrifuged at 15,000 rpm for 2 minutes at 4°C. The supernatant was treated with an equal volume of cold acetone to precipitate additional protein. The resulting precipitate was redissolved in a minimal volume of 0.05 M Tris-acetate buffer (pH 6.5) and stored at -20°C.

Dialysis and gel filtration chromatography

Dialysis was performed for 12 hours against extraction buffer using dialysis tubing to remove small molecules. The dialyzed enzyme solution was then subjected to gel filtration chromatography using a Sephadex G-100 column (1.8 × 35 cm), pre-equilibrated with 0.025 M Tris-acetate buffer (pH 6.5). The buffer was refreshed every four hours (three times in total). Fractions (2 mL) were collected at a 0.3 mL/min flow rate and analyzed for protein content and urease activity (Emmanuel et al., 2020).

Determination of optimum pH

The effect of pH on urease activity was evaluated using 0.05 M Tris-acetate buffer with pH values ranging from 5.5 to 8.0. Enzyme reactions were conducted at 60°C with 0.2 M urea as the substrate. Activity was measured as previously described.

Determination of optimum temperature

Urease solutions were incubated at various temperatures (20°C–80°C) in Tris-acetate buffer at the optimum pH. After 30 minutes, enzyme activity was assessed to determine the thermal stability profile.

Kinetic parameters (K_m and V_{max})

The kinetic constants $\mathbf{K_m}$ and $\mathbf{V_{max}}$ were determined using the Lineweaver-Burk plot, a linear transformation of the Michaelis–Menten equation (Emmanuel et al., 2020): $\mathbf{1/V = (K_m/V_{max}) \times (1/[S]) + 1/V_{max}}$

Where:

V = reaction velocity (µmol/s/mg protein),
[S] = substrate concentration (mmol/L),
$\mathbf{K_m}$ = substrate concentration at half-maximal velocity,
$\mathbf{V_{max}}$ = maximum reaction velocity.

Ammonia production ra te was used to calculate enzyme velocity.

Effect of metal ions on urease activity

The influence of selected metal ions, Ni^{2+}, Hg^{+}, Zn^{2+}, K^{+}, Ba^{2+}, Mg^{2+}, Ca^{2+}, and the chelating agent EDTA (all at 5 mM) was assessed. The enzyme was pre-incubated with each ion for 30 minutes at the previously determined optimal pH and temperature, and residual activity was measured using the standard urease assay.

Results

Protein concentration in some selected sprouted melon varieties

The results of protein level in sprouted melon seeds are represented in Figure 2. sprouting significantly increase the protein level in Blackmelon and Watermelon with optimum protein level at day 7, while Whitemelon has optimum protein level at day 8 after which the protein level began to decline.

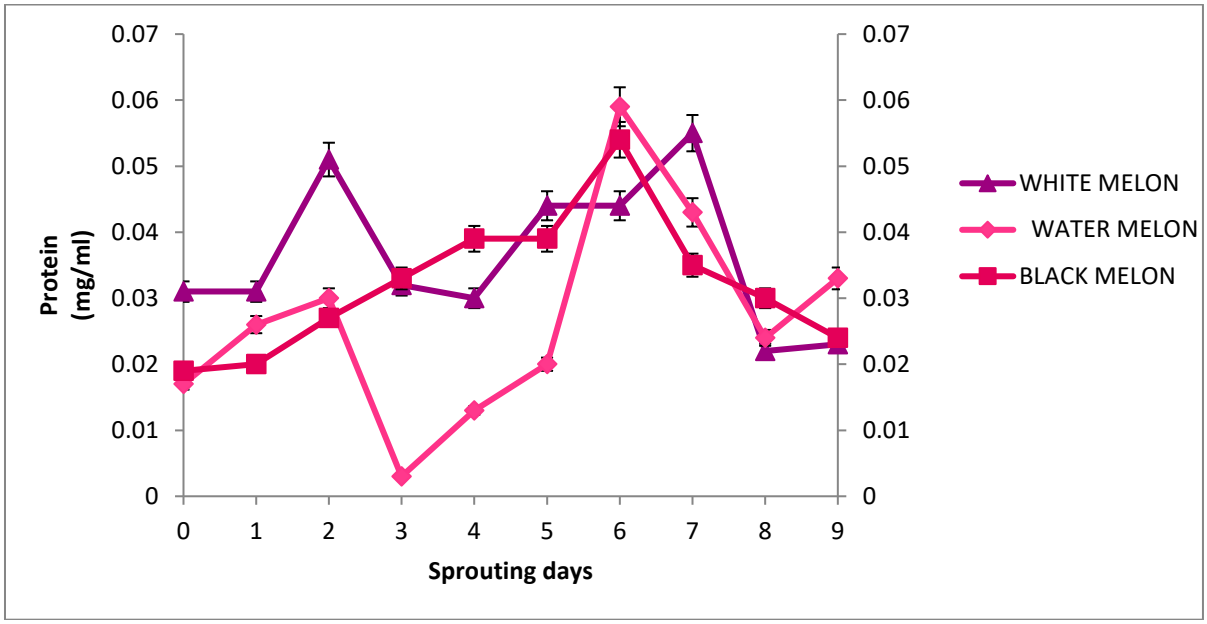

Figure 2. Sprouting effect on protein level of three melon varieties

Urease activity in three sprouted melon varieties

The study revealed that urease activity increased gradually and showed maximum activity at day 6 for White melon while Blackmelon and Watermelon's highest peak was in day 7, and then declined rapidly (Figure 3). In the present study, sprouting was optimized in day 7 for Blackmelon and Watermelon, while Whitemelon urease was optimized at day 6 and was analyzed for further experimental purpose.

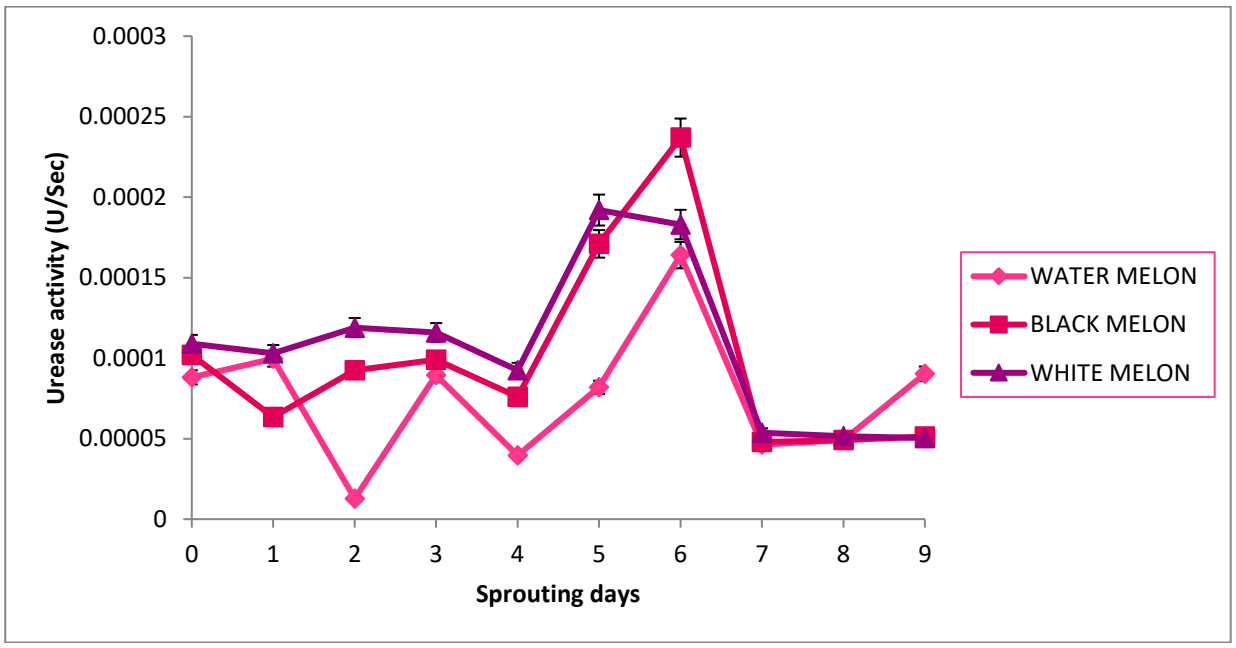

Figure 3. Sprouting effect on the activity of urease

Protein Level and Urease activity of sephadex G-100 Fractions

The results of protein level and urease activity in selected melon species after dialysis and elution are presented in Figure 4, 5 and 6 for Watermelon, Whitemelon and Blackmelon respectively. The protein level for Watermelon shows the highest peak at fraction five and the result for urease activity shows that activity was highest in fraction seven (see Figure 4). Likewise, the protein level for Whitemelon peak was observed in fraction two and that of urease activity was clearly observed in fraction three (see Figure 5). In the same vein, the protein level for Blackmelon shows its highest peak at fraction eleven while the urease activity at fraction seven shows its highest peak as observed in Figure 6.

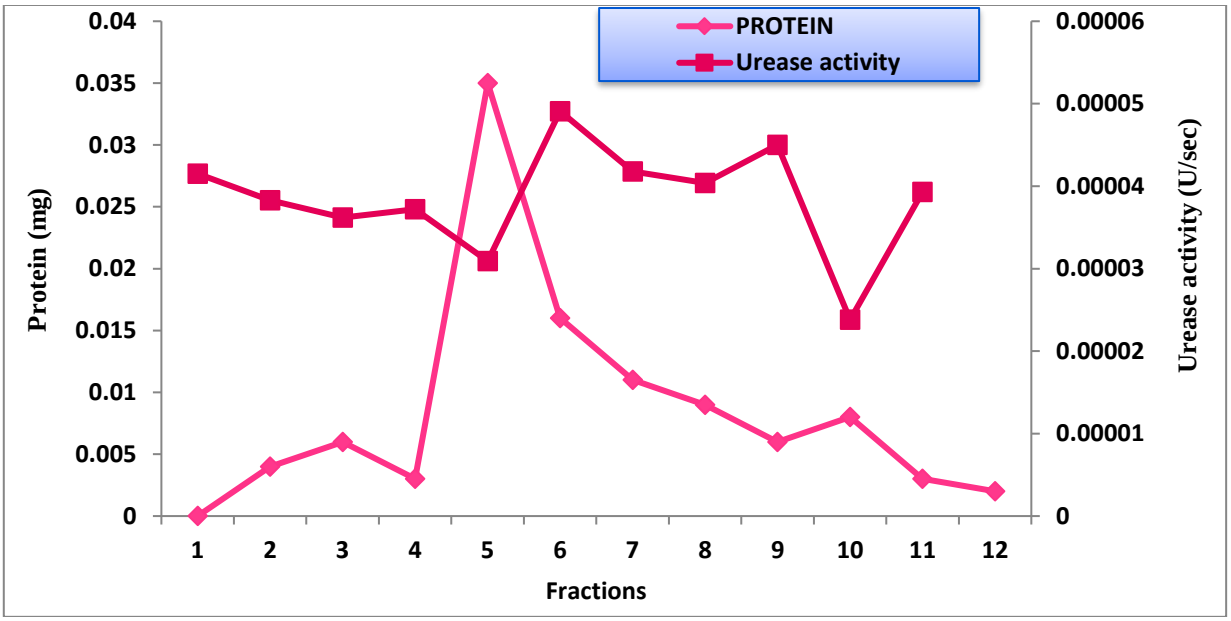

Figure 4. Elution profile of urease from Watermelon

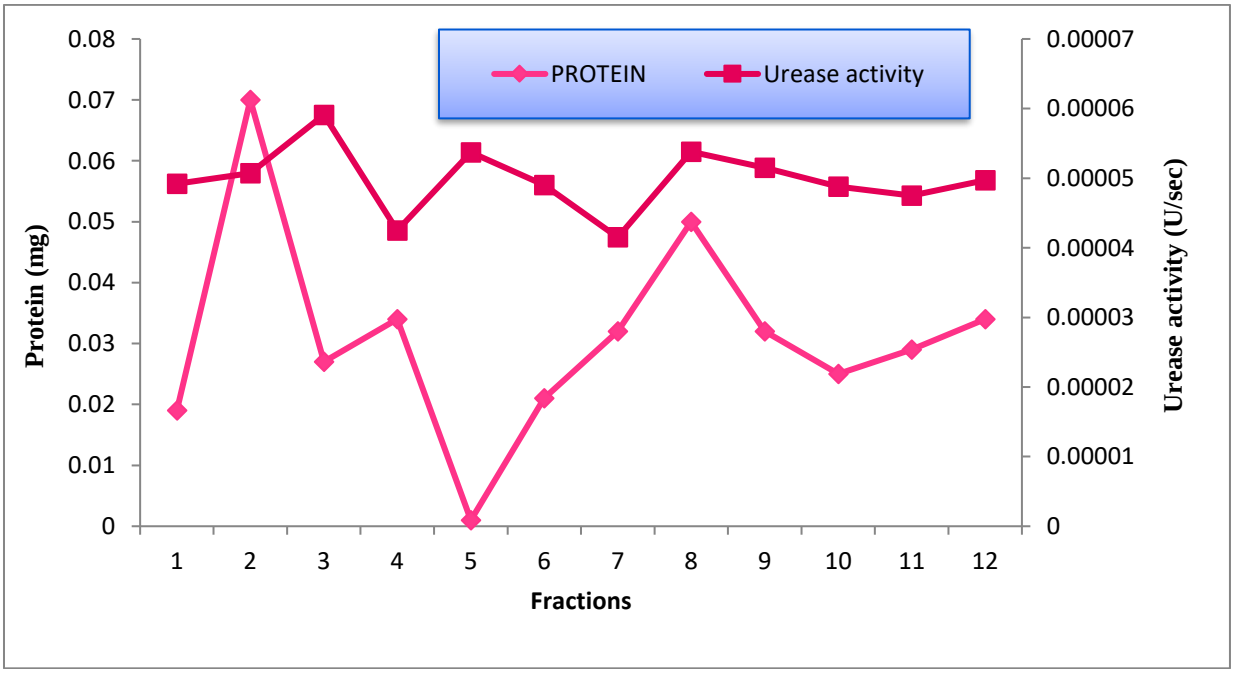

Figure 5. Elution profile of urease from Whitemelon

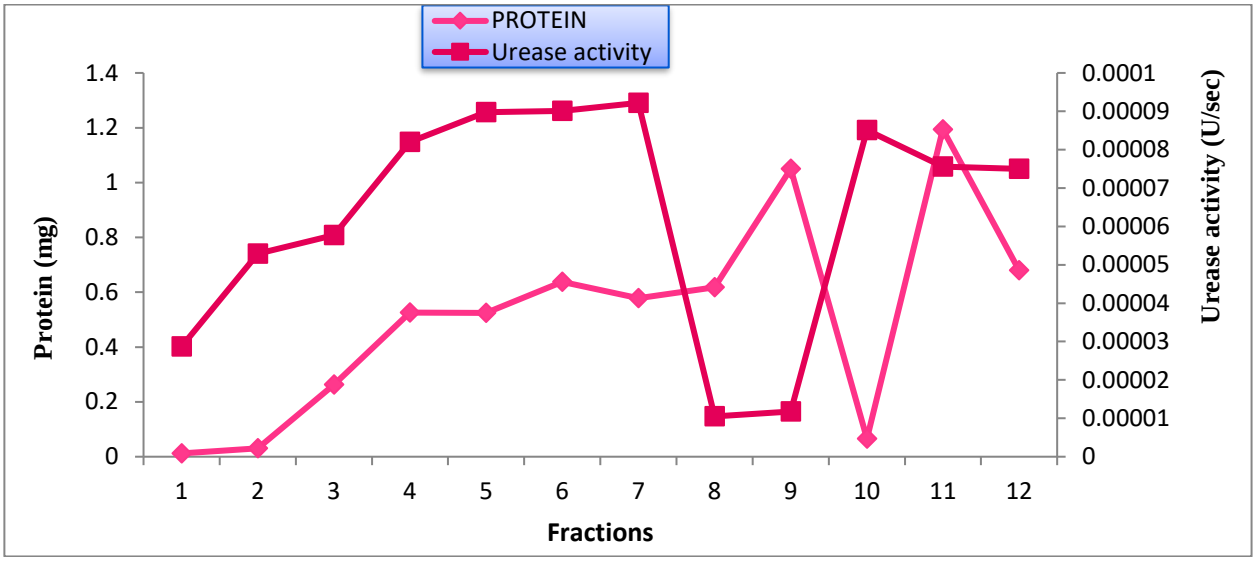

Figure 6. Elution profile of urease from Blackmelon

Optimum pH of urease extracted from three melon varieties

The results of optimum pH for urease extracted from Watermelon, Blackmelon and Whitemelon are represented in Figure 7. The results shows that the optimum pH for Blackmelon and Whitemelon was 6.0 while Watermelon has its optimum pH at 7.5.

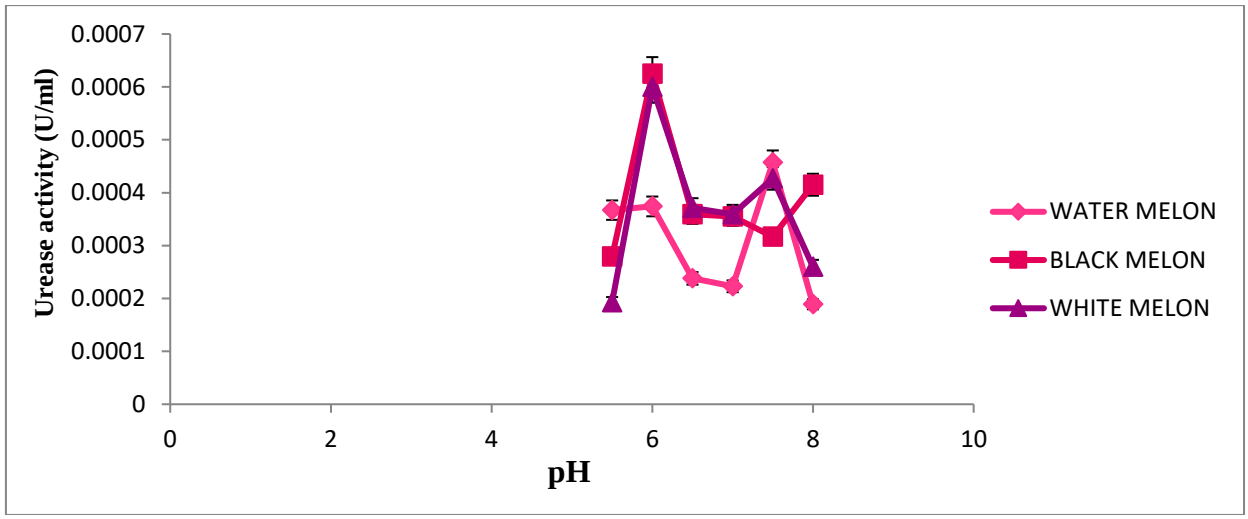

Figure 7. Optimum pH of urease extracted from three melon varieties

Table 1. Purification profile of Blackmelon, Watermelon, and Whitemelon

Purification step	Volume (ml)	Total activity (U/sec)	Total protein (mg)	Specific activity (U/sec/mg)	Percentage yield	Percentage fold
Crude						
Blackmelon	10	0.000323	0.0600	0.00538	100	1
Watermelon	10	0.000210	0.0700	0.00300	100	1
Whitemelon	10	0.000250	0.0590	0.00424	100	1
Acetone precipitation						
Blackmelon	5	0.000238	0.0300	0.00793	73.6	1.4
Watermelon	5	0.000165	0.0242	0.00681	84.2	2.2
Whitemelon	5	0.000169	0.0293	0.00576	67.6	1.5
Gel filtration on Sephadex G-100 column						
Blackmelon	3	0.0000631	0.0029	0.02175	19.5	4.0
Watermelon	3	0.0000387	0.0052	0.00744	18.4	2.5
Whitemelon	3	0.0000498	0.0082	0.00606	19.2	1.6

Optimum Temperature

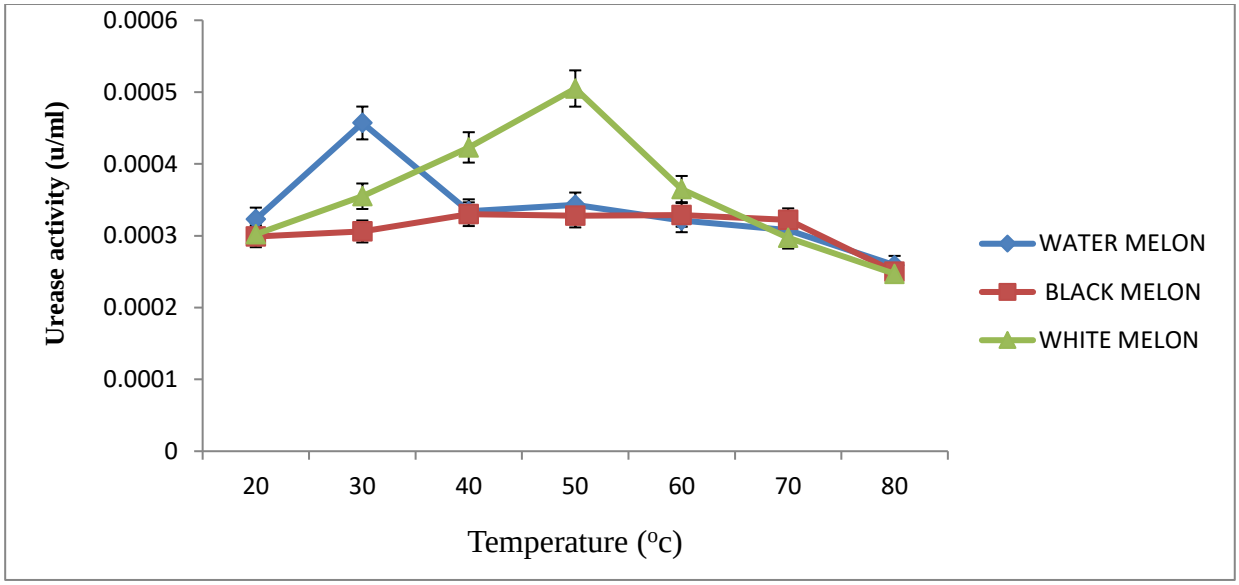

Figure 8. Optimum Temperature of urease extracted from three melon varieties

The result of effect of temperature on urease from some selected sprouted melon seeds are presented in Figure 8. The result showed that the optimum temperature for Watermelon, Blackmelon, and Whitemelon is 30℃, 40℃ and 50℃ respectively.

Effect of substrate concentration

The result for substrate effect on urease activity was presented in Figure 9. Urease from Watermelon has its optimum substrate concentration at 0.5M while that of Blackmelon and Whitemelon was observed at 0.4M.

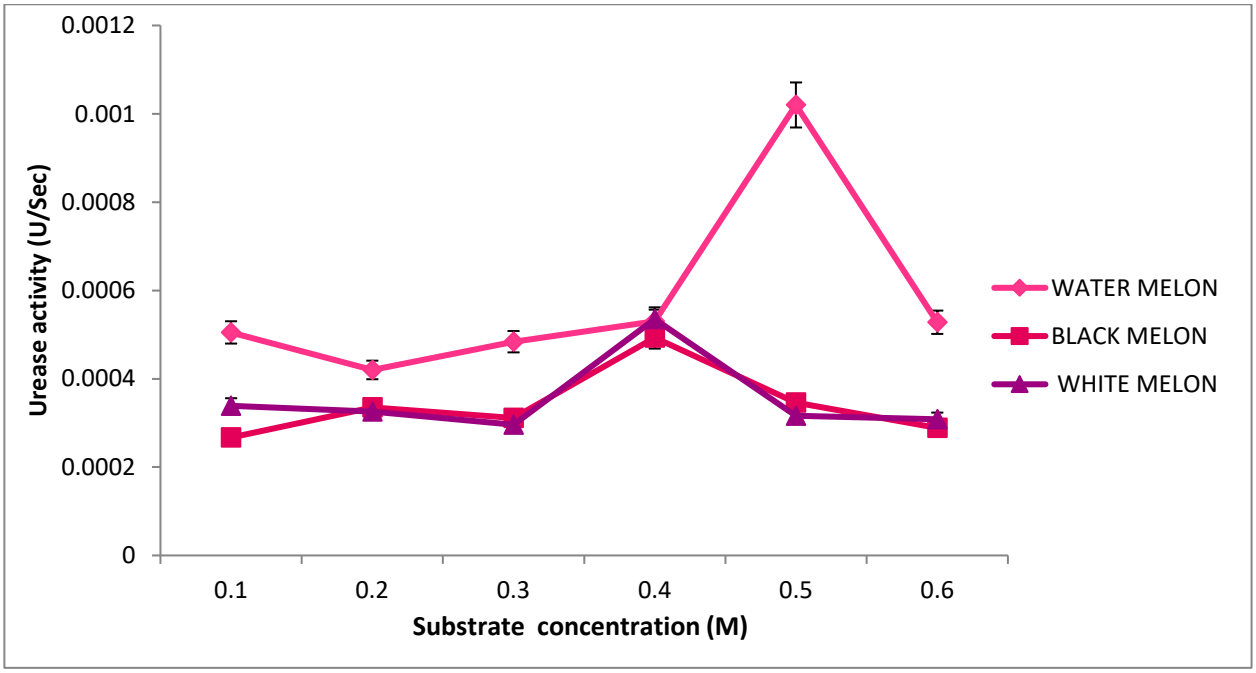

Figure 9. Substrate Concentration of urease extracted from three melon varieties

Effect of ions

The effect of ions on urease from three sprouted melon species was presented in Figure 10. The result shows that Nickel has positive effect while K, Ba, EDTA, Ca, Hg, and Mg ions have negative effect on urease activity of the melon seeds analyzed.

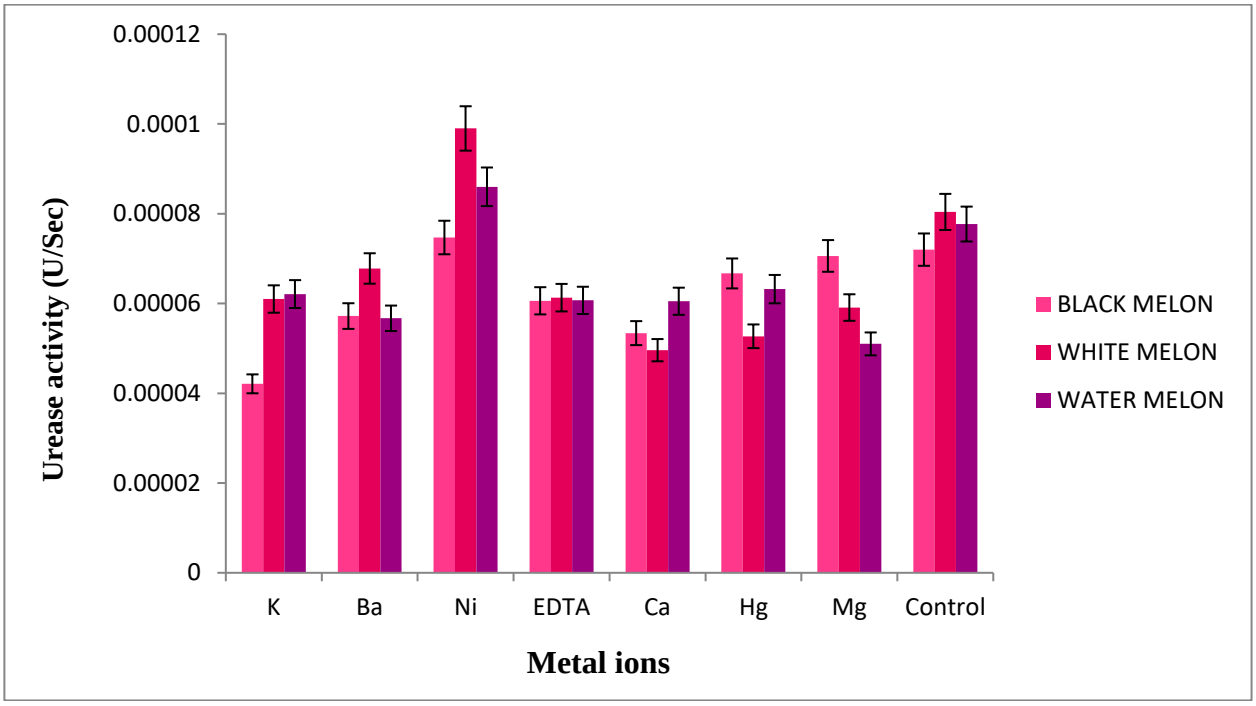

Figure 10. Effect of different ions on the activity of partially purified urease from some selected melon seeds

Electrophoresis

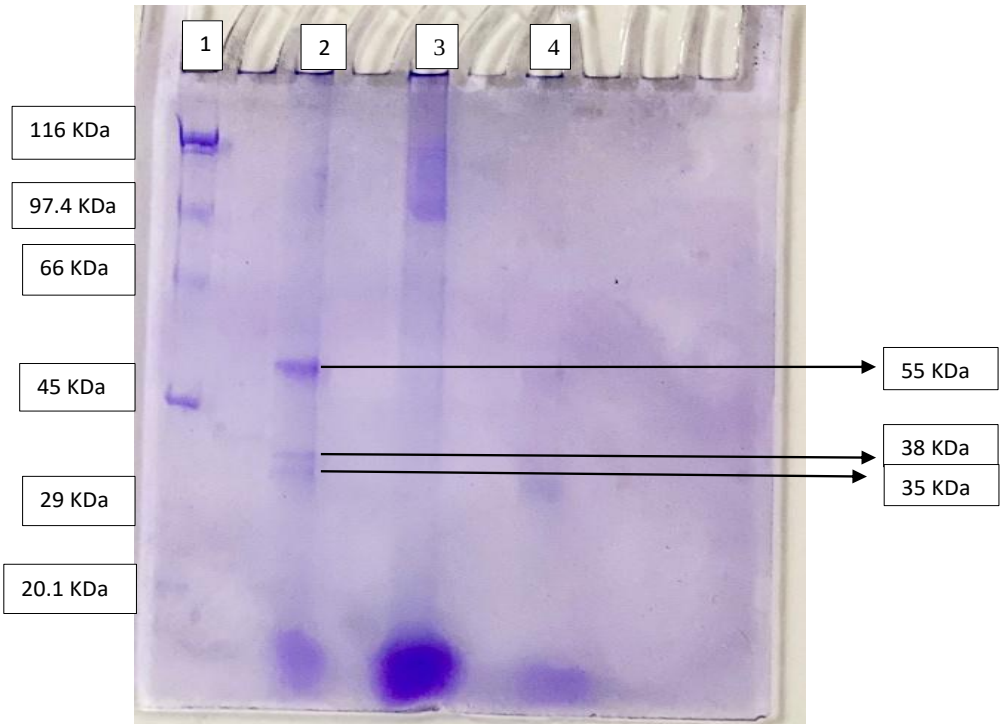

Figure 11. SDS-PAGE of the active fractions from the gel filtration (Lane 1: protein size marker, Lane 2: Whitemelon, Lane 3: Blackmelon, and Lane 4: Watermelon)

Native-PAGE of the active fractions from the gel filtration was run. As shown in Figure 11, there are three bands that appear in the well labeled 2 and 4. The molecular weight of purified urease was determined by Accu Ladder^{TM} protein size Marker. The enzyme showed three bands with the molecular weight of approximately 53kDa, 38kDa and 35kDa for Whitemelon and Watermelon. While Blackmelon has no distinct band.

Kinetic characteristics (V_{max} and K_m) of purified urease from Watermelon, Blackmelon, and Whitemelon samples

The K_m and V_{max} were calculated from the Lineweaver-Burk plot and the summary of the kinetic parameters was presented in Table 2 which shows that the V_{max} are lower than the K_m from all the ureases from the samples analyzed.

Table 2: Kinetic characteristics (V_{max} and K_m) of purified urease from Watermelon, Blackmelon, and Whitemelon samples.

Melon Variety	Vmax (U/sec)	Km (mg/mL)
Watermelon	0.000611	0.0340
Black melon	0.000410	0.0469
White melon	0.000163	0.00212

Discussion

The results of this study indicate that sprouting significantly enhanced the protein content in Blackmelon and Watermelon, with peak levels observed on day 6, recording 0.059 mg/mL and 0.055 mg/mL, respectively. For Whitemelon, the optimal protein content (0.054 mg/mL) was attained on day 7, after which a decline in protein levels was observed (Figure 2). These observations are consistent with previous findings (Dikshit & Ghadle, 2003; Rumiyati et al., 2013; Świeca et al., 2012), which reported that sprouted seeds are enriched in proteins, vitamins, minerals, amino acids, digestible energy, and phytochemicals, key components supporting germinating plants' early growth. Similar trends have been reported by Urbano et al. (2005) and Kaushik et al. (2010), who noted increased protein content in germinated grains. A more detailed mechanism was proposed by Nonogaki et al., (2010), who attributed this increase to protein synthesis initiated during the imbibition phase of germination, which is hormonally regulated. The purification profiles of urease differed among the melon varieties studied. This variation may be attributed to intrinsic differences in the total protein content of each seed type. Arinathan et al. (2009) demonstrated similar findings, showing that Mucuna pruriens possesses significantly higher protein levels than commonly consumed pulses like cowpea, pigeon pea, chickpea, black beans, and green gram. The high protein content of these sprouted melon varieties makes them promising, cost-effective plant protein sources for nutritional, agricultural, and industrial applications. Their nutritional composition supports their use in human diets, animal feed formulation, and processes that require protein-rich substrates or enzyme extraction for biotechnological use. To determine the optimal sprouting duration for maximum urease activity, seeds were germinated over a period of 0 to 9 days. The results revealed that sprouting significantly enhanced urease activity across all three melon varieties. Watermelon and Blackmelon exhibited peak urease activity on day 6, recording 0.000165 U/sec and 0.000238 U/sec, respectively. In contrast, Whitemelon reached its maximum activity on day 5 (0.000193 U/sec), after which a rapid decline was observed (Figure 3). The differences in urease activity among the melon varieties could be attributed to inherent variation in their endogenous urease content or differences in protein expression during germination. These findings are consistent with those of (Dhar & Banerjee, 2019), who reported that urease activity increases with prolonged germination time. The enhanced activity during sprouting may be linked to the synthesis of accessory proteins that assist in nickel incorporation into the enzyme's active site, a critical step in urease maturation (Polacco & Holland, 1993). Since urease plays a central role in nitrogen metabolism during early seedling development, it's up regulation during sprouting ensures efficient conversion of urea into ammonia and carbon dioxide for assimilation. Additionally, studies have shown that the upregulation of urease-specific activity in plants is often observed when urea is applied as a nitrogen source (Mobley & Housinger, 1993). In such cases, urease becomes indispensable because plants cannot utilize urea directly until it is hydrolyzed. A deficiency in urease activity can result in an accumulation of unmetabolized urea, potentially leading to leaf necrosis and toxicity in seedlings (Stebbins et al., 1991; Krogmeier et al., 1989). These findings support the potential of sprouted melon seeds, particularly on days 5–6, as optimal sources for urease extraction for both agricultural and biotechnological applications. Enzyme purification involves isolating the target protein from other cellular components and co-extracted proteins. In this study, crude urease extracts from Blackmelon, Watermelon, and Whitemelon were subjected to cold acetone precipitation. This step significantly improved the enzyme's specific activity from 0.00538 to 0.00793 U/sec, 0.00300 to 0.00681 U/sec, and 0.00424 to 0.00576 U/sec—with respective purification folds of 1.4, 2.2, and 1.5. Corresponding recovery yields were 73.6% (Black melon), 84.2% (Watermelon), and 67.6% (Whitemelon), as presented in Table 1. Following cold acetone precipitation, 3 mL of each partially purified sample was further subjected to size-exclusion chromatography using a Sephadex G-100 column. Distinct activity peaks were observed: a single peak at fraction 6 for Watermelon, fraction 3 for Whitemelon, and three peaks at fractions 5, 6, and 7 for Blackmelon (Figures 4–6). The final enzyme activities after gel filtration were 0.02175 U/sec (Blackmelon), 0.00744 U/sec (Watermelon), and 0.00606 U/sec (Whitemelon), with protein concentrations of 0.0029, 0.0052, and 0.0082 mg/mL, respectively.

The final purification steps yielded purification folds of 4.0, 2.5, and 1.6, with enzyme recovery of 19.5%, 18.4%, and 19.2% for Blackmelon, Watermelon, and Whitemelon, respectively. Specific activity across the three melon varieties reached 0.2 U/mg/sec. As expected, increased enzyme purity resulted in increased specific activity, corroborating earlier studies that link purification processes with enhanced catalytic efficiency (Javed et al., 2018). Urease activity was examined across a range of pH values to determine the optimal condition for each melon-derived enzyme. The optimum pH for urease activity was 6.0 for White melon and Black melon, and 7.5 for Watermelon (Figure 7). These findings partially agree with Evans & Adewoyin, (2013) who reported urease pH optima between 6.5 and 7.0. Differences in pH optima may be attributed to conformational changes in the enzyme's active site under varying hydrogen ion concentrations, which affect the ionization of amino acid residues essential for catalysis. The temperature dependence of urease activity was also investigated. Optimal activity was observed at 30°C for Watermelon, 40°C for Blackmelon, and 50°C for Whitemelon (Figure 8). These variations can be attributed to thermal stability and protein structure differences among the melon varieties. Although urease is known to be a thermostable enzyme (Krajewska, 2009), excessive heat

can lead to the denaturation of its tertiary structure, resulting in a loss of activity. Comparable temperature optima have been reported in other plant and microbial systems: 40°C in Chenopodium album leaves (El-Shora, 2001), 40°C in Pisum sativum L. seeds (EL-Hefnawy et al., 2014), and 55°C in *Rhizopus oryzae*. The observed temperature optima reflect the general trend in enzyme-catalyzed reactions, where the reaction rate initially increases with temperature due to greater kinetic energy, followed by a sharp decline as thermal denaturation sets in (Wolfgang, 2007). The effect of substrate concentration on urease activity was evaluated to determine the optimal concentration for catalysis. The optimum substrate concentration was 0.4 M for both Whitemelon and Blackmelon samples, while Watermelon urease peaked at 0.5 M urea (Figure 9). Urease activity increased progressively with rising substrate concentration until the optimum was reached, after which further increases in urea concentration had no additional effect. This trend is characteristic of Michaelis–Menten enzyme kinetics, where enzyme activity increases with substrate availability up to a saturation point, beyond which the enzyme's active sites become fully occupied. Once saturation is reached, the reaction rate plateaus, indicating that additional substrate no longer influences catalytic activity (Urbanowicz et al., 2021). The observed behavior confirms that urease from the three melon varieties follows classical enzyme-substrate interaction dynamics. The initial reaction rate rises proportionally with substrate concentration, reflecting the abundance of unoccupied active sites. Upon saturation, the reaction velocity remains constant due to the finite number of active sites available for catalysis. These findings are important for understanding enzyme efficiency and designing urease-based applications, such as biosensors, diagnostic tools, and bioreactors, where precise substrate concentrations are essential for optimal activity.

The influence of various metal ions on urease activity was evaluated at a uniform concentration of 5 mM. Among the ions tested nickel (Ni^{2+}), calcium (Ca^{2+}), potassium (K^{+}), barium (Ba^{2+}), mercury (Hg^{2+}), magnesium (Mg^{2+}), and the chelating agent EDTA nickel consistently produced the highest stimulatory effect on urease activity across all three melon varieties (Figure 10). This finding aligns with prior studies (El-Shora, 2009), which have established that urease is a nickel-dependent metalloenzyme, typically incorporating two Ni^{2+} ions at its active site. Nickel is essential for proper folding and activation of the enzyme, directly participating in the catalytic mechanism of urea hydrolysis. In contrast, other tested metal ions exhibited inhibitory effects, with heavy metals like mercury (Hg^{2+}) causing the most pronounced suppression of enzyme activity. This inhibitory effect is attributable to the interaction of heavy metals with thiol (-SH) groups present in the active site of urease. The enzyme contains multiple cysteine residues, including one highly conserved cysteine located within a mobile flap structure at the active site, which is crucial for its catalytic function (Magomaya et al., 2017). Heavy metal ions are known to form complexes with sulfhydryl groups, leading to conformational changes or direct blockage of the active site, thus disrupting enzymatic activity through a mechanism analogous to metal sulfide precipitation (Magomaya et al., 2017). The chelating agent EDTA also showed an inhibitory effect, likely due to its ability to sequester essential metal ions like Ni^{2+}, thereby depriving the enzyme of its catalytic cofactor. These results further reinforce the role of nickel as a cofactor essential for urease activity and highlight the enzyme's vulnerability to metal ion-induced inhibition, a critical consideration for both biotechnological applications and environmental enzyme use. The partially purified urease fractions obtained from gel filtration chromatography were further subjected to Native-PAGE analysis, as shown in Figure 11. Distinct protein bands were observed in lanes 2 and 4, corresponding to the Whitemelon and Watermelon urease fractions, respectively. Three clear bands were identified in each, with estimated molecular weights of approximately 53 kDa, 38 kDa, and 35 kDa, based on comparison with a protein molecular weight marker in lane 1. In contrast, lane 3, representing the Blackmelon urease fraction, showed no well-defined bands. The absence of visible bands in this sample may indicate protein degradation or insufficient protein concentration, which could have resulted from partial proteolysis or improper sample handling during purification. The observed variation in banding patterns and molecular weights supports the concept that urease molecular weight can vary depending on the source organism. This is consistent with findings by Singh et al. (2017), who reported that urease purified from Bacillus sphaericus, when analyzed under denaturing conditions (SDS-PAGE), yielded three subunits with molecular weights of 70 kDa, 60 kDa, and 55 kDa. Additionally, the molecular mass of urease isolated from Proteus mirabilis was reported to be as high as 155 kDa (Singh et al., 2017). These results further affirm that urease is a multimeric enzyme, and its molecular structure and subunit composition can vary significantly across species, likely influenced by evolutionary divergence and functional specialization.

The kinetic parameters Michaelis constant (K_m) and maximum velocity (V_{max}) of the partially purified urease enzymes were calculated using the Lineweaver–Burk double reciprocal plot, as shown in Figures 10, 11, and 12. The K_m and V_{max} values obtained were as follows:

- Watermelon: K_m = 0.0341 mg/mL, V_{max} = 0.000611 U/sec
- Whitemelon: K_m = 0.00212 mg/mL, V_{max} = 0.000163 U/sec
- Blackmelon: K_m = 0.0469 mg/mL, V_{max} = 0.000410 U/sec
  (See Table 2 for a summary.)

These values suggest that urease from Whitemelon exhibits the highest affinity for urea (lowest K_m), while Blackmelon shows the lowest affinity. Generally, higher K_m values indicate that a greater substrate concentration is required to achieve half of V_{max}, reflecting a lower binding affinity of the enzyme's active site for its substrate. The findings align with previous studies. For example, Singh et al. (2017) reported K_m and V_{max} values for partially purified urease from

Bacillus sphaericus MTCC 5100 as 2.0 ± 0.5 mM and 1.82 µM/min, respectively. Additionally, Ogunmolasuyi & Evans (2011) found that germination increased both K_m and V_{max} of urease in treated plant samples compared to the control, suggesting that enzyme maturation and metal ion incorporation (notably Ni^{2+}) during seedling development may enhance enzymatic efficiency. The observed increase in V_{max} values after sprouting supports the hypothesis that germination promotes structural and functional enzyme refinement possibly via enhanced nickel incorporation into the urease active site, thus facilitating improved catalytic turnover (Polacco & Havir, 1979). This is particularly relevant for plants relying on urea-based fertilization, where robust urease activity is crucial for nitrogen assimilation and plant development.

Conclusion

This study demonstrates that sprouting enhances protein content and urease activity in three melon varieties: Blackmelon, Watermelon, and Whitemelon up to an optimal point, after which activity declines. Partial purification of urease via cold acetone precipitation and gel filtration chromatography resulted in 19.5%, 18.4%, and 19.2% yields, with 4.0, 2.5, and 1.6 purification folds, respectively. The optimum pH for urease activity was 6.0 for Blackmelon and White melon, and 7.5 for Watermelon, while the optimum temperatures were 30°C, 40°C, and 50°C for Watermelon, Blackmelon, and Whitemelon, respectively. Native-PAGE analysis revealed three distinct protein bands for Whitemelon and Watermelon (approximately 55 kDa, 38 kDa, and 35 kDa), whereas Blackmelon showed no discernible bands, possibly due to protein degradation. Metal ion studies confirmed nickel (Ni^{2+}) as a positive effector of enzyme activity, while Mg^{2+}, Hg^{2+}, EDTA, Ba^{2+}, Ca^{2+}, and K^{+} exerted varying degrees of inhibition. Kinetic analysis revealed K_m values of 0.0341, 0.0469, and 0.0021 mg/mL, and V_{max} values of 0.000611, 0.000410, and 0.000163 U/sec for Watermelon, Blackmelon, and Whitemelon, respectively. These findings enhance our understanding of plant-derived urease, particularly from sprouted melon seeds, and suggest potential applications in biotechnology, agriculture, environmental diagnostics, and urea-based soil fertility management.

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Author contributions

JPS and EEC conceived the idea for this research and proposed the research design. MDA and EEC supervised the research. JPS conducted the experiment, JPS, OOJ and ATO analyzed, and interpreted the research data. OOJ carried out a critical review of the revised manuscript and was a major contributor to editing it. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Not applicable.

References

Amin, F., Bhatti, H. N., & Asgher, M. (2010). Partial purification and characterization of anacid invertase from Saccharum officinarum. *Pakistan Journal of Botany, 42*(4), 2531–2540.

Arinathan, V., Mohan, V. R., Maruthupandian, A., & Athiperumalsami, T. (2009). Chemical evaluation of raw seeds of certain tribal pulses in Tamil Nadu, India. *Tropical Subtropical. Agroecosystem, 10*, 287-294.

Bello, L. B., Gimba, A. Y., Ayodele, P. F., Farohunbi, S. T., & Eneye, A. B. (2024). Assessment of Urease Activity in Selected Varieties of Leguminous Seeds. *Thomas Adewumi University Journal of Innovation, Science and Technology*, *1*(1).

Dhar, S., & Banerjee, S. (2019). Effects of various factors on urease activity in germinating seeds of Macrotyloma uniflorum. *International Journal of Botany Studies*, *4*(1), 25.

Dikshit, M., & Ghadle, M. (2003). Effect of sprouting on nutrients, antinutrients and in vitro digestibility of the MACS-13 soybean variety. *Plant Foods for Human Nutrition, 58*(3), 1–11. https://doi.org/10.1023/B:QUAL.0000040357.70606.4c.S2CID84496987

EL-Hefnawy, M. E., Sakran, M., Ismail, A. I., & Aboelfetoh, E. F. (2014). Extraction, purification, kinetic and thermodynamic properties of urease from germinating *Pisum Sativum L.* seeds. *BMC Biochemistry, 15*, 15. https://doi.org/10.1186/1471-2091-15-15.

El-Shora, H. M. (2001). Properties and immobilization of urease from leaves of Chenopodium album (C3). *Botanical Bulletin of Academia Sinica, 42,* 251-258.

Emmanuel, M. B., Evans, E. C., Abubakar, A., Labaran, L. M., Ali, A. V., & Zabe, M. (2020). Production, Partial Purification and Characterization of Lipase Enzyme Expressed by Klebsiella pnemoniae of Vegetable Oil Contaminated Soil. *International Journal of Biochemistry and Biophysics*, *8*(2), 30-39.

Evans, C. E., & Adewoyin, M. O. (2013). Activatory Effect of Germination on Catalytic Capacity of Urease Extracted from Beans Samples (Effect of Germination on the Kinetics of Urease from Beans). *International Journal of Biochemistry Research & Review*, *3*(1), 21.

Fahmy, A. S., Mohamed, M. A., & Kamel, M. Y. (1993). Ureases in the cucurbitaceae, distribution and properties, *Phytochemistry, 35*(1), 151-154.

Hamzah, N. A. (2014). Main properties of urease partially purified from seeds of Syrian mesquite (Prosopis farcta). *Journal of Babylon University Pure and Applied Sciences*, *22*(3), 1071-1079.

Javed, S., Azeem, F., Hussain, S., Rasul, I., Siddique, M. H., Riaz, M., Afzal, M., Kouser, A., & Nadeem, H. (2018). Bacterial lipases: A review on purification and characterization. *Progress in Biophysics and Molecular Biology, 132,* 23-34.

Joseph, P. S., Musa, D. A., Egwim E. C., & Uthman A. (2022). Function of Urease in Plants with Reference to Legumes: A Review. In *Legumes Intech Open,* 1-19.

Kaushik, G., Satya, S., & Naik, S. N. (2010). Effect of domestic processing techniques on the nutritional quality of the soybean. *Mediterranean Journal of Nutrition and Metabolism, 3*(1), 39-46.

Kayastha, A. M., & Nilanjana, D. (1999). Simple laboratory experiment for teaching enzyme immobilization with urease and its application in blood urea estimation. *Biochemical Education, 27,* 114–117A.

Krajewska, B. (2009). Ureases I. Functional, catalytic and kinetic properties: a review. *Journal of Molecular Catalysis B: Enzymatic, 59*(1-3), 9-21.

Krishna, B. L., Singh, A. N., Patra, S., & Dubey, V. K. (2011). Purification, characterization and immobilization of urease from Momordica charantia seeds. *Process Biochemistry, 46,* 1486–1491.

Krogmeier, M. J., McCarty, G. W., & Bremner, J. M. (1989). Phytotoxicity of foliar-applied urea. *National Academy of Science. U.S.A. 86,* 8189-8191.

Kulshrestha, Y., & Husain, Q. (2006). Direct immobilization of peroxidase on DEAE cellulose from ammonium sulphate fractionated proteins of bitter gourd (*Momordica charantia*). *Enzyme and microbial technology*, *38*(3-4), 470-477.

Liu, L., Mo, H., Wei, S., & Raftery, D. (2012). Quantitative analysis of urea in human urine and serum by 1H nuclear magnetic resonance. *Analyst*, *137*(3), 595-600.

Magomaya, A. M., Barminus, J. T., & Osemeahon, S. A. (2017). Assessment of Metal -Induced Inhibition of Soybean Urease as a Tool for Measuring Heavy Metals in Aqueous Samples. *IOSR Journal of Applied Chemistry*, *10*(6), 61-70.

Mirbod, F., Schaller, R., & Cole, G. (2002). Purification and characterization of urease isolated from the pathogenic fungus Coccidioides immitis. *Medical Mycology, 40*(1), 35-44.

Mobley, H. L. T., & Hausinger, R. P. (1989). Microbial ureases significance, regulation, and molecular characterization. *Microbiological Reviews, 53*(1), 85-108.

Nonogaki, H., Bassel, G. W., & Bewley, J. D. (2010). Germination—still a mystery. *Plant science*, *179*(6), 574-581.

Ogunmolasuyi, A. M., & Evans, C. E. (2011). Kinetic characteristics of urease isolated from selected beans species as affected by germination. *Science Journal of Biochemistry, 2011*(1). https://studylib.net/doc/10999108/kinetic-characteristics-of-urease-isolated-from-selected-beans-species-as-affected-by-germination

Pervin, M. S., Jahan, M. G., Rana, A. Y., Sana, N. K., Rahman, M. H., & Shaha, R. K. (2013). Effects of some environmental variables on urease in germinating chickpea (Cicer arietinum L.) Seed. *Journal of Stress Physiology & Biochemistry, 9*, 345-356.

Polacco, J., & Havir, E. A. (1979). Comparism of soybean urease isolated from seed and tissue culture. *Journal of Biological Chemistry, 254*, 1707.

Polacco, J., & Holland, M. A. (1993). Roles of urease in plant cells. *International Review of Cytology, 145*, 65.

Real-Guerra, R., Staniscuaski, F., & Carlini, C. R. (2013). Soybean urease: over a hundred years of knowledge. In: Board JE, editor. A comprehensive survey of international soybean research - genetics, physiology, agronomy and nitrogen relationships. *Rijeka, Croatia: Intech Open Science*, 317-339. http://dx.doi.org/10.5772/52106

Rumiyati, R., Jayasena, V., & James, A. P. (2013). Total Phenolic and Phytosterol Compounds and the Radical Scavenging Activity of Germinated Australian Sweet Lupin Flour. *Plant Foods for Human Nutrition, 68* (4), 352–357. https://doi.org/10.1007/s11130-013-0377-6

Singh, A. K., Singh, M., & Verma, N. (2017). Extraction, purification, kinetic characterization and immobilization of urease from Bacillus sphaericus MTCC 5100, *Biocatalysis and Agricultural Biotechnology, 12*, 341-347. https://doi.org/10.1016/j.bcab.2017.10.020.

Smith, P. T., King Jr, A. D., & Goodman, N. (1993). Isolation and characterization of urease from *Aspergillus niger*. *Microbiology, 139*(5), 957-962.

Stebbins, N., Holland, M. A., Cianzio, S. R., & Polacco, J. C. (1991). Genetics test of the roles of the embryonic urease of soybean. *Plant physiology, 97*, 1004.

Świeca, M., Gawlik-Dziki, U., Kowalczyk, D., & Złotek, U. (2012). Impact of germination time and type of illumination on the antioxidant compounds and antioxidant capacity of Lens culinaris sprouts. *Scientia Horticulturae, 140*, 87-95.

Tsai, H., & Doong, R. (2005). Simultaneouse determination of urea, acetylecholine and heavy metals using array based enzymatic optical biosensor. *Biosensors and Bioelectronics, 20*, 1796-1804.

Urbano, G., Lopez-Jurado, M., Frejnagel, S., Gomez-Villalva, E., Porres J. M., Frias, J., Vidal-Valverde, C., & Aranda, P. (2005). Nutritional assesment of raw and germinated pea (Pisum Sativum L.) protein and carbohydrate by in vitro and in vivo techniques. *Nutrition, 21*(2), 230-239.

Urbanowicz, M., Sadowska, K., Paziewska-Nowak, A., Sołdatowska, A., & Pijanowska, D. G. (2021). Highly Stable Potentiometric (Bio)Sensor for Urea and Urease Activity Determination. *Membranes (Basel), 11*(11), 898. https://doi.org/10.3390/membranes11110898

Varalakshmi, V. & Devi, A. (2014). Isolation and Characterization of Urease Utilizing Bacteria to Produce Biocement. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 8*(4), 52-57. https://doi.org/10.9790/2402-08425257

Whitaker, J. R., & Granum, P. E. (1980). An absolute method for protein determination based on difference of absorbance at 260 and 280nm. *Analytical Biochemistry, 109*, 156-159.

Wolfgang, A. (2007). Enzymes in industry: Production and applications. *John Wiley & Sons, New Jersey, USA, 11*, 8-13.