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# Aloe vera leaves potential as a new source of transglutaminase

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**ABSTRACT**

*Transglutaminase is an enzyme that catalyzes cross-linking reactions between primary protein chains. This activity can be applied to improve the texture quality of protein-based food products, such as meatballs, and surimi sausage. Elasticity is an important parameter for consumers when choosing these food products. Currently, many chemical compounds of phosphate have been used to increase the elasticity of food products. Therefore, this study aimed to use plant-derived transglutaminase as a gelling agent for food products to replace chemicals. The experiment was carried out by extracting transglutaminase from aloe vera leaves for characterization. The parameters characterized include transglutaminase activity, optimum temperature and pH, molecular weight, as well as inhibiting and activating compounds. The results showed that aloe vera leaves extract had high transglutaminase activity (0.47 U.mg-1), optimum temperature of 40-50°C, pH 5-7, molecular weight of 70-75 kDa, strongly inhibited by Zn2+ ions, and activated in the presence of dithiothreitol (DTT). The high activity shows that aloe vera leaves can be applied as a gelling agent in food products.*



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## INTRODUCTION

Transglutaminase is an enzyme that catalyzes the acyl transfer reaction from a glutamine residue of a protein and requires the amine residue of another protein chain as an acyl recipient. Proteins that have the amino acid lysine can form an intermolecular bond ε-(γ-Glu)-Lys by interacting with other molecules composed of glutamine (Mahmood and Sebo 2009). The isopeptide bonds that form contribute to constructing a stable protein network and allow the formation of inter- and intramolecular cross-links. These inter-protein bonds cause high molecular weight polymers to develop, resulting in aggregation or even gelation (Djoullah et al. 2016).

In general, transglutaminase is widely used in various branches of industry, specifically food due to the ability to change the physical and chemical properties of proteins. It affects various characteristics of food, such as texture, solubility, viscosity, gelation capacity, and water retention capacity, which are reflected in multiple texture properties. Transglutaminase forms cross-links between proteins during processing, cutting, and heating (Gaspar and de Góes-Favoni 2015, Lerner and Matthias 2015). Furthermore, it is generally used in modern food technology. The enzyme increases the effectiveness in improving the quality of the desired product and also provides a high level of efficiency. Enzymatic reactions are more environmentally friendly and offer more health value than chemical food additives.

For large-scale production, transglutaminase from S. Mobaraense bacteria is still the most widely used enzyme. However, enzyme production from this bacteria also has disadvantages, including problems related to post-translational protein modification. In the context of this limitation, studies on efforts to find a significantly cheaper and more efficient source should be performed for reduced costs related to extraction, purification, storage, and application of the enzyme (Kieliszek and Misiewicz, 2014). The search for a new source of transglutaminase is needed to obtain a cheap source with high activity and enzyme characteristics that are beneficial for application. Therefore, exploration of transglutaminase from aloe vera leaves was carried out to address these challenges.

The discovery of transglutaminase a new sources from local materials will be an opportunity to produce the enzyme using local raw materials at low prices. Production with local raw materials will reduce costs, resulting in products more affordable and accessible to small industries. Therefore, this study aimed to examine the potential and characterization of transglutaminase from aloe vera leaves. The parameters evaluated include transglutaminase activity, characteristics, as activators and inhibitors.

## METHOD

### Materials and Tools

The study materials were aloe vera leaves, and the chemicals used include pure transglutaminase enzyme (Sigma Aldrich), ammonium sulfate (Merck), Zn, Fe, PMSF, EDTA (Merck), and other chemicals with pro-analysis specifications (PA). The equipment used includes transglutaminase characterization and SDS Pag equipment.

### Study Stages

#### *Extraction*

Water solvent containing NaCl salt, 0.2 M phosphate buffer, and 0.2 M acetic acid were used to extract transglutaminase from aloe vera leaves. Extraction was carried out with a 1:3 weight ratio of material to solvent, followed by blending and filtration with Whatman paper. The filtrate was centrifuged (Type GTR22-1) at 10,000g for 20 minutes at a temperature of 4 supernatant and then purified by precipitation.

#### *Ammonium sulfate purification*

The precipitation method was used to purify the crude transglutaminase extract. This process was conducted using ammonium sulfate $(NH_4)_2SO_4$, with saturation varying from 40% to 60%, followed by stirring at low speed (magnetic stirrer MS-H280pro). The purification process was carried out at a temperature of 4°C to avoid denaturation of the enzyme protein. Ammonium sulfate was separated from transglutaminase by dialysis using a cellophane bag (OrDial D80-6000-8000, Cellulose) for further purification by ion exchange chromatography. The parameters observed were transglutaminase activity and molecular weight.

***Activity testing***

The substrate consisting of 3 mM 2-mercaptoethanol, 15 µml monodansilcadaverine, 1 mg/mm N'N-dimethylated casein, 5 mM $CaCl_2$, in Tris-HCl pH 7.5, 70 mM concentration was used to test transglutaminase activity. About 0.1 ml of enzyme was incubated for 10 minutes at 37°C. The activity was measured using a spectrophotometer (Shimadzu Uv-Vis double beam) at 480 nm and 350 nm wavelengths. The parameters observed were transglutaminase activity at room temperature.

***Enzyme characteristics***

The properties of transglutaminase evaluated in this characterization are optimum pH, temperature, and substrate concentration. The variations used in the optimum temperature characterization of transglutaminase were 30, 35, 40, 45, 50, and 55, 60°C in a water bath (Mascot model DWBC 6H). Meanwhile, the pH was varied from pH 4.0, 4.5, 5.0, 5.5, 6.0, 6, 5, 7.0, 7.5, 8.0 and 8.5. It was controlled using phosphate buffer. The parameters observed were transglutaminase response to variations in treatment.

***Transglutaminase inhibitors and activators***

Some reagents can be activators or even inhibitors for enzymes. Transglutaminase inhibitors and activators were analyzed by adding PMSF and EDTA, Zn, Mg, Cl, Ca, Mn, and Fe ions at a concentration of 1 mM to the test substrate. The control was used as a comparison and was determined to be 100% activity (14). The parameters observed were the response of transglutaminase to inhibitors and activators.

***Protein profile and molecular weight of transglutaminase***

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular weight profile of transglutaminase extract from aloe vera (SDS page Mini-Protean II Gel System, Bio-Rad) according to standard procedures. After electrophoresis, the extract was stained using Coomassie Brilliant Blue R-250 and washed with a methanol-acetic acid solution.

## RESULT AND DISCUSSION

**Transglutaminase extraction**

In this study, transglutaminase extraction used a phosphate buffer to maintain pH stability. The selection of phosphate buffer was based on previous studies that used phosphate buffer to extract enzymes from plant tissue (Huang et al. 2011, Amid et al. 2012). The extraction results showed that aloe vera leaves transglutaminase extract had an activity of 0.47 U.mg-1 (Table 1). Aloe vera leaves are very thick and watery, making it easy to extract the enzyme. The extraction of transglutaminase enzyme was generally constrained by the large amount of extract absorbed in the residue (dregs). Therefore, the content in the filtrate was small.

shows that aloe vera leaves have a high protein content and transglutaminase activity. Partial purification using ammonium sulfate significantly increased transglutaminase activity at 40% and 60% saturation (p. 0.05).

Table 1 Transglutaminase activity following purification using the ammonium sulfate precipitation method

| Transglutaminase Source | Protein (mg) | Transglutaminase activity (U/mg) | Increased activity (purity) |
|---|---|---|---|
| Crude aloe vera extract | 2611a | 0.47e | - |
| 40% saturation purification | 1725c | 2.89b | 6 times |
| 60% saturation purification | 1824b | 3.67a | 7.8 times |

The average transglutaminase activity in the same column, accompanied by different letters, shows a significant difference in activity based on the Least Significant Difference (LSD) 0.05 test.

Purification through the precipitation method using ammonium sulfate is intended to separate proteins from solutions containing protein. The addition of ammonium sulfate can affect the equilibrium of protein solubility. Ammonium sulfate will replace the position of the protein-bound by water. Given that ammonium sulfate binds more strongly to water, the protein will separate from the solution system and precipitate. Changes in protein solubility due to adding ammonium sulfate leads to protein precipitation but do not cause denaturation when the process is carried out properly at cold temperatures with gentle stirring. This characteristic makes ammonium sulfate method suitbale for purifying various types of enzymes because enzyme activity is not damaged. Ammonium sulfate purification has been used to extract transglutaminase from Streptomyces, sp. (Macedo et al. 2011) and silver bet leaves (Darwish et al. 2019).

Ammonium sulfate precipitation is the most widely used method for separating and purifying proteins, including enzymes, because of several advantages. These include being highly solubilized in water solvents, inexpensive, and no adverse effect on protein bioactivity. Several other protein purification methods have good effectiveness, but often, the reagents interact with proteins, changing the nature and bioactivity. Purification using the isoelectric method or organic solvents can separate proteins but may cause protein denaturation and loss of bioactivity.

Proteins have different degrees of precipitation against ammonium sulfate solutions. Therefore, the effectiveness of separating each enzyme is also influenced by the saturation level of ammonium sulfate in the solution. Abu El-souod et al. (2014) purified crude extracts of enzyme proteins using 80% ammonium sulfate saturation. Verma and Pandey (2019) also used 80% saturation to purify enzyme proteins from Citricoccus sp. bacteria.

**Transglutaminase optimum temperature**

Statistical analysis results (p.0.05) showed that the highest aloe vera transglutaminase activity was at 40°C and not significantly different (Table 2) from 50°C. Based on these data, aloe vera leaves transglutaminase has an optimum temperature for activity in the range of 40 to 50°C, with a relative activity of 92% to 100%. At optimum temperature, the enzyme will work effectively because high temperatures provide sufficient energy for the substrate catalysis process. Additionally, at the optimum temperature, the enzyme has a suitable structure to bind the substrate. Extremely high temperatures decrease enzyme activity by causing denaturation of the protein. The results imply that aloe vera leaves transglutaminase has a high optimum temperature (thermophilic enzyme) in a wide range.

Table 2 Transglutaminase activity of aloe vera leaves at different temperature levels

| Substrate incubation temperature (oC) | Aloe vera transglutaminase activity (%) |
|---|---|
| 30 | 85b |
| 40 | 100a |
| 50 | 92a |
| 60 | 78bc |
| 70 | 65c |
| 80 | 25d |

The average transglutaminase activity in the same column and accompanied by the same letter shows no significant difference in activity based on the LSD 0.05 test.

Previous studies reported different optimum temperatures for transglutaminase. The optimum temperature for *Streptoverticilli ladakan* was reportedly 40°C (Ho et al. 2000), while for Streptomyces was 45°C (Kieliszek and Misiewicz 2014). Therefore, further exploration of enzyme sources with temperature characteristics that suit the needs is needed, considering that transglutaminase from different sources shows significant differences in response patterns to temperature.

**Transglutaminase Optimum pH**

Substrates having different pH levels with a range of pH 3-8 were used to evaluate the optimum pH of aloe vera transglutaminase. The highest activity of each source was calculated as 100% transglutaminase activity. Statistical analysis showed that aloe vera leaves had a wide optimum pH range. The activities at pH 5, 6, and 7 were 92, 100, and 95%, respectively, which were not significantly different (p. 0.05). This shows that the optimum pH was 5-7 (Table 3).

The study showed that transglutaminase activity outside the optimum pH range experienced a sharp decrease. The pH of the substrate was related to the availability of H+ ions in the substrate. The concentration of H+ ions is

strongly correlated with the conformation of the enzyme active site when binding to the substrate. At the optimum pH, the concentration of H+ ions in the substrate contributes to forming the structure of the active site in binding and catalyzing the substrate. However, excessive H+ ions (pH below 5) and alkaline substrate conditions (pH above 8) lead to protein denaturation and transglutamination loss activity. The optimum pH of aloe vera leaves transglutaminase is slightly different from several previous studies where the optimum pH of microbial ranges from 5-6 (10), and *cunang* fish 7.5 (Sidauruk et al. 2017). Meanwhile, *Streptomyces sp transglutaminase* is 6.0 (Nur'amaliyah et al. 2016)

Table 3 Aloe vera leaves transglutaminase activity at various pH levels of different substrates

| pH of substrate | Aloe vera transglutaminase activity (%) |
|---|---|
| 3.0 | 15c |
| 4.0 | 35b |
| 5.0 | 92a |
| 5.0 | 100a |
| 7.0 | 95a |
| 8.0 | 40b |
| 9.0 | 10c |

The average transglutaminase activity in the same column and accompanied by the same letter shows no significant difference in activity based on the LSD 0.05 test.

**Transglutaminase inhibitors and activators**

The study of several inhibitors and activators can be used to formulate substrates hence, maximum transglutaminase activity is obtained. This study used EDTA, PMSF, Ca2+, Zn2+, and Mg2+ to identify reagents that act as inhibitors or activators. The results obtained are shown in Table 4.

The results showed that the response of aloe vera leaves transglutaminase was not affected by the presence of ions such as PMSF, EDTA, $Mn^{2+}$, and other ions. However, this was strongly inhibited by the presence of $Zn^{2+}$, while $Fe^{2+}$ showed a slight difference in response compared to $Mg^{2+}$. Aloe vera leaves transglutaminase was not influenced by the presence of $Mg^{2+}$ ions. In contrast to most transglutaminase from animal tissue, this does not depend on the presence of Ca2+ ions in the substrate. The results also follow the character of transglutaminase from bacteria where $Zn^{2+}$ is a very strong inhibitor (Macedo et al. 2010). The substrate from Euphausia superba was also strongly inhibited by $Zn^{2+}$ (Zhang et al. 2017).

Table 4 Aloe vera leaves transglutaminase activity at various pH levels of different substrates

| Activator/inhibitor | Aloe Vera Transglutaminase Activity (%) |
|---|---|
| Control | 100 |
| $Ca^{2+}$ | 105 |
| $Mg^{2+}$ | 95 |
| $Fe^{2+}$ | 65 |
| $Mn^{2+}$ | 93 |
| $Zn^{2+}$ | 40 |
| PMSF | 98 |
| EDTA | 104 |
| DTT | 137 |

The presence of Dithiothreitol (DDT) increased aloe vera leaves transglutaminase activity with a relative activity of 137% compared to the control. This shows that the presence of sulfhydryl or thiol compounds is essential for transglutaminase activity of these two sources.

**Molecular weight profile of transglutaminase protein**

SDS-PAGE was used to determine the molecular weight profile of transglutaminase extract from aloe vera. After the electrophoresis process, staining was carried out using Coomassie Brilliant Blue R-250 followed by washing with a methanol-acetic acid solution. The results of the sample run on SDS-Page included markers at various molecular weights as standard proteins (M) and the weight of aloe vera extract protein (L) are shown in Figure 1. The weight of aloe vera transglutaminase protein migrated to produce different band positions. This shows that the extract from aloe vera has a type of protein with a different molecular weight ranging from 70-75 kDa.

The molecular weight of aloe vera leaves transglutaminase protein is close to that of *kurisi* fish, with a molecular weight of 63 kDa (Hemung and Yongsawatdigul 2008). Several previous studies have shown that transglutaminase protein can have many bands with a very wide molecular weight range, such as *cunang* fish at 27 to 172 kDa (Sidauruk et al. 2017). The molecular weight of the enzyme will affect the activity. Enzymes with

low molecular weight tend to have higher activity due to higher mobility to catalyze the substrate.

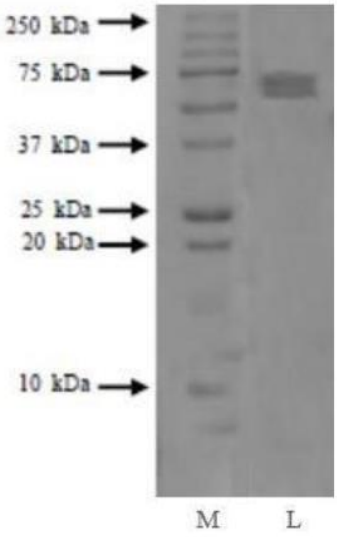


Figure 1 Transglutaminase protein pattern from aloe vera leaves extract. Purification of the extract using ammonium sulfate precipitation method. M; Standard molecular weight marker; L: aloe vera extract

## CONCLUSION

In conclusion, the results showed that aloe vera leaves transglutaminase extract had high activity (0.47 U.mg-1), an optimum temperature of 40-50 oC, an optimum pH of 5-7, and a molecular weight of 70-75 kDa. The presence of Zn2+ ions strongly inhibited transglutaminase activity, activated by the presence of DTT on the substrate. The high activity shows that transglutamination can be applied as a "gelling agent" in food products.

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