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Extraction and Purification of Protease from Ginger (*Zinger* officinalae) Using in Aged Cows Meat Tenderizing

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ABSTRACT

The purpose of this study was to tenderize cow meat using protease extracted from ginger. Protease was extracted from ginger using three extraction solutions: distilled water, sodium chloride solution and calcium phosphate solution buffer. One mole from calcium phosphate at pH 7 was the best extraction solution, giving the highest qualitative activity 4.136 units/mg. Extraction protease at 0.5% was added to the cow meat with incubation time of 30, 60 and 90 min. The sample of meat without enzyme was a control. The results showed that the meat samples treated with the enzyme were significantly superior in humidity compared to the control treatment. Sample incubated for 90 min gave highest moisture content 77.94%. The pH of meat samples treated with enzyme for 90 min significantly increased to 5.98, compared with the pH of the control sample which was 5.6. The weight of samples increased because the water holding capacity of meat samples treated with enzyme compared to the control sample was significantly increased. All treatments in the study were significant in the values of the tyrosine/total, protein and non-protein tryptophan factor compared to the control treatment, and the 90 min treatment sample recorded the highest rates which reached at 5.270, 1.325 and 3.945, respectively. It was clear that the protease enzyme from ginger affected positively the studied properties of treated meat.

Key words: Ginger, purification, protease, meat, tenderizing

INTRODUCTION

Proteases are one of the most important industrial enzymes, accounting for 60% of the total number of enzymes used in various fields of application such as medical, pharmaceutical, leather softening materials and in the manufacture of protein decomposers (Arbab *et al.*, 2022). The extract of protases is used in the field of food industries such as meat tenderizing, bread making, cheese, wine and de-turbidity of beer. Plants, animals, and microorganisms are the main natural sources to extract proteases (Murtala *et al.,* 2017).

Ginger protease (EC: 3.4.22.67) is a vegetable protease of cysteine protease and is found in ginger rhizomes (Murtala *et al.*, 2017). This enzyme has a high activity in meat tenderizing equal to 10 times the activity of papain and bromelain. It also increases both the flavour and nutritional value of meat products due to the high activity of collagenase (Bhat *et al.*, 2018). Tenderness is considered one of the most important matters in the food sciences, especially the processes of preparing the meat of animals and preparing meat of acceptable tenderness. Tenderness is the most important palatable factor for the consumer and is the most important impression a person feels when eating meat and cutting it in the mouth into small pieces (Al- Hanna, 2022; Miller, 2023).

Ginger is the dried roots or rhizomes of the *Zingiber officinale* plant, which is called Ginger or Zingiber. This plant belongs to the Zingiberaceae family Zingiberales order and Zingiber genus (Prasath *et al.*, 2021). Roots are spread horizontally and branched irregularly and the length of one branch reaches 12 cm. Also, the colour is dark yellow with a distinct aromatic smell and stinging taste (Prasath *et al.*, 2021; Kopustinskiene *et al.*, 2022). The original home of Ginger is south-east Asia. It is cultivated in India, China and Jamaica. Its roots and stems contain plant enzymes that break down muscle proteins and collagen bonding tissues (Prasath *et al.*, 2021; Amponsah *et al.*, 2022). Therefore, the objective of this study was to extract and purify the protease from the ginger for use to tenderize cow meat. Additionally, to study the physical and chemical properties of meat after adding protases.

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MATERIALS AND METHODS

Protease was extracted from fresh ginger plant obtained from the local market of Basra city. After cleaning the ginger plant was cut into small pieces to extract protases. Three different solutions were used to extract protease: distillery water, 5% sodium chloride and 0.1 ml potassium phosphate at pH 7.20 g ginger was mixed in 100 ml of each solution sportily. Mixture was digested in blender for 2 min at 4°C. After this the mixer was filtered using cheese cloth and centrifuged for 10000 x g at 4 °C for 20 min. Supernatant was crud lysate.

Protease activity was determined by using specter photo meter at 280 nm. Two milliliter of crude enzyme was added to 2 ml of catalyze solution (1:1:4) (Calcium phosphate buffer 0.2 molar at pH 7 with Casein solution (3 g of casein equipped by BDH) in 90 ml distilled water with stirring and heating at 90°C and then added 2.5 ml of solution 1 M sodium hydroxide with stirring until melting and completing the volume to 100 ml distilled water). Mix solution was incubated at 35 °C for 20 min in water bath. The reaction was stopped by added 10% TCA. The solution was centrifuged at 1911.6 x g for 20 min and the absorption was measured at 280 nm (Yadav and Upasana, 2022). The specific activity of the enzyme was estimated in each extraction solution:

Specific activity = Proteolytic activity/ Protein concentration

The method of Nikolaeva *et al.* (2022) was used to estimate protein concentration during various purification stages. A certain weight of ammonium sulfate was added to the enzymatic extract with constant stirring by magnetic stirrer in an ice bath to get a 20% saturation rate. Solution was centrifuged at 1911.6 x g for 30 min and the protease activity was estimated in the supernatant and pellet. The supernatant was saturated by adding more ammonium sulfate until t high activity of enzyme was obtained. The enzyme dialysis was performed with distilled water for 24 h, with water replacing every 12 h, the volume of the solution was estimated, and the proteolytic activity and protein concentration were estimated.

The meat obtained from the local market of Basra city was cut into cubes (3x3x3 cm) pieces. These were treated with the enzyme solution under study at a concentration of 0.5% and left for 30, 60 and 90 min, while the control treatment was left without adding the enzyme. Moisture content, pH value and Water Holding Capacity (W. H. C.) were studied along with physical and chemical properties of cow meat. Water holding capacity was used to measure water holding capacity by taking 50 g of meat treated with enzyme solution and homogenized with 50 ml of distilled water for one min, centrifuged at 5000 x g for 10 min at 4°C. Water holding capacity was calculated as:

Water holding capacity (%) = [(Weight of water added to meat - Weight of water after centrifugation)/(Weight of sample)] x 100

Meat treated with enzyme for 30, 60 and 90 min was put in polyethylene bags, closed tightly and cooked in a water bath for 90 min at a temperature of 70°C (Ortuño *et al.*, 2021). The liquid was pulled from the bag and stored in the refrigerator for 24 h. The samples were weighed after removing the liquid from the surface of the samples to calculate the loss rate as:

Cooking weight loss (%) = [(Sample weight before cooking–Sample weight after cooking)/(Sample weight before cooking)] x 100

The method mentioned by Jioe *et al.* (2023) was used to estimate the tyrosine/tryptophan factor in meat samples by adding 50 ml of distilled water to 10 g of the minced meat, mixed well and filtered through Whatman (No. 1). The supernatant was diluted four times by distilled water, the optical density was estimated by a spectrophotometer device at 280 nm after considering the dilution amount. The result adopted the tyrosine/non-protein tryptophan factor, which was estimated by taking 20 ml of supernatant and added an equal volume of 15% triace chlorotic acid and then filtered, the supernatant was used in estimation of the tyrosine/non-protein tryptophan factor at the same wavelength and subtracting it from the tyrosine/total tryptophan factor to get the tyrosine/protein tryptophan factor. The results were statistically analyzed using complete randomized design

and the data were statistically analyzed using the SPSS-2006- and the results were compared using the R. L. S. D. at P<0.05.

RESULTS AND DISCUSSION

The best solution for protease extracting from ginger was calcium phosphate solution buffer, as it gave the highest specific efficacy which was 4.136 units/mg compared to NaCl solution which gave a specific efficacy of 3.11 units/mg and distilled water which gave 2.786 units/mg (Fig. 1). These results are in agreement with Murtala *et al.* (2017) who obtained the specific efficacy of ginger protease extracted with distilled water which was 2.25 units/mg. Ginger protease was concentrated using ammonium sulfate salts with a gradient saturation from 20 to 90%. The best saturation percentage of enzyme concentration was 20-65 where the sediment resulting from centrifugation was collected and dissolved in a distilled water. It was dialyzed with distilled water to get rid of ammonium sulfate and then measured the enzyme activity and the concentration of protein in it. This step gave an enzymatic activity and quality of 8.075% units/mg and 15.189 units/mg, respectively, and thus achieved partial purification of the enzyme.

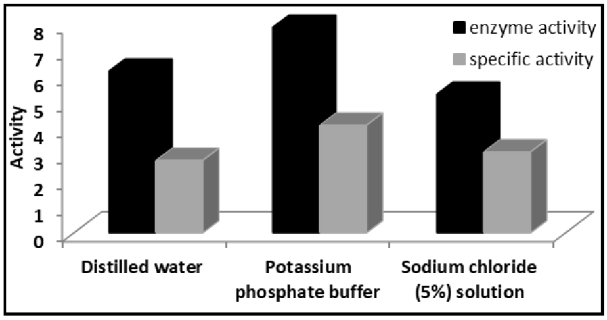

Fig. 1. A comparison of protease extract solutions from ginger.

There were significant differences in moisture content of aged cows' meat with increasing submerging periods for 30, 60 and 90 min (Fig. 2). The samples treated with ginger protease exceeded the moisture content compared to the control treatment. The meat sample treated with the enzyme for a period of 90 min recorded the highest moisture content reaching 77.94%, while the sample of meat treated with enzyme for 30 and 60 min had moisture content 76.83 and 77.31%, respectively. Contrarily, the control sample had 74.42%. These results are higher than the results obtained by Abdeldaiem *et al.* (2014) when they used ginger extract of 15, 30 and 45% to improve the tenderness of camel meat where the moisture content of the treated meat reached 57.15, 57.23 and 57.25%, respectively.

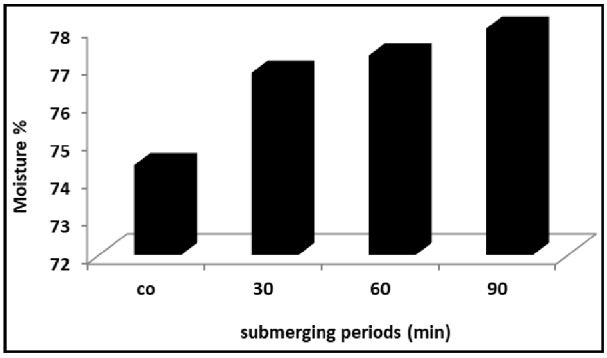

Fig. 2. The percentage of moisture content of cows' meat treated with ginger protease.

Progress in submerging periods 30, 60 and 90 minutes showed increase in the pH which reached 5.85, 5.71 and 5.98, respectively, compared to the control sample which was 5.6 (Fig. 3). This increase in pH caused an increase in water holding capacity of meat by moving away from isoelectric point of the meat which was 5.2-5.3. These results are lower than the findings of Abdeldaiem *et al.* (2014) when they used ginger extract of 15% in improving the tenderness of camel meat, where the pH value of the treated meat was 6.26.

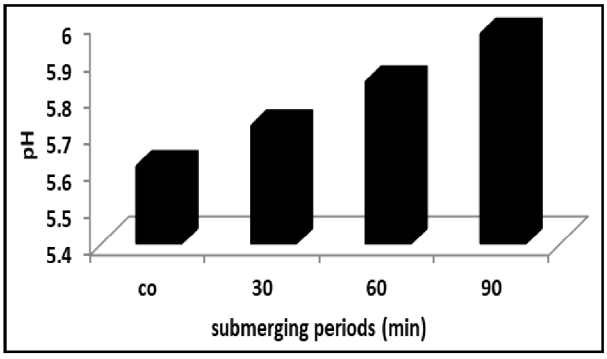

Fig. 3. The pH of cows' meat treated with ginger protease.

Progress in submerging periods 30, 60 and 90 min increased water holding capacity of treated meat samples compared to the control sample which was 25.11% (Fig. 4). The meat sample treated with enzyme for 90 min recorded the highest water holding capacity of 31.54%. The meat's water holding capacity increased as pH

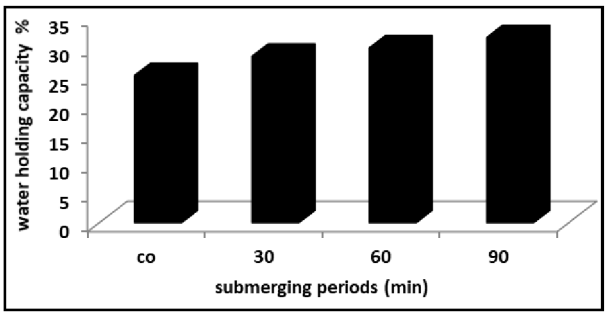

Fig. 4. Percentage of water holding capacity of cows' meat treated with ginger protease.

moved away from the isoelectric point (Nuñez *et al.*, 2020).

The percentage of weight loss during cooking decreased in submerging periods 30, 60 and 90 min for the meat treated with enzyme which was 27.33, 25.41 and 23.12%, respectively, compared to the control sample which was 30.21% (Fig. 5). The reason behind decreasing of the percentage of weight loss during cooking may be attributed to increasing water holding capacity of meat samples treated with enzymes.

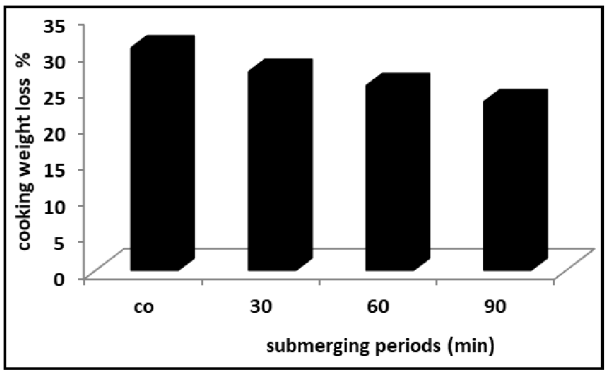

Fig. 5. The percentage of weight loss during cooking for cows' meat treated with ginger protease.

There are significant differences among the values of the tyrosine/total, protein and non-protein tryptophan factor with the progress of submerging periods (30, 60 and 90 min), as the results showed that the meat treated with ginger protease led to an increase in the values of the tyrosine/total, protein and non-protein tryptophan factor compared to control treatment (Figs. 6, 7 and 8). Total protein and non-protein tryptophan compared to the control sample, as the highest values for the meat treated for 90 min with ginger protease were 5.270, 1.325 and 3.945, respectively, and this result confirmed that ginger protease has led to increase the proteolysis of muscle proteins, which increases the amino acids percentage

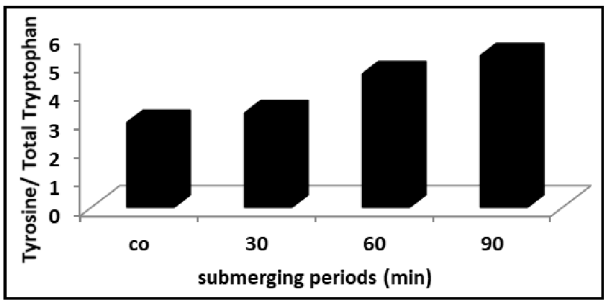

Fig. 6. Tyrosine/total tryptophan factor for cows' meat treated with ginger protease.

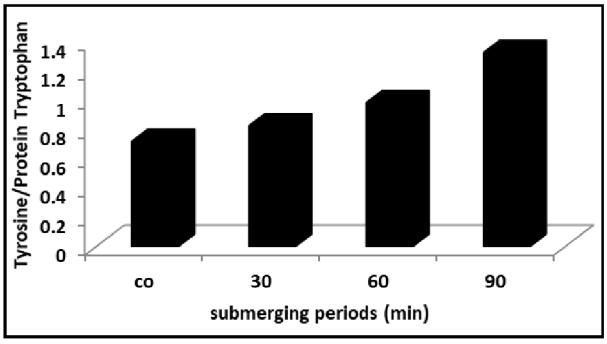

Fig. 7. Tyrosine/protein tryptophan factor for cows' meat treated with ginger protease.

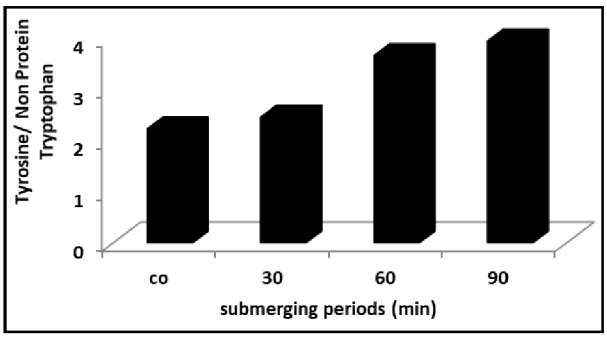

Fig. 8. Tyrosine/non-protein tryptophan factor for cows' meat treated with ginger protease.

and the values of measuring the proteolysis degree of cyclic acid tyrosine/tryptophan, where tyrosine/tryptophan factor regarded as a measure of the concentration of aromatic (cyclic) amino acids, and increasing this factor was an evidence of the occurrence of meat protein proteolysis (Jioe *et al.*, 2023). These results are also consistent with Jahanbin (2021) statement that the high percentage of amino acids resulting from the proteolysis of meat proteins was largely reflected on the increase of tyrosine/tryptophan factor which was an indicator of increasing of dissolved nitrogenous materials.

CONCLUSION

This research demonstrated that protease

extracted from ginger could be tenderized in cow meat. Calcium phosphate buffer at pH 7 gave the highest qualitative activity of the protease. The results showed the meat samples treated with the enzyme were significantly superior in humidity compared to the control treatment. Moisture content was the highest with incubation of the meat samples at 90 min. The pH of meat samples was a significant increase after incubation time. The tyrosine/total, protein and non-protein tryptophan factors significantly differed after treating the meat samples with protease enzyme.

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