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EXTRACTION AND PARTIAL PURIFICATION OF ACTINIDIN FROM KIWI FRUITS AND ITS EFFECT ON AGED CHICKEN MEAT

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ABSTRACT

The aim of this study was to extract the enzyme actinidin from kiwi fruits and purify it partially and determine the pH and the optimum temperature for the effectiveness and stability of the enzyme. Three concentrations of the purified enzyme were used for treatment of aged poultry meat for a period of 2, 4 and 6 h for the purpose of softening it. Some variables used as indicators of oxidation were monitored. The results showed that the crude enzyme had activity of 23 units/ml and specific activity of 19.65 units/mg, and the purification steps resulted in obtaining the enzyme with specific activity of 52.80. The results indicated that the optimal pH for enzyme activity and stability was 7, and the optimum temperature for enzyme activity was 40°C, and the enzyme retained its activity in the range of 20-60 °C. Treating aged chicken breast meat with enzyme led to a significant decrease in peroxide value with increasing enzyme concentration, and it increased with incubation period. The value of total volatile nitrogen was at the lowest value in the control treatment (5.44%) and increased with increasing enzyme concentration and incubation period. The values of Thio barbituric acid showed a significant increase in the control treatment and reached its highest value in the longest incubation period, and actinidin enzyme decreased the values of Thio barbituric acid at all concentrations used. The values of free fatty acids decreased in the enzyme-containing treatments, compared to the control, which gave the highest value (0.68 for an 8-h incubation period).

Key words: kiwi fruit, protease, enzyme extraction, ammonium sulfate, meat quality.

الاستخلاص والتنقية الجزئية للأكتينيدين من فاكهة الكيوي وتأثيرها على لحوم الدجاج المسنة

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الخلاصة

هدفت هذه الدراسة إلى استخلاص إنزيم الأكتينيدين من ثمار الكيوي وتنقيته جزئياً وتحديد الأس الهيدروجيني ودرجة الحرارة المثلى لفعالية وثبات الإنزيم. استخدمت ثلاث تراكيز من الانزيم المنقى لمعالجة لحوم الدواجن المسنة لمدة 2 و 4 و 6 ساعات لغرض تليينها. تم رصد بعض المتغيرات المستخدمة كمؤشرات للأكسدة. أظهرت النتائج أن الإنزيم الخام له نشاط 23 وحدة / مل ونشاط نوعي 19.65 وحدة / ملجم ، وخطوات التنقية أدت إلى الحصول على الإنزيم بنشاط نوعي 52.80. أشارت النتائج إلى أن درجة الحموضة المثلى لنشاط الإنزيم وثباته كانت 7 ، ودرجة الحرارة المثلى لنشاط

الإنزيم كانت 40 درجة مئوية، واحتفظ الإنزيم بنشاطه في نطاق 20-60 درجة مئوية. انخفاض معنوي في قيمة البيروكسيد مع زيادة تركيز الإنزيم وزيادة مع فترة الحضانة. كانت قيمة النيتروجين الكلي المتطاير أقل قيمة في المعاملة الضابطة (5.44٪) وتزداد مع زيادة تركيز الإنزيم وفترة الحضانة. أظهرت قيم حامض الثيوباربيتوريك زيادة معنوية في المعاملة الضابطة ، ووصلت إلى أعلى قيمته في أطول فترة حضانة ، وخفض إنزيم الأكتينيدين قيم حمض الثيوباربيتوريك بكافة التركيزات المستخدمة. انخفضت قيم الأحماض الدهنية الحرة في المعاملات المحتوية على الإنزيم مقارنة بالمجموعة الضابطة التي أعطت أعلى قيمة (0.68 لمدة حضانة لمدة 8 ساعات).

الكلمات المفتاحية: فاكهة الكيوي ، البروتييز ، استخلاص الإنزيم ، كبريتات الأمونيوم ، جودة اللحوم.

INTRODUCTION

Kiwi is the most popular crop of the genus Actinidia which have fruits with unique flavor, high nutritional value, and beneficial health effects **(Wu *et al.*, 2019)**. China is the richest natural resource in production of kiwi fruits, accounting for nearly 50% of the total world production **(Madhusankha & Thilakarathna, 2021)**. The fruits of the Actendines produce fifteen types, but the commercially available and edible kiwis are three types, which are the green kiwi A. deliciosa, the yellow kiwi A. chinensis, and the hard kiwi A. arguta **(Kaur& Boland, 2013)**. Actinidin enzyme is the dominant enzyme and is abundantly present in kiwi species, with a ratio of (10.7-1.6 mg / 100 gm of fruit juice) **(Gong *et al.*, 2020)**. Previous studies have shown through *in vivo* experiments that this enzyme improves the digestion of protein in the stomach and small intestine. Kiwi contains a simple but important protein that consists of both soluble and insoluble forms **(Montoya *et al.*, 2014; Bagheri *et al.*, 2019)**.

Actinidin is a protein extracted from edible kiwi fruit and Chinese gooseberry **(Dhiman *et al.*, 2021).** The enzyme is also obtained commercially from a variety of organisms **(Chao, 2016)**. It is part of the family of sulfhydryl proteases and its main use is as a meat tenderizer. It used than the rest proteases in meat tenderization. Actinidin forms up to 30-60% of the soluble protein in kiwi fruit, and the immunological detection results showed that kiwi fruit contains 3800 times more actinidin than leaves **(Peyrot des & Breslin, 2016)**. Actinidin has a high potential for commercial use in meat tenderization and milk curdling, and is included in health-promoting diet supplements (**Bagheri *et al.*, 2019)**, and this enzyme is used to help the digestive system in digesting protein food in particular. It is also involved in the production of compounds that help in the process of digesting food, especially in humans with digestive problems such as enteritis **(Peyrot des & Breslin, 2016).**

Considering that actinidin protease is capable of digesting many enzymes, including alpha-amylase, which is an essential enzyme found in saliva and pancreatic juice, responsible for the decomposition of large, insoluble starch into simple parts of sugar, and this affects the rate of carbohydrate digestion and absorption **(Martin *et al.*, 2017)**, and treatment Type 2 diabetes which involves inhibition of the alpha-amylase enzyme by the enzyme actinidin **(*Mahdian et al.*, 2022; Bagarinao, 2020)**. It is a globular protein that accounts for 50-40% of soluble proteins **(Aworh & Nakai, 1986).**

The aim of the recent study was to extract the enzyme actinidin from kiwi fruit, partially purify it, determine the optimum pH and temperature for the activity.

MATERIALS AND METHODS

Kiwi fruits used in the experiment.

Fresh kiwi fruit was purchased from the local markets in the capital, Baghdad, and was used in the experiment after peeling, washing with distilled water, drying it with blotting paper, and then cutting it into small pieces, about 2 cm in size.

Enzyme extraction

In the extraction process, the method described befor was adopted. One hundred g of kiwi fruit pulp was taken and cut into small pieces of 2 cm in size. The pieces were soaked with the extraction solution previously prepared, consisting of (6% sodium chloride + 2% boric acid) in a ratio 4:1 (v/v). After 24 h at room temperature (20°C), the soaked pieces were macerated in electrical mixer for 5 minutes and the mixture was filtered through cotton. The pulp was re-extracted and the whole filtrate was centrifuged at 3000 × g for 20 min. Supernatant was used raw extract.

Enzyme activity and protein concentration

In a test tube 0.95 mL of previously prepared cysteine solution and 0.1 mL of enzyme extract was mixed, left at 35 °C for 3 min, 0.95 mL of previously prepared casein solution was added, and the mixture was kept at a temperature 35°C for 10 min. The reaction was stopped by adding 3 ml of TCA solution at a concentration of 5% at room temperature for 60 min. Centrifugation at 3000 × g for 20 min was done, the liquid was separated quietly, and the absorbance was measured at a wavelength of 280 nm. Blank solution was prepared by following the aforementioned steps, except that the TCA solution was added to the substrate solution before adding the enzyme **(Chao, 2016)**. The enzyme activity was measured according to the equation below:

Enzyme activity (unit / mole) = Absorbance at 280nm / (0.1×10 × 0.01)

The unit of enzyme activity was defined as the amount of enzyme in milliliters that gives an increase of 0.01 in the absorbance at a wavelength of 280 nm per minute under the experimental conditions.

For Estimation of protein concentration, the method mentioned priviously **(Segel, 1976)**.

Enzyme purification

The enzyme was concentrated by the gradual addition of ammonium sulfate to the crude enzyme extract with continuous stirring on the magnetic stirrer in an ice bath until reaching 20% saturation. Centrifugation was carried out at 11000 × g for 20 min. The lytic activity of the enzyme in the filtrate and the sediment was estimated. Then the filtrate was taken, and a new weight of ammonium sulfate was added to the filtrate in order to reach a saturation percentage at which the enzyme activity in the filtrate decreases **(Horwitz, 1975)**. A saturation percentage was used to concentrate the actinin enzyme from (20%, 40%, 60% and 80%) respectively.

The precipitate was dissolved in a small amount of previously prepared sodium phosphate buffer solution. The precipitate representing the crude enzyme extract was collected and the dialysis process was performed using sodium phosphate solution at 4°C for 24 h with the replacement of the solution (phosphate buffer) every 4 hours after which the methylated

extract was collected and the final volume and analytical activity of the enzyme were measured. The enzymatic activity of the remaining enzyme was measured using of method of **Horwitz, (1975)**.

Factors affecting enzyme production.

For determination of the optimum pH for the activity of the purified enzyme, several tubes of buffer solutions with different pH numbers ranging from (4-9) were prepared previously by adding 3 ml of all solutions in different tubes. 0.1 ml of purified enzyme solution was added to tubes containing 0.3 ml of buffer solutions with different pH numbers. The tubes were incubated for 20 min at a temperature of 37°C, then 0.95 ml of casein solution prepared at a concentration of 1% and a pH of 7 was added. Then the reaction was stopped, the enzyme activity was estimated, and the relationship between the pH and the remaining activity of the enzyme was drawn to determine the optimal pH for enzyme activity. The tubes were incubated for another 20 min at a temperature of 37 °C for determination of the optimum pH for the stability of the purified enzyme **(Horwitz, 1975)**. The same procedures with certain modifications were adopted for determination of optimum temperatures for enzyme activity and stability.

Treatment of chicken breast meat with enzyme solution

After lyophilization, the partially purified enzyme was dissolved and diluted with an amount of deionized water for the purpose of immersing the chicken breast pieces, and the dilution ratio was (0.25,0.75 and 1) %, where 200 g of chicken breast was immersed for every 100 ml of diluted enzyme The samples were incubated on the optimum temperature for enzyme action for different periods of time (0, 2, 4, 6) h. After the incubation period, these samples were placed directly in an electric oven for 20 minutes to inactivate the enzyme at 120 °C, cooled and kept in the refrigerator for the purpose of conducting tests.

Qualitative tests

Peroxide value (PV)

The peroxide value was estimated according to the A.O.A.C method **(Witte *et al.*, 1970)** using the following equation:

Peroxide number (meq) = S×N×1000 / g

Where S = ml of $Na_2S_2O_3$ sodium thiosulfate, N = sodium thiosulfate molar and g = number of grams of oil.

Thio barbituric acid (TBA)

Thio barbituric acid was estimated for the purpose of measuring lipid oxidation for each sample separately and it was accomplished according to (**Bekhit *et al.*, 2021)** using the equation:

TBA Value (mg MDA/kg) = A530 × 5.2

Where: A530 is the absorbance of reaction mixture at 530 nm.

Total Volatile Nitrogen (T.V.N)

The method adopted by (**Ahamed & AL-Abadi, 2015)** was used for estimation of the percentage of total volatile nitrogen in chicken breast meat samples treated with the enzyme in addition to the control sample. 100 g was taken from each sample, homogenized with 300 ml of 5% trichloroacetic acid (TCA) solution, filtered to obtain a clear extract and transferred to 5

mL of a 2 M sodium hydroxide solution. The mixture was heated until distillation into a receiving flask containing 4% boric acid. Few drops of methyl red dye indicator and bromocresol green was added. Then titration was performed for the mixture using hydrochloric acid with a concentration of 0.01 M. The amount of volatile nitrogen was calculated according to the following equation.

Amount of volatile nitrogen (mg nitrogen / 100g) = 500 / v (300 + mo) × 14

Free Fatty Acid (FFA)

Free fatty acids were estimated according to (**Cunniff, 1995)**. the method **(Pizarro *et al.*, 2013)**. The method is summarized by extracting the fat by the cold method, taking 10 g of fat and adding 25 ml of ethanol at a concentration of 95% neutral and one ml of phenolphthalein reagent and treating it with 0.1 M sodium hydroxide until the solution becomes pink. The percentage of free fatty acids was calculated as oleic acid:

Acid value = NaOH (ml) × 5.61/ Sample weight (g)

% FFA = Acid value/2

Non-protein Nitrogen (NPN)

Non-protein nitrogen was estimated in chicken breast samples according to (**SAS, 2018**). The method mentioned in 1995. Five g of each sample was weighed and placed in a 100 ml glass container, 5g of pure sand was added to it, the sample was mixed well using a glass rod and 25 ml of pure sand was gradually added to it. Distilled water is warm while continuing to mix, then 25 ml TCA 24% was added, and the solution was left for 10 min. The solution was filtrated through Whatman No.1 filter paper. One ml was transferred to the digestion vial of the microkeldahl device, and 10 ml of concentrated H_2SO_4 was added to it. The digestion process was completed using the Buchi 430 apparatus and distillation using the Buchi 320 apparatus.

Statistical analysis

The statistical program System Statistical Analysis (**SAS- 2018)** was used to analyze the data to study the effect of different factors (concentration and time) on the studied traits according to a complete random design (CRD). Significant differences between means were compared with a less significant test (Least significant difference –LSD).

RESULTS AND DISCUSSION

Enzyme extraction

The aforementioned extraction solution was used to extract the actinidin enzyme, and this solution was composed of sodium chloride and boric acid, as it gave an enzyme activity of 23 units/ml and a specific activity of 19.6 units/mg protein, and the protein concentration was 1.17 mg/ml as shown in (Table ,1). This result was similar to the results obtained by **(Chao, 2016)**, when the enzyme actinidine was extracted using a phosphate buffer with a pH7, as the enzymatic activity reached 59.67 units / ml and the specific activity was 56.82 units/ mg. Sharma and Vaidya **(Zhu *et al.*, 2018)** used a phosphate buffer to extract the enzyme actinidin, as the enzymatic activity was 0.22 units/ml, the specific activity was 0.55 units / mg protein, and the protein concentration was 0.4 mg / mol. If a phosphate buffer with a pH8 was used, the

enzymatic activity was 220 unit / ml and the specific activity was 0.52 mg: protein and the protein concentration was 0.42 mg / ml.

The difference in the specific activity values from one method to another may be due to the difference in the solubility of the proteins present in the fruit or due to the difference in the extraction solutions that were reflected in the protein concentration, which affected the specific activity of the enzyme under study. All enzymes are extracted and purified at 4°C, although the temperature reduces the solubility of proteins, but in return it reduces the loss of enzymatic activity to the maximum extent, and then preserves the enzyme with the highest activity **(Dhaher *et al.*, 2022)**.

Shows Table,1 the step of gradual saline precipitation of the crude extract using gradual saturation rates of ammonium sulfate that ranged from 20% to 80%, as the enzymatic activity reached the highest value (52.4 units / ml) at a saturation rate of 60%. The sediment resulting from centrifugation was collected, and membrane aspiration was carried out in against phosphate buffer every 6 hours for 24 h and at 4 °C. After that, the enzymatic activity was estimated to be 84 units/ml. The specific activity was 52.8 units/mg, the protein concentration was 1.6 mg/ml, purification folds 2.69, and the enzyme yield was 73.04%, as shown in Table1.

The results differed according to the different sources when using ammonium sulfate for the purpose of enzyme concentration and sedimentation. The results were similar with Sharma and Vaidya. **(Zhu *et al.*, 2018)**, when using ammonium sulfate with a saturation of 60% to precipitate and purify the enzyme actinidin from kiwi fruit, then membrane permeability, as it obtained a specific activity of 0.86. mg/protein, enzymatic activity of 190 units/ml, enzyme yield of 86%, and purification folds of 1.65. It also agreed with (**Chao, 2016)**, when using ammonium sulfate with a saturation of 60% to precipitate and purify the actinidin enzyme, and then membrane permeability, as it obtained a specific efficiency of 88.54 units / mg, enzymatic activity 244 units / ml, enzymatic yield 23% and purification folds 1.55. Ammonium sulfate is used in most studies with different saturation rates because of its positive advantages, including its high solubility, cheap price, and lack of toxicity **(Seifter & Englard, 1990**). and that the reason for the difference in the results in different sources is due to the use of different concentrations of ammonium sulfate used to modify the charges on the surface of the enzyme and to cause disruption of the water layer surrounding the protein, and hence reduce the solubility of the protein and its precipitation **(Zou *et al.*, 2018; Nidhal, 2013)**.

Table (1): Steps of purification of actinidin enzyme from kiwi fruit.

Purification step	volume (ml)	activity units/ml	protein mg/ml	Specific activity Units/mg	Total activity units	Purification folds	Yield %
Crude extract	1000	23	1.170	19.65	23000	1	100
Precipitation with Ammonium sulfate (20–80%) and Dialysis	200	84	1.592	52.8	16800	2.693	73.04

Optimum pH for the activity of the partially purified enzyme

The optimal pH for the activity of the purified actinidin enzyme from kiwi fruit was set with a range of pH numbers ranging from 4-9. It was shown from the results shown in Figure1

that the optimum pH of the purified actinidin from kiwi fruit was 7, as it was found that the highest activity of the enzyme 30 units / ml, and it is noted that the activity decreases at pH numbers 8-9. The reason of the decrease in the pH in the acidic and basic ranges is due to the effect of the groups present in the active site of the enzyme or the substrate or both, and the reason for the change in the ionic state of these groups and its reflection on the ability of the enzyme to bind to the subject matter **(Al-Zubaidy, 2017)**. This result was consistent with what was found by **(Al-Mahdawi, 2022)**, as the optimum pH of the purified actinidin enzyme from kiwi fruit is 7, and identical to **(Chao, 2016)**, where the optimum activity of the actinidin enzyme was at a pH7.

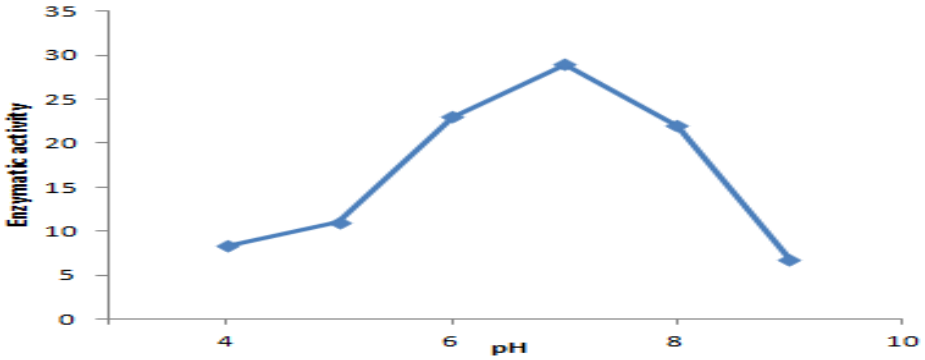

Figure (1): Optimum pH curve for activity of purified actinin enzyme from kiwi fruit

Optimum pH for the stability of the purified enzyme

The results shown in Figure 2 showed that the optimal pH for enzyme stability is between 6 and 8, as it was observed that the enzyme retained its effectiveness at pH 6 by 95%, while at pH 8 it was 91.1%, and the highest stability of the enzyme was at pH 7, while the enzyme activity was low in the extreme values of acidic and basic pH. The reason for the decrease in the enzymatic activity at the acidic and basic pH values is due to the change in the secondary and tertiary structure of enzyme that leads to the push amino acids in the active site away from each other **(Zhang *et al.*, 2017)**. These results were also close to the results of **(Kazeem *et al.*, 2013)** as they indicated that the optimal pH for the stability of the enzyme extracted from kiwi fruit is between 3 and 7. Also, the results of **(Chao, 2016)** showed that the purified actinidin enzyme showed high activity within a pH range between 6 and8 for sodium phosphate buffer solution.

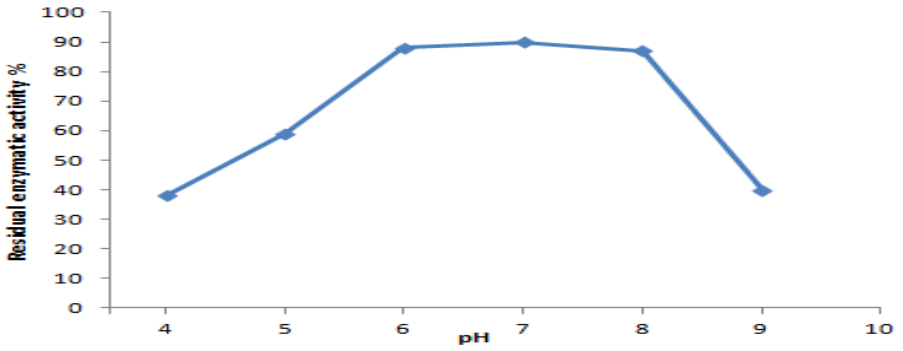

Figure (2): Optimum pH curve for the stability of purified actinidin enzyme from kiwi fruit

Optimum temperature for enzyme activity

The reaction of purified actinidin enzyme from kiwi fruit was done at different temperatures to determine the optimum temperature for the enzyme activity, as these temperatures ranged between 20 and 60 for 10 minutes. Figure 3 shows that at a temperature of 40, the enzymatic activity reached 27 units/ml, then the enzymatic activity decreased to 25.5 units/ml at a temperature of 50, and reached the lowest level of activity at 60, reaching 4.6 units/ml. The increase in temperature increases the speed of enzymatic reactions, and the reason is due to the increase in the thermal energy of the molecules, and then the increase in the collisions between the enzyme molecules and the molecules of the substrate, as it reaches the degree at which the reaction speed reaches its maximum value. After that the enzymatic activity decreases due to the denaturation of protein at high temperatures which changes the secondary, tritiary and tetratiary structures of protein **(Horwitz, 1975)**. These results agreed with **Kazeem, *et al.*, (2013)** who found that the optimum temperature for the action of the purified enzyme extracted from kiwi fruit was 40 degrees Celsius, after which the activity lost a large percentage at 60 degrees Celsius**(Lee *et al.*, 2010)** stated that the optimum temperature for the activity of the actinidin enzyme extract is 65 °C, and this contradicts what was confirmed by **Zhang *et al.*, (2017)** who stated that the optimum temperature for the actinidin enzyme is 40°C.

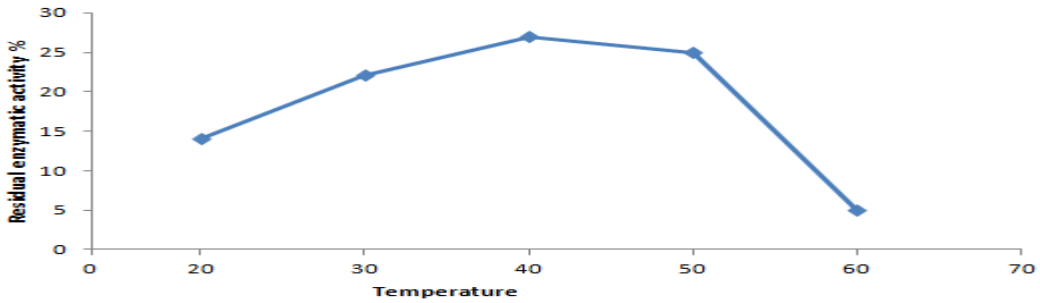

Figure (3): Optimum temperature curve for activity of purified actinin enzyme from kiwi fruit.

Optimum temperature for enzyme stability

The results in Figure 4 showed that the temperatures of enzyme stability ranged between (20-40) for a period of 20 minutes, where the enzyme retained its maximum activity, which ranged from (96.3-93.0)%, after which the enzyme activity decreased with the increase in temperature to reach 51.6% at a temperature 50 ºC, and the effectiveness continued to decrease with the increase in temperatures, to drop to 23% at 60 ºC. These results agreed with what was indicated by other authors **(Kazeem, *et al.*, 2013; Lee *et al.*, 2010**), as the enzyme retained its full activity at a temperature of 20-40 °C, then lost it by 50% at 50°C. The activity continued to decrease with the increase in temperature, dropping to only 10 % at 60°C.

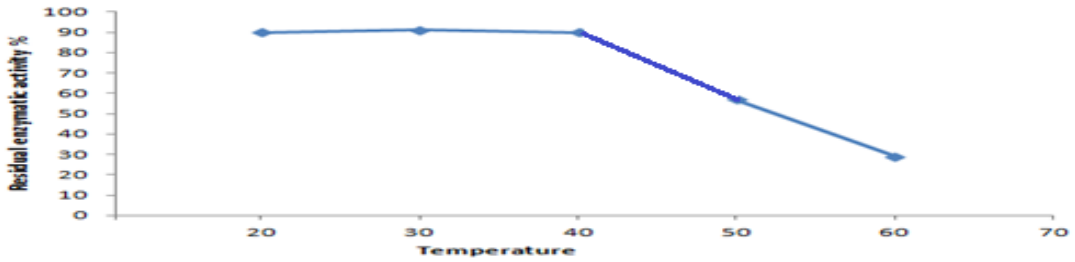

Figure (4): Optimum temperature curve for the stability of purified actinin enzyme from kiwi fruit

Qualitative tests

Peroxide value (PV)

Table 2 shows the effect of partially purified actinidin enzyme on peroxide values of aged chicken breast fortified with three concentrations (0.25, 0.75 and 1.00) % during incubation periods (0, 2, 4 and 6) h at 40 °C. Significant decreases ($p \leq 0.05$) were noted in peroxide values for the three treatments compared to the control. The higher the concentration, the lower the value of peroxide in the treatment samples during the same incubation period. The average of PV values for the control was 3.65, then it decreased after an incubation period of 2 hours to become (2.85, 3.05, 3.22), for concentrations (0.25, 0.75,1.00) %, respectively, but when comparing the control with one concentration during different incubation periods (0, 2, 4, 6) hours, higher PV values were recorded for the enzyme-treated samples compared to the control. It reached the highest value at a concentration of 0.25% and during the longest incubation period of 6 h, reaching 4.98. Through these results, it was found that the PV value is affected by two important factors, namely the incubation period and concentration. The higher the enzyme concentration, the lower the PV value in chicken breast samples treated with the purified enzyme. At the same time PV increases with increase the incubation period, as the average value of peroxide for the treatment at a concentration of 0.25% and for an incubation period of 6 h was 4.98, which is the highest value compared to other treatments except control. These results agreed with what was stated by Poona **(Abdelrahman *et al.*, 2023)** which applied kiwi juice applied to the chicken breast meat and observed significant differences in the peroxide values between the treatments compared to the control.

Table (2): Effect of actinidin enzyme on peroxide value in chicken breast samples at incubation periods (0, 2, 4, 6) hours with three concentrations (0.25, 0.75 and 1.00%).

Time	Control	0.25%	0.75%	1%
2	3.65	3.22	3.05	2.85
4	4.54	4.11	3.40	3.19
6	5.66	4.98	4.25	4.02
LSDvalue	**0.994***			
***(p≤0.05)**				

Total volatile nitrogen (TVN)

Table 3 shows the effect of three concentrations (25, 0.75, 1.00) % for different storage periods (0, 2, 4, 6) h at 40°C on TVN values for chicken breast samples treated with partially purified actinidin enzyme. Significant increases (p≤0.05) in the values of TVN for the samples treated with the enzyme, where the value of TVN for the control sample was 5.44, while it was in the three treatments at concentrations (0.25, 0.75, 1.00) % (5.87, 6.00, 6.25) during the same period 2 h, respectively. It was also observed a rise in the values of TVN with increasing concentration and with the increase of the incubation period, where the highest value of TVN was 8.09 at the incubation period of 6 hours at a concentration of 1%. These results agreed with **(Abd Elrahman *et al.*, 2023)** who found an increase in TVN values of chicken breast meat treated with kiwi juice at a concentration of 5-7 + pineapple juice 5-7) % and (kiwi juice 5% + pineapple juice 5%) during a storage period of 3 months.

Table (3): Effect of actinidin enzyme on the value of total volatile nitrogen in chicken breast.

Time	Control	0.25%	0.75%	1%
2	5.44	5.87	6.00	6.25
4	5.80	6.08	6.33	7.00
6	6.05	6.44	6.74	8.09
LSDvalue	2.061*			
***(p≤0.05)**				

Thio barbituric acid (TBA)

Table 4 shows the effect of three concentrations of the enzyme (0.25, 0.75 and 1.00)% on TBA value of chicken breast samples treated with partially purified actinidin enzyme for different storage periods (0, 2, 4, 6) h at 40 °C. Significant decreases (p≤0.05) in the TBA values of the enzyme-treated samples were observed, where the TBA value for the control sample was 0.036, while it was (0.030, 0.026, 0.021) for the treatments at concentrations (0.25, 0.75, 1.00)% during the same incubation period 2h, respectively It was also observed a rise in the TBA values with a decrease in concentration with the incubation period, as the highest value in the treatment reached 0.050 during the longest incubation period of 6 hours at the lowest concentration 0.25%. Through these results, it was found that the value of TBA was affected by two important factors, namely the incubation period and concentration. The lower enzyme concentration the lower TBA value in chicken breast samples treated with purified enzyme.

As shown by **(Abdel-Naeem & Mohamed, 2016)**, there was a significant decrease in the TBA values when treating camel meat with ginger extract and papain compared to the

control sample, and there was no significant difference between the same treatments after soaking the meat with ginger extract at a concentration of 7% and papain at a concentration of 0.01% and a mixture of ginger extract and papain at a concentration of 0.01%. The decrease in the value of TBA is attributed to the activity of ginger extract in inhibiting the action of the peroxidase enzyme, which can reduce the release of unsaturated fatty acids and the oxidation of unsaturated fatty acids.

These results were confirmed by **(Naveena *et al.*, 2001)** when using ginger extract on smoked chicken meat. From these results it can be conclude that proteolytic enzymes possess antioxidant activities **(Richardson *et al.*, 2018).**

Table (4): The effect of actinin enzyme on the value of Thio barbituric acid (TBA) in chicken.

Time	Control	0.25%	0.75%	1%
2	0.036	0.030	0.026	0.021
4	0.052	0.035	0.030	0.027
6	0.069	0.050	0.042	0.035
LSDvalue	**0.022***			
***(p≤0.05)**				

Free fatty acids (FFA)

(Table, 5) shows the effect of partially purified actinidin enzyme on the values of free fatty acids in chicken breast during the incubation period of (0, 2, 4, 6) h at 40 °C for three concentrations (0.25, 0.75 and 1.00) %. Significant decreases ($p \leq 0.05$) were observed in the value of free fatty acids (FFA) for meat samples at three concentrations and at the same incubation period compared to the control sample, where its value was 0.35, while the averages of the treatments were (0.31, 0.26, 0.22) for the three concentrations (0.25, 0.75, 1.00) %, respectively. However, when comparing the control sample to a single concentration during different incubation periods, an increase in the FFA value of the enzyme-treated samples can be seen compared to the control sample. These data showed that the highest average of FFA was when the concentration treatment was 0.25% and for an incubation period of 6 h.

These results agreed with **(Pooona & Singh 2020)**, when soaking chicken breast pieces in kiwi juice and during different refrigerated storage periods, as the value of free fatty acids decreased during the storage period.

Free fatty acids are among the main evidence for judging the quality of fat in meat and its various products. It is an indicator of spoilage, especially the volatile ones. There may be a decomposition of glycerides by the action of the lipase enzyme and the production of fatty acids. This decomposition is rapid in the presence of light and air and is accompanied by rancidity **(Auda& Khalifa, 2019; Hassan, *et al.*, 2022; Ibrahim, 2022; Fradi, 2022).**

Table (5): The effect of actinidin enzyme on the value of free fatty acids (FFA) in chicken.

Time	Control	0.25%	0.75%	1%
2	0.35	0.31	0.26	0.22
4	0.47	0.37	0.31	0.27
6	0.68	0.48	0.40	0.35
LSDvalue	0.219*			
***(p≤0.05)**				

CONCLUSION

The enzyme actinidin can be extracted and partially purified from kiwifruit with good activity. This enzyme acts as an antioxidant beside its basic function which is tenderizing agent. These results suggest the potential use of this enzyme in tenderization of aged meat. Further studies may be required to improve extraction and purification steps and to apply the enzyme in other processing treatments which utilize enzymes.

CONCLUSIONS

1. Actinidine enzyme is one of the most important enzymes present in kiwi fruit. It constitutes 60% of the protein. It can be extracted, precipitated, and purified with ammonium sulfite salt at a temperature of 4 °C.
2. The results of the qualitative tests confirmed that the enzyme actinidine is a natural antioxidant that can provide protection for the treated mites according to several factors, the most important of which are concentration, incubation period and temperature.
3. It was found that the optimum pH for enzyme activity was 7 and the stability ranged between 6-8

RECOMMENDATIONS

1. A study of the effect of actinidine as an anti-cancer and anti-inflammatory
2. Study of the effect of partially purified actinidine as an antimicrobial agent for food contaminants
3. The use of the enzyme in the manufacture of easy-to-digest protein foods for sensitive groups

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