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المجلة العراقية لبحوث السوق وحماية المستهلك

THE USE OF BROMELAIN ENZYME EXTRACTED FROM PINEAPPLE CROWN AS ANTI-BROWNING IN APPLE JUICE AND ANTI-BACTERIAL

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ABSTRACT

Lyophilized bromelain enzyme was obtained from the Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad.

Some properties of the enzyme were determined, the optimum temperature and pH for activity and stability, as it used a range of graduated temperatures from 25 °C to 85 °C, as well as a range of pH degrees from 4 to 9. The results showed that the best pH for activity was 7, as it reached the enzymatic activity was 42.3 units/ml, and the best pH range for stability was 7-5. The best temperature for the activity was 45°C, as the enzymatic activity reached 59.3 units/ml, and the enzyme retained its full activity after being incubated at a temperature of 25 - 45 °C.

The anti-browning enzyme activity was tested by using apple juice by adding graded concentrations of the enzyme of 15, 30, 45, and 60 mg/ml of bromelain enzyme. It was found that bromelain has the ability to reduce the amount of enzymatic browning, as the absorbance reached 0.987, 0.973, 0.891, 0.831, and 0.817 at zero time, and after 30 minutes the absorbance reached 1.315, 1.243, 1.132, 1.124, and 1.113, but after 60 minutes, the absorbance reached 1.412 And 1.312, 1.192, 1.133, and 1.124, and after 90 minutes it reached 1.623, 1.378, 1.211, 1.157, and 1.135, to reach after 120 minutes to 1.858, 1.416, 1.243, 1.182, 1.182, and 1.14 for the control treatment, the first treatment (15 mg enzyme/ml), the second treatment (30 mg enzyme/ml), the third treatment (45 mg enzyme/ml) and the fourth treatment (60 mg enzyme/ml), respectively.

The ability of the bromelain enzyme to inhibit the growth of some types of Gram-negative and Gram-positive bacteria was studied, with an antibacterial effect, as the diameters of the inhibition zones were 4.91, 6.55, 12.85, and 2.8 mm for *Staphylococcus aureus, B. cereus, E. coli, and Salmonella*, respectively, when using the concentration of 15 mg/ml of bromelain enzyme, but when using 30 mg/ml, the diameters of the inhibition zones were 7.05, 8.5, 13.85, and 6.85 mm, respectively.

Keywords: bromelain, anti-browning activity, antibacterial activity.

استخدام إنزيم البروميلين المستخرج من تاج الأناناس كمضاد للأسمرار في عصير التفاح ومضاد للبكتيريا

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الخلاصة:

تم الحصول على إنزيم البروميلين المجفف بالتجميد من قسم علوم الأغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، وتم تحديد بعض خصائص الإنزيم، ودرجة الحرارة المثلى والرقم الهيدروجيني الأفضل للفعالية والثبات، اذ استخدمت مجموعة من درجات الحرارة المتدرجة من 25 85 م، فضلا عن مجموعة من درجات الرقم الهيدروجيني من

9-4، وأظهرت النتائج أن أفضل رقم هيدروجيني للفعالية كان 7، اذ بلغت الفعالية الانزيمية 42.3 وحدة/مل، وأفضل نطاق رقم هيدروجيني للثبات كان 5-7، كانت أفضل درجة حرارة للفعالية هي 45 م، اذ بلغت الفعالية الانزيمية 59.3 وحدة/مل، واحتفظ الإنزيم بكامل فعاليته بعد حضنه عند درجة حرارة 25- 45 م.

تم اختبار نشاط الإنزيم المضاد للاسمرار الانزيمي باستخدام عصير التفاح عن طريق إضافة تراكيز متدرجة من إنزيم البروميلين بلغت 15 و30 و45 و60 ملغم/مل، وجد أن البروميلين لديه القدرة على تقليل الاسمرار الانزيمي من خلال قياس درجة اللون البني اذ بلغت الامتصاصية 0.987، 0.973، 0.891، 0.831، و 0.817 في وقت الصفر، وبعد 30 دقيقة بلغت الامتصاصية 1.315، 1.243، 1.132، 1.124، و 1.113، ولكن بعد 60 دقيقة وصلت الامتصاصية الى 1.412 و 1.312، 1.192 و 1.133 و 1.124 ، وبعد 90 دقيقة وصلت إلى 1.623 و 1.378 و 1.211 و 1.157 و 1.135، لتصل بعد 120 دقيقة إلى 1.858، 1.416، 1.243، 1.182، 1.182، و 1.14 لمعاملة السيطرة والمعاملة الأولى (15 ملغم إنزيم/مل) والمعاملة الثانية (30 ملغم إنزيم/مل) والمعاملة الثالثة (45 ملغم إنزيم/مل) والمعاملة الرابعة (60 ملغم إنزيم/مل) على التوالي

تمت دراسة قدرة إنزيم البروميلين على تثبيط نمو بعض أنواع البكتيريا السالبة والموجبة لصبغة كرام، اذ بلغت أقطار مناطق التثبيط 4.91، 6.55، 12.85، و2.8 مم لبكتريا *Staphylococcus aureus* و *B. cereus* و *E. coli* و *Salmonella* على التوالي عند استخدام تركيز 15 ملغم/مل من إنزيم البروميلين، اما عند استخدام تركيز 30 ملغم/مل فقد كانت أقطار مناطق التثبيط 7.05، 8.5، 13.85 و6.85 ملم للبكتريا أعلاه على التوالي.

الكلمات المفتاحية: انزيم البروميلين، الفعالية المضادة للاسمرار الانزيمي، الفعالية المضادة للبكتريا.

INTRODUCTION

Bromelain is a mix of proteolytic enzymes extracted from the parts of the pineapple plant through successive steps such as filtration, centrifugation, sedimentation, and lyophilization (**Nelson *et al.*, 2022**) which represent 60 % of the total commercial enzymes worldwide. Bromelain is one of the most important industrial enzymes (**Feijoo & Villa, 2010**) Bromelain is used in many medical applications. It also has a role in food processing and is included in other industries such as cosmetics and textile industries. Bromelain was classified by the US Food and Drug Administration (FDA) in 2006 as a safe food additive (**Tochi *et al.*, 2008**). Bromelain enzyme was proposed and presented as a therapeutic compound (**Kelly, 1996**). Bromelain is used in the treatment of acute infections and sports injuries and is also used in clinical applications, and it has the ability to prevent enzymatic browning caused by the activity of polyphenol oxidase (PPO) to prolong the shelf life of fruits to keep them fresh and not spoil. Bromelain works as a good enzymatic anti-browning agent when it works alone or when combined with aloe vera extract.

Aminan *et al.* (2020) reported that bromelain has a high inhibition power for enzymatic browning in fresh fruits. **Ramalingam *et al.* (2012)** observed that bromelain prevented phenol oxidation and thus the enzymatic browning of fruits, and pineapple juice was used as an inhibitor of enzymatic browning in bananas as its study reported a 100 % inhibitory effect of bromelain towards the oxidation of polyphenols (PPO).

Mynott *et al.* (1997) observed that bromelain is considered an antibacterial agent by inhibiting the growth of intestinal bacteria, such as *Vibrio cholera* and *E. coli*. And stops the production of enterotoxins by *E. coli* bacteria and prevents diarrhea caused by them, and its infection can be eliminated by using bromelain as a prophylactic and the synergistic effect of bromelain has been observed when used in conjunction with antibiotics. Bromelain can also be considered an antifungal and used to treat fungal infections (**Brakebusch *et al.*, 2001**).

Bromelain significantly increases the effectiveness of antibiotics in a variety of conditions (**Gighe *et al.*, 2010**). The minimum inhibitory concentration of bromelain has been tested on isolated strains of *Streptococcus mutans, Enterococcus faecalis, Aggregatibacter actinomycetemcomitans,* and *Porphyromonas gingivalis* (**Praveen *et al.*, 2014**).

Rashid *et al.* (2016) observed the antitoxin activity, as it found inhibition of bacterial toxin secretion (35-62 %) by various types of microorganisms in animals treated with bromelain. This was explained by either the inhibition of cAMP and cGMP that are activated by bacterial toxins or the enzymatic modification of the glycoprotein receptor binding sites with *E. coli* present on the intestinal mucosa, thus preventing bacterial adhesion to intestinal cells.

Mynott *et al.*, (1999) observed that bromelain has an anthelmintic effect against gastric and intestinal nematodes. The results obtained indicate that the proteolytic properties are responsible for the anti-parasitic activity of bromelain, as bromelain acts by enzymatic digestion of structural proteins present in nematodes, leading to a definitive loss of motility by the parasite. Therefore, bromelain has demonstrated significant antibacterial, anthelmintic, and antimicrobial effects either alone or in combination with an antibiotic in the treatment of various symptoms and diseases and can be considered a reliable, effective, and safe treatment. Bacterial resistance to antibiotics has become a major clinical problem, as bacteria are able to develop resistance to antimicrobial agents (**Nawwab, 2007; Alshamary & Ahmad, 2019**).

In view of what was mentioned earlier and the wide range of industrial and commercial uses of this enzyme compared to its extraction sources, which are non-valuable industrial wastes such as pineapple peel and crown, this study aimed to characterize the enzyme and measure the effectiveness of the enzyme as an anti-tanning enzyme and test the activity of the enzyme as an antibacterial.

MATERIALS AND METHODS

Determination of the optimal pH for enzyme activity.

Three types of buffer solutions were used in determining the optimal pH for enzyme activity with a concentration of 0.01 M in order to obtain different values of pH, which are sodium acetate buffer with pH numbers 5.0 and 5.5, and sodium phosphate buffer solution with pH numbers 6.0 and 6.5,7.0,7.5,8.0, and Tris-HCL buffer with pH 8.5,9.0,9.5, and 10.0.

A solution of the casein was prepared with different pH degrees ranging between 5-10 with a difference of half a degree, at a temperature of 35 for 30 minutes, then the enzyme activity was estimated.

Determination of the optimal pH for enzyme stability.

Incubating the enzyme solution with buffer solutions at pH 3-8 and a concentration of 0.1 M in test tubes at a temperature of 35 ° C for 30 minutes, then the incubated enzyme was added to tubes containing the casein, then the enzyme activity was estimated.

Determination of the optimum temperature for the enzyme activity.

The optimum temperature was determined by incubating the tubes of the enzyme substrate at different temperatures ranging from 25-85 °C, with a difference of 10 degrees for each tube, then the enzyme activity was measured. (**Sedrah & Ahmaed, 2016**)

Determination of the optimum temperature for enzyme stability.

the enzyme was incubated at 25-85 C for 10 minutes, then the tubes were cooled in an ice bath, after which the enzyme was added to the substrate tubes, and incubated at 35 C for 30 minutes, then the activity of the enzyme was measured (**AL-doori *et al.*, 2015**)

Determination of enzyme activity as an anti-enzymatic browning reaction.

Apple fruit was chosen as a model for measuring the browning reaction. Apple juice was distributed among five tubes containing 10 ml of juice. Then graduated concentrations of

the enzyme solution were added, which are 15,30,45, and 60 mg/ml, and the first tube was left without addition as a control treatment.

Measurement of enzyme activity as an antibacterial

After activation of bacterial isolates belonging to the *Bacillus cereus*, *E. coli*, *Staphylococcus aureus*, and *Salmonella* attended the center Mueller Hinton agar according to the manufacturer's instructions. The diffusion method was used by diffusion to detect the inhibitory activity of the enzyme extract, three holes with a diameter of 4 mm were made inside the medium after hardening, then the enzyme solution was added with two concentrations of 15 and 30 mg/ml in the holes, and sterile distilled water was placed in the third as a control treatment. The plates were incubated for 48 hours at a temperature at 38 °C, after which the diameters of the inhibition zones were measured. (**Mamo & Assefa, 2019**).

RESULTS AND DISCUSSION

Determination of the optimum pH for activity

The effect of pH on the activity of the purified enzyme was studied at pH numbers ranging between 3-9 as Figure 1 shows that the optimal pH for enzyme activity was 7.0, when the enzyme activity reached 42.4 units/ml, while the enzyme activity witnessed a decrease at pH numbers Acidity 5-6 and basicity 8-9.

The effect of the pH on the enzyme activity is attributed to its effect on the enzyme complex and the base material, which depends on the type of side chains (R-groups) of the amino acids, which are responsible for the formation of the active site and other supporting effective groups and the corresponding groups present in the base material, where the chances of binding are reduced, and the formation of the complex by the loss of these aggregates and the case reaches the occurrence of identification of the enzyme at the pH numbers (**Garret & Grisham, 2005; Grzonka *et al.*, 2001**).

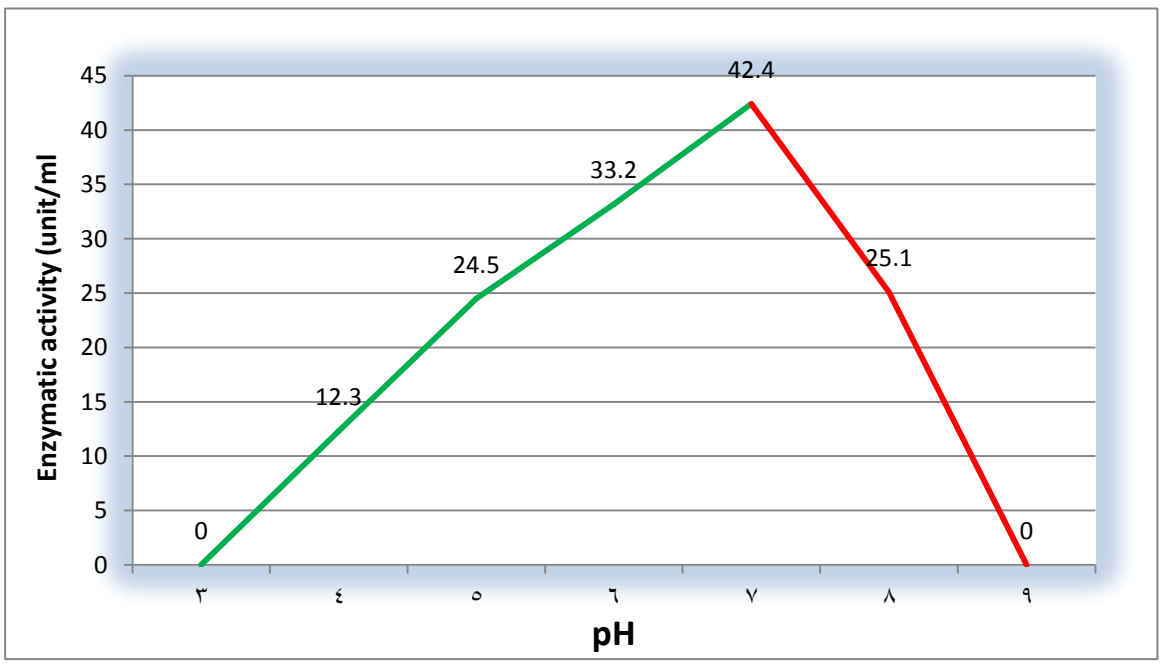

Figure (1): optimum pH for the activity of the bromelain enzme extracted from the crown of the pineapple fruit.

Determination of the optimum pH for stability.

It is clear from Figure 2 that the optimal pH for the stability of the bromelain enzyme is between 5 - 8 when incubated at 37 C for a of 15m and in buffer solutions with pH ranging from 3-9 The results showed that the enzyme retained about 90% of its activity in pH between 5-8. It also notes a clear decrease in its effectiveness at pH 8.0. On the other hand, the enzyme retained about 42% of its activity at pH between 4-5, and the enzyme lost all effectiveness at pH 9.0. The loss of enzyme activity may be attributed to the effect of the ionic groups responsible for the stability of the enzyme, and thus the enzyme's loss of its structure and the possibility of denaturation of the enzyme as a result of these numbers. extreme pH (**Berg, 2007; Mahajan *et al.*, 2010**).

It is clear from what has been mentioned that the enzyme has high stability at the optimum pH for activity, and it was distinguished by its sensitivity towards extreme pH numbers, both in the acid and alkaline, and that it prefers a neutral pH. Because of the large change that occurs in the charges of the ionizable side chains, which leads to the destruction disruption of the triple structure of the enzyme molecule to create a more random structure instead and a change in the effective site and thus loss of effectiveness (**Palmer, 1985**).

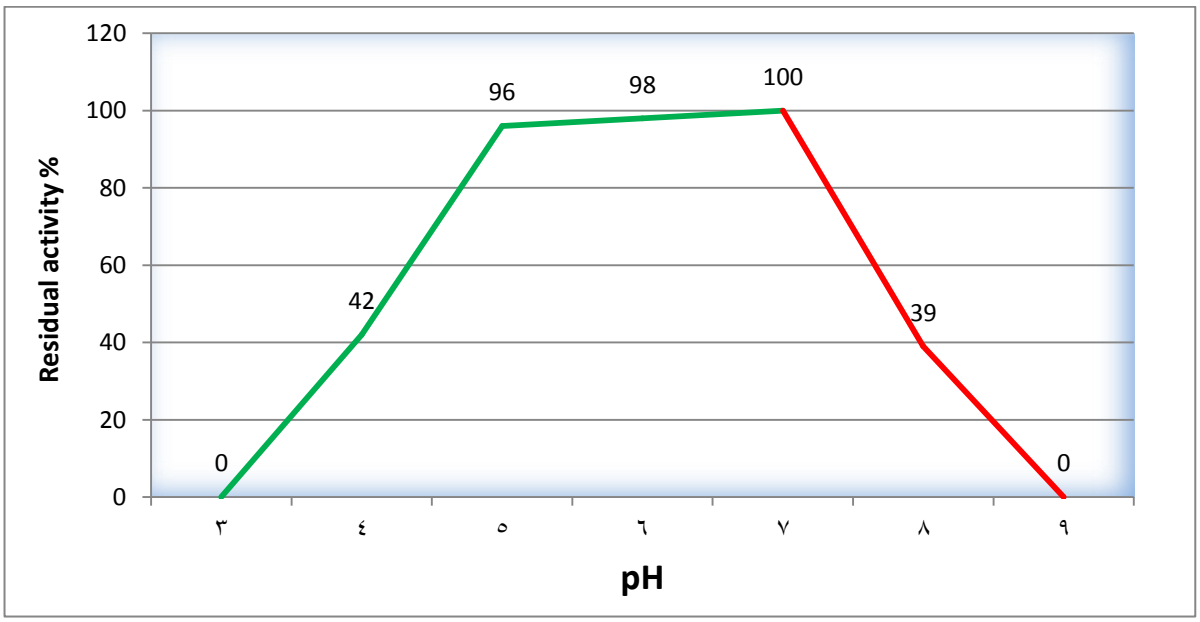

Figure (2): the PH for the stability of the bromelain enzyme extracted from the crown of the pineapple fruit.

Determination of the optimum temperature for activity.

It is noted in Figure 3 that an increase in enzyme activity occurs with the rise in temperature, reaching its maximum value of 71.5 units/ml at 55°C, which represents the optimum temperature for activity. Then it deteriorated to reach 32.1 units/ml at 75°C, which constitutes 50% of the maximum activity, and then the enzyme completely lost its activity at 85°C, this is due to the phenomenon of self-digestion of the enzyme, which often reaches its strongest at high temperatures (**AL-jumaili *et al.*, 2009**).

The difference in the optimum temperatures for proteases is due to the different sources, as well as the different conditions for estimating the effectiveness of a base material

and the pH, in addition to the different methods of estimation used (**Whitaker, 1994; ALhaidari *et al.*, 2020**).

The increase in enzyme activity with the rise in temperature is due to the increase in kinetic energy for both the base material and the enzyme, which leads to an increase in the chances of collision between them (**Segel, 1976**), but after exceeding the optimal temperature, a break will occur in the hydrogen bonds, which will affect the enzyme's composition, then the enzyme will lose its ability to retain its integral structure with a higher temperature, to gradually lose its effectiveness, and a collapse may occur in the enzyme's composition and its natural properties (**Murray *et al.*, 2000; ALeasawi, 2020**)

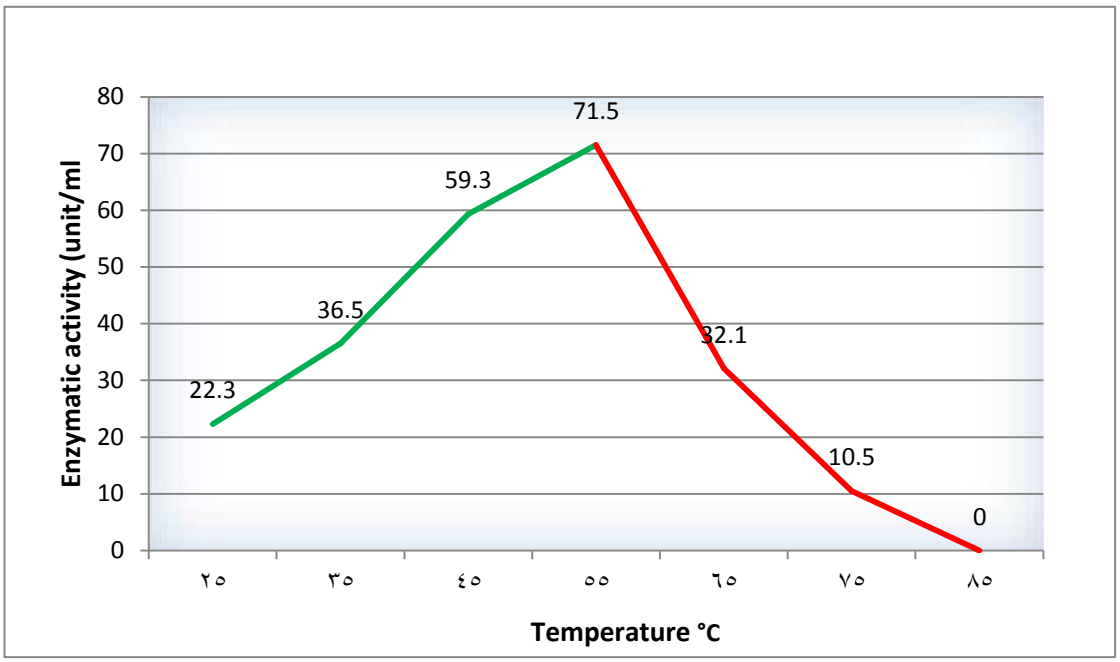

Figure (3): optimum temperature for the activity of bromelain enzyme extracted from the crown of pineapple fruit

Determination of the optimum temperature for bromelain stability

The thermal stability of the enzyme was estimated at temperatures between 25 - 85°C with a difference of 10 degrees from the preceding temperature when incubated for 15 minutes at pH 7.0. Then the residual activity was estimated against a 1 % casein solution prepared in phosphate buffer. The results in Figure 4 indicate that the bromelain enzyme retained about 100 % of its activity at 55 °C and with the rise in temperature, the stability of the enzyme began to gradually decrease, while the enzyme retained 45% of its activity when incubated at a temperature of 65 °C, while the enzyme lost most of its activity at 85°C, which is expected to occur denaturation of the enzyme due to the high temperature. This variation in temperatures is due to a difference in the number and type of amino acids constituting the enzyme structure, in addition to the number and locations of the sulfur bridges binding the enzyme molecule (**Bisswanger, 2008; Whitaker, 1994**)

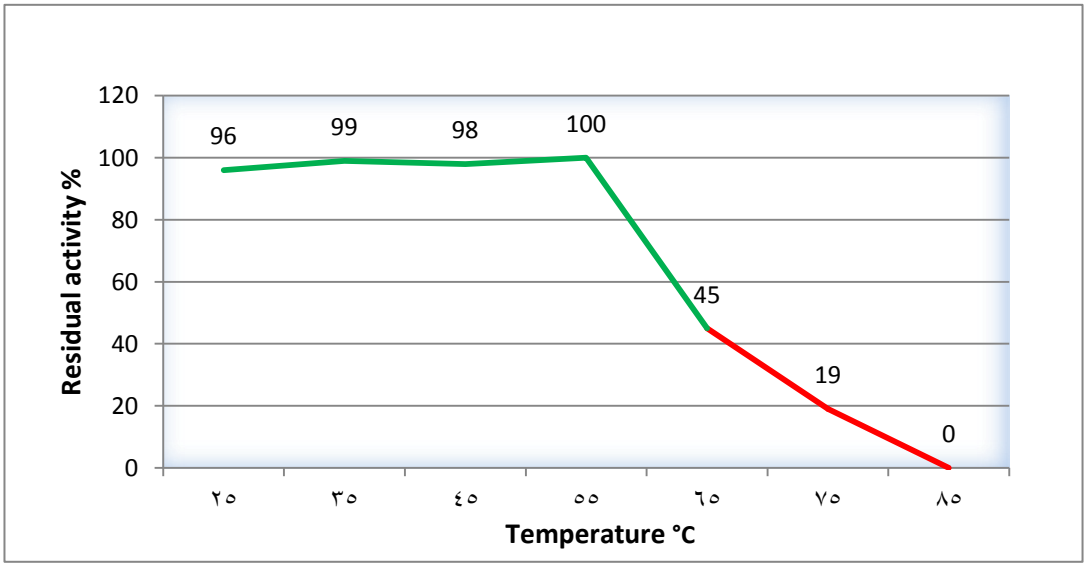

Figure (4): optimum temperature for the stability of the bromelain enzme extracted from the crown of the pineapple fruit.

Enzyme activity as an inhibitor of enzymatic browning.

It is noted from Figure 5 that the degree of enzymatic browning decreased in apple juice samples with an increase in the concentration of the added enzyme compared to the control treatment, which amounted to 0.987, 0,973, 0.891, 0.831 and 0.817 for the control treatments, the first treatment (15 mg/ml), the second treatment (30 mg/ml), the third treatment (45 mg/ml) and the fourth treatment (60 mg/ml) respectively, while the readings of the treatments reached at a wavelength of 450 nm after an incubation period at a temperature of 35 °C after 120 minutes 1.858, 1.416, 1.243, 1.182, and 1.148 respectively.

It is clear from the above results that the deterioration and decline of the polyphenol enzyme (PPO), is primarily responsible for the appearance of enzymatic browning. The bromelain enzyme acts as an inhibitor of the enzyme (PPO) responsible for the appearance of brown color in fruit juice for enzymatic browning. Despite the use of commercial agents against enzymatic browning on a large scale, the Food and Agriculture Organization restricts some of them and bans them, such as sulfates, even with the presence of many effects There are negative features associated with the use of sulfates, which lead to a decrease in consumer acceptance. Therefore, there is a need for natural anti-browning agents that can be used in the food industry, such as the bromelain enzyme. The bromelain enzyme is the best enzymatic anti-browning agent compared to some commercial agents available as an anti-browning agent (**Bhagavathy *et al.*, 2019; Chaisakdanugull *et al.*, 2007**).

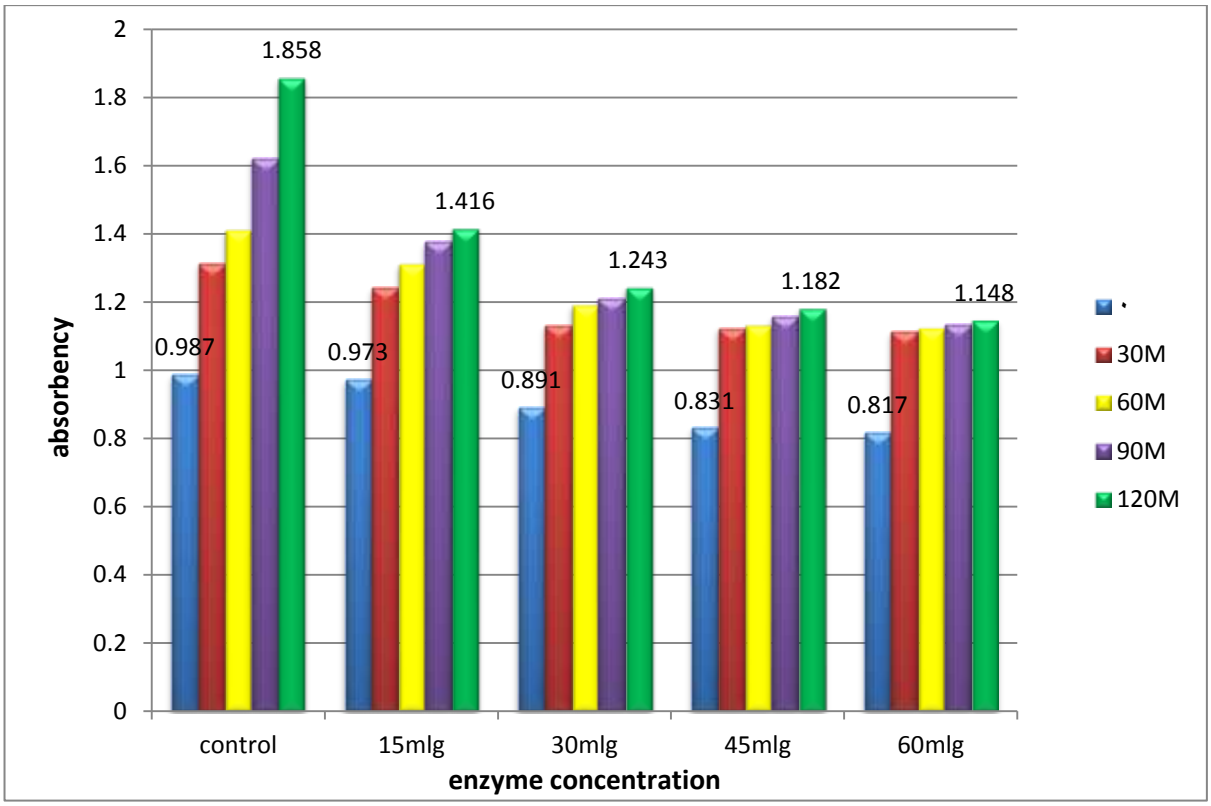

Figure (5): Enzyme activity as an anti-browning agent

Bromelain enzyme activity as an antibacterial

Figure 6 shows that the bromelain enzyme has antibacterial activity, this activity led to forming a clear zone around the enzyme when growing *Staphylococcus* 4.91 mm and 7.05 mm when using an enzyme concentration of 15 mg/ml and 30 mg/ml, respectively, but in the case of *B. cereus* the diameter of the clear zone was 6.55 mm and 8.5 mm, respectively. In the case of *E. coli* reached to 12.55 mm and 13.85 mm. While *Salmonella*, the diameter of the inhibition zone reached 6.35 and 6.85.

This anti-bacterial activity is explained by the fact that The bromelain enzyme has the ability to analyze some of the peptide chains that make up the cell walls of bacteria (**Tochi, *et al.*, 2008**), thus damaging the cell wall and allow leakage and cell death. Depending on the cell walls from one sex to another, the effect of the bromelain enzyme varies from one type to another. The bromelain enzyme has high effectiveness against Gram-negative bacteria much more. Gram-positive bacteria (**Sparso & Moller, 2002**). The bromelain enzyme has high activity against Gram-negative bacteria, Compared with Gram-positive bacteria Sparso & (**Moller, 2002; Musa & Alsamarrai, 2010**).

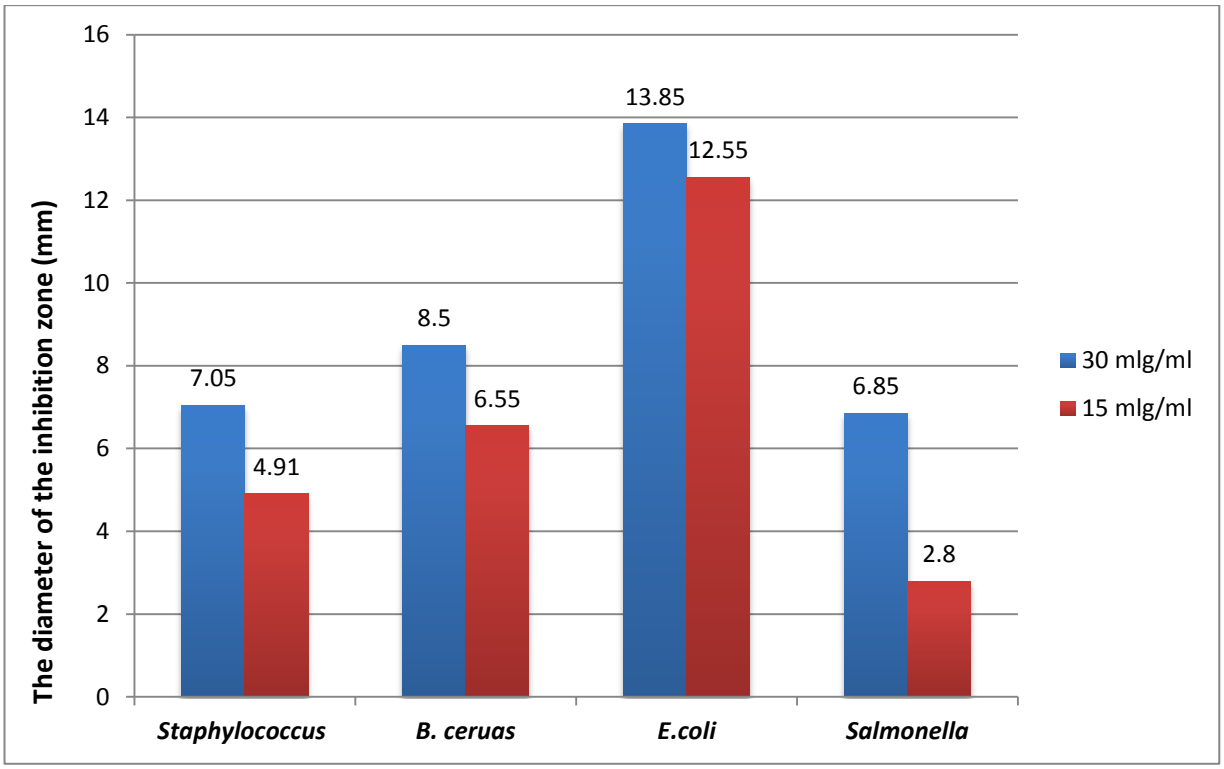

Figure (6): Activity of bromelain enzyme extracted from the crown of pineapple fruit as an antibacterial agent.

CONCLUSION

Through this study, we concluded that bromelain works in a wide range of temperatures from 25-55 °C, as well as being effective at physiological pH 7. It is hoped that the enzyme will be developed to be an alternative to industrial enzymatic browning inhibitors, as the enzyme showed anti-enzymatic browning activity as well. Effective activity against bacteria. It was noted that the increase in the concentration of the enzyme is directly proportional to the inhibition of both bacteria and the activity of enzymatic browning.

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