RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSIONS

4.1. Chemical composition of unripe fruits, fresh and dried latex of papaya (*Carica papaya*):

Chemical composition of unripe, fruits, fresh and dried latex of papaya (*Carica papaya*) are shown in Table (2). The unripe fruits of papaya contain 1.18 % protein , 26.97 % carbohydrates, 0.99 % ash, 0.49 % fat and 70.37 % moisture. While fresh latex contain 14.10 % protein, 3.14 % carbohydrates, 2.91 % ash, and 79.85 % moisture. However, freeze-dried latex contain 69.85 % protein , 16.71 %carbohydrates, 9.53 % ash and 3.9 % moisture (Table 2). The chemical composition of papaya was carried out by Marilyn and Carol (1994) at the 3 different stages of ripening (unripe, semiripe and ripe. They reported that no significant differences were observed in moisture, lipid or ash contents for the tested samples. Howevr, the protein content of unripe was seen to be significantly higher (1.02 %) than that for either semiripr (0.93 %) or ripe fruit (0.81%) Chnges in protein content have been observed for other fruits, but these changes have not been shown to be a dependable index of maturity for several fruits including avocados (Baile and Young, 1971). Although significantly lower protein contents were observed in ripening pawpaws, conclusive evidence that associates protein content with pawpaw ripening is lacking.

The compositional analysis of dried latex material was studied by Gutavo et al (1990) . They found that dried latex contain 67 % protein, and

17 % carbohydratws. The final product has 4 % residual water, a level which is difficult to reduce owing to the hugroscopic propertieds of the product. The water content of crude latex was ranged from 78 to 82 % .

They added also that the measured protein content (67 %) agrees closely with the value reported for commercial preparations, i.e 70 % protein for a similar commercial product, 80 % protein for 2x crystalline papain. By contrast, the water content of fresh latex of *Carica candamarcensis* 80 % (w/w). This is 8 % lower when compared with the water content of *Carica papaia* fresh latex. The differential water content is partly reflected in the fruit size.

Table 2.Chemical composition (%) of unripe fruits, fresh and dried latex of papaya (*Carica papaya*).

Components	Unripe fruits	Fresh latex	Dried latex
Moisture	70.37	79.85	3.90
Protein	1.18	14.10	69.85
Ash	0.99	2.91	9.53
Fat	0.49	—	—
Carbohydrates*	26.97	3.14	16.71

* Calculated by differences.

4.2. Papain yields as affected by different factors:

4.2.1. Effect of incisions on papain yields:

Data presented in Table (3) show the resultant yield of papain latex as affected by number of incisions. Results indicated that papain yields in fresh latex from the initial tapping was higher with ten cuts being 8.93 g/fruit. After 7 and 14 days , the yield decrease either using six or ten cuts. It could be noticed that the total yields of collected papain from the initial tapping upto 14 days was higher in the fruit with ten incisions (16.67 g/fruit) than that of six incisions (13.83 g/ fruit). The total fresh yields when freeze dried gave about 3.43 and 4.18 g/fruit for six and ten incisions, respectively. The number of incisions made on a fruit also affect the yield; the more the incisions, the higher the yield (Castro, 1981). In other experiments in Sri Lanka, showed that 8 incisions given to fruit gave higher yield than 3 incisions , but the latter is more practical since sufficient space is available on the fruit to continue the lancings over longer period untile the fruit becomes ripe and ceases to give latex (Charavanapvan, 1952).

Table 3. Effect of incisions on fresh and freeze-dried papain yields.

Tapping time (day)	Fresh latex yield (g/fruit)		Dried latex yield (g/fruit)	
	Six incisions	Ten incisions	Six incisions	Ten incisions
first day	6.82	8.93	1.83	2.06
7 days	4.74	4.84	0.98	1.13
14 days	2.27	2.91	0.61	0.99
Total yields	13.83	16.67	3.43	4.18

4.2.2. Effect of fruit sizes on papain yields:

Latex can be tapped continuously from a papaya fruit as long as it is unripe. In this experiment 24 fruits of different sizes were selected and divided into three groups (8 fruits for each group) as follows: I. 5 - 15 cm long, II. 16 - 25 cm long, and III. 26 - 35 cm long. Each fruit was tapped three times a week by six or ten longitudinal incisions each time. The collected latex was weighed, dried, powdered and weighted again. This was done for 14 days or until the fruit ripens. Table (4) gives the yield of latex per fruit /day as affected by fruit sizes. Results proved that fresh and dry _ latex yields increased gradually by increasing the fruit size. The amount of latex collected per day was quite variable and papain was usually 20 - 25 % by weight of fresh latex. The highest yield of dry papain after 14 days was 3.46 g/fruit was produced by the biggest fruits. These fruits, however, can be tapped for a short period since they are ready to ripen. These results were confirmed with that reported by Castro (1981).

Who reported that the total yield of latex after 14 days were 1.44, 3.25, and 3.72 g/dry fruit for the different sizes of 6 - 15, 16 -25 and 26 - 35 cm long, respectively. He added also that the highest yield of dry latex and papin after one month were 4.94 and 1.17 g/ fruit, respectively which was produced by these fruits, however this can only tapped for a short period since they are ready to ripen. On the other hand eperiments in India showed that any stage between 70 and 110 days from fruit-set could be regarded as optimum for high yields. Beyond these limits, the latex yields were less than the general mean (Seemanthani and Balakrishnan, 1964).

Table 4. Yield of latex from papaya fruits of different sizes (g / fruit):

Tapping time (day)	Fruit size 5 - 15 cm (long) Yield of		Fruit size 16 - 25 cm (long) Yield of		Fruit size 26 - 35 cm (long) Yield of	
	Frsh latex	Dry latex	Fresh latex	Dry latex	Fresh latex	Dry latex
First day	3.42	0.92	6.35	1.71	8.31	1.91
7 days	2.05	0.48	4.57	0.90	3.98	0.81
14 days	1.83	0.42	2.78	0.69	2.75	0.74
Total yields	7.31	1.82	13.71	3.30	15.04	3.46

4.2.3.Effect of fruit age and time of tapping on the activity of yielded latex:

Twenty four fruits of papaya (*Carica papaya*) were selected with sizes ranging from 26 - 35 cm long with ages of 1.5 - 2.0 , 2.0 - 2.5, and 2.5 - 3.0 monthes. Three fruits were tapped with ten incisions each for different collected times according to the following schedule: (a) 9:00 - 10:00 am , (b) 10:00 - 12:00 noon, (c) 12:00 - 2:00 pm and (d) 2:00 - 4:00 pm. The collected latex was freeze dried and powdered.

The results of the experiments on the best collection time and age of fruits are given in Table (5). Maximum yield and activity of fresh and dried papain (latex) was obtained from the 10:00 am to 12:00 noon, while, the minimum yield and activity were found at 2:00 - 4:00 pm. On the other hand, the best fruit ages and maximum yield of fresh and dried papain was obtained from the fruit of 2.5 - 3.0 months. These results are in a good agreement with that obtained by Madrigal et al (1980) who reported that the yields of fresh and crude papain increase with the fruit age. However, Castor (1981) found that the maximum yield of papain was obtained from 10:00 am - 12:00 noon collection while maximm activity was at 8:00 - 10:00 am. Maximum yield and activity, on the other hand, was at 2:00 - 4.:00 pm. The optimum time of collection, therefore, would be in the morning. Contrary to the results reported by (Cheshire, 1966), we have found it impractical to tap before the sun rises especially, during cold days because latex becomes viscous and coagulates rapidly on the skin of the fruit.

Table 5. Effect of fruit age and the collection time on the activity and latex yield.

Collection time	Fruit age (month) 1.5 - 2.0				Fruit age (month) 2.0 - 2.5				Fruit age (month) 2.5 - 3.0			
	A	B	C	D	A	B	C	D	A	B	C	D
9:00 - 10:00 am	2.86	8.41	0.71	4.26	3.52	7.27	0.88	3.68	4.85	6.90	1.35	3.49
10:00 - 12:00 noon	3.95	8.79	0.98	4.45	5.03	7.81	1.36	3.95	5.93	7.64	1.65	3.87
12:00 - 2:00 pm	2.03	6.84	0.51	3.47	3.23	6.57	0.20	3.33	4.16	6.49	1.12	3.29
2:00 - 4:00 pm	1.61	6.00	0.40	3.30	2.70	5.57	0.67	2.82	3.41	5.25	0.85	2.66

Where:

A : Fresh latex yield (g / fruit).
B : Activity of fresh latex (u / mg).
C : Dry latex yield (g / fruit).
D : Activity of dry latex (u / mg).

From the afformentioned results it could be concluded that the number of incisions made on the fruit play an important role in the yield of latex; the more the incisions , the higher the yield. The bigger the fruit, the greater the yield of latex. The amount of latex collected per day was quite variable and papain was usually 20 - 25 % by weight of fresh latex. The best time of collection was in the morning from 10:00 - 12:00 noon. The yields of fresh and dried latex increased with the increasing the fruit age.

4.3. Factors affecting the proteolytic activity of *Carica papaya* latex:

4.3.1. Proteolytic activity of *Carica papaya* latex from different organs:

Proteolytic activity of papaya latex from different organs (leaves and skin) was carried out, and the obtained results are summarized in Table (6) .Data revealed that the highest activity was observed in the fresh latex (7.64 u/mg) followed by 5.74 and 3.97 u/mg in the leaves and skin extracts, respectively. It could be noticed also, drying process caused a reduction in the proteolytic activity of different latex extracted from different organs compared with their respective fresh latex.

Table 6. Proteolytic activity of fresh and dried latex extracted from different organs of *Carica papaya.*

Organs	Activity (u/ mg fresh weight)	Activity (u/mg dried weight)
Latex	**7.64**	**3.87**
Leaves	**5.74**	**2.90**
Skin	**3.97**	**2.76**

Gustavo et al (1990) compared the proteolytic activity of juice (soluble fraction) and extract (insoluble fraction) from different organs of Carica plant (stem , trunk, leaves and fruit skin). They reported that the greatest activity was observed in the stem in both juice and extract

fractions. Juice from trunk and leaves has an activity 1.5 times higher when compared with a similar fraction from the trunk. They noticed also , the proteolytic activity found in the ripened fruit is the lowest of all confirming the low content of proteinases remaining in the mature fruit.

4.3.2. Effect of different methods of skin drying of papaya and freezing process on the proteolytic activity of extracted latex.

Data presented in Table (7) show the effect of different methods of skin drying of papaya and freezing process on the proteolytic activity of extracted latex. Results indicated that proteolytic activity decreased as affected by different drying methods (sun dried, oven dried, vacuum drying and freez drying) compared with the fresh skin latex. It could be observed that freez dried skin exhibited a higher activity (2.76 u/mg dry skin), while sun - dried latex showed a lower activity (1.01 u/mg dry lskin) compared with other drying methods. On the other side, freeze drying preserve most of the original activity followed by vacuum drying which retains 70 % of the original activity and finally oven drying, which results in the largest losses of all the product retaining only 56 % of the original activity. However, the proteolytic activity of latex extracted from frozen skin (paste) was higher (2.17) than those by different methods except freeze-dried, but still lower than the control sample (fresh).

The reduction in the proteolytic activity as affected by sun drying may be due to the effect of ultraviolet light on the histidine in papain, which is essential for enzyme activity . The sun dried product is

darker in color and smell strongly, since microbial contamination is enhanced by the longer drying time (Arnon, 1970). However Castro (1981) observed that sun-dried papain had a lower activity than oven-dried. Other workers have shown , however, that sun-dried samples may have a higher activity than oven-dried samples (Hinkel, 1951). Although their activities may be comparable, sun-drying is a slower process and produces a lower grade product due to exposure to dirt and dust. Papain oven-dried at 40 C° gave the highest activity, while that oven-dried at 60 C° and 80 C° gave the lowest activity. However, there were no significant differences among the three temperatures used showing that papain can be dried upto 80 C° without loss of activity (Castro, 1981). It has been shown that papain can still retain its activity even after being heated to 105 C° (Hwang and Ivy, 1951). Papain ,therefore, is thermostable and can tolerate a wide range of temperature.

The previuos results indicated that the proteolytic activity of extracted latex affected by the organs and the higher activity was observed in latex either fresh or dried compared with that extracted from leaves and skin. On the other hand, the proteolytic activity decreased as affected by different drying methods compared with the fresh latex. Freeze-dried latex showed a higher activity, while sun-dried samples showed a lower activity.

Table 7. Effect of different methods of skin drying of papaya and Freezing process on the proteolytic activity of extracted latex.

Drying methods	Activity (u/mg dry skin)
Fresh skin latex	**3.97**
Frozen skin (paste)	**2.17**
Dryin methods:	
Sun drying	**1.01**
Oven drying (55C°/6hr)	**1.56**
Vacuum drying (55C°/6hr)	**1.93**
Freeze drying	**2.76**

4.4.Chemical composition of unripe figs (*Ficus carica*) and crude ficin

Table (8) shows the chemical composition of unripe fig fruits and crude ficin. Unripe fig fruits contain 74.26 % moistur, 4.53 % ash, 2.32 % protein, 1.5 % fat and 17.39 % carbohydrates. These results were slightly higher than that reported by Englund et al (1968) who found that the unripe fig fruits contain 1.5 % protein, 11 % carbohydrates, 79 % moisture, 1.0 % fat and 0.7 % ash. Data indicated also that crude ficin contain 71.83 % protein, 31.71 % carbohydrates, 0.59 % ash and 4.94 % moisture. While, El-Gharbawi and Whitaker (1963) found that crude ficin contain 4.34 % moisture, 79.1 % protein and 0.66 % ash.

Table 8.Chemical composition of unripe fig fruits and crude ficin .

Components (% on as is basis)	Unripe fig fruits	Crude ficin
Moisture	**74.26**	**4.94**
Ash	**4.53**	**0.59**
Protein	**2.32**	**71.83**
Fat	**1.50**	**0.94**
Carbohydrates*	**17.39**	**31.71**

* Calculated by differences.

4.5. Proteolytic activity of crude ficin extracted from fig leaves:

Results presented in Table (9) indicate that proteolytic activity of extracted ficin from fig leaves was lower than that of latex either in fresh or dried samples . Gustavo et al (1990) reported that the proteolytic activity of extracted latex from leaves was lower compared with the latex extracted from unripe fruits.

Table 9. Proteolytic activity of crude ficin extracted from fig leaves.

Sources	Proteolytic activity (u/mg fresh weight)	Proteolytic activity (u/mg dried weight)
Latex	**3.82**	**2.66**
Leaves	**0.76**	**0.47**

4.6. Chemical composition of bovine spleen , kidneys, liver and lung:

Results presented in Table (10) show that the highest protein levels was found in liver (19.87 %) followed by spleen (17.13 %) and kidneys (15.97 %), while kidneys were found to contain a higher levels of fat (6.79 %) followed by liver tissues (3.94 %). On the other hand, lung tissues contain a lower levels of ash and carbohydrates (0.98 and 1.05 %, respectively) . These results are in a good agreement with that obtained by Fruton and Irving (1941). Who reported that bovine spleen contain 18.50%

protein, 1.87 % carbohydrates, 76.17 % moisture, 1.90 % fat and 1.54 % ash. While the kidneys contain 16.63 % protein, 1.61 % carbohydrates, 73.12 % moisture , 7.26 % fat and 1.38 % ash. However, Gutmann and Froton (1948) found that liver contain 20.38 % protein, 2.34 % carbohydrates, 71.25 % moisture, 4.37 % fat and 1.66 % ash ,while lung contain 13.68 % protein, 0.60 % carbohydrates, 2.01 % fat, 1.00 % ash and 82.71 % moisture.

Table 10. Chemical composition of bovine spleen , kidneys, liver and lung.

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)*
Spleen	77.50	17.13	1.80	1.40	2.17
Kidneys	74.82	15.97	6.79	1.17	1.25
Liver	69.76	19.87	3.94	1.48	5.25
Lung	81.68	13.91	2.38	0.98	1.05

* Calculated by differences.

4.7. Proteolytic activity of crude extracts from different animal organs:

Proteolytic activity of crude extracts from different animal organs (spleen, kidneys, liver and lung) was carried out and the obtained results are summarized in Table (11). Data revealed that the highest activity was observed in the bovine spleen extract (5.73 u/mg protein) followed by 3.64, 2.15 and 2.01 u/mg protein in the kidneys,liver and lung extracts, respectively. These results are confirmed with that obtained by Mycek (1970) who reported that the higher proteolytic activity was found in spleen extract followed by kidney extracts. He added also that lung and liver had about one-third of the activity of spleen.

Table 11.Proteolytic activity of crude extracts from different animal organs

Organs	Activity (u/mg protein)
Spleen	5.73
kidneys	3.64
Liver	2.15
Lung	2.01

4.8 Effect of pH ,temperature, substrate and enzyme concentrations on the proteolytic activity of proteolytic enzymes:

4.8.1. Effect of pH:

Results presented in Table (12) and illustrated in Fig (1) indicate that the crude papain had its maximum activity in the pH range of 7.5 to 8.0 with the activity extending in the wide pH range 6.0 to 9.0. Kimmel and Smith (1957) reported that the activity over wide range was characteristic of papain. They also stated that the optimum pH range of commercial papain depends on the substrate and on the treatments previously given to the enzyme during preparation.

Table 12. Effect of pH on the proteolytic activity of crude papain

pH	Proteolytic activity (Enzyme units/ml)
4.5	2.00
5.0	2.09
5.5	3.01
6.0	3.25
6.5	3.45
7.2	3.82
7.5	3.95
8.0	4.10
8.5	3.61
9.1	2.81
9.4	2.18
9.9	2.05

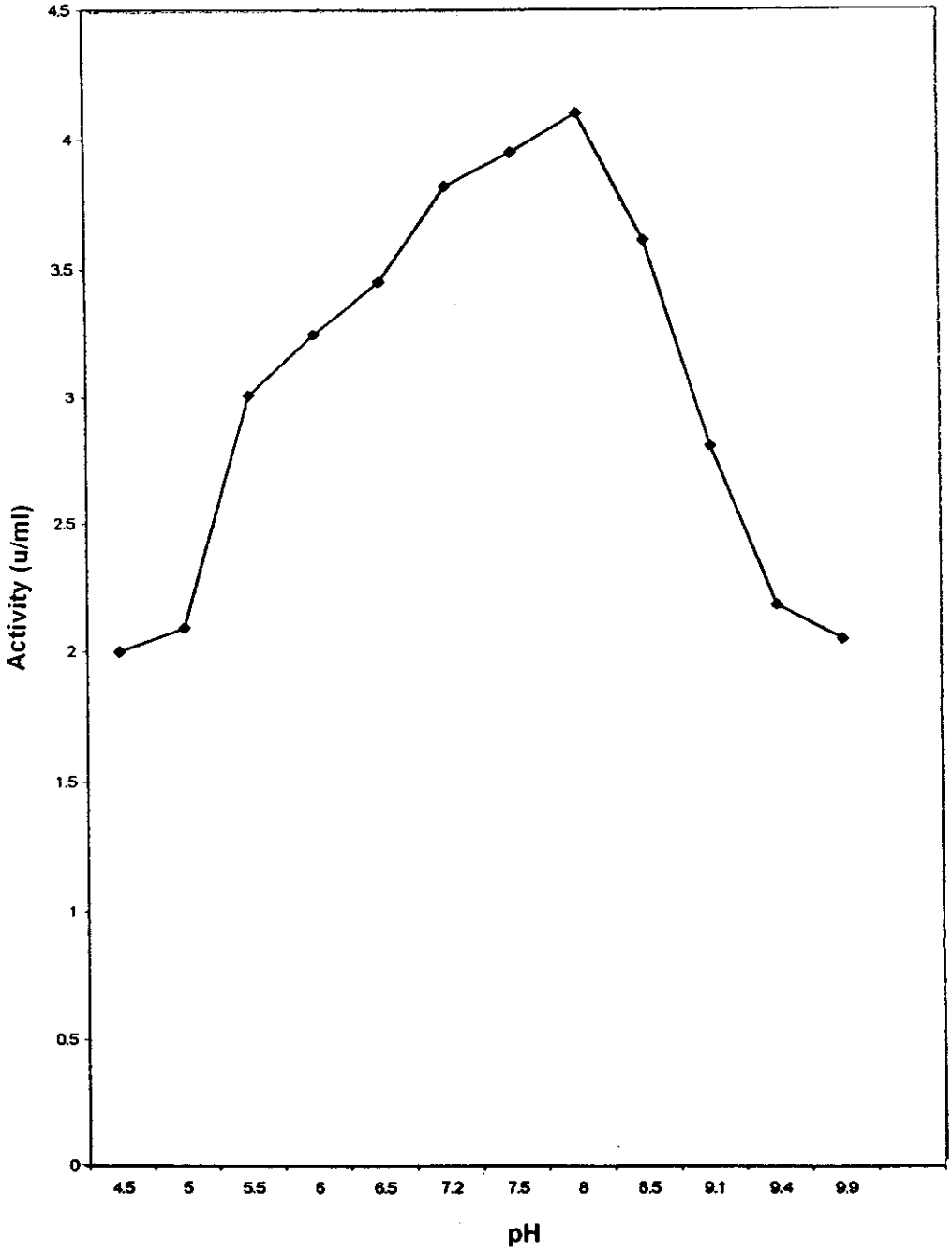

Fig (1): Effect of pH on the proteolytic activity of crude papain.

The proteolytic activity of crude ficin as affected by pH are found in Table (13) and Fig (2). It is clear that there are two peaks of activity, one near pH 6.7 and the other near pH 9.5. The proteolytic activity near pH 6.7 was obtained in the presence of phosphate buffer whereas that near pH 9.5was found in the presence of borate buffer. The possibility that the buffer ions might have an influence on the proteolytic activity was tested in two ways. First, the proteolytic activity at pH 6.7 and 9.5 was determined in the presence of mixed phosphate-borate buffers and secondary, the proteolytic activity in the pH range of 7.5 to 8.5 was determined with both phosphate and borate buffers.No essential differences in activity was obtained.

Table 13. Effect of pH on the proteolytic activity of crude ficin.

pH	Proteolytic activity (Enzyme unit /ml)
2.0	0.98
3.0	1.29
4.0	2.40
5.0	3.02
6.0.	3.29
6.5	3.42
6.7	3.47
7.0	3.33
8.0	2.93
9.0	3.11
9.5	3.33
10.0	3.24
11.0	2.71
12.0	0.98

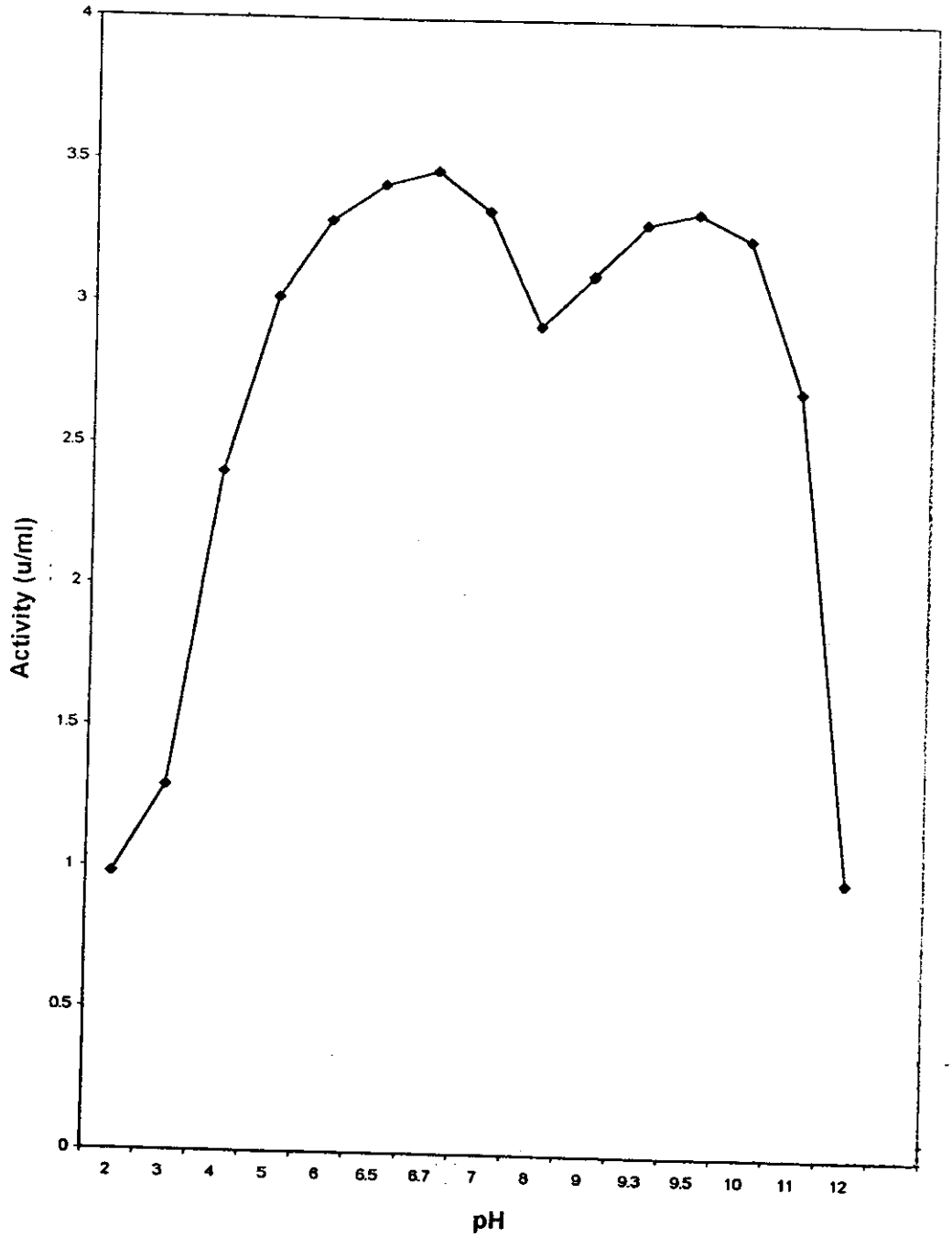

Fig (2): Effect of pH on the proteolytic activity of crude ficin.

On the basis of the differences in behavior of ficin at different pH values it was postulated that two proteolytic enzymes are present in crude ficin (John, 1957). However, Liener and Friedenson (1970) reported that ficin showed maximum stability in a broad region between pH 4.5 and 9.5 except for a minimum in a narrow zone around pH 8.0.

The effect of pH on proteolytic activity of the crude catheptic extract are shown in Table (14) and Fig (3). The optimum pH of the activity was found in the range of 5.0 - 5.5. Fukushima et al (1971) reported that the catheptic enzyme was most stable at pH range from 4 to 5 and 90 % of the initial activity remained at pH 4.3.

4.8.2. Effect of temperature :

The proteolytic activity of the papain was determined at temperature ranging between 30 and 90 C°. Results in Table (15) and Fig (4) show that the maximum activity of papain was found at 80C°. These results agree with those reported by Kimmel and Smith (1957) ; Castro (1981) who showed that papain exhibited maximum activity at high temperatures. However, Hwang and Ivy (1951) stated that papain can still retain its activity even after being heated to 105 C°. The higher optimum temperature of papain in comparison with those of the oryzae proteases makes the enzyme very convenient for meat tenderization particularly during the early stages of cooking.

Table 14. Effect of pH on the proteolytic activity of crude catheptic.

pH	Activity (u/mg protein)
2.0	0.30
2.5	0.91
3.0	1.10
3.5	1.30
4.0	1.51
4.5	3.10
5.0	5.10
5.5	5.59
6.0	4.01
6.5	3.21
7.0	1.20
7.5	0.61
8.0	0.50
8.5	0.39
9.0	0.39
9.5	0.30

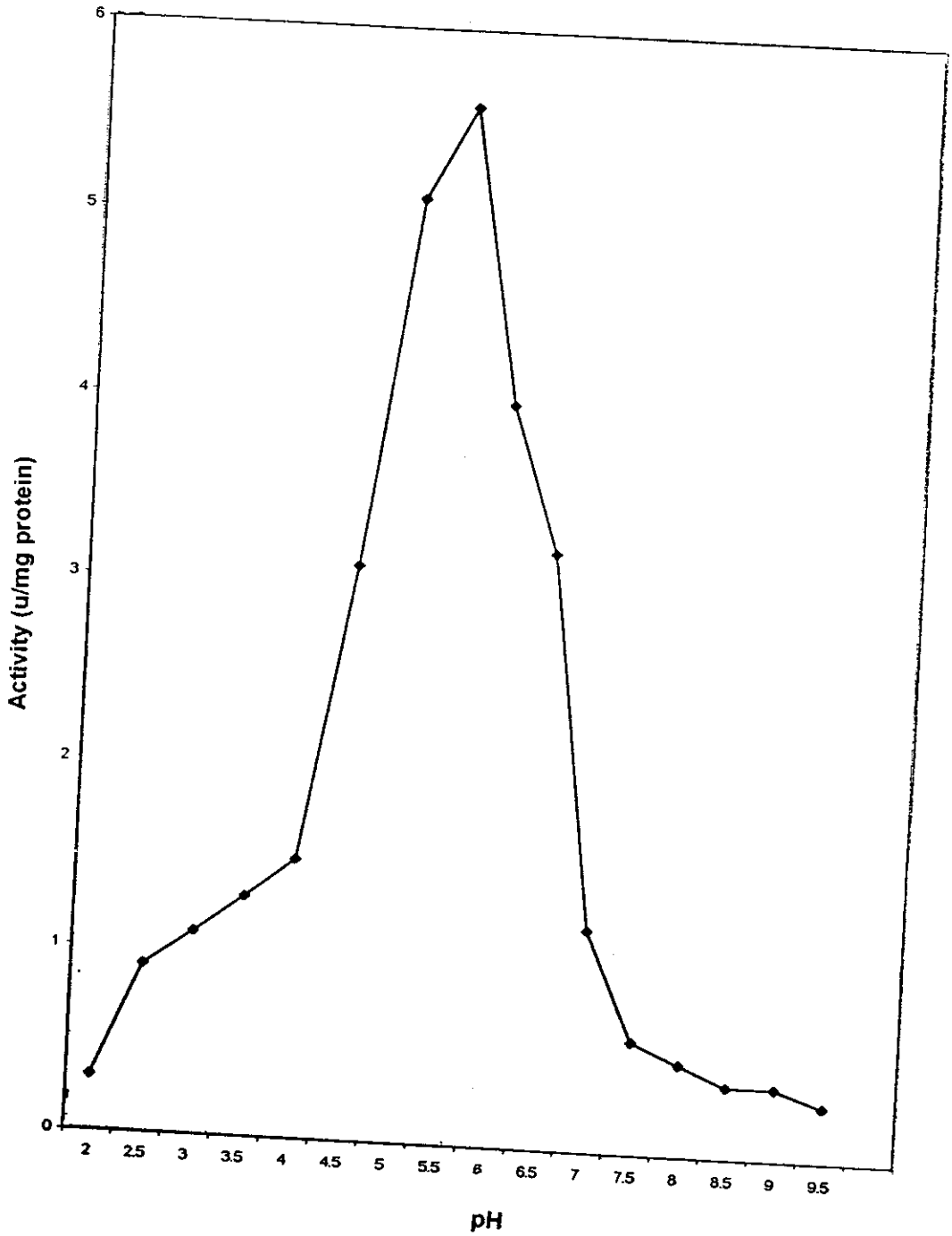

Fig (3): Effect of pH on the proteolytic activity of crude catheptic.

Table 15. Effect of temperature on the proteolytic activity of crude papain.

Temperature C°	Activity (Enzyme units/ml)
30	5.35
35	5.43
40	5.76
45	6.13
50	6.50
55	6.86
60	6.99
65	7.33
70	7.40
75	7.69
80	7.86
85	7.72
90	7.29

The effect of temperature on the proteolytic activity of crude ficin was determined at 50, 55 and 60 C° for different periods. Table (16) and Fig (5) indicated that the maximum activity of ficin (87.5 %) was observed at 50 C°/20 min. while, Liener and Friedenson (1970) reported that ficin showed a maximum stability at 50 C° for 2 hr, while the latex of Ficus carica var. kodata revealed that ,retained at least 75 % of their activity after one hour at pH 7 and 55 C°.

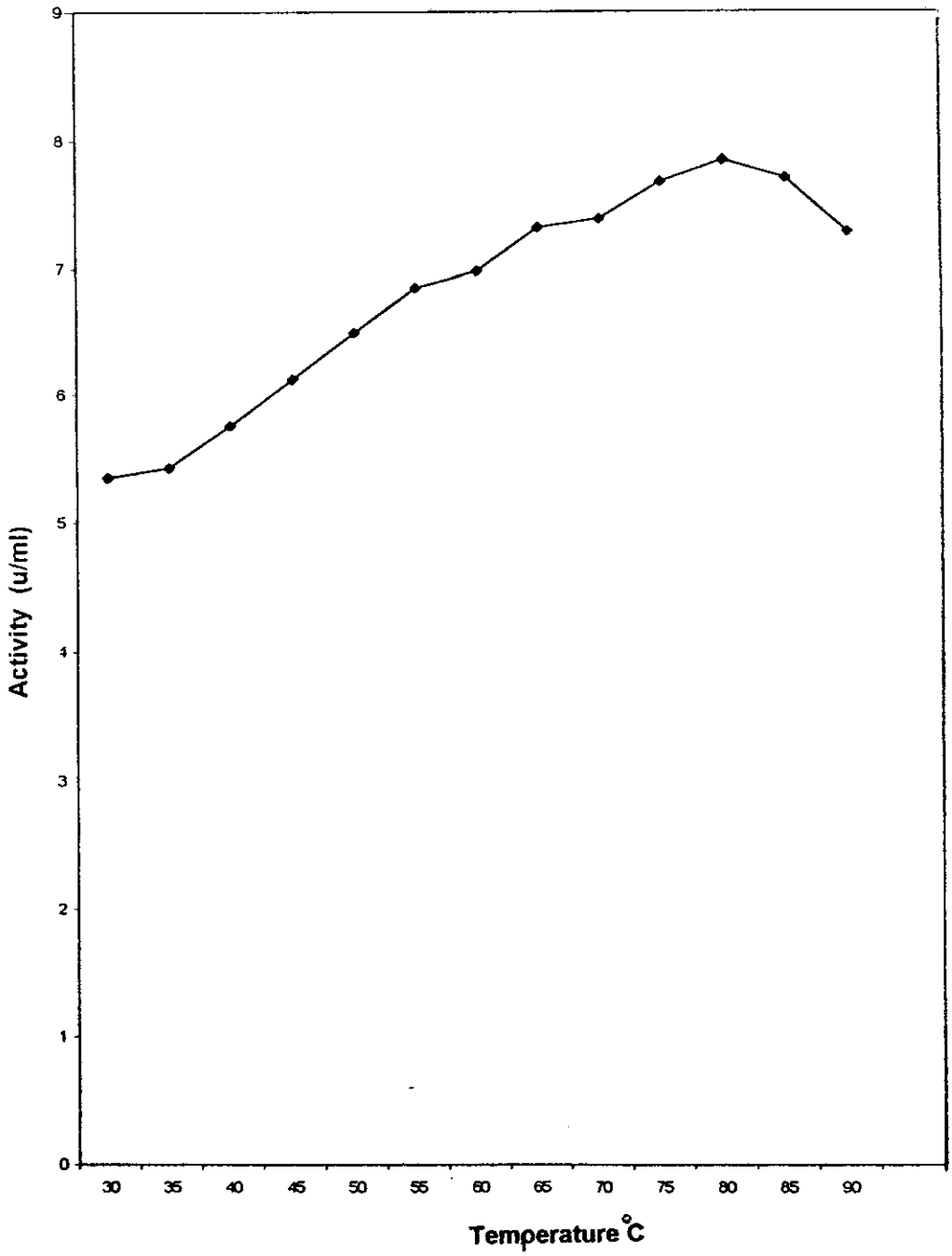

Fig (4): Effect of temperature on the proteolytic activity of crude papain.

Table 16.Effect of time- temperature on the proteolytic activity of crude Ficin.

50 C°			55 C°			60 C°		
A	B	C	A	B	C	A	B	C
20	2.32	87.5	20	2.13	80	20	1.73	65
40	2.12	80	40	1.84	69	40	1.22	46
60	1.97	74	60	1.62	61	60	0.93	35
80	1.84	69	80	1.41	53	80	0.64	24
100	1.76	66	100	1.28	48	100	0.35	13
120	1.70	64	120	1.17	44	120	0.16	6
140	1.73	65	140	1.09	41	140	0.08	3
160	1.78	67	160	1.28	46	—	—	—
180	1.70	64	180	1.25	47	—	—	—
200	1.90	63	200	1.20	45	—	—	—

Where:

A: Time / min.

B: Activity (u/mg protein).

C: Activity %.

As shown in Table (17) and Fig (6), the optimum temperature of the crude catheptic enzyme for hydrolysis of denatured casein was observed at 50 C°. Deng and Lillard (1973) found that the optimal temperature of catheptic is 47.5 C° when the reaction mixture was incubated for 90 min. When the enzymes were held at 45 and 50 C°, the activity of the enzymes decreased linearly as the length of heat treatment increased. On the other

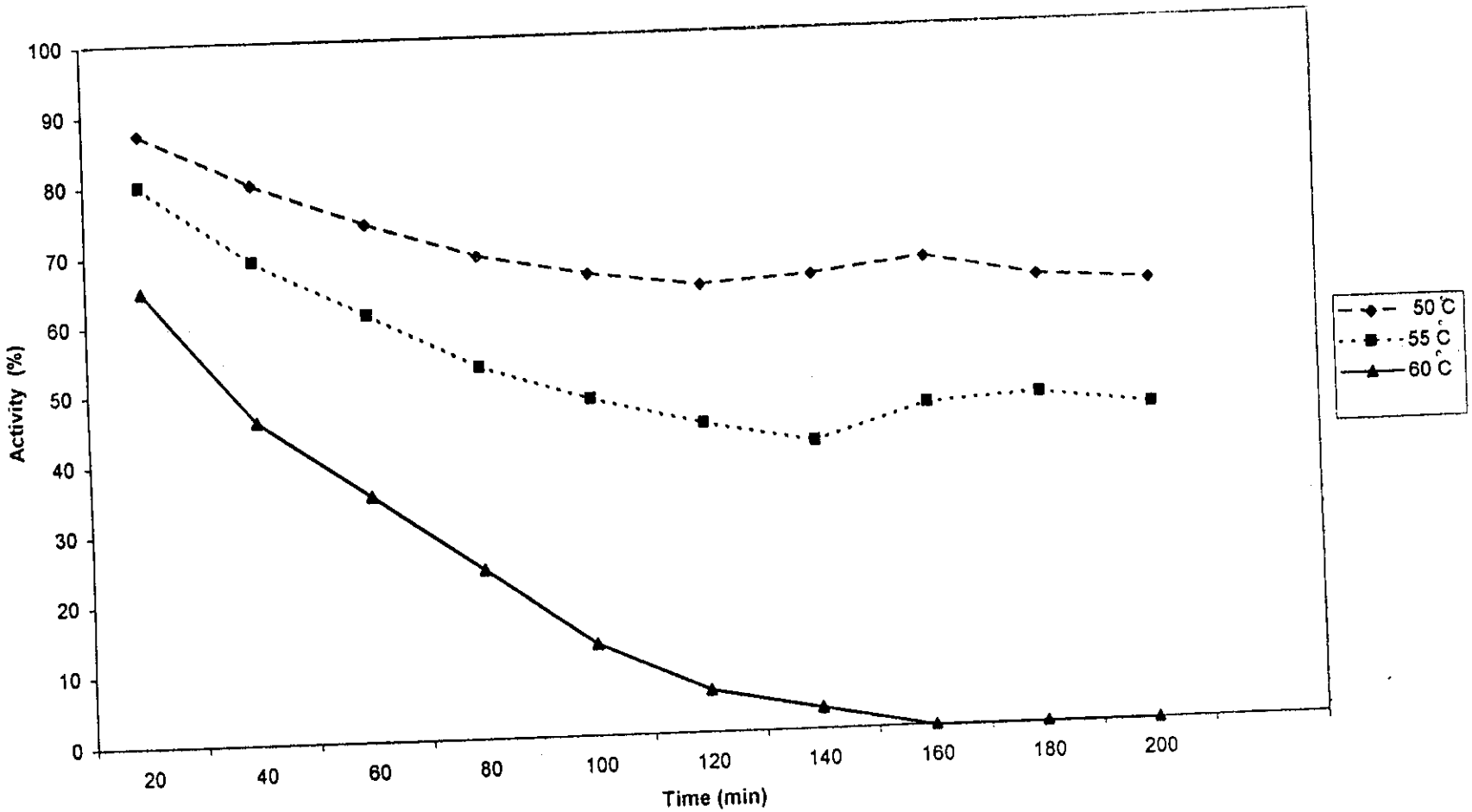

Fig (5): Effect of temperature on the proteolytic activity of ficin

hand, Draper and Zeece (1989) reported that the increasing the temperature of incubation resulted in decreased enzyme activity, with a sharp decline at temperatures above 55 C°. Similar trends of decreasing activity with increasing temperature were observed at pH 4.5 and 5.5 with activities at pH 5.5 being the lowest. Approximately 43 % of the activity remained at 55 C° (pH 5.5). Preliminary experiments with cathepsin, approximately 51 % of its activity remained after preincubation at 50 C° (pH 5.5). The optimum temperature of catheptic was also observed at 40 C° under atmospheric pressure, while at temperature above 40 C°,the activity decreased and at 60 C°was well below the level at 30 C°. This probably indicates thermal denaturation of the enzyme.

Table 17.Effect of temperature on the proteolytic activity of crude Catheptic enzyme.

Temperature C°	Activity (u/mg protein)
10	1.06
20	1.11
30	3.63
40	5.25
50	5.49
60	1.50

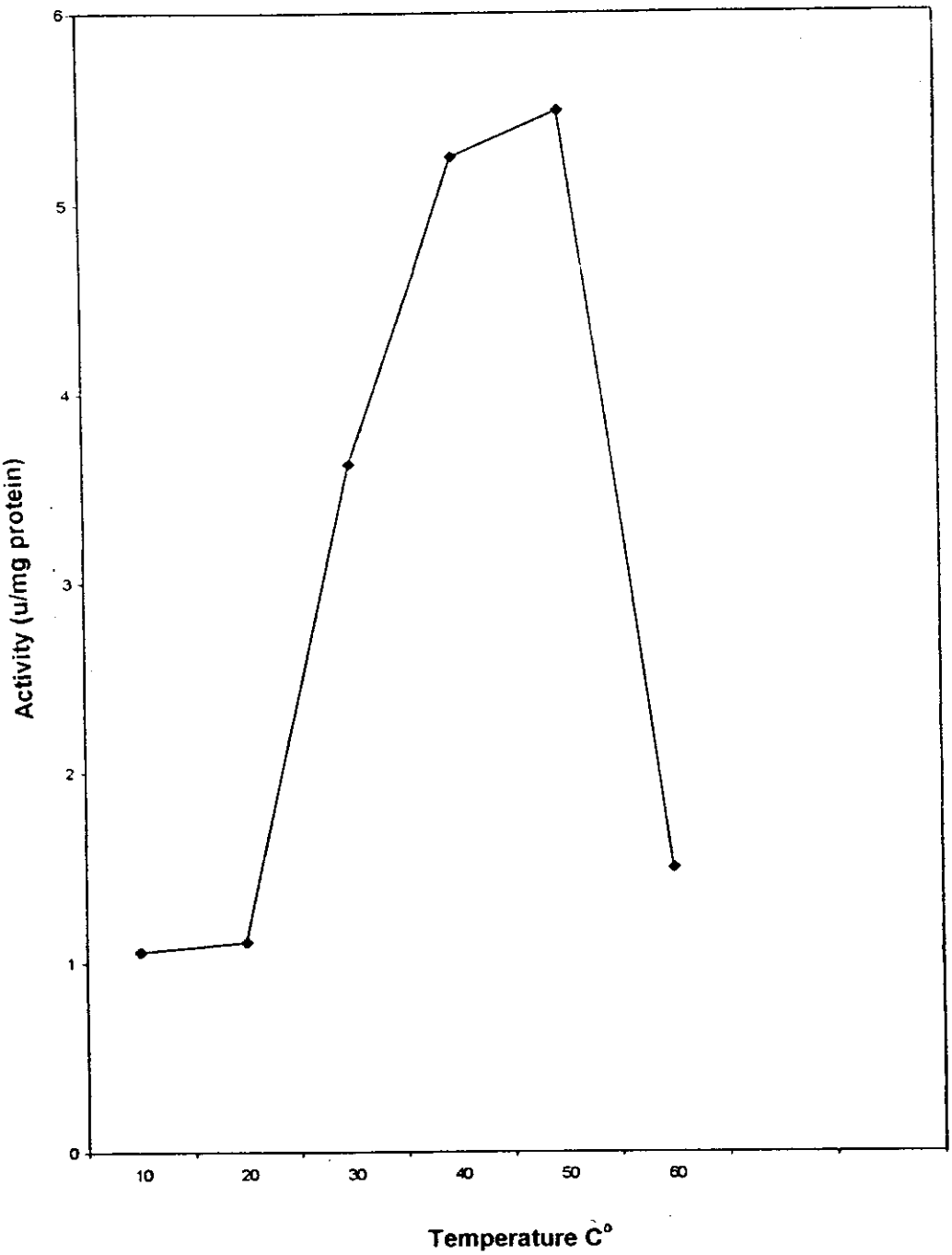

Fig (6): Effect of temperature on the proteolytic activity of crude catheptic

4.8.3. Effect of substrate concentration:

Data presented in Table (18) and Fig (7) show the effect of substrate (casein) concentrations on the activity of crude ficin. It could be noticed that the activity of ficin increased gradually with increasing the substrate (casein) concentrations upto 20 mg either at pH 6.7 or pH 9.5, then decreased with increasing the substrate concentrations. The maximum activity was observed at pH 6.7 and 9.5 with casein concentrations being 15 and 20 mg , respectively. These results are in a good agreement with that obtained by John (1957) who reported that, the maximum activity of ficin was found in the presence of 10 mg of casein and the activity decreased at higher concentrations of substrate. However, at pH 9.5, the maximum activity was observed in the presence of 25 mg of casein.

Table 18. Effect of substrate concentration on ficin activity.

Substrate conc. (mg) casein.	Activity (u/mg protein)	
	pH 6.7	pH 9.5
5	1.78	1.56
10	2.53	1.69
15	2.76	2.18
20	2.72	2.36
25	2.30	2.33
30	2.44	2.31
35	2.22	2.22
40	2.02	2.13
45	2.00	2.04
50	1.51	1.93

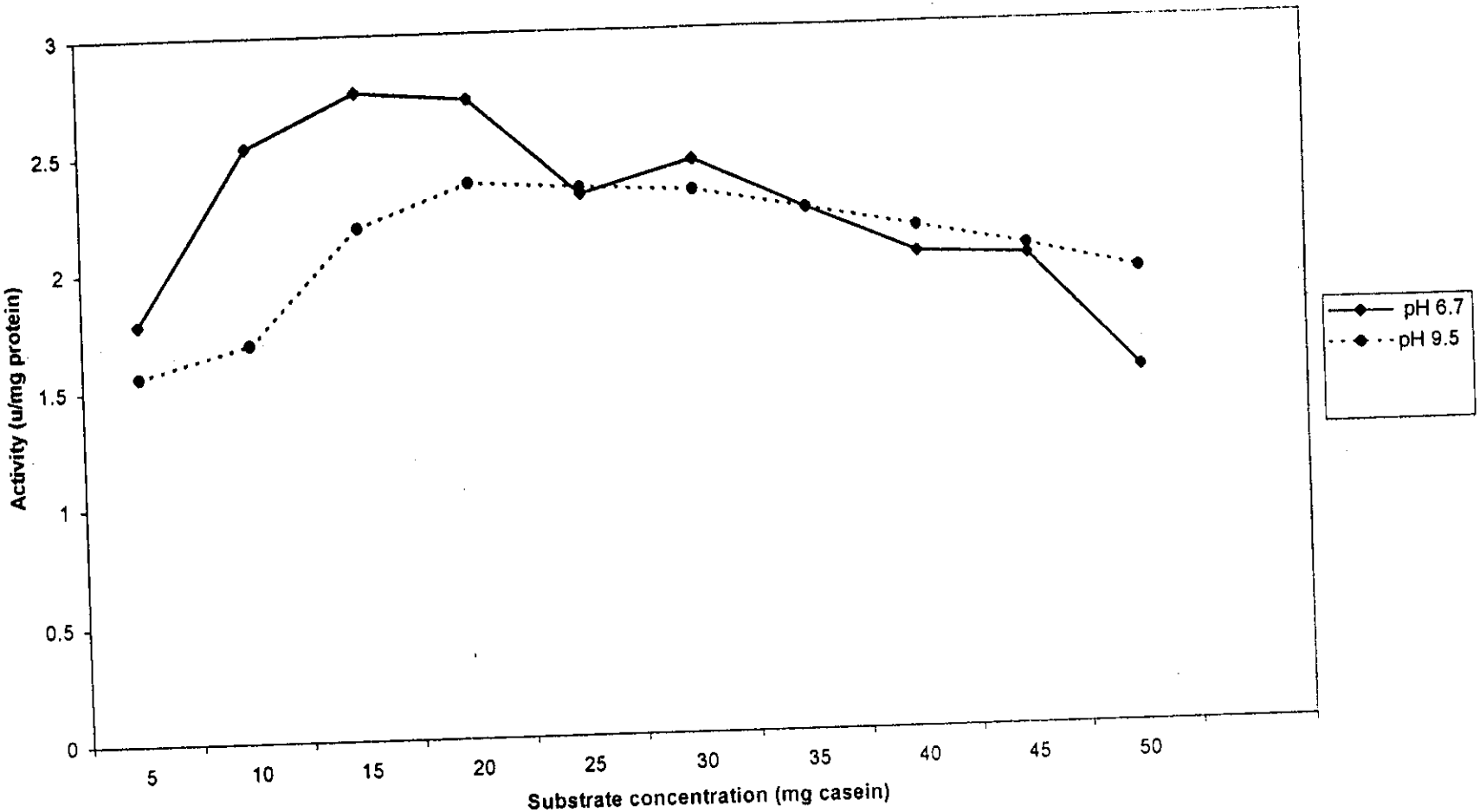

Fig (7): Effect of substrate concentration on ficin activity.

Results presented in Table (19) and Fig (8) show that the maximum activity of catheptic was noticed in the presence of 1.0 mg casein/ml (2.14u/mg protein). On the other hand, the activity of catheptic decreased gradually by increasing the substrate levels. These results confirmed with that obtained by Walter and Frieden (1963) who stated that the reaction was linear at low concentrations and attained a maximum at a substrate concentration of 1.2 mg. Above this concentration there was a decrease in activity.

Table 19. Effect of substrate concentration on catheptic activity.

Substrate conc. (mg) casein	Activity (u/mg protein)
0.4	0.93
0.6	1.43
0.8	1.93
1.0	2.14
1.2	2.07
1.4	2.00
1.6	1.79
1.8	1.43
2.0	1.14

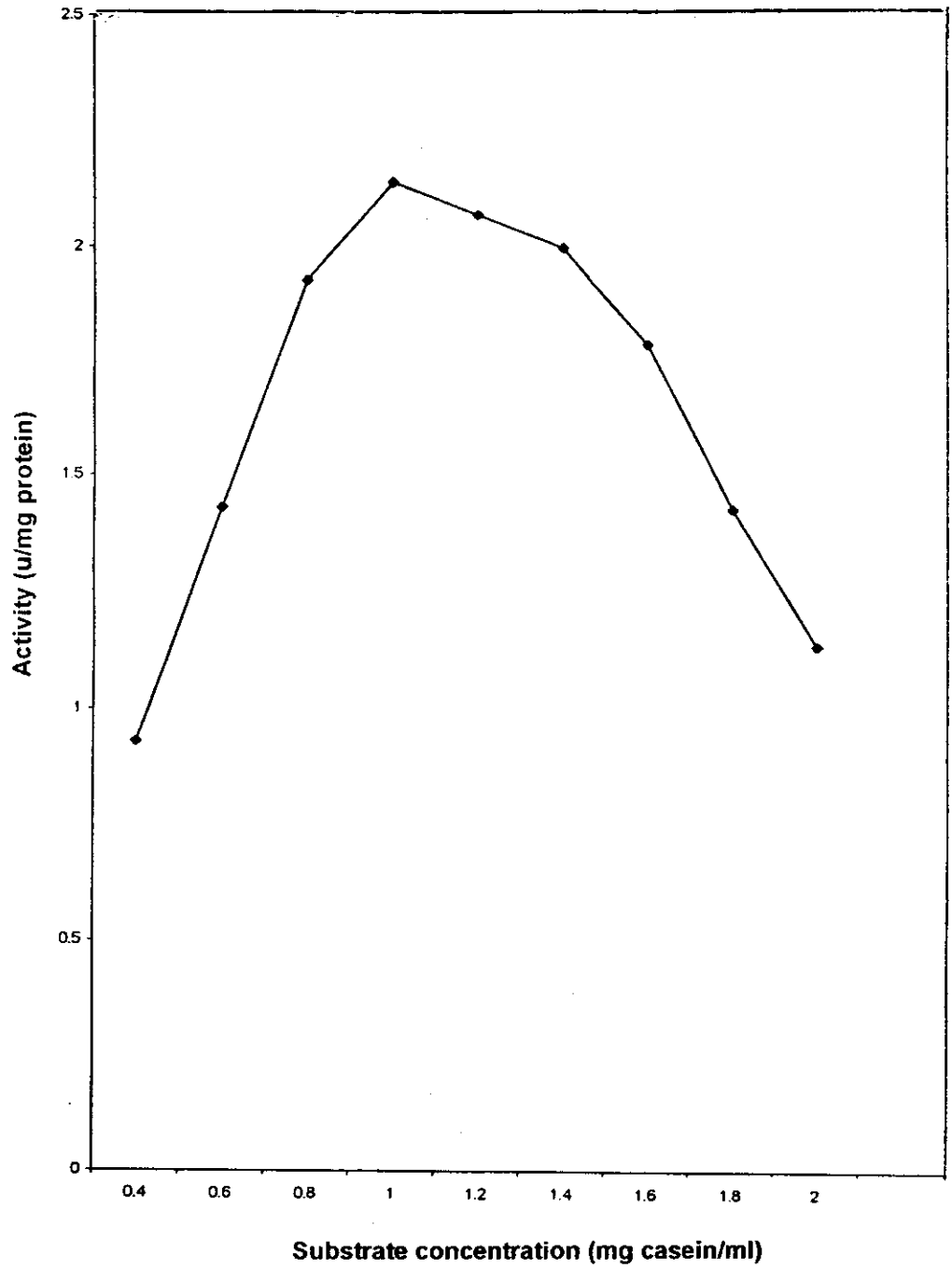

Fig (8): Effect of substrate concentration on catheptic activity.

4.8.4. Effect of enzyme concentration:

Table (20) and Fig (9) show the effect of ficin concentrations on the proteolytic activity. Data indicate that the maximum activity of ficin increased gradually at pH 6.7 as the levels of enzyme increased upto 5.0 mg ficin, then decreased . While, the proteolytic activity of ficin at pH 9.5 increased gradually upto 7.0 mg ficin being (1.81 u/mg protein). These results are in a good agreement with that obtained by John (1957) who reported that at pH 6.72, the maximum activity of ficin was obtained in the presence of 3.5 mg ficin. However, at pH 9.5, the maximum activity of ficin was obtained in the presence of 6.2 mg ficin.

Table 20. Effect of ficin concentration on the proteolytic activity.

Enzyme conc. (mg) ficin.	Activity (u/mg protein)	
	pH 6.7	pH 9.5
1.0	0.22	0.64
2.0	0.44	0.96
3.0	0.59	1.32
4.0	0.67	1.49
5.0	0.87	1.63
6.0	0.80	1.76
7.0	0.67	1.81

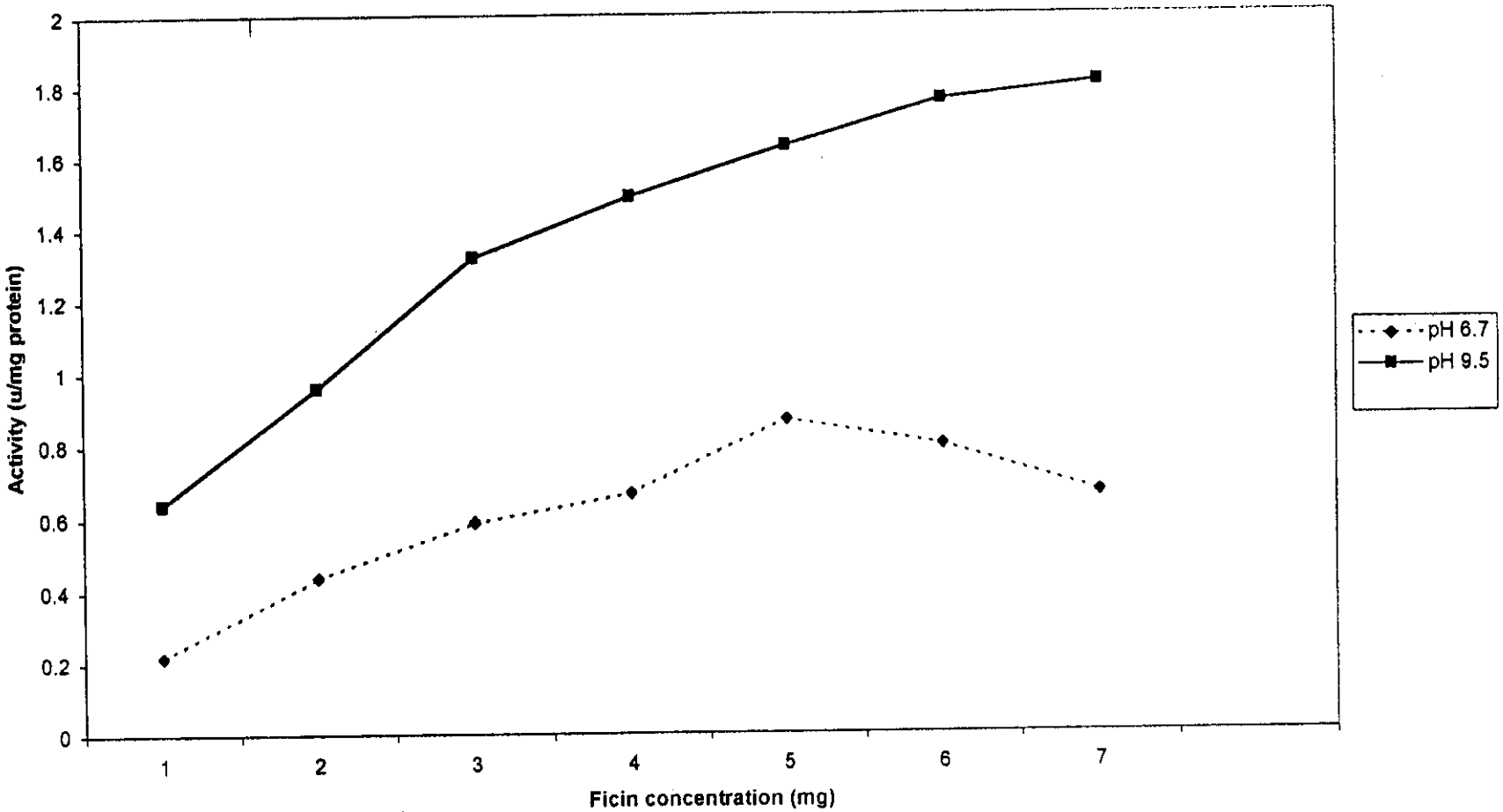

Fig (9): Effect of ficin concentration on the proteolytic activity.

Results presented in Table (21) and Fig (10) indicated that the maximum activity of catheptic increased with increasing the concentrations of enzyme (9.1 x 10^{-3} mg protein/ml). These results are confirmed with that obtained by Elias et al (1991c) who reported that a good linear response for catheptic activity was found when adding 0 to 6 mg of protein per cubic centimeter.

Table 21. Effect of catheptic concentration on the proteolytic activity.

Enzyme conc. (mg protein/ml)	Activity (u/mg protein)
1.3 x 10^{-3}	0.65
2.6 x 10^{-3}	0.99
3.9 x 10^{-3}	1.51
5.2 x 10^{-3}	1.78
6.5 x 10^{-3}	2.36
7.8 x 10^{-3}	2.59
9.1 x 10^{-3}	2.83

From the afformentioned results, it could be noticed that, the maximum activity of papain was found at pH range of 7.5 - 8.0, and temperature of 80 C°. The maximum activity of ficin was noticed at pH 6.7 and pH 9.5. The proteolytic activity of ficin near pH 6.7 was obtained due to the presence of phosphate buffer whereas, that near pH 9.5 due to the presence of borate buffer. Moreover , the maximum activity of ficin was found at temperature of 50 C°/ 20 min. Maximum activity of catheptic enzyme was found at pH range of 5.0 - 5.5 and temperature of 50 C°.

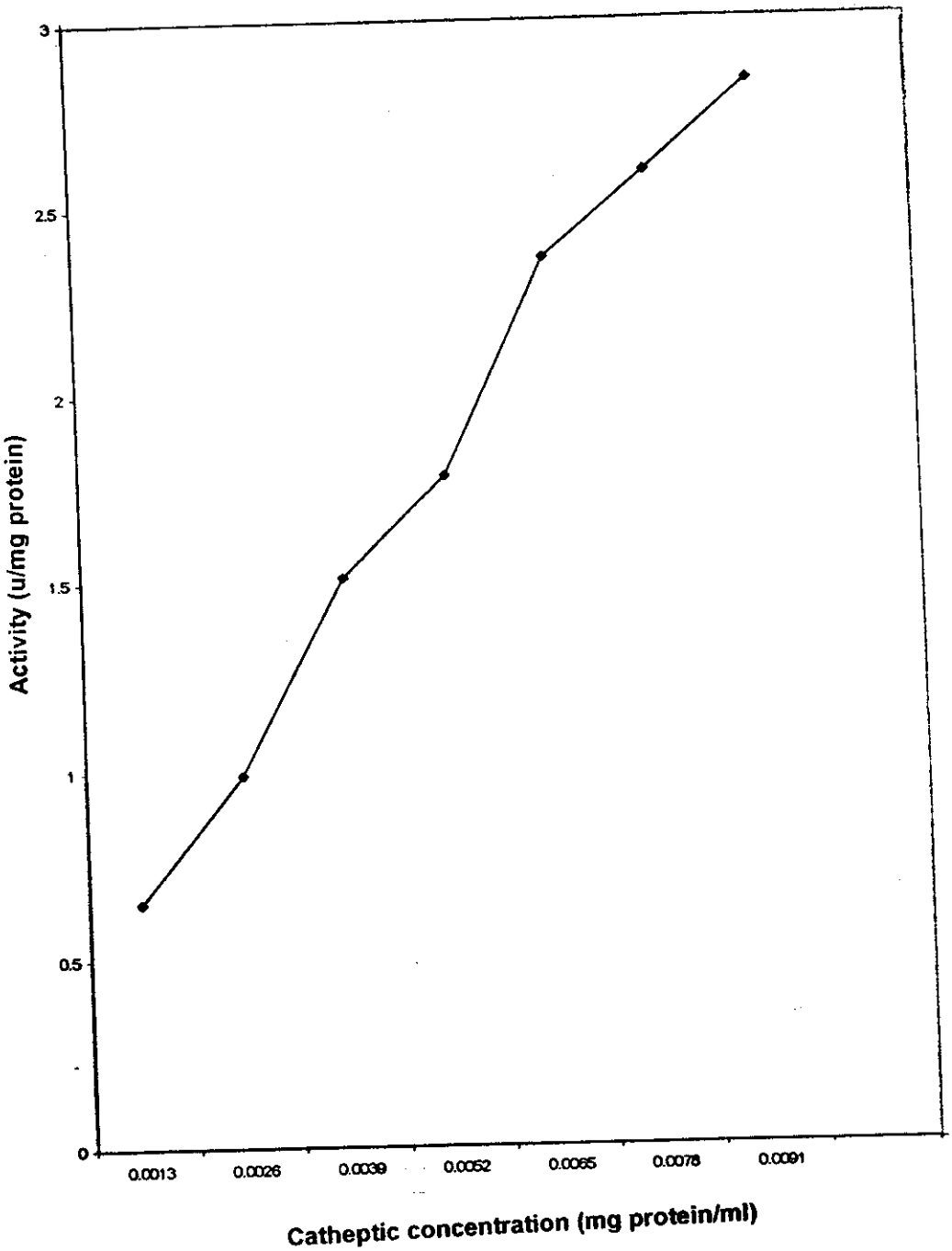

Fig (10): Effect of catheptic concentration on the proteolytic activity.

4.9. Chemical composition of spent hen muscles,camel and buffalo meats:

Breast and leg of spent hen muscles were analyzed for their moisture, ash, fat, protein and carbohydrates. Table (22) show that breast meat of spent hen contained relatively higher moisture content than leg meat. The low levels of moisture in spent hen is generally responsible for tough, dry and sinewy (Federal Register, 1980; Devitre and Cunningham, 1985 and Kondaiah and Panda, 1987). Ash was lower in leg (1.17 %) than the breast (1.24 %) which due to higher contents of fat in leg (6.30 %). However, protein was higher in breast (21.79 %) than leg meats (19.28 %). These results are in a good agreement with Kondaiah and Pands (1987) who found that protein percentage was higher in breast than in leg meat. While, carbohydrates was higher in leg (0.82 %) than breast meats. These results were closed with that obtained by Haiam (1994).

With respect to the camel and buffalo meats it could be noticed that ash, fat, protein and carbohydrate contents of camel meat was higher than those of buffalo meat (Table 22). These results are in a good agreement with that obtained by Hosny (1982) who showed that protein and fat contents of camel meat was higher than that of buffalo meat. Meanwhile, both ash and carbohydrates content revealed almost the same values in the two types of meat.

fractions (TSN, SPN and NPN) increased gradually by increasing the concentration of proteolytic enzymes (papain, ficin and catheptic) in both breast and leg meats of spent hen.However, stroma nitrogen decreased with increasing of enzyme concentrations, compared with their respective control (without enzyme). Similar results were obtained by El-Gharbawi and Whitaker (1963); McCready and Cunningham (1970); Youn et al (1973) and Balaz et al (1974). On the other hand, a reduction in TSN, SPN,and NPN was observed in both breast and leg meats when treated with papain, ficin and catheptic enzymes with concentrations more than 0.100, 0.008 and 0.375 % (w/v), respectively (Table 23).

Huffman et al (1967) found that antemortem injections of 35, 55 and 35 ppm of crude papain resulted in more tender or over tenderized chicken meat. Bawa et al (1981) injected enzyme solutions of papain and bromelin at 50, 75 and 100 ppm into the vascular system of birds for five min prior to slaugher. The results showed that tenderness increased with increased enzyme and that papain resulted in greater tenderization than bromelin.

The changes in the different protein fractions as affected by ficin may be due to its effect on the hydrolysis of actomyosin protein to peptides and amino acids as concluded by Delaney (1965) and K'osev et al (1971). On the other hand, Dawson and Wells (1969) found that the optimum enzyme concentrations for production of suitable tenderness in freeze-dried old hens were 0.02, 0.0008, .002 and .002 % for Rhozyme p-11, ficin, bromelin and papain, respectively.

The connective tissues of proteolytic enzyme treated meat decreased during 40-60 min at 25 C°. This might be due to the possibility that the action of proteolytic enzymes of plant origin was preferentially against the connective tissue in the muscle, as they first break up the mycopolysaccharides of the ground substance matrix, then progressively reduce the connective tissue fibers to an amorphous mass (Youn and Yong, 1974). While, Gassman et al (1976) concluded that such proteolytic enzymes do not attack native collagen but rather act upon the collagen denatured by heat during cooking.

Table 23. Effect of enzyme concentration on the protein fractions of tenderized spent hen meat.

Enzyme	Enzyme concent. (%w/v)	Breast Protein fractons(% dry wt.) TSN	SPN	NPN	Stroma	Leg Protein fractions(% dry wt.) TSN	SPN	NPN	Stroma
Control*	**0.00**	**2.00**	**0.61**	**1.39**	**2.89**	**1.53**	**0.39**	**1.14**	**5.45**
Papain	**0.050**	**2.19**	**0.67**	**1.52**	**2.78**	**1.77**	**0.45**	**1.32**	**4.43**
	0.075	**2.34**	**0.71**	**1.63**	**2.22**	**1.90**	**0.49**	**1.41**	**4.09**
	0.100	**2.59**	**0.88**	**1.71**	**2.21**	**2.14**	**0.55**	**1.59**	**3.71**
	0.125	**2.39**	**0.71**	**1.68**	**2.01**	**2.13**	**0.54**	**1.59**	**3.72**
Ficin	**0.004**	**2.18**	**0.67**	**1.51**	**2.89**	**1.70**	**0.44**	**1.26**	**4.41**
	0.006	**2.31**	**0.71**	**1.60**	**2.75**	**1.88**	**0.48**	**1.40**	**4.05**
	0.008	**2.50**	**0.77**	**1.73**	**2.20**	**1.97**	**0.51**	**1.46**	**3.68**
	0.010	**2.50**	**0.72**	**1.66**	**1.98**	**1.79**	**0.45**	**1.34**	**3.69**
Catheptic	**0.125**	**2.17**	**0.49**	**1.68**	**2.87**	**1.66**	**0.38**	**1.28**	**4.38**
	0.250	**2.30**	**0.45**	**1.85**	**2.73**	**1.69**	**0.32**	**1.37**	**4.02**
	0.375	**2.34**	**0.45**	**1.89**	**2.18**	**1.85**	**0.30**	**1.55**	**3.63**
	0.500	**2.34**	**0.49**	**1.85**	**1.97**	**1.83**	**0.28**	**1.55**	**3.65**

*** Control without enzyme.**

TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen.
Cooking time: 60 min.

4.10.1.2.Tenderization temperature:

Table (24) show the effect of tenderization temperature on the nitrogenous compounds of tenderized spent hen breast and leg treated with papain, ficin and catheptic enzymes. Data indicate that nitrogenous compounds (TSN, and NPN) increased as the temperature increased for both control (untreated) of spent hen breast and leg,while a graduall reduction of stroma nitrogen was observed. It can be also noticed that the best tenderization temperature was found at 60 C° for all three studied enzymes. However, there is no increases in TSN and NPN was noticed at the temperature more than 60 C°. While, SPN increased gradually upto 80 C°. These results were confirmed by Tappel et al (1956). Moreover Weiner et al (1957) found that the optimum temperature for enzymatic reaction was related to the time of the reaction. In general, short digestion times required high optimum temperatures for the reaction. Optimum temperatures for ficin, bromelin, papain and Rhozyme p-11 were reported as 30-50, 30-60, and 43-60 C°, respectively (Anonymous, 1963).

4.10.1.3. pH of tenderization:

Data presented in Table (25) revealed that TSN, SPN and NPN of untreated samples (control) increased gradually as increasing the pH of tenderization increased upto pH 7.0, and decreased by increasing the pH upto 9.0 for both breast and leg. While, stroma nitrogen decreased gradually.However, the protein fractions (TSN and NPN) of hen tenderized breast and leg increased gradually as the pH increased upto 7.0, then decreased upto pH 9.0. Moreover, SPN increased gradually upto pH

Table (24) : Effect of tenderization temperature on spent hen breast and leg protein fractions.

Enzyme	Tenderization temperature °C	Breast								Leg							
		Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt.)				Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Papain	40	1.25	0.60	0.65	3.15	2.11	0.42	1.69	2.92	1.77	0.38	1.39	3.90	1.98	0.37	1.61	2.93
	50	1.17	0.53	0.64	3.06	2.20	0.48	1.72	2.70	1.75	0.41	1.34	3.00	2.05	0.42	1.63	2.68
	60	1.36	0.58	0.78	2.90	2.38	0.51	1.87	2.18	1.88	0.42	1.46	2.85	2.18	0.41	1.77	2.14
	70	1.35	0.52	0.83	2.62	2.27	0.55	1.72	2.25	2.01	0.40	1.61	2.53	2.14	0.45	1.69	2.23
	80	1.38	0.51	0.87	2.53	2.23	0.58	1.65	2.38	2.09	0.39	1.70	2.45	2.11	0.49	1.62	2.35
Ficin	40	1.25	0.60	0.65	3.15	2.01	0.39	1.62	2.95	1.29	0.40	0.89	3.11	1.89	0.35	1.54	2.90
	50	1.17	0.53	0.64	3.06	2.13	0.47	1.66	2.71	1.18	0.38	0.80	3.03	1.92	0.39	1.53	2.61
	60	1.36	0.58	0.78	2.90	2.31	0.45	1.86	2.20	1.98	0.45	1.53	2.87	2.09	0.40	1.69	2.10
	70	1.35	0.52	0.83	2.62	2.23	0.49	1.74	2.29	2.00	0.42	1.58	2.59	2.01	0.43	1.58	2.21
	80	1.38	0.51	0.87	2.53	2.21	0.51	1.70	2.41	2.05	0.41	1.64	2.49	1.99	0.44	1.55	2.31
Catheptic	40	1.25	0.60	0.65	3.15	1.36	0.41	0.95	2.98	1.17	0.51	0.66	3.13	1.28	0.39	0.89	2.94
	50	1.17	0.53	0.64	3.06	1.41	0.45	0.96	2.73	1.16	0.46	0.70	3.05	1.35	0.37	0.98	2.70
	60	1.36	0.58	0.78	2.90	1.68	0.41	1.27	2.22	1.20	0.49	0.71	2.88	1.54	0.35	1.19	2.24
	70	1.35	0.52	0.83	2.62	1.63	0.39	1.24	2.30	1.19	0.42	0.77	2.61	1.50	0.31	1.19	2.26
	80	1.38	0.51	0.87	2.53	1.46	0.43	1.03	2.45	1.19	0.41	0.78	2.52	1.40	0.36	1.04	2.35

*** Control without enzyme.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

Table (25) : Effect of tenderization pH on spent hen breast and leg protein fractions.

Enzyme	Tenderization pH	Breast								Leg							
		Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt.)				Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Papain	4	2.23	0.50	1.73	2.81	2.15	0.43	1.72	2.26	2.10	0.49	1.61	2.80	2.01	0.39	1.62	2.24
	5	2.27	0.53	1.74	2.89	2.23	0.40	1.83	2.34	2.11	0.52	1.59	2.87	2.11	0.32	1.79	2.32
	6	2.52	0.57	1.95	2.94	2.59	0.57	2.02	2.26	2.38	0.53	1.84	2.93	2.31	0.31	2.00	2.24
	7	2.61	0.73	1.88	2.66	2.72	0.41	2.31	2.02	2.43	0.65	1.78	2.65	2.47	0.47	2.00	2.00
	8	2.34	0.71	1.63	2.78	2.68	0.58	2.10	2.14	2.22	0.62	1.59	2.74	2.35	0.48	1.87	2.11
	9	2.28	0.66	1.62	2.75	2.31	0.59	1.72	2.18	2.12	0.57	1.55	2.72	2.29	0.49	1.80	2.15
Ficin	4	2.23	0.50	1.73	2.81	1.90	0.49	1.41	2.24	2.10	0.49	1.61	2.80	1.59	0.35	1.24	2.19
	5	2.27	0.53	1.74	2.89	1.97	0.51	1.46	2.32	2.11	0.52	1.59	2.87	1.69	0.39	1.30	2.28
	6	2.52	0.57	1.95	2.94	2.33	0.45	1.88	2.24	2.38	0.53	1.84	2.93	1.89	0.40	1.49	2.18
	7	2.61	0.73	1.88	2.66	2.50	0.45	2.05	2.00	2.43	0.65	1.78	2.65	2.11	0.43	1.66	1.94
	8	2.34	0.71	1.63	2.78	2.38	0.57	1.81	2.12	2.22	0.62	1.59	2.74	2.01	0.45	1.58	2.06
	9	2.28	0.66	1.62	2.75	2.29	0.56	1.73	2.16	2.12	0.57	1.55	2.72	2.08	0.46	1.62	2.10
Catheptic	4	2.23	0.50	1.73	2.81	1.60	0.39	1.21	2.22	2.10	0.49	1.61	2.80	1.46	0.39	1.07	2.21
	5	2.27	0.53	1.74	2.89	1.71	0.41	1.30	2.30	2.11	0.52	1.59	2.87	1.51	0.40	1.11	2.31
	6	2.52	0.57	1.95	2.94	1.86	0.44	1.42	2.22	2.38	0.53	1.84	2.93	1.82	0.37	1.45	2.20
	7	2.61	0.73	1.88	2.66	2.35	0.44	1.91	1.98	2.43	0.65	1.78	2.65	1.90	0.38	1.52	1.97
	8	2.34	0.71	1.63	2.78	2.27	0.49	1.86	2.10	2.22	0.62	1.59	2.74	1.75	0.42	1.33	2.09
	9	2.28	0.66	1.62	2.75	2.03	0.58	1.45	2.14	2.12	0.57	1.55	2.72	1.67	0.41	1.26	2.13

*** Control without enzyme.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

9.0. On the other hand, stroma nitrogen decreased upto pH 7.0 then slightly increased upto pH 9.0 (Table 25) for both tenderized breast and leg by papain , ficin and catheptic enzymes. These results agree with that reported by Dawson and Wells (1969) who reported that the protein solubility ratio decreased with the increases of pH value above 7.0.

4.10.1.4. Salt concentrations:

Different concentrations of sodium tripolyphosphate (NaTPP) ranged from 0.5 to 2.0 % and 1 % sodium chloride (NaCL) were added to the breast and leg of spent hen during tenderization using papain, ficin, and catheptic . The effect of different concentrations of the previous salts on the nitrogenous compounds were analyzed. It could be noticed that from data presented in Table (26), TSN and NPN increased gradually as increasing the concentrations of NaTPP upto 1.5 % in both breast and leg meats, either control (without enzyme) or tenderized. At 2.0 % NaTPP concentration, a slight reduction in TSN and NPN was observed. On the other hand, SPN increased by increasing NaTPP upto 2% ,while stroma nitrogen decreased in both breast and leg either control or tenderized. Improving tenderness of spent hen meats using NaTPP and NaCL without adding papain, ficin and catheptic (control) may be due to the ability to increase charge, pH and subsequent hydration of protein (Monk et al, 1964; Farr and May, 1970; Wood and Richards, 1974; Hale et al, 1977; El-Samahy and Shehata, 1977). Results indicated that proteolytic enzyme in combination with NaTPP and NaCL play an important role for tenderization of meat compared to enzyme alone . This might be due to

Table (26) : Effect of salt concentration on spent hen breast and leg protein fractions.

Enzyme	Salt concentration	Breast								Leg							
		Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt.)				Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Papain	0.5% NaTPP & 1% NaCl	2.22	0.91	1.31	2.90	1.93	0.41	1.52	2.18	2.01	0.82	1.19	2.86	1.73	0.37	1.36	2.13
	1.0% NaTPP & 1% NaCl	2.26	1.01	1.25	2.82	2.51	0.40	2.11	2.06	2.03	0.99	1.04	2.89	2.14	0.34	1.80	2.03
	1.5 % NaTPP & 1% NaCl	2.38	1.07	1.31	2.69	2.60	0.38	2.22	1.83	2.16	1.02	1.14	2.65	2.20	0.31	1.89	1.80
	2.0% NaTPP & 1% NaCl	2.33	1.12	1.21	2.66	2.55	0.41	2.14	1.87	2.15	1.05	1.10	2.63	2.17	0.39	1.78	1.83
Ficin	0.5% NaTPP & 1% NaCl	2.22	0.91	1.31	2.90	1.89	0.45	1.44	2.15	1.94	0.68	1.26	2.83	1.83	0.29	1.54	2.16
	1.0% NaTPP & 1% NaCl	2.26	1.01	1.25	2.82	2.47	0.42	2.05	2.04	1.98	0.80	1.18	2.76	1.99	0.39	1.60	2.05
	1.5 % NaTPP & 1% NaCl	2.38	1.07	1.31	2.69	2.58	0.35	2.23	1.81	2.09	0.83	1.26	2.62	2.12	0.38	1.74	1.82
	2.0% NaTPP & 1% NaCl	2.33	1.12	1.21	2.66	2.54	0.46	2.08	1.84	2.02	0.87	1.15	2.58	2.11	0.45	1.66	1.85
Catheptic	0.5% NaTPP & 1% NaCl	2.22	0.91	1.31	2.90	1.86	0.43	1.43	2.12	2.09	0.75	1.34	2.80	1.71	0.40	1.31	2.10
	1.0% NaTPP & 1% NaCl	2.26	1.01	1.25	2.82	2.46	0.40	2.06	2.02	2.15	0.82	1.33	2.73	1.88	0.39	1.49	2.00
	1.5 % NaTPP & 1% NaCl	2.38	1.07	1.31	2.69	2.54	0.33	2.21	1.80	2.20	0.85	1.35	2.60	2.10	0.31	1.79	1.78
	2.0% NaTPP & 1% NaCl	2.33	1.12	1.21	2.66	2.51	0.45	2.06	1.82	2.18	0.83	1.35	2.55	2.08	0.35	1.73	1.80

* Control without enzyme.

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).

Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

higher water binding capacity and increased tenderness (Rao and Panda, 1984b). Chilling the carcasses in 3 % and 7.5 % solution of polyphosphates for 6 hr (May et al, 1962; Spencer and Smith, 1962), 5 % mixture solution of sodium triphosphate and sodium pyrophosphate for 22 hr (Klose et al , 1963) improved the tenderness of light and dark poultry meats. Peterson (1977) found that the toughening effect of cutting chicken broiler breast muscle within 1 hr after slaughter could be prevented by injecting sodium polyphosphates into the muscle at 20 min postmortem. Palladino and Ball (1979) studied the effect of 15 inorganic salts, except calcium salts, had a tenderizing effect on spent hen muscle.

4.10.1.5.Tenderization time:

The effect of tenderization time on protein fractions of tenderized spent hen (breast and leg) are given in Table (27) . The results show that the highest protein fractions were obtained when the samples were tenderized with papain , ficin and catheptic for 45 min. However , there is no increases in protein fractions was observed at the tenderization time more than 45 min. Weiner et al (1957) stated that the optimum temperature for enzymic reaction was colsely related to length of time which that reaction covered. Dawson and Wells (1969) found that a direct linear increase in non protein nitrogen occurred in samples held upto 60 min of incubation for ficin. On the other hand, the pre-treatment of samples before cooking with proteases, to reduce the myofibrillar strength to its minimum was done by Gassman et al (1976). The NPN content of the cooked breast muscle was not significantly influenced by soaking time within the papain concentration, except that the breast muscle soaked in 0.1 % papain for 3

Table (27) : Effect of tenderization time on spent hen breast and leg protein fractions.

Enzyme	Tenderization time / minute	Breast				Leg			
		Protein fractions (% dry wt.)				Protein fractions (% dry wt.)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Control*	60	2.09	0.66	1.43	2.91	2.09	0.53	1.56	2.90
Papain	15	2.09	0.45	1.64	2.76	2.18	0.41	1.77	2.75
	30	2.17	0.41	1.76	2.52	2.11	0.38	1.73	2.51
	45	2.35	0.48	1.87	2.20	2.13	0.42	1.71	2.20
	60	2.33	0.46	1.87	2.23	2.10	0.37	1.73	2.21
Ficin	15	2.08	0.42	1.66	2.75	1.95	0.39	1.56	2.73
	30	2.16	0.40	1.76	2.50	2.03	0.37	1.66	2.49
	45	2.33	0.45	1.88	2.19	2.09	0.41	1.68	2.16
	60	2.30	0.43	1.87	2.20	2.05	0.38	1.67	2.18
Catheptic	15	2.06	0.44	1.62	2.78	1.75	0.39	1.36	2.70
	30	2.15	0.40	1.75	2.54	1.86	0.37	1.49	2.46
	45	2.31	0.45	1.86	2.22	2.05	0.42	1.63	2.13
	60	2.29	0.43	1.86	2.26	2.04	0.41	1.63	2.15

*** Control without enzyme.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

hr had higher NPN than that soaked for 1 hr. The NPN content in thigh muscle of cooked carcass also appeared unaffected by soaking time within enzyme concentration, but increased with increase in enzyme concentration within any soaking time (Miyada and Tappel, 1956; Davey and Gilbert, 1969). They added also the increase of NPN was in proportion to the increase of enzyme concentration, reaction temperature and soaking time. However, Rao and Panda (1984a) soaked spent hen carcasses in 0.05, 0.075 and 0.10 % papain solution for 1, 2, and 3 hr. The results indicated that the solubility of protein, water and salt soluble nitrogen as well as non protein nitrogen increased with increase the enzyme concentration especially in cooked samples while the collagen nitrogen decreased.

4.10.1.6. Cooking time:

The previous results indicated that the best conditions for spent hen tenderization by the proteolytic enzymes were as follow: the best enzyme concentrations were 0.10, 0.008 and 0.375 % (w/v) for papain, ficin and catheptic, respectively ; the optimum temperature was 60 C° at pH 7.0 and salt concentration was 1.5 % sodium tripolyphosphate + 1 % sodium chloride for 45 min as tenderization time. Therefore, the optimum conditions were used as standared for tenderization of both breast and leg of spent hen muscles which were then cooked for different time (30, 40, 50 and 60 min) in order to assess the best cooking time for tenderization. Results in Table (28) revealed that the use of papain , ficin and catheptic lowered the time required for spent hen cooking. Meanwhile, the same results further indicated that increasing the time of cooking at the same

Table (28) : Effect of cooking time on tenderized spent hen breast and leg protein fractions.

Enzyme	Cooking time / minute	Breast				Leg			
		Protein fractions (% dry wt.)				Protein fractions (% dry wt.)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Control*	60	2.07	0.66	1.41	2.92	2.06	0.51	1.55	5.50
Papain	30	2.34	0.37	1.97	2.21	2.19	0.26	1.93	4.22
	40	2.42	0.38	2.04	2.15	2.31	0.37	1.94	3.83
	50	2.51	0.31	2.20	1.84	2.31	0.26	2.05	3.71
	60	2.56	0.35	2.18	1.86	2.39	0.35	2.04	3.74
Ficin	30	2.32	0.35	1.97	2.70	1.96	0.41	1.55	4.18
	40	2.40	0.37	2.03	2.13	2.03	0.42	1.65	3.76
	50	2.50	0.30	2.20	1.81	2.07	0.39	1.68	3.62
	60	2.55	0.34	2.21	1.83	2.03	0.37	1.66	3.65
Catheptic	30	2.24	0.27	1.97	2.23	1.75	0.34	1.41	4.20
	40	2.32	0.28	2.04	2.17	1.81	0.31	1.50	3.80
	50	2.41	0.25	2.16	1.86	1.95	0.28	1.67	3.69
	60	2.46	0.30	2.16	1.88	1.92	0.27	1.65	3.70

*** Control without enzyme.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

temperature influenced the changes encountered in the tenderized spent hen samples. Moreover, the results given in the same table show that the characteristics of tenderized samples were clearly affected by using different cooking time. The changes were clearly detected through the increase in NPN and TSN and was further evidenced through the decrease in the stroma nitrogen. Thus , the best cooking time of tenderized spent hen breast and leg was found at 60 min compared with other cooking times.

From the affomentioned results, it could be concluded that protein fractions (TSN, SPN and NPN) of spent hen meats increased gradually by increasing the concentration of proteolytic enzymes (papain, ficin and catheptic) in both breast and leg. The best concentrations of proteolytic enzymes of tenderization was 0.10, 0.008 and 0.375 % (w/v) for papain, ficin and catheptic, respectively. The best temperature of tenderization was observed at 60 C° for the three studied enzymes and the optimum pH was noticed at pH 7.0. On the other hand, the best concentrations of salt solution was found at the concentration of 1.5 % NaTPP+1 % NaCL for 45 min. Finally, the best cooking time of tenderized spent hen meat under the optimum conditions was observed at 50 C° and 60 C° for 60 min. Moreover, papain was found to be more effective under specific conditions as meat tenderizer than other two enzymes (ficin and catheptic).

4.10.2.Tenderized camel and buffalo meats:

4.10.2.1. Enzyme concentration:

Table (29) summarize the effect of different concentration of papain (3.87 u/mg protein), ficin (2.66 u/mg protein) and catheptic (3.14 u/mg protein) on different fractions of proteins (TSN, SPN, NPN and stroma nitrogen) in both camel and buffalo meat muscles. Results indicate that the increase in papain, ficin and catheptic concentrations resulted in an increase in both TSN and NPN, while induced a reduction effect on the SPN in two different types of meat under investigation compared with the untenderized (control) upto the concentration of 0.10, 0.008 and 0.375 % (w/v) for papain, ficin and catheptic, respectively. Similar results were given by many workers, El-Gharbawi and Whitaker (1963) studied the effect of ficin and bromelin concentration on the disappearance of beef proteins in 60 min at pH 5.6 and 60 C°, they found that over 80 % of the collagen and elastin disappear at the high enzyme concentrations while only about 55 % of the NaOH-soluble protein fraction disappear.

Abdel Baki et al (1957) showed that the tenderness of camel meat cuts, treated with papain and ficin increased as the concentration of enzyme increased. The enzyme treatment caused the decrease of alkali insoluble protein, which indicates that the connective tissue proteins undergo marked changes by the effect of proteolytic enzymes. However, ficin treatment increased NPN due to the destruction of protein molecules .

Table (29) : Effect of tenderizing proteolytic enzyme concentration on camel and buffalo meat protein fractions.

Enzyme	enzyme concentration % (w/v)	Camel meat Protein fractions (% dry wt.)				Buffalo meat Protein fractions (% dry wt.)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Control*	-------	2.06	1.08	0.98	2.94	2.01	1.15	0.85	2.71
Papain	0.050	2.02	1.02	1.00	2.91	2.19	1.07	1.12	2.60
	0.075	2.39	0.99	1.40	2.58	2.89	1.06	1.83	2.39
	0.100	2.99	1.07	1.92	1.95	2.96	1.04	1.92	1.83
	0.125	2.97	1.17	1.80	1.95	2.95	1.05	1.90	1.85
Ficin	0.004	2.07	1.05	1.02	2.23	2.32	1.06	1.26	2.19
	0.006	2.90	1.01	1.89	2.07	2.89	1.01	1.88	1.87
	0.008	2.91	1.01	1.90	1.90	2.90	1.00	1.90	1.83
	0.010	2.90	1.02	1.88	1.89	2.87	1.00	1.87	1.86
Catheptic	0.125	2.35	1.15	1.20	2.20	2.36	0.95	1.41	2.15
	0.250	2.55	1.14	1.41	1.98	2.65	1.13	1.52	1.81
	0.375	2.63	1.13	1.50	1.83	2.66	1.16	1.50	1.76
	0.500	2.61	1.13	1.48	1.85	2.64	1.15	1.49	1.75

*** Control without enzyme.**

TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen
Cooking time: 60 min.

The obtained results generally revealed a decreasing effect on stroma nitrogen in both camel and buffalo meats. Youn and Yong (1974) observed that the connective tissues of proteolytic enzyme treated meat decreased during 40 - 60 min at 25 C°. This might be due to the possibility that the action of proteolytic enzymes of plant origin was preferntially against the connective tissue in the muscle, as they first break up the mycopolysaccharides of the ground sbstance matrix, then progressively reduce the connective tissue fibers to an amorphous mass. Gassman et al (1976) concluded that such proteolytic enzymes do not attack native collagen but rather act upon the collagen denatured by heat during cooking. On the other hand, Delaney (1965) used TSN, SPN, NPN and connective tissue content as indication parameters to meat tenderness in meat samples treated with proteases. They found that TSN and NPN increased, while SPN and connective tissues content decreased in tenderized meat samples.

4.10.2.2.Tenderization temperature:

Table (30) show the effect of tenderization temperature on the nitrogenous compounds and stroma nitrogen of tenderized camel and buffalo meats tenderized by papain, ficin and catheptic. Results reveal that with the increase of tenderization temperature there is an increase in TSN, SPN and NPN for both the control and tenderized camel as well as buffalo meats and decrease in stroma nitrogen.It can be also noticed that 60 C°was the best temperature of tenderization for both camel and buffalo meats tenderized by papain , ficin and catheptic. Tappel et al (1956); Dawson and Wells (1969) reported that the optimum tenderization temperature of

Table (30) : Effect of tenderization temperature on tenderized camel and buffalo meat protein fractions.

Enzyme	Tenderization temperature °C	Breast								Leg							
		Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt.)				Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Papain	40	1.85	0.51	1.34	2.90	2.12	0.61	1.51	1.92	1.98	0.57	1.31	2.70	2.15	0.65	1.50	1.88
	50	1.88	0.60	1.28	2.85	2.35	0.72	1.63	1.88	1.91	0.62	1.29	2.65	2.40	0.75	1.65	1.81
	60	2.01	0.66	1.35	2.82	2.59	0.88	1.71	1.85	2.11	0.68	1.43	2.61	2.61	0.90	1.71	1.71
	70	2.12	0.67	1.45	2.75	2.51	0.82	1.69	1.84	2.18	0.70	1.48	2.58	2.55	0.87	1.68	1.76
	80	2.22	0.69	1.53	2.69	2.48	0.78	1.70	1.81	2.28	0.73	1.55	2.57	2.51	0.80	1.71	1.73
Ficin	40	1.85	0.51	1.34	2.90	1.95	0.51	1.44	2.00	1.98	0.57	1.31	2.70	1.99	0.56	1.43	1.92
	50	1.88	0.60	1.28	2.85	2.01	0.72	1.29	1.98	1.91	0.62	1.29	2.65	2.10	0.75	1.35	1.89
	60	2.01	0.66	1.35	2.82	2.61	0.79	1.82	1.91	2.11	0.68	1.43	2.61	2.65	0.95	1.70	1.85
	70	2.12	0.67	1.45	2.75	2.53	0.88	1.65	1.92	2.18	0.70	1.48	2.58	2.57	0.91	1.66	1.83
	80	2.22	0.69	1.53	2.69	2.51	0.91	1.60	1.95	2.28	0.73	1.55	2.57	2.55	0.82	1.73	1.82
Catheptic	40	1.85	0.51	1.34	2.90	1.91	0.47	1.44	1.99	1.98	0.57	1.31	2.70	1.95	0.50	1.45	1.90
	50	1.88	0.60	1.28	2.85	2.00	0.56	1.44	1.95	1.91	0.62	1.29	2.65	2.06	0.65	1.41	1.89
	60	2.01	0.66	1.35	2.82	2.46	0.77	1.69	1.90	2.11	0.68	1.43	2.61	2.52	0.81	1.71	1.83
	70	2.12	0.67	1.45	2.75	2.39	0.63	1.76	1.89	2.18	0.70	1.48	2.58	2.47	0.79	1.68	1.81
	80	2.22	0.69	1.53	2.69	2.35	0.59	1.76	1.87	2.28	0.73	1.55	2.57	2.53	0.71	1.82	1.79

*** Control without enzyme.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

papain was found at 50 C°, while it was ranged from 60-80 C° for tenderized beef meats (Weiner et al , 1957). They stated also that the optimum tenderization temperature for any enzymatic reaction was colsely related to the length of time which that reaction covered. In general, short degestion times required high optimum temperatures for the reaction. Optimum temperatures for ficin, bromelin, papain and Rhozyme p-11 were reported as 30-60, 30-60 , 43-60 and 60-85 C°, respectively (Anonymous, 1963).

4.10.2.3. pH of tenderization:

From the data presented in Table (31) , it was cleared that the increase of pH values there was an increase in protein fractions for both control and tenderized meats (camel and buffalo) by papain , ficin and catheptic upto pH 7.0 which gave the highest values of protein fractions for both camel and buffalo meats. At the same time there was a decrease in protein solubility with the increases of pH value above 7.0. These results are in a good agreement with that obtained by El-Gharbawi and Whitaker (1963) who reported that the maximum solubilization of all beef protein fractions occurs at pH 7.0 (80 C°) for ficin, papain and bromelin. However, ficin showed a wide range of pH activity (pH 5.0 to 9.0) with greater activity at pH 5.0 and pH 7.0 at 70 C° (Yatco-Manzo and Whitaker, 1962). While Anonymous (1963) found that the optimum pH of ficin to hydrolyze elastin was found at pH 5.0 to 5.5 at 55 C°.

Table (31) : Effect of tenderization pH on tenderized camel and buffalo meat protein fractions.

Enzyme	Tenderization pH	Breast								Leg							
		Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt.)				Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Papain	4	1.42	0.47	0.95	2.80	1.93	0.58	1.35	1.86	1.48	0.51	0.97	2.60	2.01	0.62	1.39	1.94
	5	1.61	0.53	1.08	2.81	2.21	0.64	1.57	1.97	1.67	0.57	1.10	2.66	2.28	0.68	1.60	1.96
	6	1.85	0.61	1.24	2.88	2.41	0.79	1.62	1.99	1.95	0.66	1.29	2.68	2.50	0.77	1.73	1.97
	7	2.01	0.66	1.35	2.61	2.61	0.91	1.70	1.95	2.10	0.70	1.40	2.51	2.67	0.98	1.69	1.90
	8	2.00	0.63	1.37	2.65	2.57	0.88	1.69	1.96	2.03	0.67	1.36	2.55	2.60	0.91	1.69	1.92
	9	1.95	0.60	1.35	2.72	2.49	0.79	1.70	1.96	1.99	0.65	1.34	2.58	2.51	0.81	1.70	1.93
Ficin	4	1.42	0.47	0.95	2.80	1.88	0.43	1.45	1.91	1.48	0.51	0.97	2.60	1.95	0.45	1.50	1.86
	5	1.61	0.53	1.08	2.81	2.04	0.48	1.56	1.93	1.67	0.57	1.10	2.66	2.15	0.51	1.64	1.87
	6	1.85	0.61	1.24	2.88	2.35	0.61	1.74	1.95	1.95	0.66	1.29	2.68	2.42	0.67	1.75	1.89
	7	2.01	0.66	1.35	2.61	2.59	0.90	1.69	1.90	2.10	0.70	1.40	2.51	2.62	0.83	1.79	1.83
	8	2.00	0.63	1.37	2.65	2.51	0.83	1.68	1.93	2.03	0.67	1.36	2.55	2.58	0.79	1.79	1.84
	9	1.95	0.60	1.35	2.72	2.45	0.70	1.75	1.94	1.99	0.65	1.34	2.58	2.48	0.65	1.83	1.85
Catheptic	4	1.42	0.47	0.95	2.80	1.85	0.39	1.46	1.87	1.48	0.51	0.97	2.60	1.90	0.43	1.47	1.80
	5	1.61	0.53	1.08	2.81	2.01	0.45	1.56	1.88	1.67	0.57	1.10	2.66	2.08	0.49	1.59	1.83
	6	1.85	0.61	1.24	2.88	2.33	0.61	1.72	1.89	1.95	0.66	1.29	2.68	2.40	0.65	1.75	1.88
	7	2.01	0.66	1.35	2.61	2.46	0.78	1.68	1.85	2.10	0.70	1.40	2.51	2.51	0.81	1.70	1.76
	8	2.00	0.63	1.37	2.65	2.40	0.70	1.70	1.86	2.03	0.67	1.36	2.55	2.48	0.75	1.73	1.78
	9	1.95	0.60	1.35	2.72	2.38	0.59	1.79	1.86	1.99	0.65	1.34	2.58	2.43	0.61	1.82	1.79

* Control without enzyme.

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

4.10.2.4. Salt concentrations:

Different concentrations of NaTPP ranged from 0.5 to 2 % and 1 % NaCL were added to the camel and buffalo meats during tenderization using papain, ficin and catheptic , and the nitrogenous compounds were analyzed. Data presented in Table (32) indicate that improving tenderness of camel and buffalo meats by using NaTPP and NaCL without adding enzymes (control) due to the ability to increase charge, pH and subsequent hydration of protein (Wood and Richards, 1974; Hale et al, 1977; El-Samahy and Shehata, 1977).

Data also show that papain, ficin and catheptic in combination with NaTPP and NaCl appeared better for tenderization of meat compared to enzyme alone. These results due to higher water binding capacity and increased tenderness (Rao and Panda, 1984b). It could also be noticed that with the increase of NaTPP there was an increase in protein fractions upto 1.5 % salt concentration.

From the previous results , it could be observed that 1.5 % NaTPP and 1 % NaCl was the best concentration for gaining the best tenderized camel and buffalo meats.

Table (32) : Effect of salt concentration on tenderized camel and buffalo meat protein fractions.

Enzyme	Salt concentration	Breast								Leg							
		Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt.)				Control (protein fractions % dry wt)*				Tenderized sample (protein fractions % dry wt)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Papain	0.5% NaTPP & 1% NaCl	2.40	0.91	1.49	2.90	2.10	0.58	1.52	2.00	2.42	0.95	1.47	2.70	2.15	0.65	1.50	1.96
	1.0% NaTPP & 1% NaCl	2.45	1.03	1.42	2.88	2.95	0.56	2.39	1.98	2.48	1.08	1.40	2.65	2.99	0.62	2.37	1.90
	1.5 % NaTPP & 1% NaCl	2.50	1.17	1.33	2.78	2.55	0.49	2.06	1.95	2.55	1.20	1.35	2.61	2.61	0.53	2.08	1.85
	2.0% NaTPP & 1% NaCl	2.43	1.15	1.28	2.73	2.45	0.45	2.00	1.96	2.48	1.18	1.30	2.56	2.52	0.50	2.02	1.86
Ficin	0.5% NaTPP & 1% NaCl	2.40	0.91	1.49	2.90	2.01	0.56	1.45	1.99	2.42	0.95	1.47	2.70	2.09	0.61	1.48	1.88
	1.0% NaTPP & 1% NaCl	2.45	1.03	1.42	2.88	2.42	0.52	1.90	1.95	2.48	1.08	1.40	2.65	2.55	0.59	1.96	1.86
	1.5 % NaTPP & 1% NaCl	2.50	1.17	1.33	2.78	2.53	0.45	2.08	1.90	2.55	1.20	1.35	2.61	2.61	0.51	2.10	1.83
	2.0% NaTPP & 1% NaCl	2.43	1.15	1.28	2.73	2.48	0.47	2.01	1.91	2.48	1.18	1.30	2.56	2.58	0.49	2.09	1.84
Catheptic	0.5% NaTPP & 1% NaCl	2.40	0.91	1.49	2.90	2.00	0.53	1.47	1.91	2.42	0.95	1.47	2.70	2.03	0.55	1.48	1.83
	1.0% NaTPP & 1% NaCl	2.45	1.03	1.42	2.88	2.38	0.50	1.88	1.89	2.48	1.08	1.40	2.65	2.41	0.52	1.89	1.79
	1.5 % NaTPP & 1% NaCl	2.50	1.17	1.33	2.78	2.49	0.44	2.05	1.85	2.55	1.20	1.35	2.61	2.52	0.48	2.04	1.76
	2.0% NaTPP & 1% NaCl	2.43	1.15	1.28	2.73	2.42	0.42	2.00	1.86	2.48	1.18	1.30	2.56	2.45	0.45	2.00	1.77

* Control without enzyme.

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).

Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

4.10.2.5.Tenderization time:

Effect of tenderization time on protein hydrolysis of ternderization of camel and buffalo meats are given in Table (33) . The results show that the highest protein fractions were obtained when the samples were tenderized with papain, ficin and catheptic for 60 min in both types of meat under investigation. Weiner et al (1957) stated that the optimum temperature for any enzymatic reaction was closely related to the length of time which that reaction covered. Dawson and Wells (1969) found that a direct linear increase in NPN occurred in samples held upto 60 min of incubation for proteases. On the other hand, the pre- treatment of samples before cooking with proteases to reduce the myofibrillar strength to its minimum was carried out by Gassman et al (1976).

4.10.2.6. Cooking time:

The previous results indicated that 0.10, 0.008 and 0.375 % (w/v) of papain, ficin and catheptic, respectively; 60 C° , pH 7.0, 1.5 % NaTPP and 1% NaCL ; and 60 min tenderization time could be considered the optimum conditions for camel and buffalo muscles tenderization. Therefore, the optimum conditions were used as standard for tenderization of meats which were then cooked for different cooking time (30, 40, 50 and 60 min), in order to asses the best cooking time for tenderization of camel and buffalo meats. Results presented in Table (34) show that the use of papain , ficin and catheptic lowered the time required for camel and buffalo meats cooking. Meanwhile, the same results further indicated that increasing the time of cooking at the same temperature influenced the

Table (33) : Effect of tenderization time on tenderized camel and buffalo meat protein fractions.

Enzyme	Tenderization time / minute	Camel meat				Buffalo meat			
		Protein fractions (% dry wt.)				Protein fractions (% dry wt.)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Control*	60	2.20	0.70	1.50	2.91	2.22	0.76	1.46	2.70
Papain	15	2.15	0.59	1.56	2.25	2.20	0.62	1.58	2.16
	30	2.24	0.53	1.71	2.00	2.28	0.57	1.71	1.95
	45	2.47	0.57	1.90	1.95	2.50	0.60	1.90	1.85
	60	2.50	0.60	1.90	1.94	2.55	0.61	1.94	1.84
Ficin	15	2.10	0.47	1.63	2.08	2.15	0.50	1.65	2.01
	30	2.21	0.45	1.76	1.98	2.25	0.48	1.77	1.89
	45	2.42	0.49	1.93	1.90	2.45	0.52	1.93	1.83
	60	2.44	0.50	1.94	1.88	2.49	0.55	1.94	1.81
Catheptic	15	2.07	0.45	1.62	2.05	2.10	0.48	1.62	2.00
	30	2.15	0.41	1.74	1.91	2.17	0.45	1.72	1.85
	45	2.33	0.47	1.86	1.85	2.38	0.49	1.89	1.76
	60	2.39	0.49	1.90	1.83	2.40	0.53	1.87	1.73

*** Control without enzyme.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

Table (34) : Effect of cooking time on tenderized camel and buffalo meat protein fractions.

Enzyme	Cooking time / minute	Camel meat				Buffalo meat			
		Protein fractions (% dry wt.)				Protein fractions (% dry wt.)			
		TSN	SPN	NPN	Stroma (N)	TSN	SPN	NPN	Stroma (N)
Control*	60	2.20	0.70	1.50	2.91	2.22	0.75	1.47	2.70
Papain	30	2.43	0.51	1.92	2.01	2.45	0.55	1.90	1.95
	40	2.46	0.55	1.91	1.98	2.47	0.57	1.90	1.89
	50	2.55	0.57	1.98	1.95	2.60	0.61	1.99	1.85
	60	2.60	0.52	2.08	1.94	2.67	0.60	2.07	1.84
Ficin	30	2.40	0.40	2.00	2.00	2.43	0.42	2.01	1.89
	40	2.43	0.43	2.00	1.95	2.46	0.45	2.01	1.88
	50	2.53	0.47	2.06	1.90	2.50	0.49	2.01	1.83
	60	2.59	0.51	2.08	1.88	2.56	0.50	2.06	1.81
Catheptic	30	2.35	0.38	1.97	1.97	2.37	0.40	1.97	1.88
	40	2.38	0.41	1.97	1.90	2.40	0.42	1.98	1.78
	50	2.41	0.45	1.96	1.85	2.43	0.48	1.95	1.76
	60	2.48	0.48	2.00	1.84	2.50	0.49	2.01	1.74

*** Control without enzyme.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

changes encountered in the tenderized meats. The changes were clearly detected through the increase in NPN and TSN and was further evidenced through the decrease in the stroma nitrogen. Thus,these results proved that proteolytic enzymes caused a reduction in cooking time that required for cooking camel and buffalo meats, and the cooking time of 50 and 60 min were considerably better than cooking for 30,40 and 50 min . On the other hand, papain had a higher effect under the optimum conditions of tenderization followed by ficin and catheptic enzymes.

4.11.Organolyptic evaluation of tenderized meats:

4.11.1. Organolyptic evaluation of cooked spent hen meats as affected by proteolytic enzymes.

The platability characteristics, i.e. tenderness, flavor, juiciness and overall acceptability of tenderized spent hen meats (breast and leg) were organolyptically evaluated after cooking. Different concentration of papain (0.050, 0.075, 0.100, and 0.125 % w/v), ficin (0.004, 0.006, 0.008 and 0.010 w/v) and catheptic (0.125, 0.250, 0.375 and 0.500% w/v) were used and the obtained results are given in Table (35). Results indicate that the average score of tenderness, flavor, juiciness and overall acceptability was increased by increasing the enzyme concentrations upto 0.100, 0.008, 0.375 % (w/v) for papain , ficin and catheptic ,respectively for both breast and leg meats. These results proved that the use of proteolytic enzymes improved the organolyptic characteristics of meats, hence it could be used as meat tenderizers. The obtained results are in a good agreement with that reported by Miller et al (1988) who found that low enzyme concentration were necessary in chicken meat to produce acceptable tenderness.

Table (35) : Mean organoleptic score of cooked spent hen breast and leg tenderized by papain, ficin and catheptic at different enzyme concentrations.

Enzyme	Enzyme concentration % (w / v)	Breast				Leg			
		Score**				Score**			
		Tend-erness	Flavor	Juici-ness	Overall accept-ability	Tend-erness	Flavor	Juici-ness	Overall accept-ability
Control*	------	3.56	7.00	5.47	4.98	3.27	7.13	4.83	5.32
Papain	0.050	4.75	7.11	6.17	5.38	5.10	7.16	5.38	5.61
	0.075	6.22	7.19	7.00	6.83	6.00	7.18	6.00	6.38
	0.100	7.18	8.21	7.75	7.68	8.11	7.76	8.40	8.10
	0.125	7.16	8.17	6.22	6.43	7.27	6.88	7.51	7.89
Ficin	0.004	5.18	7.15	6.83	6.00	4.88	7.15	5.28	6.18
	0.006	7.30	7.30	7.82	7.38	6.41	7.22	7.31	6.57
	0.008	7.28	7.30	7.78	7.18	7.32	7.35	7.52	7.30
	0.010	7.28	7.27	7.71	7.18	7.32	7.32	7.50	7.30
Catheptic	0.125	5.00	4.00	6.62	5.26	5.20	7.20	5.28	5.58
	0.250	6.28	6.36	7.20	6.61	6.30	6.81	7.00	6.71
	0.375	6.50	7.39	7.50	7.14	8.11	7.42	7.21	7.26
	0.500	6.50	5.41	6.71	6.22	7.52	6.48	7.00	6.68

*** Control without enzyme.**

**** Means of ten panilests.**

TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen
Cooking time: 60 min.

4.11.2. Organolyptic evaluation of tenderized spent hen meats as affected by cooking time:

The platability characteristics i.e. tenderness, flavor, juiciness and overall acceptability of tenderized spent hen breast and leg as affected by cooking time (30, 40, 50 and 60 min) were organolyptically evaluated. Results in Table (36) indicate that the overall acceptability obtained for both breast and leg of spent hen samples cooked for 60 min, were considerably higher and better than that obtained for the same samples cooked for 30, 40 and 50 min. This means that the overall acceptability increased with increasing the cooking time of tenderized spent hen meats by proteolytic enzyme under the optimum conditions.

4.11.3. Organolyptic evaluation of camel and buffalo meats as affected by proteolytic enzymes:

The results given in Table (37) indicate that the average scores of taste-panel for tenderness, flavor, juiciness and overall acceptability of both camel and buffalo meats treated with different concentrations of proteolytic enzymes (papain, ficin and catheptic) were improved compared with the control samples. Besides, the proteolytic enzymes were found to have improving effect on the other organolyptic characteristics of camel and buffalo meats, i.e, juiciness and flavor, the improving effect on the tenderness, flavor, juiciness and overall acceptability of camel and buffalo meats were found to increase with increasing the concentrations of proteolytic enzymes applied from 0.05 to 0.10% (w/v for papain), from 0.004 to 0.008 (w/v for ficin) and from 0.125 to 0.375 % (w/v for

Table (36) : Effect of cooking time on tenderized spent hen breast and leg organoleptic score.

Enzyme	Cooking time / minute	Breast				Leg			
		Score**				Score**			
		Tend-erness	Flavor	Juici-ness	Overall accept-ability	Tend-erness	Flavor	Juici-ness	Overall accept-ability
Control*	——	3.62	7.67	5.70	5.53	3.47	7.60	5.52	5.37
Papain	30	6.22	6.00	6.77	6.38	7.11	6.27	6.82	6.74
	40	6.26	6.17	6.96	6.47	7.26	7.10	6.76	7.14
	50	7.00	7.88	7.28	7.39	8.00	7.65	7.10	7.59
	60	7.01	7.86	7.28	7.38	8.02	7.64	7.10	7.58
Ficin	30	6.21	7.56	6.22	6.17	6.28	7.55	5.83	6.17
	40	7.11	7.62	7.58	6.38	7.32	7.61	6.18	6.22
	50	8.38	7.79	8.21	8.30	8.28	7.68	7.82	8.14
	60	8.38	7.81	8.21	8.30	8.35	7.68	7.82	8.23
Catheptic	30	5.21	6.22	6.76	6.16	5.38	6.21	6.71	6.11
	40	6.28	6.15	7.55	6.62	6.22	7.51	5.88	5.56
	50	8.00	7.86	8.21	8.12	7.12	8.10	7.22	7.49
	60	8.00	7.85	8.21	8.11	7.10	8.11	7.22	7.48

*** Control without enzyme.**

**** Means of ten panilests.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

Table (37) : Mean organoleptic score of cooked camel and buffalo meats tenderized by proteolytic enzymes at different enzyme concentration.

Enzyme	Enzyme concentration % (w / v)	Camel meat Score**				Buffalo meat Score**			
		Tend-erness	Flavor	Juici-ness	Overall accept-ability	Tend-erness	Flavor	Juici-ness	Overall accept-ability
Control*	———	5.00	5.30	5.00	5.20	5.80	6.00	6.00	6.10
Papain	0.050	6.10	6.00	7.00	6.37	7.50	7.80	8.00	7.77
	0.075	8.30	8.00	8.00	8.20	9.00	8.80	9.00	8.93
	0.100	9.60	8.70	9.00	9.11	10.00	9.50	9.65	9.72
	0.125	8.80	8.50	8.00	8.44	10.00	8.80	9.50	9.43
Ficin	0.004	7.30	8.00	7.50	7.70	7.30	7.00	7.80	7.38
	0.006	9.00	7.50	8.00	8.17	10.00	9.00	9.00	9.30
	0.008	9.00	8.00	9.00	8.68	9.50	9.00	9.50	9.36
	0.010	9.00	7.00	7.50	7.83	10.00	8.00	8.80	8.94
Catheptic	0.125	7.10	7.50	6.40	7.10	8.00	7.00	7.00	7.35
	0.250	9.40	7.50	6.00	7.68	9.80	8.00	8.00	8.66
	0.375	9.50	8.00	8.00	8.48	10.00	9.00	8.50	9.18
	0.500	9.40	7.00	6.00	7.49	10.00	7.50	7.00	8.17

*** Control without enzyme.**

**** Means of ten panilests.**

TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen
Cooking time: 60 min.

catheptic). Meanwhile, the higher scores for tenderness, flavor, juiciness and overall acceptability were obtained by using 0.10, 0.008 and 0.375 % (w/v) of papain , ficin and catheptic , respectively for both camel and buffalo meats. It could be also noticed from the same results that there was no clear differences in the tenderness scores between the camel and buffalo meats treated with the optimal concentrations of proteolytic enzymes. While, the juiciness and flavor scores were slightly lower in the case of camel meat than in buffalo meat samples treated with the same concentration of the tested enzymes. This might be an indication of the proteolytic enzymes acted only on the protein fractions, leading to an increase in the tenderness scors, while on the other hand, it did not affect the juiciness or the flavor scores (Huffman et al, 1967). These results are in agreement with those reported by Culler et al (1978) on bovine longissimus dorsi. On the other hand, the same results (Table 37) further show that the greatest effect of proteases on the organolyptic characteristics of camel and buffalo meat samples was exhibited by plant proteases i.e, papain and ficin followed by animal proteases i.e, catheptic, when such enzymes were used at their optimal concentrations.

4.11.4. Organolyptic evaluation of tenderized camel and buffalo meats as affected by cooking time:

The platability characteristics i.e, tenderness, flavor, juiciness and overall acceptability of tenderized camel and buffalo meats were organolyptically evaluated as affected by different cooking times (30, 40, 50, and 60 min) and tenderized by papain , ficin and catheptic enzymes under the optimum conditions. Results in Table (38) indicate that the

Table (38) : Effect of cooking time on tenderized camel and buffalo meats organoleptic score.

Enzyme	Cooking time / minute	Camel meat Score**				Buffalo meat Score**			
		Tend-erness	Flavor	Juici-ness	Overall accept-ability	Tend-erness	Flavor	Juici-ness	Overall accept-ability
Control*	-----	5.00	5.41	5.20	5.00	6.00	5.81	6.00	6.20
Papain	30	5.11	6.22	6.00	5.88	6.60	6.70	6.11	6.28
	40	5.70	7.00	6.38	6.37	6.84	7.00	6.50	6.31
	50	8.00	8.20	8.25	8.18	8.11	8.00	7.00	7.73
	60	7.61	7.60	7.75	7.66	7.72	7.50	6.65	7.28
Ficin	30	6.20	6.66	6.10	6.35	6.24	6.91	6.11	6.28
	40	6.60	7.00	6.18	6.60	6.82	7.00	6.20	6.38
	50	7.73	8.11	7.58	7.83	8.10	8.10	7.00	7.76
	60	7.32	7.21	6.27	6.95	7.38	7.00	7.00	7.13
Catheptic	30	5.70	7.00	6.00	5.96	6.12	6.00	6.51	6.53
	40	5.87	7.44	6.11	6.49	6.52	6.71	6.70	6.96
	50	7.71	8.30	7.70	7.93	7.71	7.79	7.71	7.70
	60	7.00	7.55	7.00	7.20	7.44	7.00	7.00	7.16

*** Control without enzyme.**

**** Means of ten panilests.**

Papain concentration: 0.100% (w/v).
Ficin concentration: 0.008% (w/v).
Catheptic concentration: 0.375% (w/v).
Cooking time: 60 min.
TSN:Total soluble nitrogen.
SPN: Soluble protein nitrogen.
NPN: Non protein nitrogen

cooking time of 50 and 60 min had higher tenderness scores compared with that cooked at 30 and 40 min for all the samples treated with the three enzymes under investigation in both camel and buffalo meats.

Concerning flavor of camel and buffalo meats, it was noticed that as the cooking time increased the flavor deteriorated. Samples cooked for 50 and 60 min had higher scores of flavor than those cooked for 30 and 40 min for both camel and buffalo meats . However, juiciness and overall acceptability was found to be higher scored in the camel and buffalo meats cooked for 60 min compared with other samples cooked for 30 , 40 and 50 min.

4.12. Production of proteolytic enzymes on the laboratory scale:

The yields of proteolytic enzymes from different sources (plant or animal) on the laboratory scale was carried out and the obtained data could be summarized as follow:

* In this investigation we used unripe fruits of *Carica papaya* from the trees (1-2 years in age), the average number of fruits was ranged from 10-12 fruit/tree, the average weight of fruit ranged from 250-1500 gm. The latex were collected from 9:00 am to 4:00 pm. We used 30 trees containing 360 fruits in average and the collected fresh latex equal 1880 gm (1625 ml), this gave 600 gm after drying. The average yield of one fruit equal 5.24 gm fresh latex (1.73 gm dry latex).

* Unripe fig fruits were also used as a source of proteolytic enzymes. We used about 20 kg of fig fruits and the resultant extract after drying was about 300 gm.

* Bovine spleen was used as an animal source of proteolytic enzymes. The weight of fresh spleen was 28.04 kg and it was 16.70 kg after preparation for extraction. The yield of crude catheptic equal 20 gm dry extract.

Generally, it could be concluded that:

1. The proteolytic enzymes i.e , plant (papain and ficin) and animal (catheptic) , improved clearly not only the tenderness , but also the flavor, juiciness and overall acceptability of tenderized meats.
2. The quality characteristics of tenderized camel and buffalo meats were exhibited by the increase of taste -panel scores for flavor , juiciness, tenderness and overall acceptability.
3. The improving effect of proteolytic enzymes on the quality characteristics of tenderized meats differed with the type and concentrations of each enzyme applied.
4. The proteolytic activity differed also with each group of enzymes.
5. The optimal tenderizing concentration of the tested enzymes under the specific conditions were 0.100, 0.008 and 0.375 % (w/v) for papain ficin and catheptic, respectively.