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THE EFFECT OF HEAT TREATMENT OF FRESH FROZEN VELLS ON RENNIN EXTRACTABILITY

by

LeEsther Mifflin Holm

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1972

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To the New Zealand Cooperative Rennet Company for making the funds and opportunity available for this study.

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LeEsther M. Holm

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ABSTRACT

THE EFFECT OF HEAT TREATMENT OF FRESH
FROZEN VELLS ON RENNIN EXTRACTABILITY

by

LeEsther Mifflin Holm, Master of Science
Utah State University, 1972

Major Professor: Dr. C. A. Ernstrom
Department: Nutrition and Food Sciences

A procedure was developed for the extraction of rennet from fresh frozen vells. Frozen vells were minced in a Model VCM-25 Hobart Vertical Cutter/Mixer. Dry sodium chloride was added until the salt concentration in the moisture of the tissue was 10%. Salted tissue was placed in galvanized steel cells and heated in a water bath to 49, 54, 57 and 60 C for 0, 15, 30, 45 and 60 minutes. The heated tissue was dried to about 4% moisture at ambient temperature with the aid of an electric fan. The dried salted tissue was extracted with a sodium chloride solution adjusted to give a salt concentration of 10% in the extraction solution. Salted vell tissue heated to 49 C for 60 minutes and to 54 C for 30 minutes prior to drying produced 82 and 55%, respectively, more recoverable enzyme activity than unheated salted tissue. However, tissue which was salted, heated and not dried prior to extraction produced less recoverable activity than unheated samples.

Unsalted vell tissue which was heated to 49, 54, 57 and 60 C for 15, 30, 45 and 60 minutes and extracted without being dried yielded lower recoverable activity than unheated samples.

Heating unsalted vell tissue to 54, 57 and 60 C for 15, 30, 45 and 60 minutes prior to drying did not increase recoverable enzyme activity over unheated samples.

Unsalted tissue heated to 49 C produced a slightly higher recovery than unheated samples.

(76 pages)

INTRODUCTION

Rennin has been used as a milk coagulant in cheese making for many years. The cheese industry uses rennin in the form of a crude extract (22), paste (21) or powder (22) called rennet.

Rennet is extracted from the fourth or true stomach (abomasum) of the milk-fed calf (11). Prior to extraction, calves' stomachs (vells) are generally prepared by either the dry-blown or flat-salted process (11). In the dry-blown process, used mostly in Europe and New Zealand, the contents of the stomach are removed and the vell is tied, inflated with air and dried at about 43 C. The presence of mucins, which form part of the lining of the stomach, make it necessary to store dry-blown vells up to one year prior to extraction. The slimy properties of the mucins make extraction of rennet from new vells impractical. Because dry-blown vells must be stored for long periods, large inventories must be maintained which increase costs associated with rennet processing. In the flat-salted process, used in the United States, the vells are opened at the slaughterhouse and washed and packed in dry salt for shipment (11). There is less cost in preparing flat-salted vells at the slaughterhouse, but higher costs are encountered at the rennet plant because the wet-salted vells must be washed and dried. However, drying in the presence of saturated salt destroys some of the mucins and makes the tissues more readily extractable.

Extraction of fresh or frozen tissues has been limited because the soluble mucins increase the viscosity of extracts and decrease the extractability of rennin.

Ernstrom (unpublished data 1955) found that heat applied to flat-salted vells during, before or after drying increased their extractability.

The objective of this study was to determine whether a heat treatment could be applied to fresh frozen vell tissue in the presence or absence of added sodium chloride that would improve the extractability of rennin without destroying enzyme activity.

LITERATURE REVIEW

Sources of rennet extract

Rennin is the main milk-clotting enzyme secreted by the abomasum (fourth stomach) of the suckling calf. Rennet is the name given to a preparation containing the enzyme rennin along with other proteinateous materials (6).

The stomach of the bovine species has four compartments: The rumen or paunch, reticulum or honeycomb, omasum or manifold and the abomasum or rennet (vell) (18). Only the fourth compartment contains rennin.

Rennin in the calf stomach is gradually replaced by pepsin as the calf grows and consumes increasing quantities of solid feed (23). Leitch (15) reported that the enzymatic secretion of the very young calf is predominantly rennin, but that at the age of five months, the calf's stomach yields pepsin almost to the exclusion of rennin. Linklater (16) found commercial rennet extracts to contain 0-3.7% pepsin, but since older calves are now used this percentage is undoubtedly higher.

Due to a world rennet shortage, extraction of adult bovine stomachs has been suggested (1). Stomachs from beef animals up to 2 years of age have been extracted on an experimental basis. Lamb stomachs have also been extracted. However, because of low enzyme yields these sources have proved to be uneconomical (1).

Berridge et al. (3) reported a method of obtaining rennet from living calves. A gastric solution was obtained by means of an abomasal fistula described by Fomin (12). Abomasal juice from calves about 2 weeks old was removed through the fistula half an hour after allowing the animals to drink diluted whey. This juice was then tested for rennin activity. It was concluded that for the amount of

rennin obtained, this method was too expensive in time, labor and cost of feed (3).

In the flat-salted process the calf is gutted and the vell carefully cut free from the paunch. The vell is then cut open and any curdled milk or other feed is removed. Vells are stretched as much as possible and are then piled flat, one over the other, with one-half inch of salt between them (17). Flat-salted vells are prepared for extraction by washing the tissue with a saturated sodium chloride solution. They are then stretched, defatted and hung on dowels in a circulating air oven at 43.3 C for 20 hours (22).

In preparing dry-blown vells, the small end of the vell is cut such that a few inches of duodenum remain connected to the vell. The contents of the vell are expelled through the gut end by passing the vell between the forefinger and thumb (19). Fat is trimmed from the vell, but care is excercised to not puncture the vell. A string is tied between the vell and omasum and then both reticulum and omasum are removed. A tube is inserted into the gut end and the vell is inflated to the approximate size of a basketball. Blown vells are tied off and hung in a circulating air oven at 37 to 43 C. After vells are properly dried, they are packed in bundles for shipment to the processor (17).

Manufacture of rennet extract

Placek, Bavisotto and Jadd (22) described a method for extracting flat-salted vells in which dried vells were ground in a hammer mill with excelsior or glass wool in the ratio of 3 to 1. Glass wool or excelsior acted as an inert support without which mucin's ropy characteristics would keep the extraction fluid from percolating. Material from the hammer mill was placed in 2000-gallon wooden extraction vats. Each vat held the equivalent of 10,000 to 15,000 vells. Extraction was carried out at 2 to 5 C with an

extraction solution adjusted such that an overall sodium chloride concentration of 10% was achieved. The liquid was circulated for 2 days by countercurrent flow (22).

Since a considerable amount of prorennin was present in the extract, the extract was activated to rennin by adjusting the pH to 4.6 with 18% hydrochloric acid. Activation required 18 to 36 hours, after which the pH was raised to 5.7 by adding sodium bicarbonate as a water slurry. Propylene glycol was added to hold down bacterial growth. Caraway flavor, caramel color, preservatives and diatomaceous earth were added before filtering the extract. The extract was filtered twice in bags made of canvas and lined inside with muslin. The extract was checked for enzymatic activity, pH, salt concentration, color and clarity. A final bacteriological test was performed prior to packaging (22).

Currently the wooden extraction vats have been replaced by fiberglass tanks. Fiberglass is also used for the finishing and storage tanks (8).

Filtering operations have been improved upon from the old process of using canvas filters. Filter presses are now in use in which rennet is filtered through a series of disposable paper filters (9).

A method of extraction has been reported in which dried vells are cut into small pieces, mixed with filler and extracted in fiberglass columns. A brine solution containing 10% sodium chloride is pumped through each of the columns to extract the rennet. After extraction the rennet is filtered and various quality control tests performed (1).

Pretreatment of vells prior to extraction

Freezing vell tissue is an effective means of stabilizing rennin, and fresh frozen vells have been used for rennet extraction (1). Clarke (7) developed a method of extracting rennet from fresh frozen vells. Partially thawed frozen

vells were minced and treated with 2% potassium alum and allowed to stand for 20 hours. Enough 0.45 M disodium phosphate was added to bring the pH of the mixture to 5.7. Rennet was extracted with a solution containing 10% sodium chloride plus 0.75% sodium benzoate. Untreated ground tissue as well as tissue treated with 0.68 to 3.40% potassium alum was batch extracted. Extract from treated tissue had a much lower viscosity than untreated tissue. It was concluded that rennet could be extracted from fresh frozen vells treated with 2 to 3% alum (7).

Potassium alum forms precipitates of aluminum phosphate which adsorb non-rennin impurities, including mucins, very strongly (4). Clarke (7) demonstrated the binding of potassium alum by vell tissue and by mucin solutions. A mucin solution was prepared by extracting 150 g of wet vell tissue with 400 ml of 10% sodium chloride solution. This extract was then dialyzed against distilled water containing 1.0% sodium benzoate at 4 C to remove the sodium chloride. Weighed samples of vell tissue and mucin solution were dialyzed at 4 C against a solution containing a known concentration of potassium alum. After five days, the materials inside and outside the dialysis casings were weighed and analyzed for aluminum and moisture. The amount of aluminum, expressed as milligrams of aluminum per milliliter of water, inside the dialysis casing was compared to the aluminum outside the dialysis casing. A higher concentration of aluminum in the water inside the dialysis casing indicated the aluminum was being bound by tissue or mucins. This was used to calculate bound aluminum per gram of tissue or mucins. Results showed that more than ten times as much aluminum was bound to mucins than to vell tissue per gram of dry matter (7).

Continuous extraction was used by Clarke (7) on dry minced vell tissue and on wet vells treated with 2% alum. After 20 hours the alum treated tissue was neutralized to

pH 5.7 ± 0.2 with 0.45 M disodium phosphate. Tissue was mixed with coarse pumice in a volume ratio of 1 to 2 and placed in extractor columns. Countercurrent flow was employed, and extraction with an overall concentration of 10% sodium chloride was performed for four to six days before discarding the spent contents. The results indicated that the potassium alum treatment was comparable to drying fresh frozen vells at 25 C ± 1 C except for a slower release of enzyme from the tissue. A smaller volume of extract was required by the dried tissue to yield an enzyme activity near that recovered from alum-treated tissues.

METHODS AND PROCEDURE

Preparation of vell tissue

Vells from 3 to 6 day old milk-fed calves were obtained through the courtesy of the New Zealand Rennet Company. They were packed in plastic bags and frozen at the abattoir. Each bag contained approximately 21 vells and weighed about 16.8 kg. Frozen tissues were sent by air cargo to Logan, Utah, and stored at -23 C. The contents of 1½ bags was used for each experiment. Tissues were ground in a Model VCM-25 Hobart Vertical Cutter/Mixer until completely homogeneous. Samples of about 120 g were used for each treatment.

Moisture analysis

Duplicate samples were analyzed for moisture by an official procedure for moisture in meat products (2).

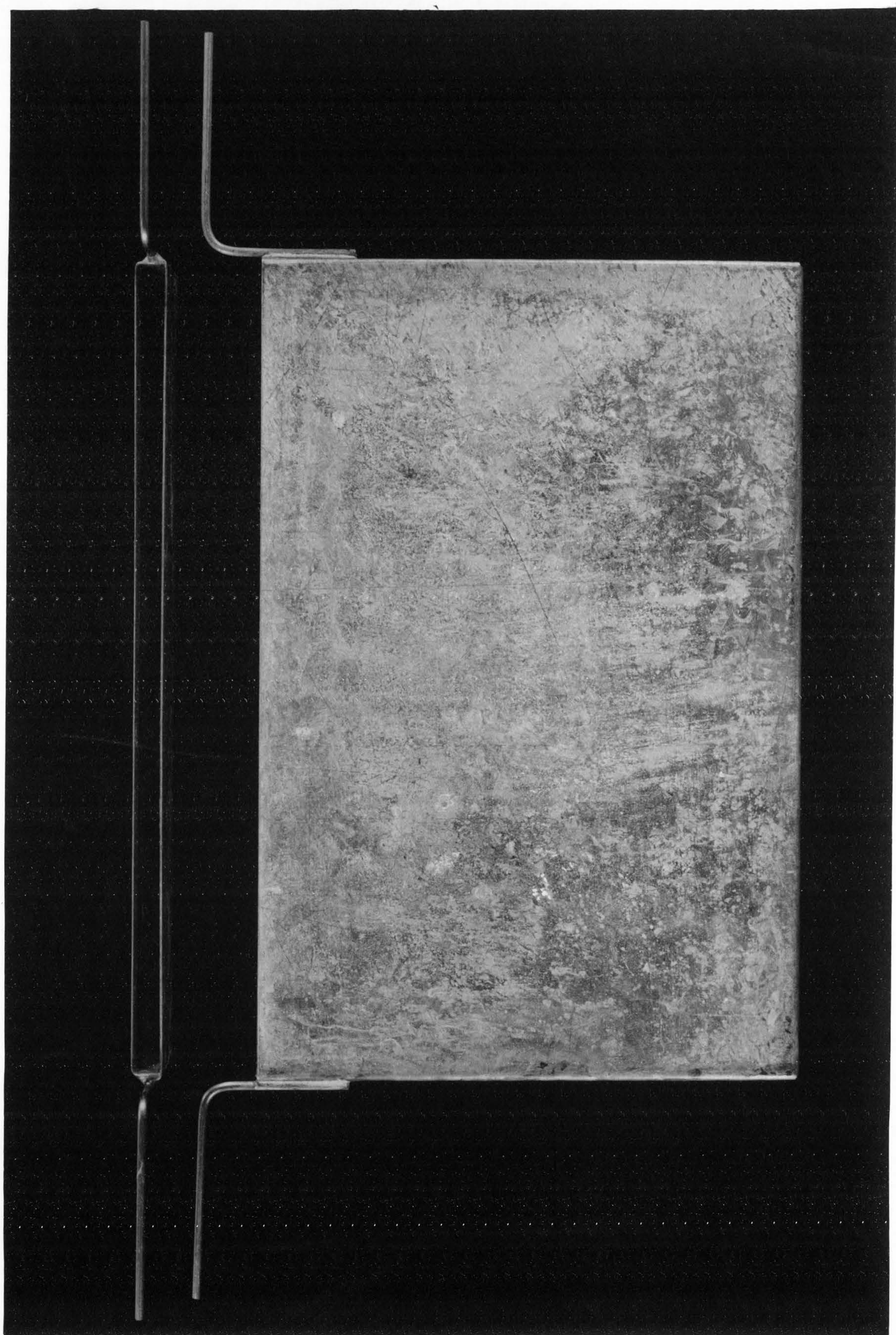
Heat treating vell tissue

Mixed ground tissues were placed in galvanized steel cells 20.3 cm long X 12.7 cm deep X .6 cm wide (Figure 1). Wire supports were soldered to each end of every cell and served the purpose of suspending the cells in the heating bath. Tissues within the cells were brought to the desired temperature in 4 min. Duplicate samples were heated to 49, 54, 57 and 60 C for 0, 15, 30, 45 and 60 min. After samples were heated for the specified time and temperature, they were immediately cooled in cold water to 12 C.

Drying vell tissue

One hundred-gram samples of heated tissue were spread in a thin layer on 8½ X 11 inch typing paper and dried at ambient temperature with the aid of an electric fan. Control samples were treated in like manner. Drying required 20-24

Figure 1. Galvanized steel cell (20.3 cm long X 12.7 cm deep X .6 cm wide) used for heating vell tissue.



hours. The dried tissue-covered paper was then cut into small pieces and used for extraction.

Extraction procedure

Samples representing 100 g of wet tissue were placed in labeled pint Mason jars and mixed with excelsior and pebble-sized pumice. Two hundred milliliters of extraction solution were added to each pint jar. The extraction solution contained 10% sodium chloride plus 0.5% sodium benzoate. Extraction was performed at 2 to 4 C.

At the end of a two day period the pint jars were uncapped, stirred and the extract drained into beakers for 1 hour. The volume of extract was measured and an equal volume of extraction solution added back to replace the extract drained. Four consecutive extractions were performed except for vells which had been heated to 57 and 60 C. Heating tissue to these temperatures destroyed a large percentage of activity.

pH of extract

The pH of extracts was measured on a Corning Model 10 pH meter.

Activation of prorennin

One hundred milliliters of extract were placed in a 100 ml screw-cap sample bottle, and 1 ml of concentrated hydrochloric acid was added. The sample was shaken and allowed to stand for 30 min before measuring enzyme activity. The pH of the activated solution was 1.2 $\pm$ .1.

Measuring enzyme activity

Rennin activity was determined by the milk clotting test described by Ernstrom (10). In this test Berridge (5) substrate was used in connection with the apparatus

described by Sommer and Matsen (24). Samples were run in 125 ml wide-mouth bottles containing 25 ml of substrate. The bottles rotated at 8 revolutions per minute, and electric counters on the apparatus operated at 20 counts per minute. Unknown samples were compared to a known standard*. The standard was arbitrarily assigned a value of 100 rennin units per milliliter (R.U./ml) and had an activity such that 1 ml of a 1:250 dilution produced coagulation in 120 $\pm$ 5 revolutions in 25 ml of Berridge (5) substrate at 30 C.

Activities of unknown samples were calculated.

$$R.U./ml = 100 \frac{Tk}{Tuk} \times \frac{Dil\ uk}{Dil\ k}$$

where R. U. = rennin units

Tk = coagulation time of standard

Tuk = coagulation time of unknown

Dil uk = dilution of unknown

Dil k = dilution of known (always 250)

Calculations

Activities for all samples, including controls, were expressed as R.U./ml. Activity per milliliter of extract was multiplied by the volume to obtain total activity in the extract. The sum of rennin units per extraction gave the total rennin units recovered. Since 100 g wet tissue were used, the total rennin units recovered was divided by 100 g to give rennin units per gram of tissue. Activities were expressed as a percentage of unheated control samples.

Statistical analysis

Standard deviation of enzyme activities was calculated by the procedure of Ostle (20).

---

* Supplied by Dairyland Food Laboratories, Waukesha, Wisconsin

RESULTS

Effect of heating fresh vell tissue before drying on the recovery of activity in rennet extracts

Figures 2 through 5 show the effect of heating time and temperature on extractable enzyme activity recovered from vell tissue that had been heated before drying, then dried to 4% moisture. Figure 2 shows the effect of heating fresh vell tissue to 49, 54, 57, and 60 C for 15 min on recovery of enzyme activity. A treatment of 49 C gave a 26% increase in extractable enzyme activity over the unheated control samples. A slight decrease in extracted activity occurred when the tissue was heated to 54 C, but a substantial loss was sustained when the fresh tissue was heated to 57 and 60 C. The loss in activity at 57 and 60 C amounted to close to 90% of the control. Figure 3 represents the effect of heating fresh vell tissue to 49, 54, 57 and 60 C for 30 min on recovery of enzyme activity. There was a gradual decrease in activity recovered as the heating temperature of the tissue was increased from 54 C to 60 C. A 16% loss in activity occurred at 54 C while there was 100% loss at 60 C. Figure 4 indicates the effect of heating fresh vell tissue to 49, 54, 57 and 60 C for 45 min on recoverable activity. The 49 C treatment showed about the same activity as unheated samples. There was 22% loss in activity at 54 C but nearly 100% loss at 57 C. Figure 5 illustrates the effect of heating fresh vell tissue to 49, 54, 57 and 60 C for 60 min on recovery of enzyme activity. Heating tissue to 49 C produced a higher activity than unheated controls although, at 54 C there was a loss of 31%.

Standard deviations for 49 and 54 C were calculated from six determinations made for each treatment. Two determinations were made for 57 and 60 C readings, but no standard deviations were calculated.

Figure 2. Effect of heating fresh vell tissue for 15 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)

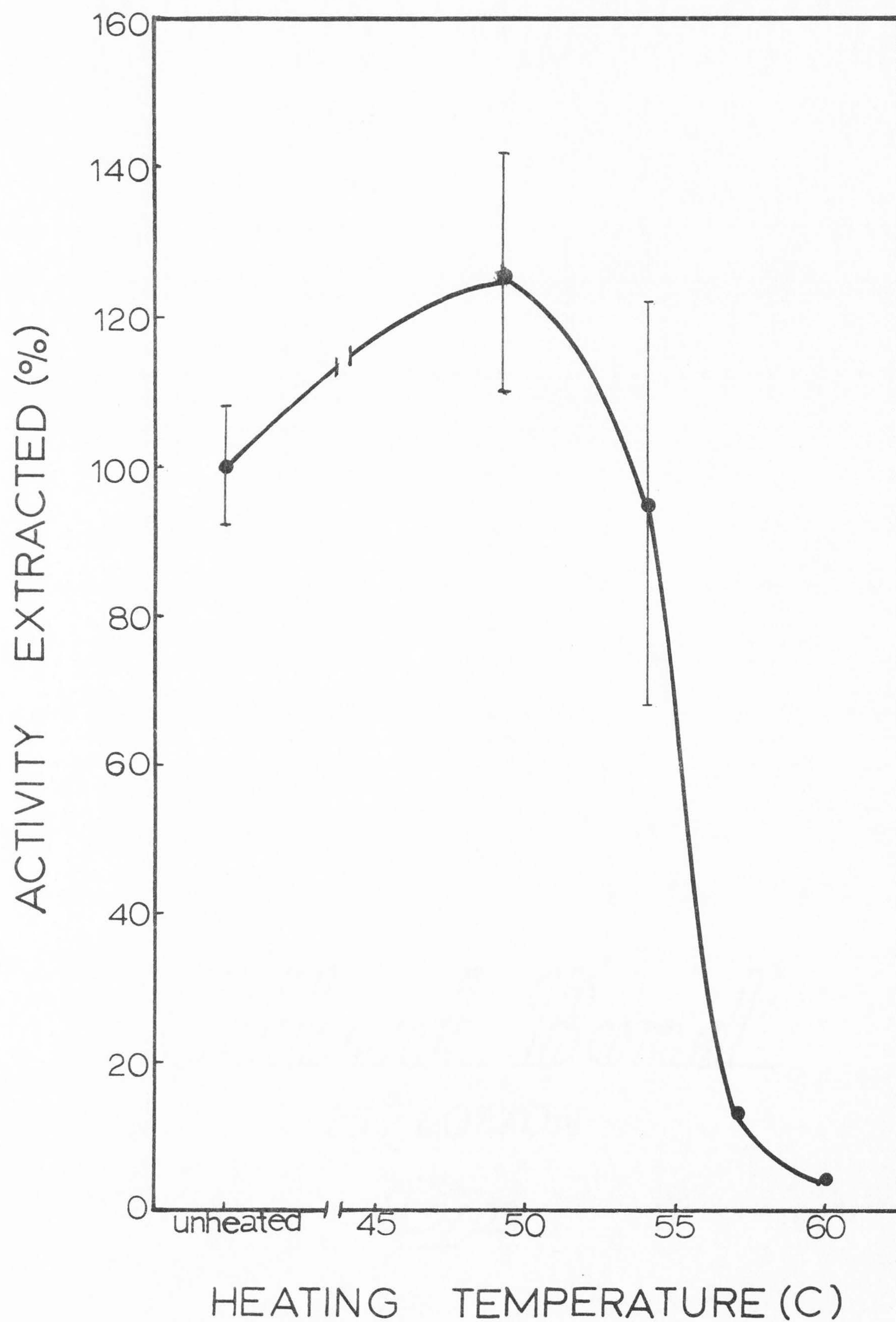

160
140
120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 3. Effect of heating fresh vell tissue for 30 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)

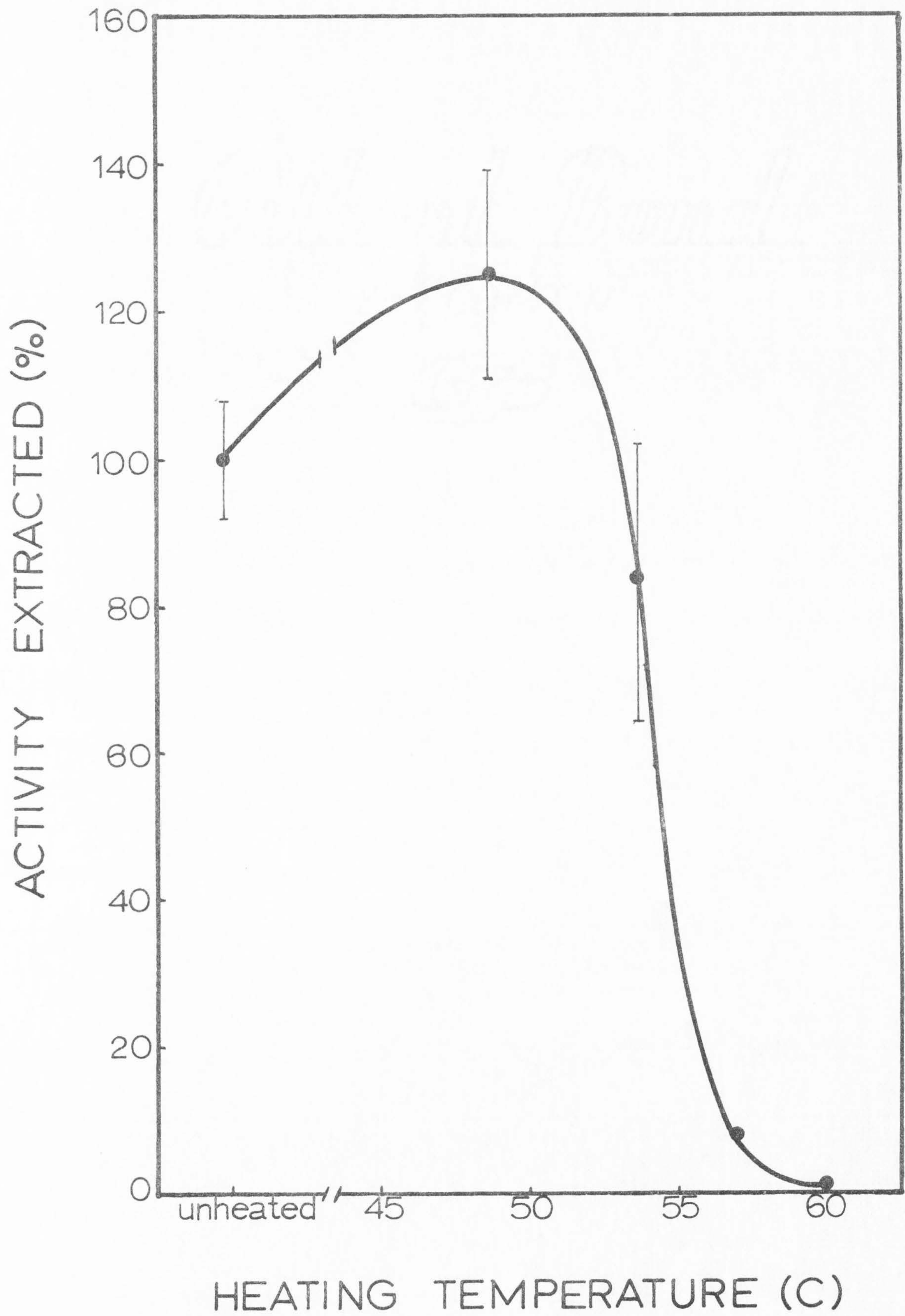

160
140
120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 4. Effect of heating fresh vell tissue for 45 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)

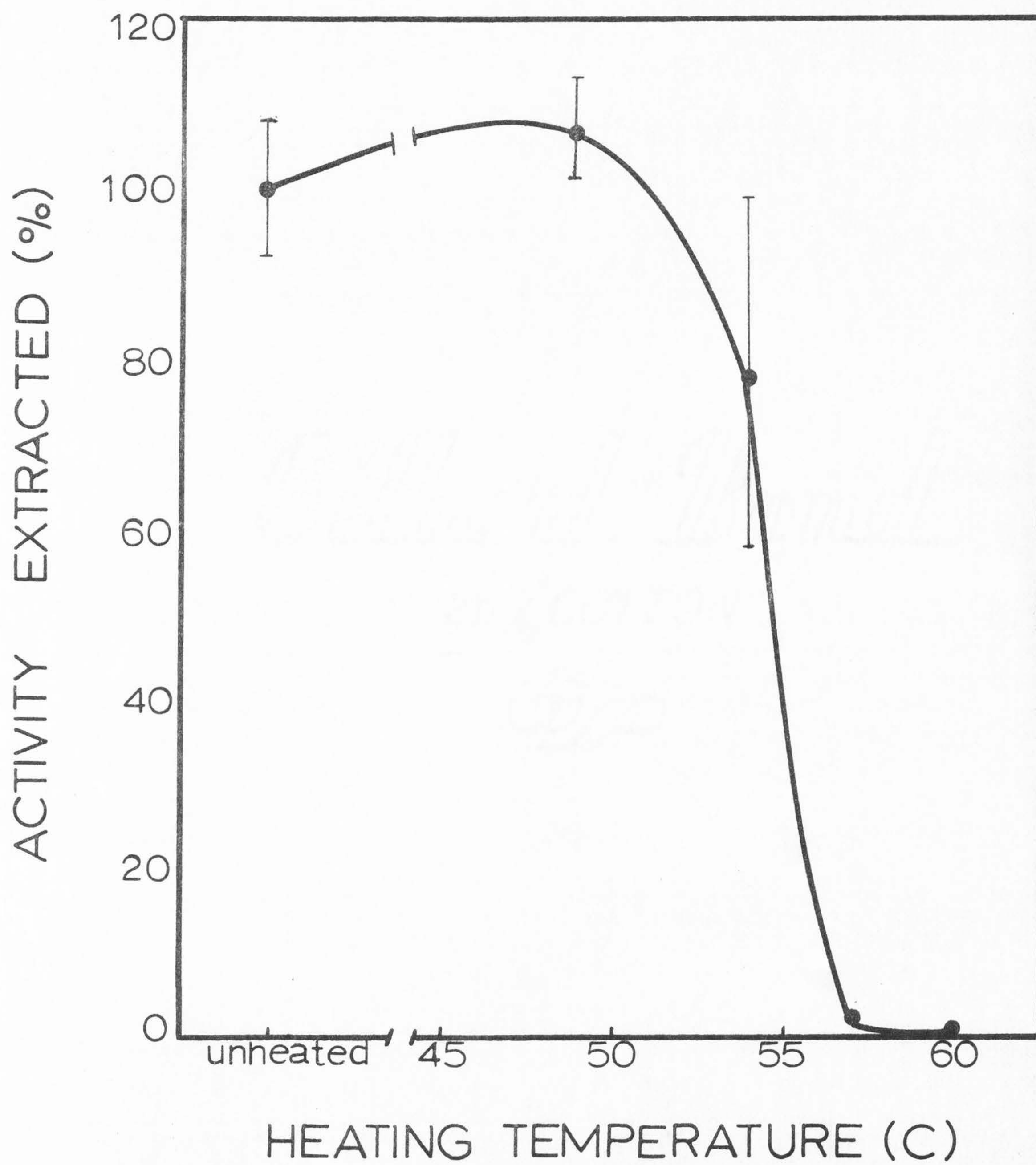

120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 5. Effect of heating fresh vell tissue for 60 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)

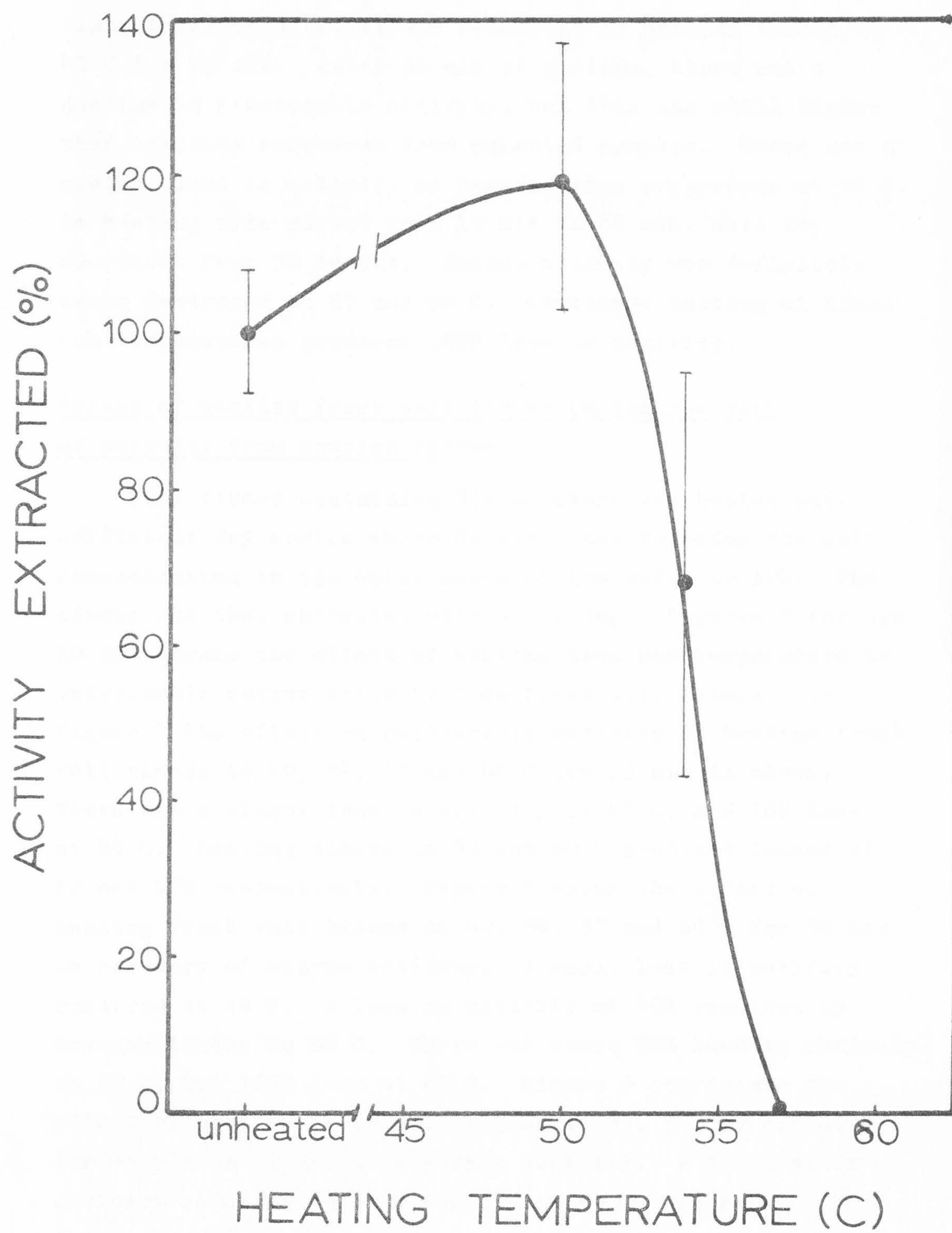
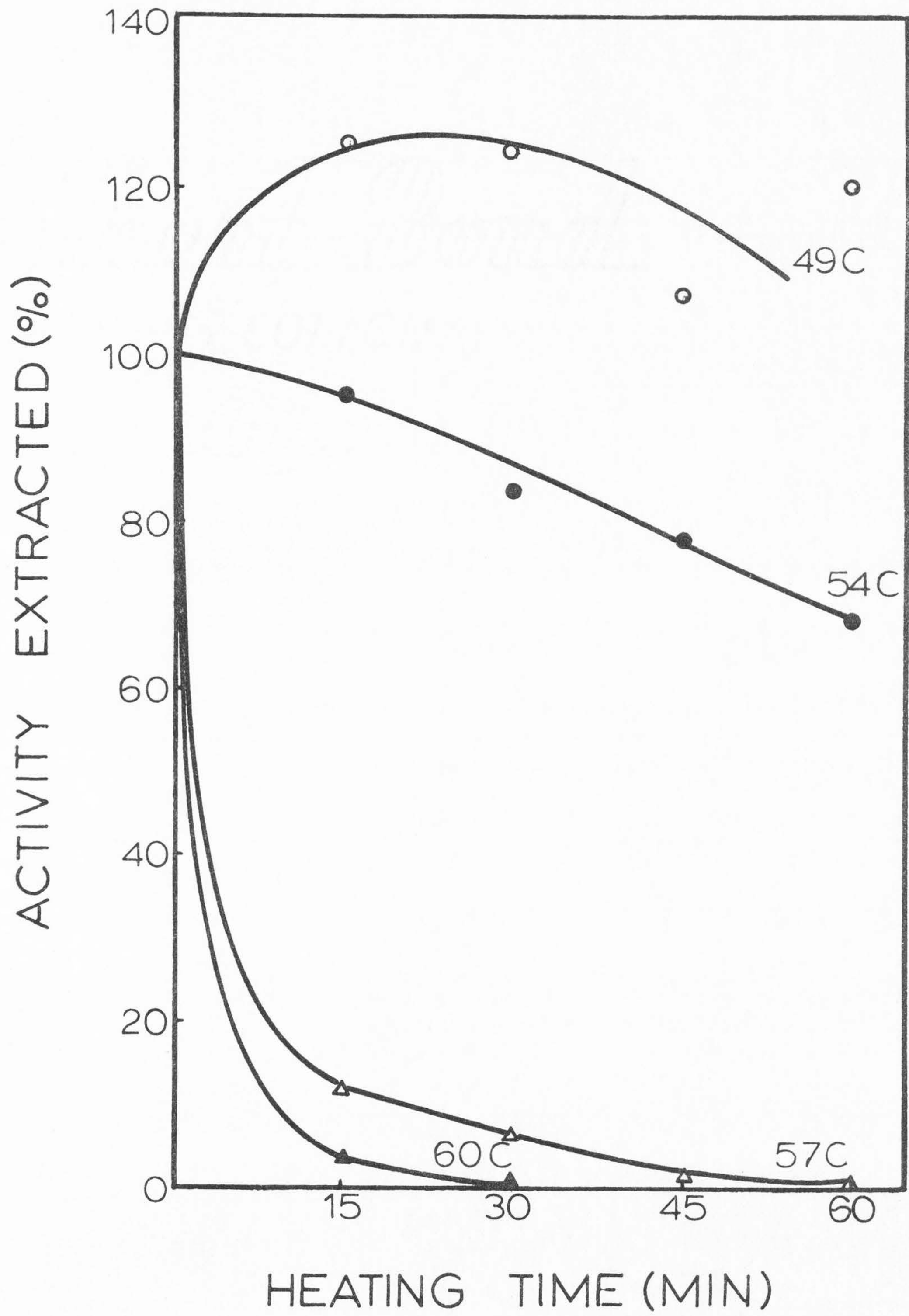

140
120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 6 summarizes the enzyme activity obtained after heating vell tissue to various temperatures before drying. Highest enzyme activity was recovered by heating tissue to 49 C for 30 min. After 30 min of heating, there was a decline in recoverable activity, but this was still higher than activity recovered from unheated samples. There was a gradual loss in activity as heating time progressed at 54 C. As heating time varied from 15 min to 60 min, activity decreased from 5% to 31%. Enzyme activity was definitely being destroyed at 57 and 60 C. Continued heating at these two temperatures produced 100% loss in activity.

Effect of heating fresh vell tissue on the recovery of activity from undried tissue

Vell tissue containing 83% moisture was heated and sufficient dry sodium chloride was added to bring the salt concentration in the water phase of the vells to 10%. The tissue was then extracted without drying. Figures 7 through 10 illustrate the effect of heating time and temperature on extractable enzyme activity from fresh vell tissue. In Figure 7 the effect on recoverable activity of heating fresh vell tissue to 49, 54, 57 and 60 C for 15 min is shown. There was a slight loss in activity at 49 C, and 38% loss at 54 C. Heating tissue to 57 and 60 C produced losses of 82 and 97% respectively. Figure 8 shows the effect of heating fresh vell tissue to 49, 54, 57 and 60 C for 30 min on recovery of enzyme activity. A small loss in activity occurred at 49 C. A loss in activity of 49% resulted by heating tissue to 54 C. There was about 90% loss in activity at 57 C, but 100% loss at 60 C. Figure 9 represents the effect of heating fresh vell tissue to 49, 54, 57 and 60 C for 45 min on recoverable enzyme activity. A 15% loss in activity occurred at 49 C and 52% at 54 C. There was nearly 100% loss in activity at 57 C. Figure 10 shows the effect of heating fresh vell tissue to 49, 54, 57 and 60 C

Figure 6. Effect of heating fresh vell tissue before drying on the recovery of activity in rennet extracts. (Activity extracted from unheated samples is 100%.)



ACTIVITY EXTRACTED (%)
140
120
100
80
60
40
20
0
49C
54C
60C
57C
15
30
45
60
HEATING TIME (MIN)

Figure 7. Effect of heating fresh vell tissue for 15 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)

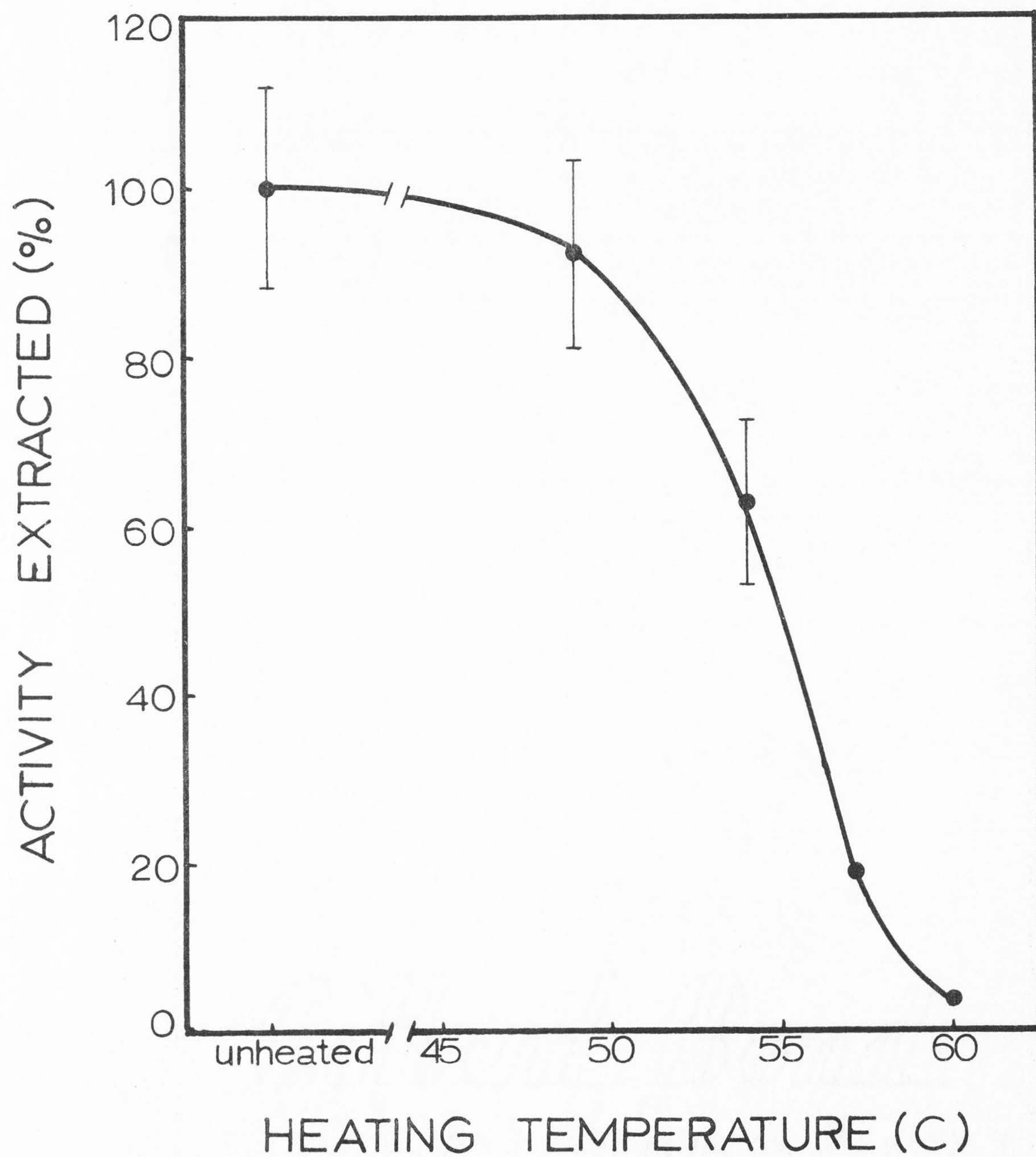
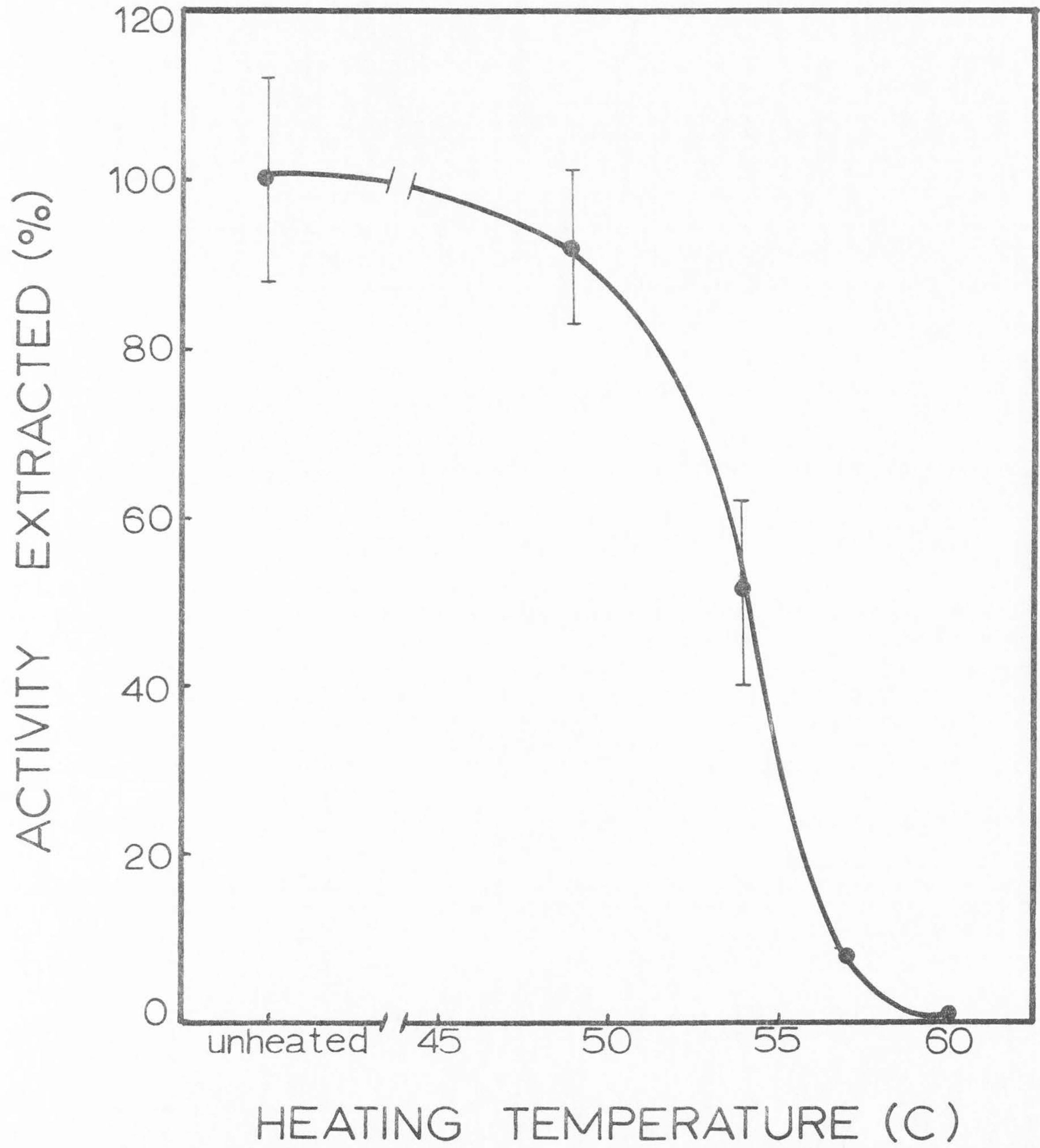

ACTIVITY EXTRACTED (%)
120
100
80
60
40
20
0
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 8. Effect of heating fresh vell tissue for 30 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)



120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 9. Effect of heating fresh vell tissue for 45 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)

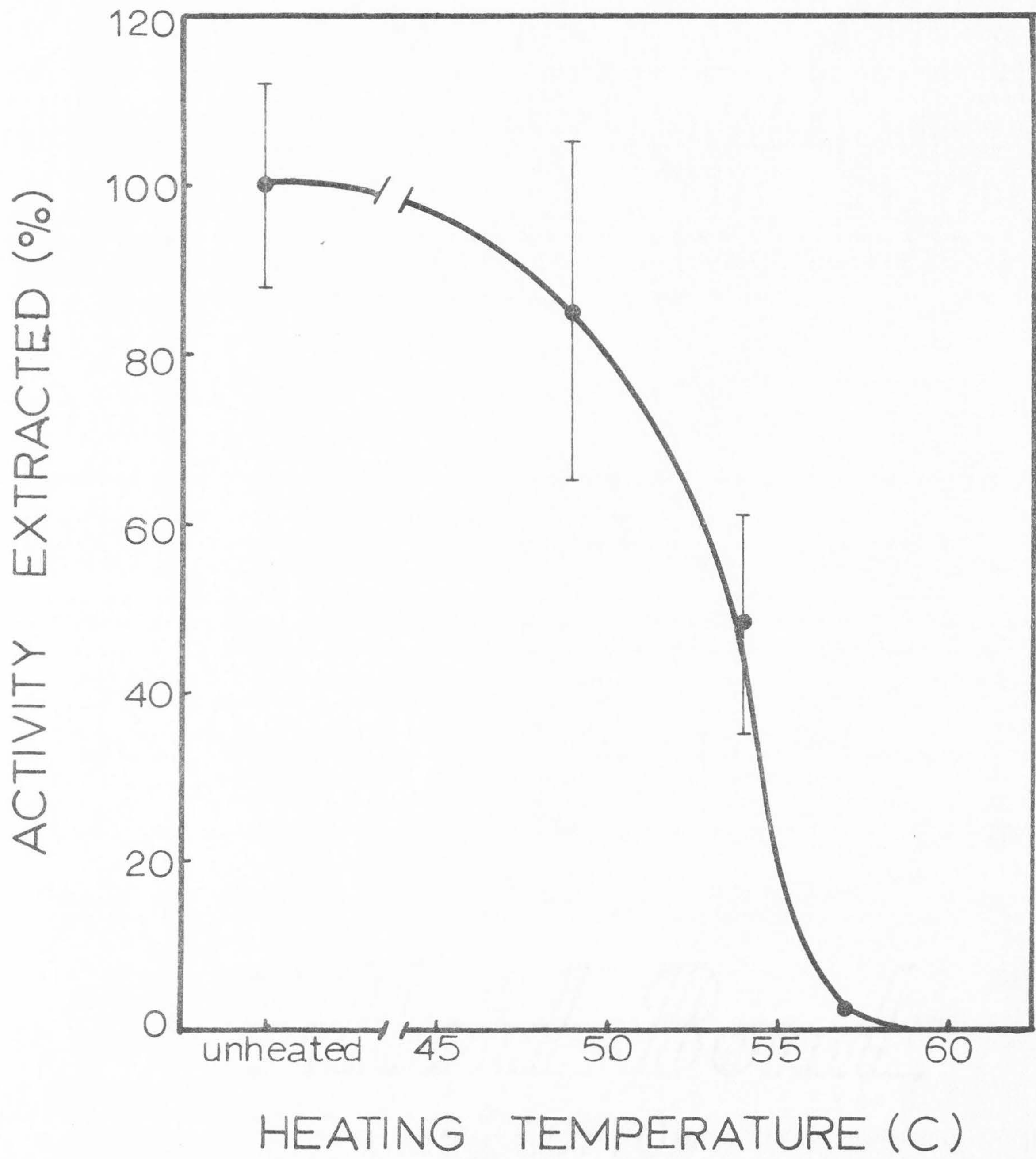
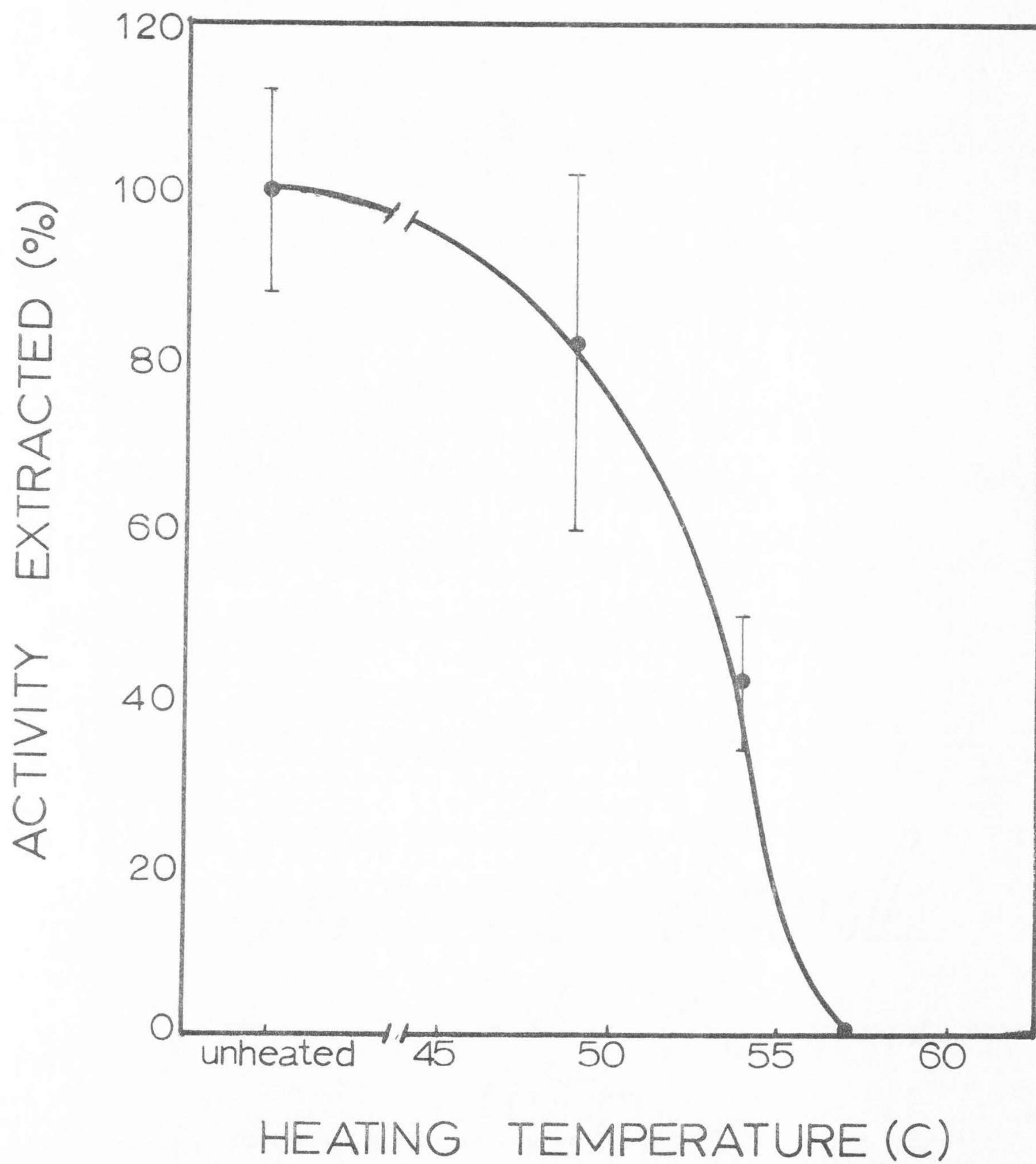

120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 10. Effect of heating fresh vell tissue for 60 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)


120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

for 60 min on recovery of enzyme activity. Activity loss of 18% resulted by heating tissue to 49 C while 58% loss occurred at 54 C.

Figure 11 is a summary of the enzyme activity obtained after heating fresh tissue to 49, 54, 57 and 60 C for various lengths of time. There was a loss in activity at all temperatures tested. The 49 C treatment produced a rather gradual loss in activity with length of heating. Activity losses for 15 min and 30 min were about the same. There was a slight decrease in activity after 45 min, but no significant further decrease at 60 min. A pronounced decrease in activity resulted at 54 C. At this temperature each heating time produced a steady decrease in activity. Heating tissue to 57 and 60 C had a detrimental effect on activity even for only 15 min.

Four determinations were made for each treatment at 49 C and six determinations at 54 C. No standard deviations were calculated for 57 and 60 C treatments because only two determinations were made for each point.

<u>Effect of heating fresh salted vell tissue on the recovery of activity from undried tissue</u>

Fresh vell tissue containing 83% moisture had enough dry sodium chloride added to give a 10% salt concentration in the aqueous phase of the tissue. The effect on recoverable activity of heating fresh salted vell tissue is shown in Figures 12 through 15. Figure 12 shows the effect of heating fresh salted tissue to 49, 54, 57 and 60 C for 15 min on recoverable activity. There was an insignificant change in activity by heating tissue to 49 C. However, there was almost 20% loss at 54 C. A 35% loss in activity occurred at 57 C while 73% loss resulted at 60 C. Figure 12 illustrates the effect of heating fresh salted vell tissue to 49, 54, 57 and 60 C for 30 min. There was a slight decrease in activity at 49 C while about 20% at 54 C. At 57 and 60 C there were

Figure 11. Effect of heating fresh vell tissue on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)

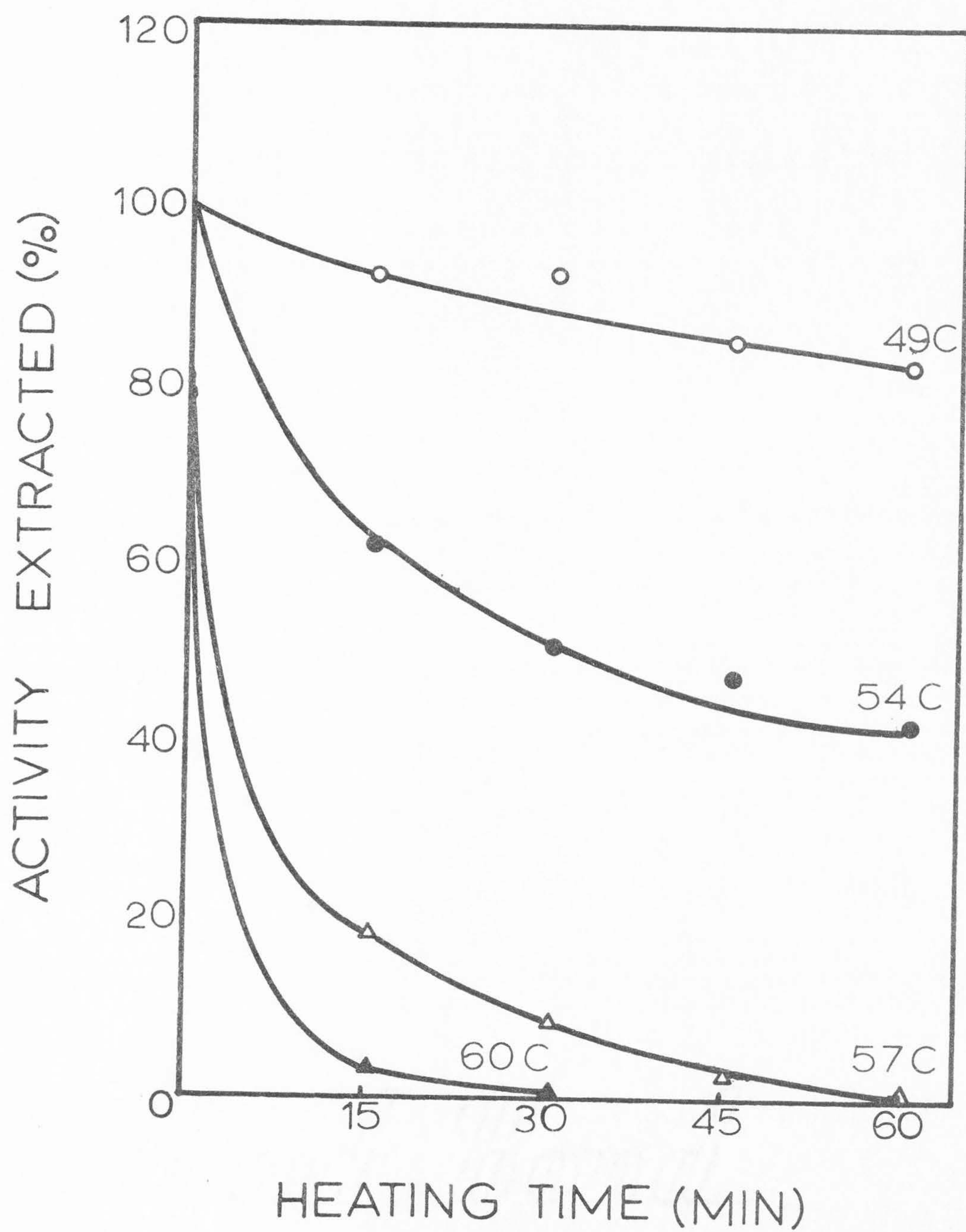
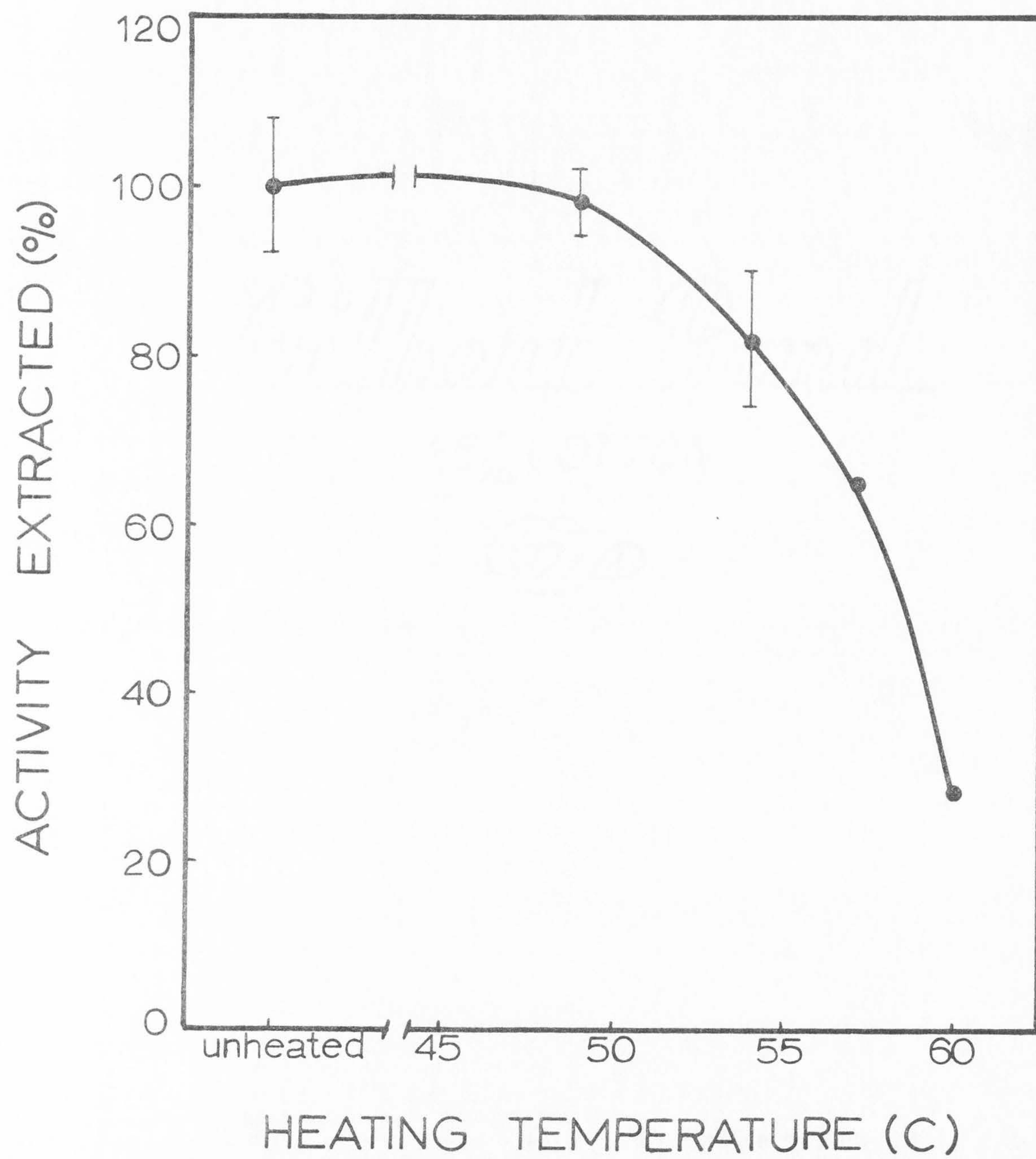
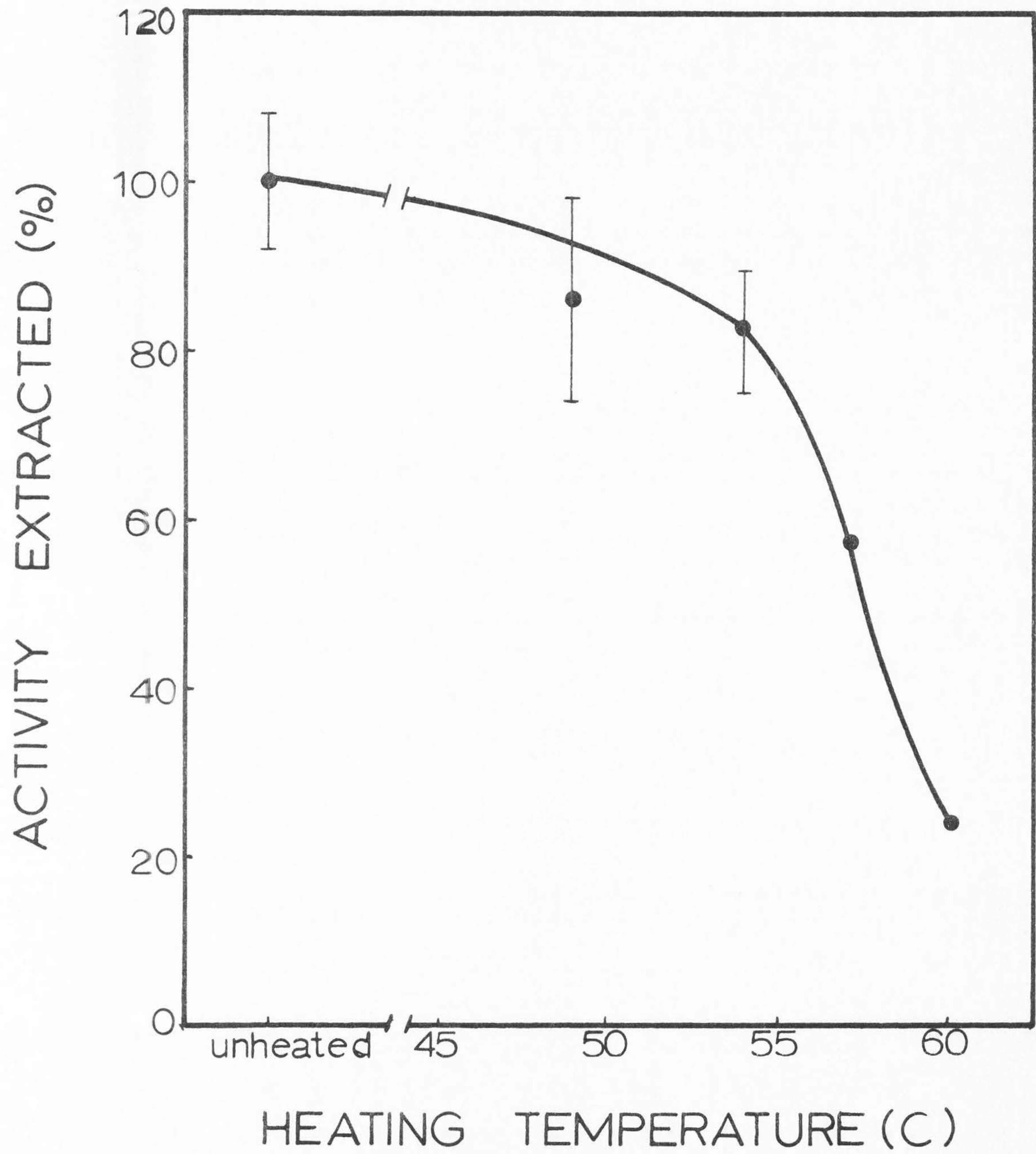

120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
49C
54 C
60 C
57 C
15
30
45
60
HEATING TIME (MIN)

Figure 12. Effect of heating fresh salted vell tissue for 15 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)



120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 13. Effect of heating fresh salted vell tissue for 30 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)



120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 14. Effect of heating fresh salted vell tissue for 45 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)

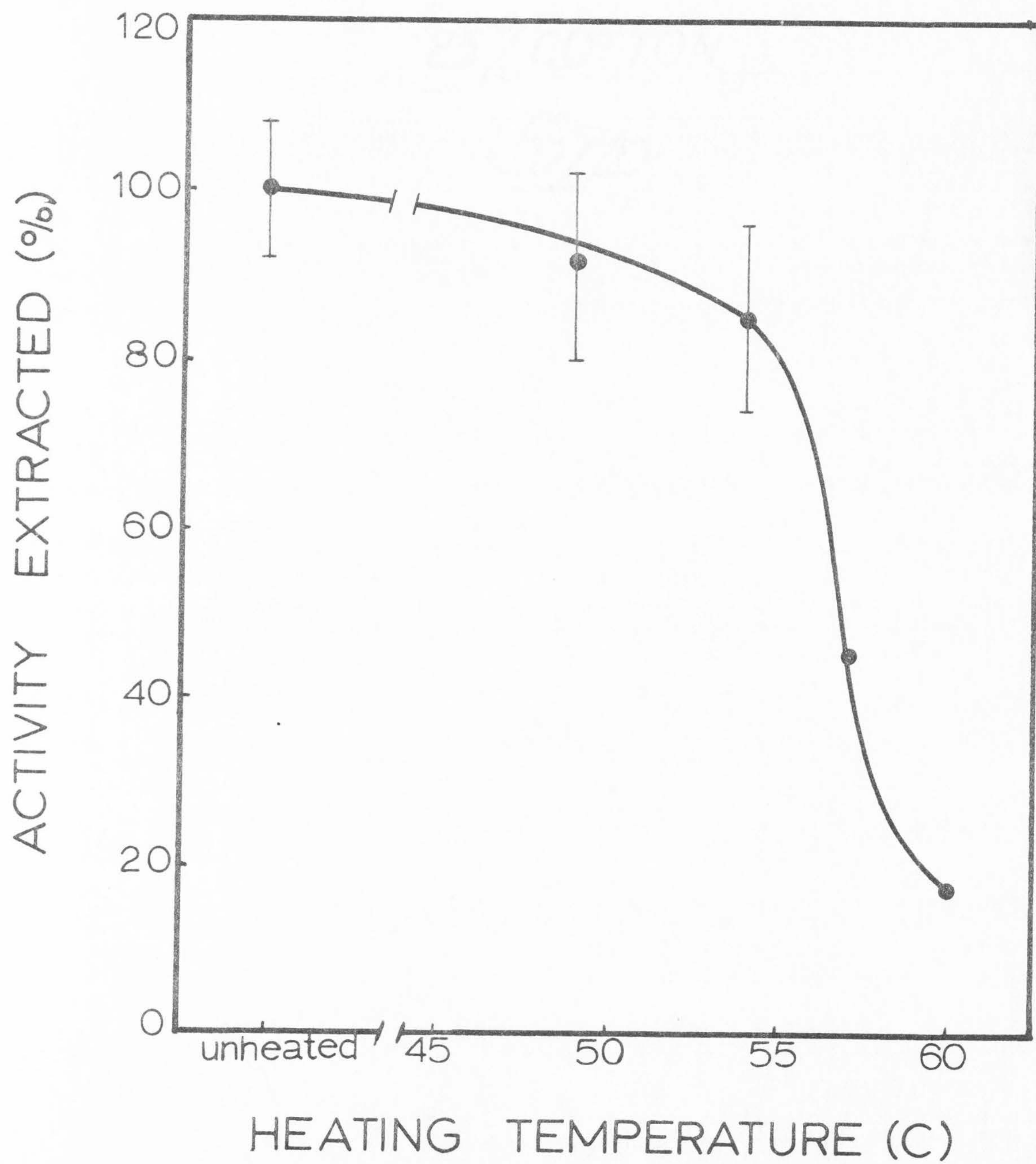
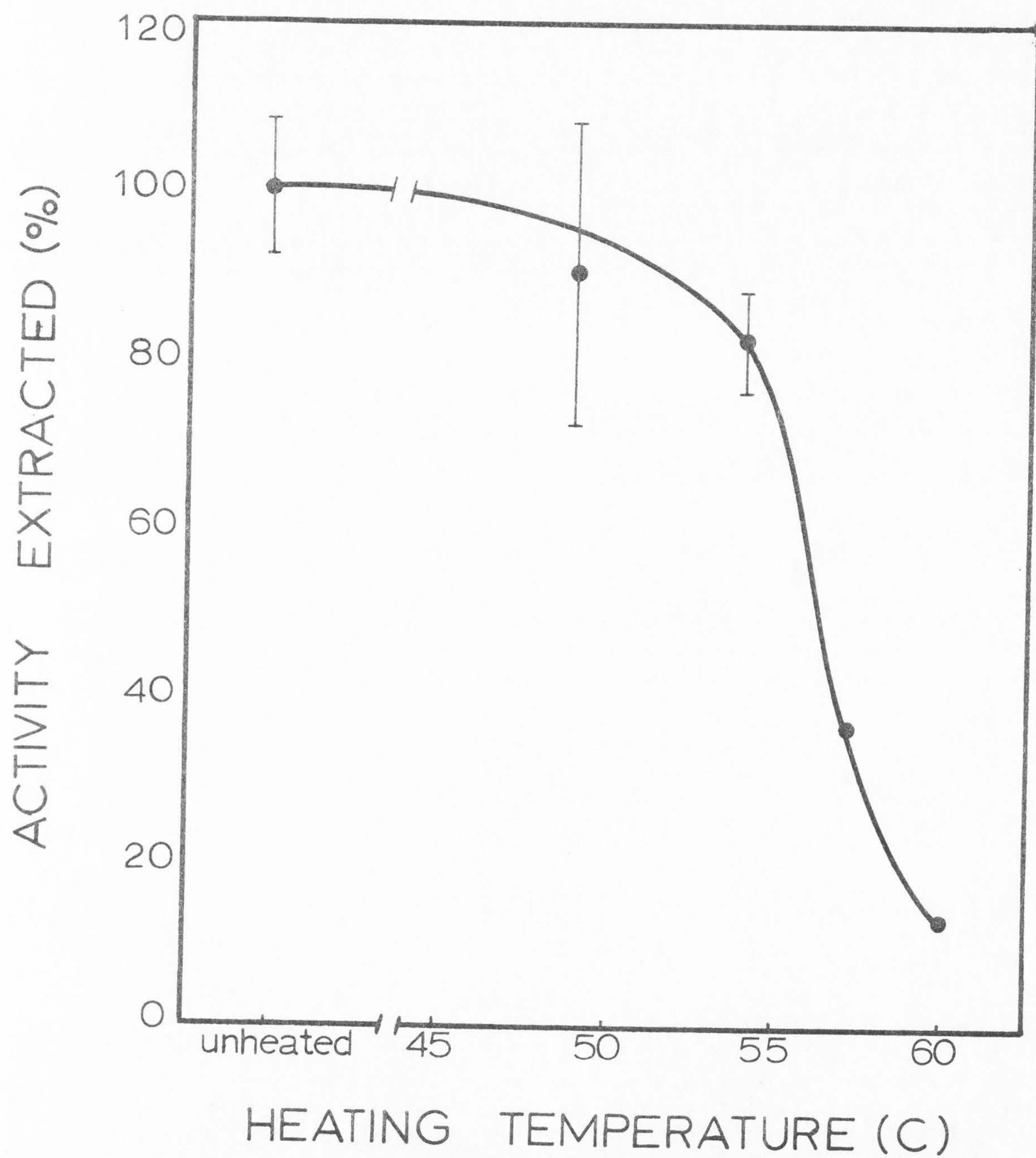

120
100
80
60
40
20
0
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 15. Effect of heating fresh salted vell tissue for 60 min on recovery of activity from undried tissue. (Activity extracted from unheated samples is 100%.)



ACTIVITY EXTRACTED (%)
120
100
80
60
40
20
0
unheated
45
50
55
60
HEATING TEMPERATURE (C)

losses of 44 and 77% respectively. Figure 14 shows the effect of heating fresh salted vell tissue to 49, 54, 57 and 60 C for 45 min. About 10% loss in activity occurred at 49 C and 15% loss at 54 C. A loss in activity of 55% occurred at 57 C and 83% at 60 C. Figure 15 represents the effect of heating fresh salted vell tissue to 49, 54, 57 and 60 C for 60 min on recoverable activity. Nearly 10% loss resulted from heating tissue to 49 C while about 20% occurred at 54 C. There was a 65% loss in activity at 57 C and 87% loss at 60 C.

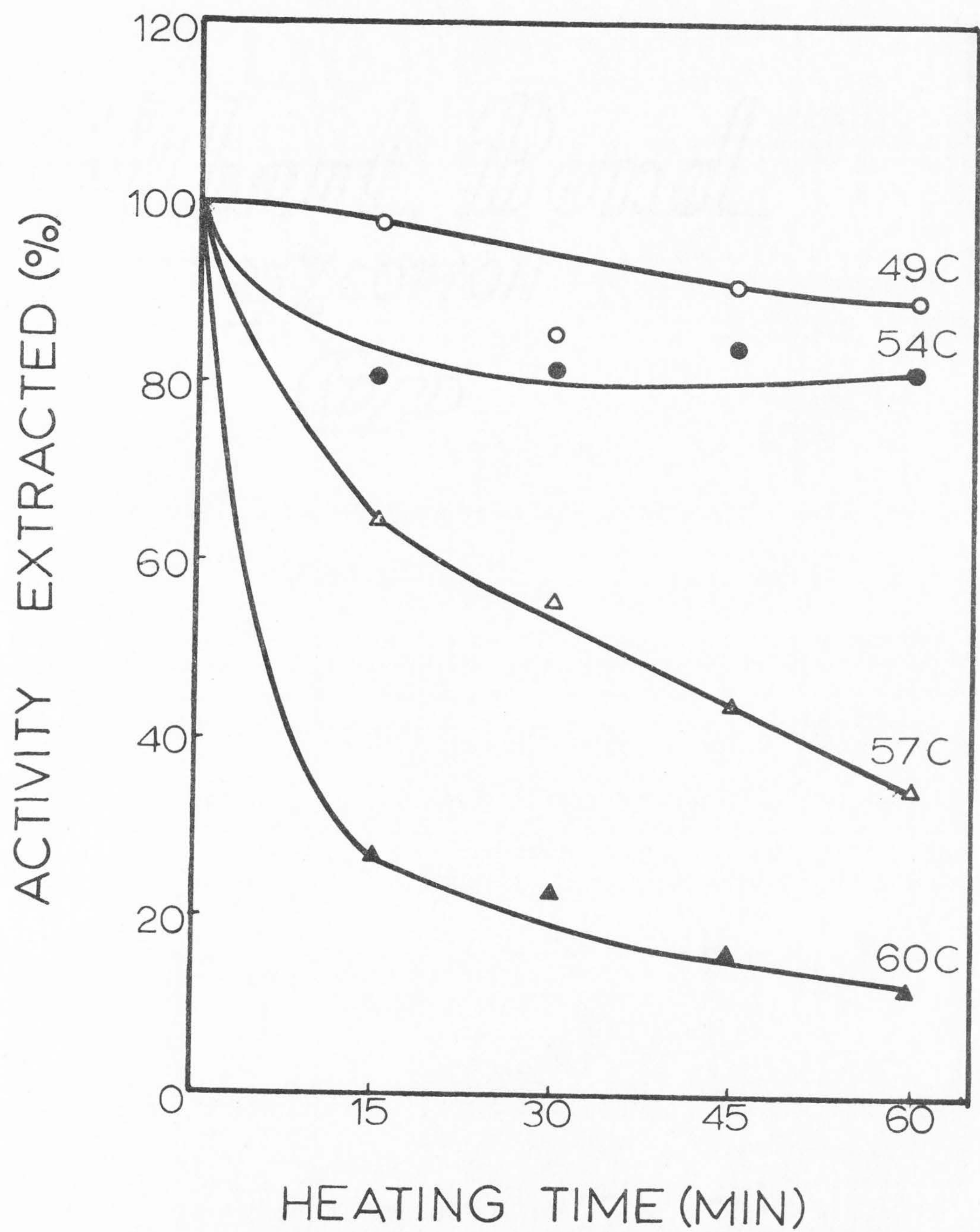
Figure 16 presents a summary of the effect on recoverable activity of heating fresh salted vells to 49, 54, 57 and 60 C for various lengths of time.

Effect of heating fresh salted vell tissue before drying on the recovery of activity in rennet extracts

Results from the above experiments suggested a separate experiment. This time fresh salted tissue was heated and then dried before being extracted. Dry sodium chloride was added to wet tissue such that a 10% salt concentration existed in the aqueous phase on the vells. Fresh salted vells had the same heating schedule used in the above experiments. Since salt was contained in the dried tissue, a 5.4% sodium chloride extraction solution was initially used to give an overall salt concentration of 10% in the extraction solution.

Figures 17 through 20 show the effect of heating time and temperature on extractable enzyme activity recovered from fresh salted tissue that had been heated and dried to 4% moisture. Figure 17 represents the effect of heating fresh salted vell tissue to 49, 54, 57 and 60 C for 15 min on recovery of enzyme activity. An increase in activity of 23% occurred at 49 C. A further rise in temperature to 54 C gave an even higher recovery of enzyme. This amounted to a 37% increase in activity. At 57 C there was still an

Figure 16. Effect of heating fresh salted vell tissue on recovery of activity in rennet extracts. (Activity extracted from unheated samples is 100%.)



ACTIVITY EXTRACTED (%)
120
100
80
60
40
20
0
15
30
45
60
HEATING TIME (MIN)
49C
54C
57C
60C

Figure 17. Effect of heating fresh salted vell tissue for 15 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)

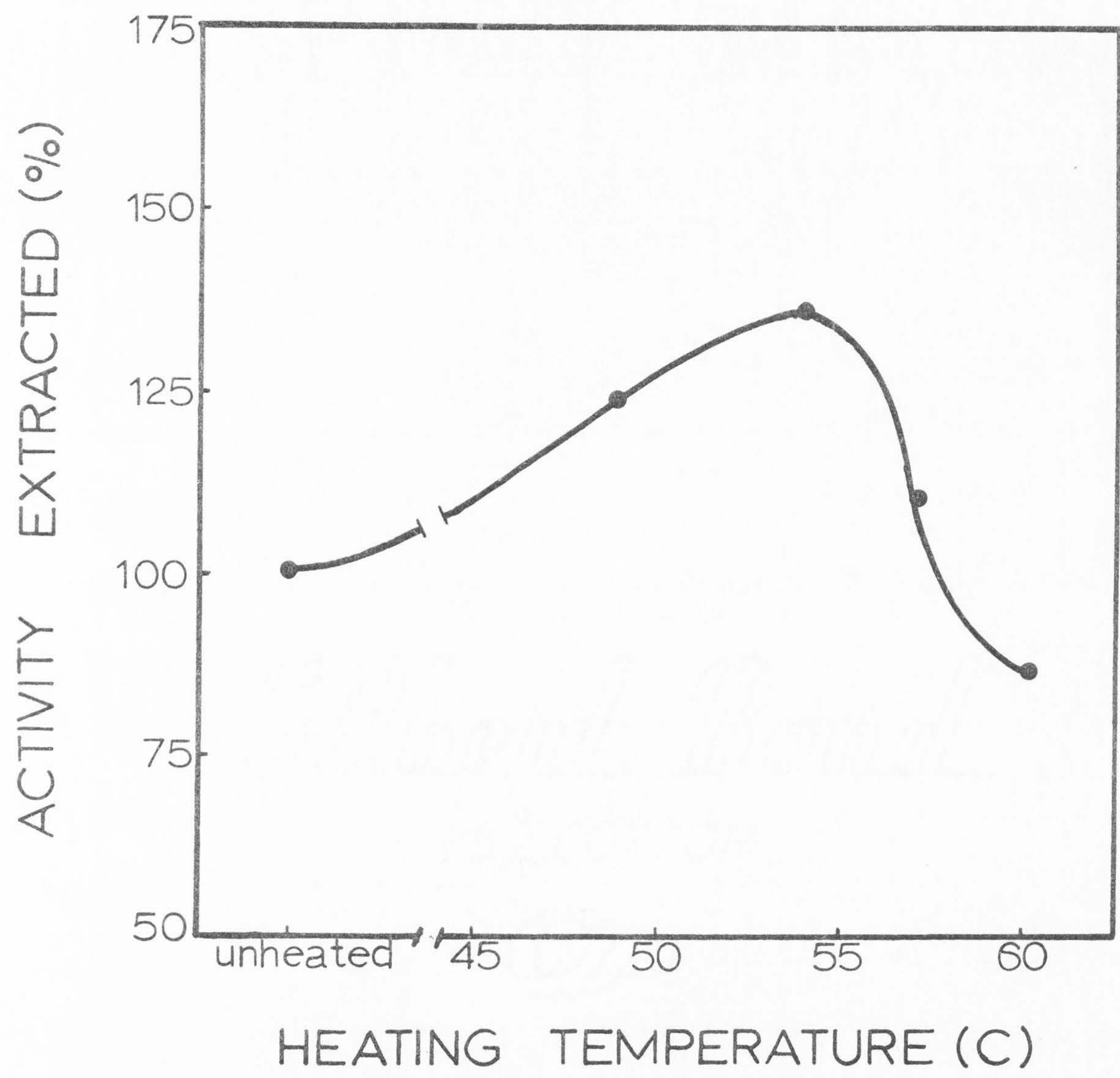

175
150
125
100
75
50
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 18. Effect of heating fresh salted vell tissue for 30 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)

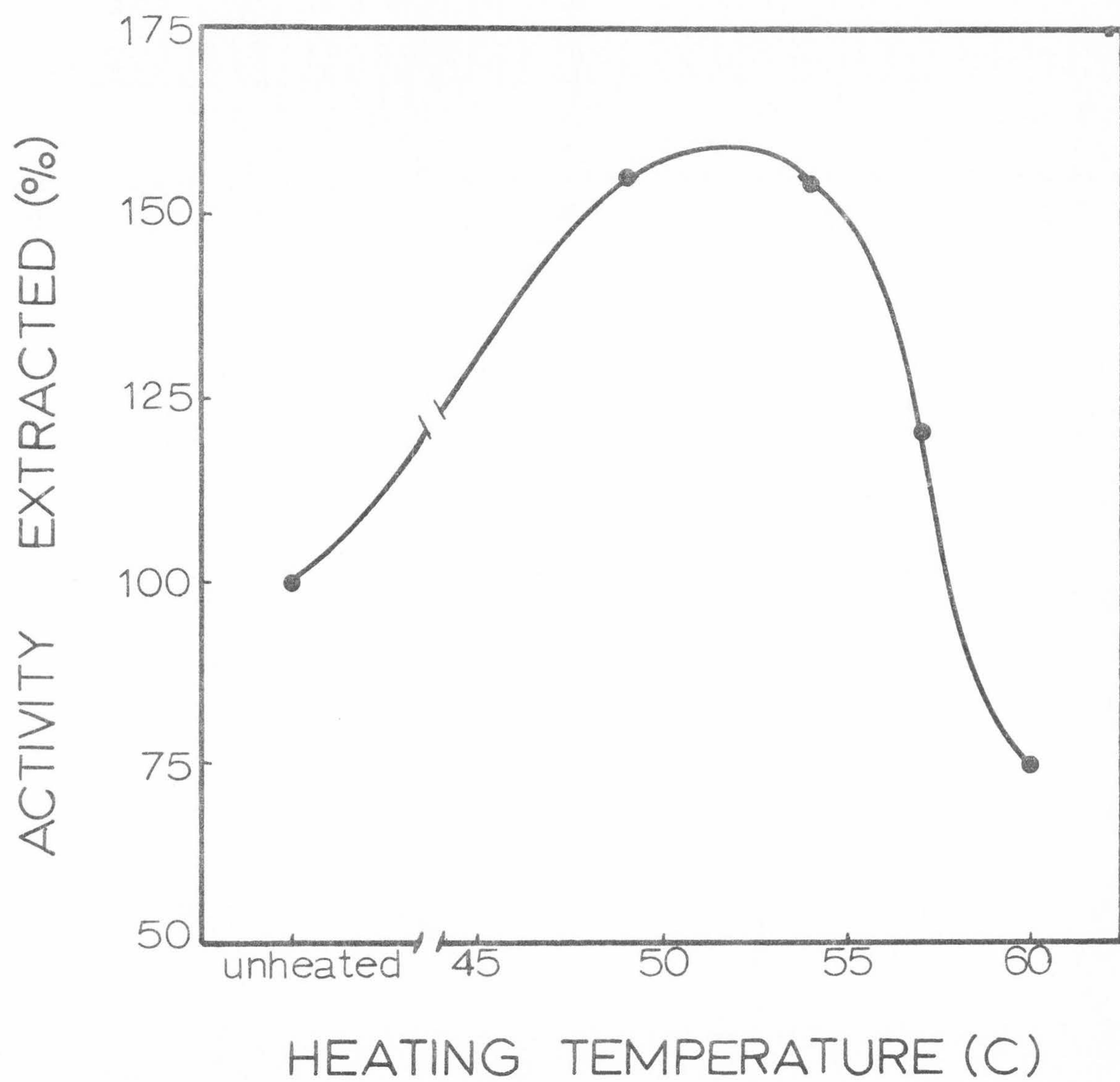

175
150
125
100
75
50
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 19. Effect of heating fresh salted vell tissue for 45 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)

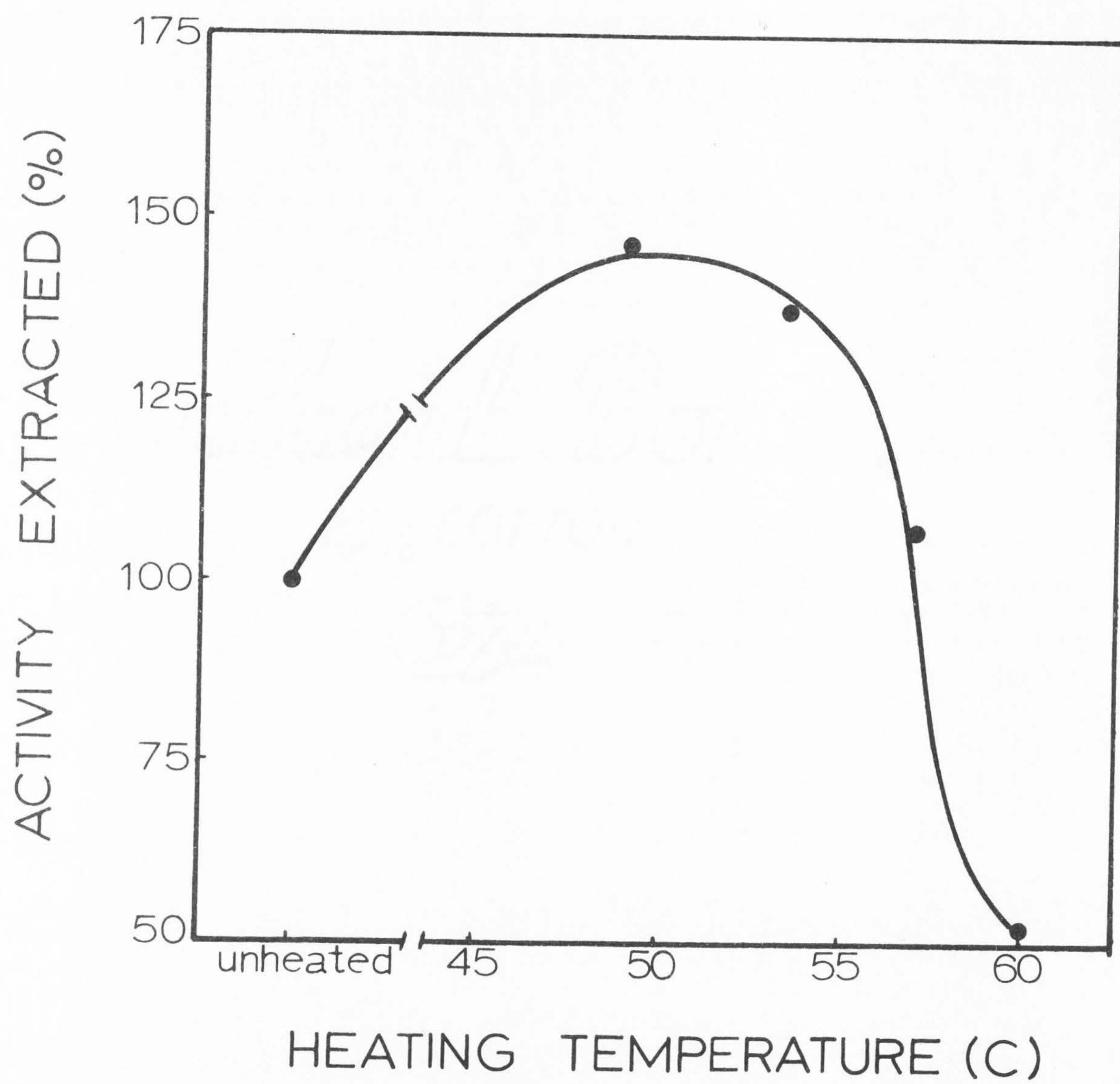
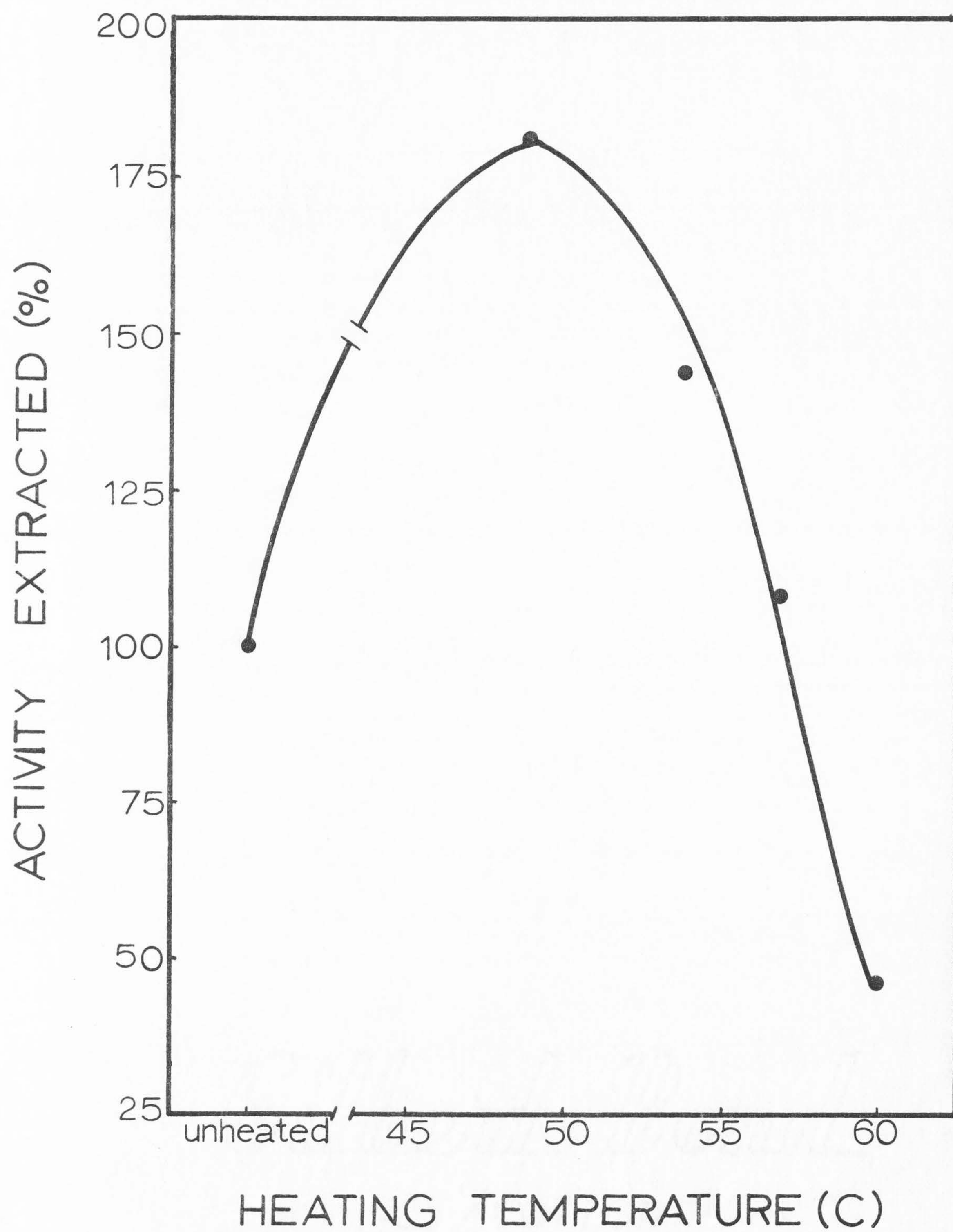

ACTIVITY EXTRACTED (%)
175
150
125
100
75
50
unheated
45
50
55
60
HEATING TEMPERATURE (C)

Figure 20. Effect of heating fresh salted vell tissue for 60 min on extraction of activity from dried vell tissue. (Activity extracted from unheated samples is 100%.)


200
175
150
125
100
75
50
25
ACTIVITY EXTRACTED (%)
unheated
45
50
55
60
HEATING TEMPERATURE (C)

increase in activity (10%), but there was a loss of 14% at 60 C. Figure 18 indicates the effect of heating fresh salted vell tissue to 49, 54, 57 and 60 C for 30 min on recovery of enzyme activity. Treatments of 49 and 54 C showed a similar increase in activity of about 55% over unheated samples. An increase of 21% in activity was noted at 57 C and a loss in activity of 26% at 60 C. Figure 19 represents the effect of heating fresh salted vell tissue to 49, 54, 57 and 60 C for 45 min on recovery of enzyme activity. There was an increase in activity of 47% at 49 C while at 54 C there was 38% increase. At 57 C an increase over unheated samples of 7% occurred. A decrease of 46% in activity resulted at 60 C. Figure 20 illustrates the effect of heating fresh salted vell tissue to 49, 54, 57 and 60 C for 60 min on recovery of enzyme activity. As much as 82% more activity was recovered by heating salted tissue to 49 C than by not heating them at all. At 54 C an increase in activity of 44% was recovered while 57 C showed 8% increase. A loss of 54% resulted at 60 C.

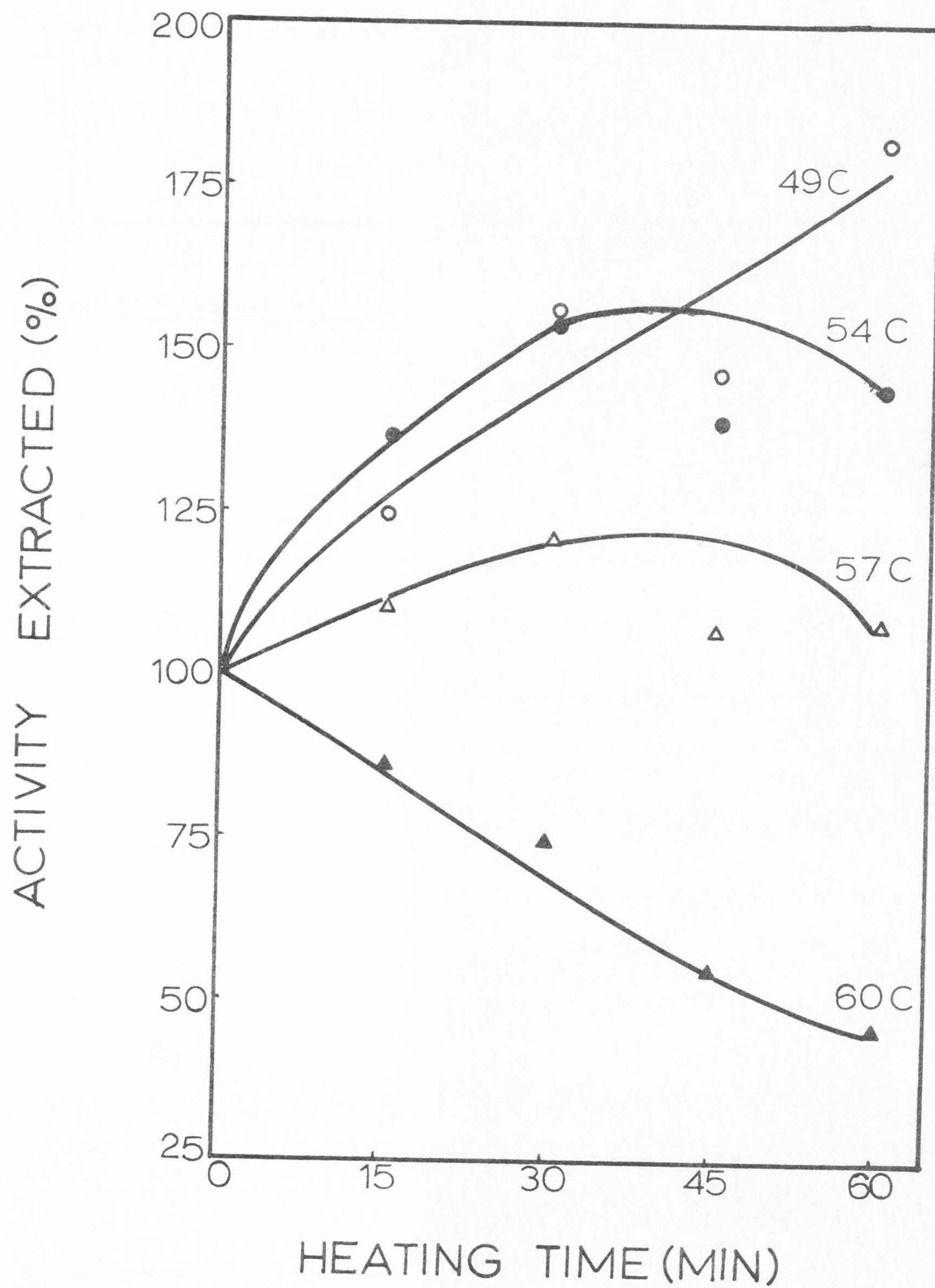
Figure 21 summarizes the effect on recoverable enzyme activity of heating fresh salted vells to 49, 54, 57 and 60 C for various times prior to drying. There was an increase in recoverable enzyme activity when fresh salted vells were heated to 49, 54 and 57 C. Recoverable activity continued to increase with length of heating at 49 C. Both 54 and 57 C treatments brought recovery of increased activity until 45 min of heating was achieved. After the 45 min period there was a slight decrease in activity. The 60 C treatment produced a loss in activity for each heating time.

Two determinations were made for each treatment with no standard deviations calculated.

Effect of heating fresh vell tissue before drying on the average volume of extract per extraction

Table 1 shows the effect on extract volume of heating fresh vell tissue before drying to 4% moisture. Heated

Figure 21. Effect of heating fresh salted vell tissue before drying on the recovery of activity in rennet extracts. (Activity extracted from unheated samples is 100%.)



200
175
150
125
100
75
50
25
ACTIVITY EXTRACTED (%)
49C
54 C
57C
60C
0
15
30
45
60
HEATING TIME (MIN)

samples gave a higher extract volume than unheated samples. Temperatures of 57 and 60 C yielded higher extract volumes than temperatures of 49 C or 54 C.

Table 1. Effect of heating fresh vell tissue before drying on the average volume of extract per extraction for 100 g of tissue

Heating temperature	Heating time	Average volume of extract per extraction
(C)	(min)	(ml)
49	0	77
	15	87
	30	88
	45	94
	60	98
54	0	70
	15	90
	30	94
	45	98
	60	96
57	0	96
	15	114
	30	112
	45	111
	60	111
60	0	78
	15	109
	30	107
	45	109
	60	115

<u>Effect of heating fresh vell tissue on the average volume of extract per extraction of undried vells</u>

Table 2 represents the effect on extract volume of heating fresh vell tissue. A marked increase in extract volume occurred at 57 and 60 C. Heating to 54 C gradually increased the amount of extract with length of heating. Vell tissue heated to 49 C produced a small increase in extract volume with length of heating.

Table 2. Effect of heating fresh vell tissue on the average volume of extract per extraction for 100 g of undried tissue

Heating temperature	Heating time	Average volume of extract per extraction
(C)	(min)	(ml)
49	0	134
	15	137
	30	146
	45	151
	60	152
54	0	112
	15	125
	30	133
	45	137
	60	139
57	0	112
	15	146
	30	174
	45	166
	60	161
60	0	124
	15	166
	30	173

Table 2. Continued

Heating temperature	Heating time	Average volume of extract per extraction
(C)	(min)	(ml)
60	45	171
	60	174

Effect of heating fresh salted vell tissue on the average volume of extract per extraction of undried vells

The effect on extract volume of heating fresh salted vell tissue is shown in Table 3. Extract volume was not improved by heating at 49 C. There was an increase in the volume at 54 C for 15 min of heating, but thereafter volume remained unchanged with longer heating times. Samples heated to 57 and 60 C showed a slight increase in extract volume with length of heating.

Table 3. Effect of heating fresh salted vell tissue on the average volume of extract per extraction for 100 g of undried tissue

Heating temperature	Heating time	Average volume of extract per extraction
(C)	(min)	(ml)
49	0	127
	15	117
	30	111
	45	117
	60	117

Table 3. Continued

Heading temperature	Heating time	Average volume of extract per extraction
(C)	(min)	(ml)
54	0	97
	15	120
	30	121
	45	120
	60	121
57	0	112
	15	126
	30	135
	45	131
	60	140
60	0	121
	15	136
	30	139
	45	143
	60	140

Effect of heating fresh salted vell tissue before drying on the average volume of extract per extraction

Table 4 shows the effect on extract volume of heating salted tissue and drying to 4% moisture. Extract volume did not increase to any degree for salted vells heated at 49 C. However, extract volume increased slightly with length of heating for salted vells heated at 54 C before drying. Heating salted tissue to 57 and 60 C produced a higher extract volume than unheated samples, but volume remained rather constant after 15 min heating.

Table 4. Effect of heating fresh salted vell tissue before drying on the average volume of extract per extraction for 100 g of tissue

Heating temperature	Heating time	Average volume of extract per extraction
(C)	(min)	(ml)
49	0	97
	15	88
	30	94
	45	87
	60	103
54	0	97
	15	100
	30	104
	45	106
	60	113
57	0	97
	15	108
	30	107
	45	102
	60	111
60	0	97
	15	112
	30	112
	45	114
	60	114

pH and viscosity of rennet extract

The pH of extracts ranged from 5.65 to 6.20.

It was observed that as extraction volume increased the viscosity of the extract decreased.

DISCUSSION AND CONCLUSION

The main difficulty in the extraction of rennet from vell tissue is the presence of mucins. This study was an attempt to find a way to destroy the mucins without destroying the enzyme.

It was apparent that drying heated vell tissue increased the yield of enzyme over tissue heated to the same temperatures and extracted wet. Since mucins are associated structurally with water shells, drying of vell tissue causes a partial collapse of this structure (14). Clarke (7) observed that mucins in dried vells became less soluble during storage.

Heating fresh tissue to 49 C prior to drying produced an increase in recoverable activity over unheated samples. Obviously at this temperature there was destruction of mucins occurring, thus allowing an improved extractability of the enzyme.

Heating fresh salted vells produced more recoverable enzyme than fresh vells heated without sodium chloride. The sodium chloride added to the vell tissue appeared to protect the enzyme from heat destruction. Fresh salted vells which were heated and then dried gave a better enzyme recovery than salted vells heated and extracted wet. This is in agreement with commercial practices in which flat-salted vells are heated and dried to inactivate the mucins.

It was concluded from all the performed experiments that heating fresh salted vell tissue to 49 C for 60 min prior to drying gave the best recovery of enzyme. This amounted to an 82% increase in activity over unheated control samples. The next best recovery of enzyme occurred when fresh salted vell tissue was heated to 54 C for 30 min prior to drying. This produced 55% more recoverable activity than unheated samples.

The following procedure for the extraction of fresh frozen vells is recommended.

1. Vells should be frozen at the slaughterhouse as soon as possible after removal from the calf.

2. Mince the frozen vells in a meat grinder or silent cutter.

3. Add dry sodium chloride to the vell tissue to achieve a 10% concentration in the moisture of the vells.

4. Heat the fresh salted vells to 49 C for 60 min.

5. Dry heat-treated salted tissue to about 4% moisture.

6. Extract the enzyme initially with an adjusted sodium chloride extraction solution such that the enzyme is extracted with 10% salt concentration.

As vell tissue was heated, there were two reactions occurring simultaneously at different rates. These two reactions were: (1) heat destruction of mucins and (2) heat destruction of the enzyme. The application of heat to vell tissue was causing enzyme destruction, but for some treatments the reaction in which mucins were being destroyed proceeded at a much faster rate. When this was the case, there was an improvement in enzyme recovery over unheated samples.

Heating vell tissue in the presence of sodium chloride protected the enzyme somewhat from heat destruction and decreased the solubility of the mucins. As a result of this, enzyme recovery was improved over heating tissue in the absence of sodium chloride.

Salted tissue which was heated prior to drying produced the best improvement in rennin extractability. However, it appeared that extending the heating period beyond 60 min may have increased enzyme recovery even further.

On an industrial scale, the heating and drying steps could be combined into one operation. Fresh frozen vell tissue could be ground and have added to it dry sodium

chloride. The salted tissue could then be spread in a thin layer on conveyor belts and dried by circulating warm air. Circulation of warm air will increase the rate of evaporation of moisture in the vell tissue. The heat required to vaporize the water in the tissue must come from the external surrounding or from the remaining tissue. The process of evaporation is so rapid when warm air is circulated that sufficient heat cannot be obtained from the surrounding bodies. Therefore heat is removed from the tissue and in doing so the temperature of the tissue is lowered. With this in mind, a higher drying temperature than 49 C may be needed to maintain a temperature of at least 49 C throughout the tissue.

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