

# A DIFFERENTIAL THERMAL ANALYZER FOR MEASURING THE RELATIVE AMOUNT OF UNDENATURED COLLAGEN IN LARGE REPRESENTATIVE SAMPLES OF COMMUNUTED HIDE

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## ABSTRACT

A differential thermal analyzer was developed which is capable of measuring the relative content of undenatured collagen in a comminuted cattle hide. The technique is based on the endothermic phase change which takes place when collagen is heated in the presence of excess water. The analyzer was designed to study large (75 g.) representative samples of comminuted hide. It was evaluated by testing tailor-made samples containing known percentages of heat-denatured hide collagen. The data indicate that the precision of the measurements was ten percent or better.

—x—x—

## INTRODUCTION

Competition is forcing the cattle hide processors to look for new outlets for their products. One possible use is to comminute the cattle hide and use it in food preparations (1). Fibrous collagen, the chief solid component of comminuted hide, has inherent properties that no other protein possesses. Thus, one of its most important functions may be in producing physical features in edible products which are highly desirable but unattainable with other proteins or additives. One unique characteristic of undenatured collagen is that it undergoes a transition from the fibrous to a gelatinous form at a specific temperature, depending upon the amount of water and other entities present. It is known that, during processing (2), pieces of hide may be subjected to denaturing temperatures in spite of efforts to keep the cutting head temperature of the apparatus at 34°F. It therefore becomes very important that the amount of undenatured collagen in large-scale preparations and products be known.

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The object of this investigation was to develop a procedure for measuring the relative amount of undenatured collagen in representative samples of comminuted hide with reasonable precision. It has been demonstrated that a convenient and rapid method for measuring the change that occurs in hide materials in the presence of excess water when subjected to programmed heating is the technique known as differential thermal analysis (DTA) (3). Unfortunately, the available commercial instruments for making these measurements were designed for handling relatively small samples, about 50 mg. maximum. It is impossible to obtain a representative sample of this size from a comminuted non-homogeneous cattle hide. Therefore, it was deemed necessary to design and construct an instrument for measuring samples weighing 50 to 100 g. This report describes such an instrument and the precision attainable with it.

### THEORY

DTA involves the continuous comparison of the temperature in two materials, a sample and a reference, as both are heated or cooled at a uniform rate. The reference is so selected that it does not undergo a physical or chemical change in the temperature range investigated. If the size and heat capacity of the sample and reference are approximately the same, there will be no temperature differential unless a physical or chemical change occurs in the sample. The temperature differential is usually measured by means of thermocouples wired in opposition.

### INSTRUMENTATION

Figure 1 is a block diagram of the differential thermal analyzer. All components are commercially available except the DTA cell, which was especially fabricated for use with large samples of comminuted hide. Shown in Figure 2 is

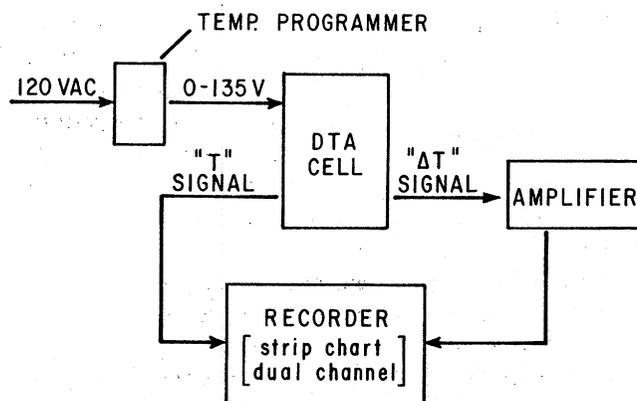


FIGURE 1.—Block diagram — programmer adjustable autotransformer; DTA cell, see Figure 2; amplifier input range 1–300 MV, adjustable output level  $0 \pm 1$  V, zero offset  $\pm 300$  MV; recorder dual channels 5 & 100 MV.

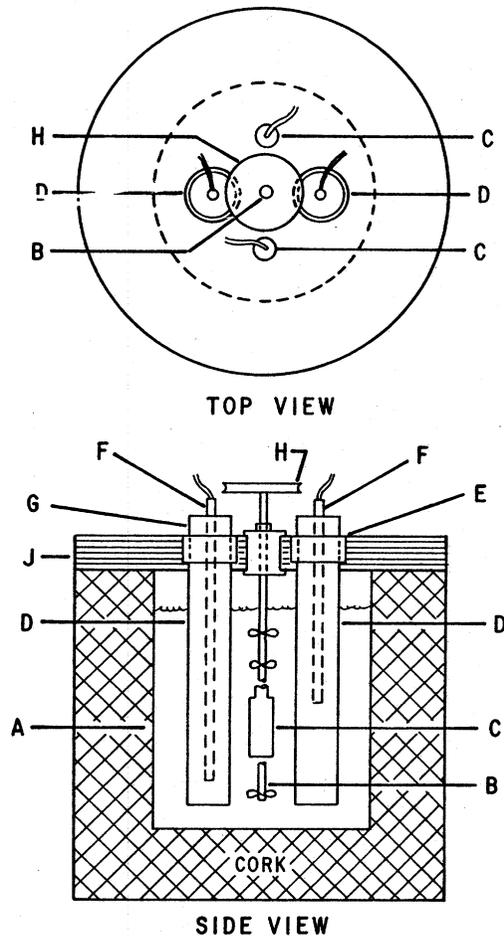


FIGURE 2.—DTA cell — (A) lagged canister  $5\frac{7}{8}$  in. diam. x 7 in. high; (B) stirrer — five-bladed axial thrust type; (C) two 125 W immersion heaters; (D) holders — one in. nominal copper tubing  $7\frac{5}{8}$  in. long capped at bottom end and a nylon restraining collar on top end; (E) collar; (F) sample and reference thermoelements; (G) thermoelement centering plug; (H) pulley; (J) lid for bath and support for components.

a sketch of the DTA cell. It is simply an adiabatic water bath with a sample and reference holder, and heaters arranged symmetrically around a stirrer. The stirrer rotates in a nylon bearing and is belt-driven. It is permanently installed in the bath lid and only the belt need be disengaged from the pulley when the lid is removed (handles are provided to make this convenient). (See Figure 3.)

The bath performs the same function as the metal block oven used in many conventional DTA instruments. When it is effectively stirred, it helps minimize temperature gradients in the large sample and has the added advantage over a

metal block oven that it can be quickly cooled after a run by the simple expedient of pouring the water out and replacing it with a cooler supply.

The one inch tube size of the holders was selected after some experimentation on the relation of transducer response to holder diameter. This size was considered about optimum for the physical setup of the analyzer. In general, a small diameter sample will have less temperature gradient across it, resulting in a more accurate and rapid initial response of the detector to the endothermic change taking place within it. However, the length of the holder did pose a problem of detecting the temperature change throughout the whole body of the sample. The problem was solved by employing an eight junction thermocouple arrangement (thermopile), four in the sample and four in the reference, connected in series.

The junctions of the sample thermoelement are equally spaced along the axis of the sample in the holder. This has the effect of dividing the sample into four overlapping zones, each of which contributes to the thermoelectric signal (Figure 4). This arrangement gives a more representative measure of the transition than does a single junction. It also provides a strong initial signal, owing to the summing characteristic of a series thermopile which reduces the amplification

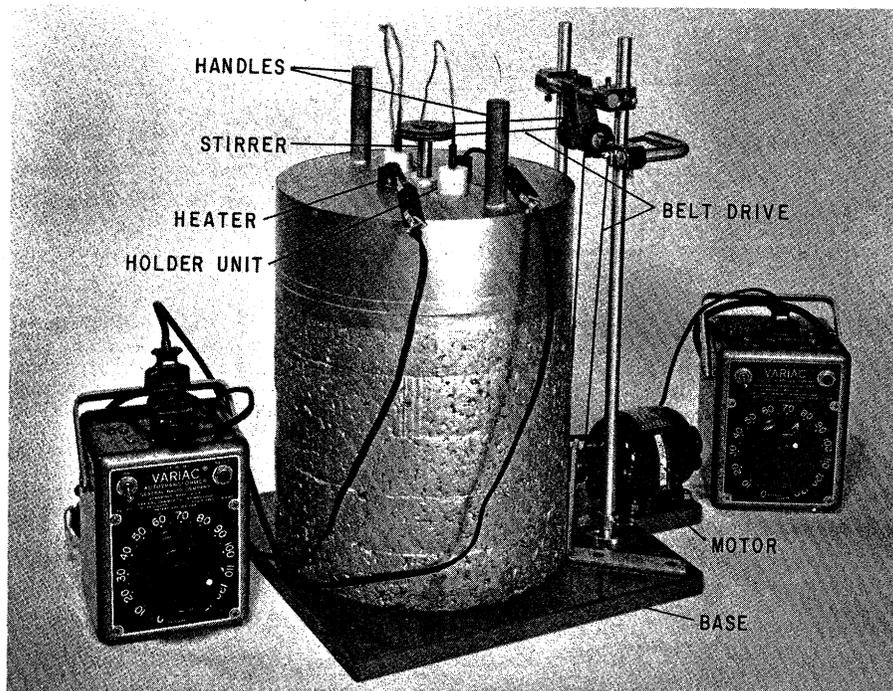


FIGURE 3.—Photograph of cell showing stirrer drive.

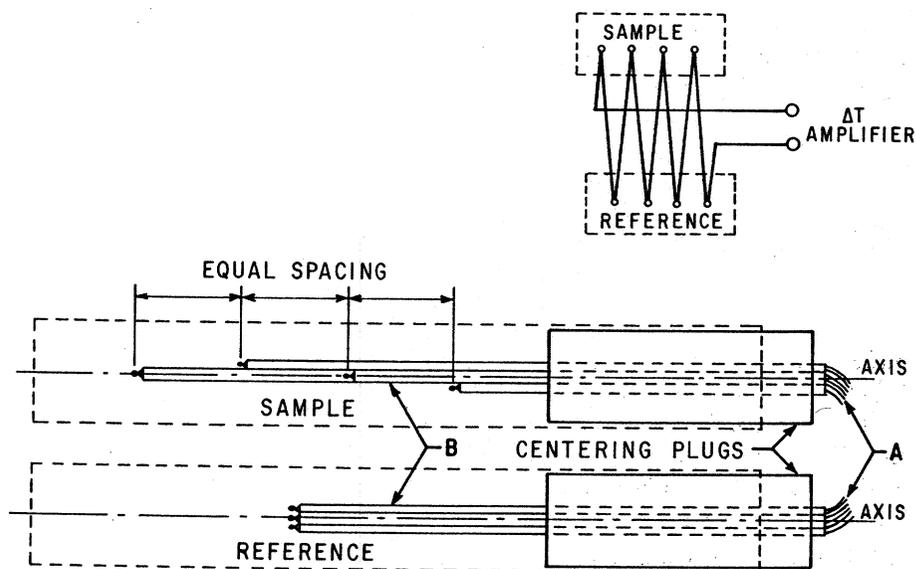


FIGURE 4.—Sketch and schematic of thermopile — (A) #30 enameled cotton covered iron-constantan thermocouple wire; (B) 15 gage hypodermic needle tubing. Thermocouple wire exposed in tubing.

needed to drive the recorder and results in a favorable signal-to-noise ratio. The junctions of the reference element are also located on the axis at the midpoint of the length of the reference material. No advantage is gained by staggering. Care must be taken to locate the thermoelements along the axes of the materials in the holders. This was achieved by axially mounting the thermoelements in nylon centering plugs, three inches long, that fit the copper tube holders very snugly. The plugs are also functional as sample and reference material compacters. A standard two-junction thermocouple with melting ice reference is used to monitor the temperature at the center of the sample.

#### Operation of the DTA Apparatus

First the bath is filled with water (ambient temperature) to a level line on the inside of the canister (2.25 liters). Next, the sample of comminuted hide is weighed out (75 g.). Since the testing technique employed is of a comparative nature, samples must have equal solids content; therefore, care must be exercised in handling samples so as not seriously to alter their moisture content. The sample is loaded into the sample holder in piecemeal fashion, about 10 g. at a time, each portion being thoroughly compacted before the next is added. This procedure is necessary to insure a minimum of voids, which can affect the accuracy of the test. The compacted sample is pierced along its cylinder axis with a  $\frac{1}{8}$  inch dowel to provide a well for the thermoelement. About  $\frac{1}{4}$  ml. of water is added

to this hole prior to inserting the thermoelement to insure good thermocouple contact. After the thermoelement is inserted in the sample, hand pressure is exerted on the centering plug to compact the sample further and obtain good contact for the thermocouple junctions.

The reference holder is filled to approximately the same height as the sample with dry asbestos fiber, using the same technique as described above. One or two runs may be required to stabilize the reference; however, once stabilized, it should not be disturbed.

The filled sample and reference holders and the immersion heaters are inserted in the lid-support and then all electrical connections are completed to make the system operable. The rate of heating must be determined experimentally by adjusting the autotransformer. All data reported were obtained from tests run at 1°C./min. and programmed from ambient to 80°C. The optimum level for the differential signal amplifier is also experimentally obtained. The gain of the amplifier of the described apparatus was 138:1. The stirrer speed is adjusted with an autotransformer to ~1,480 rpm.

Before starting a test, the system is equilibrated with the heaters off for ten to 20 minutes. At equilibrium, both pen traces will run parallel to the time axis of the chart. Theoretically, the  $\Delta T$  trace should track along the zero line of the strip chart, but practically this is seldom the case.

The test is started (after equilibration) by turning on the temperature programming autotransformer. After several minutes the  $\Delta T$  trace will stop its rapid upscale excursion and will have leveled off (at this point the base line begins to develop and it almost always has a negative slope). When this point of the test is reached, it is prudent to move the  $\Delta T$  trace back toward the lower end of the recorder scale to insure that the differential peak has space to develop on the chart. The zero offset is provided for this purpose.

The temperature is programmed only from ambient to 80°C., since the expected transition takes place within this range.

### Evaluation of the Recorded Data

Shown in Figure 5 is a typical recording of an actual run. The data are recorded in units of MV and time. One tracing shows the sample temperature (millivolts), which increases linearly with time and has a slight inflection at the transition temperature of collagen. The other tracing results from the temperature difference between the sample (comminuted hide) and inert reference (asbestos) as a function of time and increasing temperature. It shows a large deviation from the base line in the temperature range 53 to 61°C., indicating an absorption of heat by the sample in this region. This is the region in which undenatured collagen undergoes a transition, which is the basis for this technique. The amount of deviation from the base line to the peak height provides

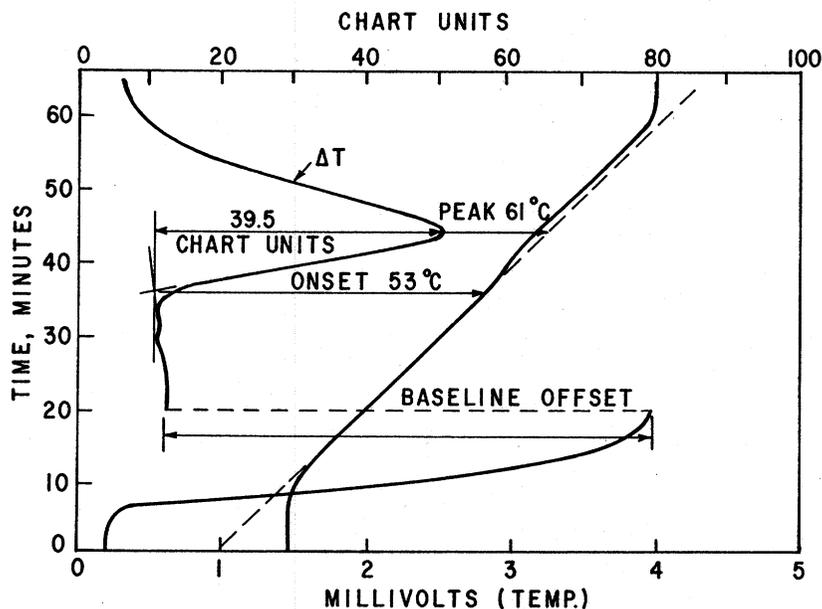


FIGURE 5.—Typical differential trace ( $\Delta T$ ) and temperature ( $T$ ).

a means for making a comparative determination of the amount of undenatured collagen present in the sample.

Quantitation of the recorded differential trace can be accomplished in a number of ways. The one selected for this study is simple and reproducible. It consists first of drawing a line that is parallel to the time axis of the strip chart and tangent to the onset inflection and then measuring the distance from it to the peak in the curve in strip chart units (peak height). This is done for both the unknown and a control, whose content of denatured collagen was assumed to be minimal. The ratio of the sample peak height to the control peak height times 100 is calculated. This ratio represents the percentage of undenatured collagen in the sample relative to that in the control. To insure accuracy, it is important that all phases of the procedure be the same for each run.

#### Experimental Check of the Apparatus

The apparatus was evaluated using 75 g. samples containing known amounts of undenatured and denatured comminuted hide. The hide used as the control and assumed to be 100 percent undenatured collagen was lime unhaird and its pH was adjusted to 6.5 before comminuting. Comminuting to granules and short fibers was carried out as described by Elias *et al.* (2). The comminuted hide was subsequently stored under refrigeration until used. Samples were prepared by mixing the appropriate portions of the control comminuted hide with

heat-denatured collagen (dry), and water. The transitions of four compositions containing 100, 90, 75, and 50 percent undenatured collagen were carried out as described above. Six runs were made on each of the four compositions. Shown in Table I is the variation in peak height with undenatured collagen content for the six test runs.

TABLE I  
PEAK HEIGHT AS A FUNCTION OF PERCENT  
CONTROL COMMUNUTED HIDE

Run	Peak Height in Chart Units			
	% Comminuted Hide			
	100	90	75	50
1	38.0	31.5	29.5	19.0
2	38.5	32.5	27.5	19.5
3	38.5	34.0	26.5	18.5
4	39.5	34.0	26.0	17.0
5	37.5	35.0	28.5	18.5
6	35.5	36.0	26.5	19.0

The test data were fitted with a straight line by the method of least squares. The calculated intercept was not significantly different from zero. This result agrees with theoretical predictions that a sample which was 100 percent denatured would give a chart height of zero. Recalculating the equation with a zero intercept gave a slope of 0.3754 chart units per percent undenatured collagen, with a standard deviation ( $\hat{\sigma}$ ) of 1.362 chart units. In practice, our technique will be used to estimate the parameter  $z = \frac{x}{y}$ , where  $z$  is the undenatured collagen fraction of an unknown,  $x$  is the chart value of the unknown, and  $y$  is the chart value of the control (minimal denatured). The error in  $z$  depends on the errors in  $x$  and  $y$ . From the theory of propagation of variance

$$\text{var}(z) = \hat{\sigma}_z^2 = \frac{1}{\bar{y}^2} \text{var}(x) + \frac{\bar{x}^2}{\bar{y}^4} \text{var}(y)$$

where  $\bar{x}$  and  $\bar{y}$  are the mean values of  $x$  and  $y$ . From the linear regression  $\bar{y} = 37.54$ . Assuming the  $\text{var}(x) = \text{var}(y) = \hat{\sigma}^2$

$$\hat{\sigma}_z = 0.036 (1 + z^2)^{1/2}$$

Values of the expected standard deviation,  $\hat{\sigma}_z$ , are given in Table II for representative values of  $z$ . Note in the table that, at the 95 percent confidence limit, the precision of the apparatus is ten percent or better.

TABLE II  
STATISTICAL ANALYSIS OF DATA

z	Single Determinations Confidence Interval			Duplicate Determinations Confidence Interval		
	$\hat{\sigma}_z$	95%	99%	$\hat{\sigma}_z$	95%	99%
1.0	.051	.10	.13	.036	.07	.09
0.9	.048	.09	.12	.034	.07	.09
0.75	.045	.09	.12	.032	.06	.08
0.50	.040	.08	.10	.028	.05	.07
0.00	.036	.07	.09	.025	.05	.06

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