

STUDIES ON INTERMEDIATE MOISTURE

BEEF MEAT PATTIES

by

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## ABSTRACT

Recent studies have shown that intermediate moisture foods, while stable to microbial growth, are susceptible to chemical changes resulting in loss of quality. This study observed the effects of different water activities, citric acid contents, fat contents, and pH on lipid oxidation and non-enzymatic browning of a meat product.

An intermediate moisture beef patty was produced by equilibrating and cooking the raw meat in various glycerol, water, citric acid and sodium chloride solutions.

Experimental results showed that peroxide values increase with increasing water activities between  $A_w$  0.655 and 0.818. Citric acid did not affect the formation of peroxides but prevented peroxide breakdown.

There was a loss of the brown colour of the cooked meat patty and an increase in yellowness possibly due to oxidation. The loss of colouring and yellowing was greatest at low pH and high water activities.

Non-enzymatic browning was not apparent. Lipid oxidation was the primary cause of the product deterioration. The shelf life was limited to three to four months by the development of rancid odours.

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## INTRODUCTION

Intermediate moisture foods (IMF) do not have a rigid definition. Characteristically they have reduced moisture contents to prevent microbial spoilage but retain enough moisture to present a soft moist texture. Typical examples are dried fruits, honey, maple syrup, some dried sausages, and fruit cake.

Intermediate moisture foods are therefore attractive because of several properties:

1. They do not require refrigeration or thermal processing. With a water activity ( $A_w$ ) below 0.80 they are stable to bacterial, yeast and most mold growth. (29)

2. They do not require rehydration before consumption, as is the case with freeze-dried products. Their moisture content is in the range of 20% to 50%, but they still retain a soft moist texture.

3. The packaging requirements are not strict; cheap, flexible materials are sufficient. If the package is broken IMF are not susceptible to spoilage. (31)

If an IMF was manufactured by dehydration to the desired moisture content, most products would have a dry, brittle texture, even at an  $A_w$  of 0.85. The only products capable of retaining a soft, moist texture are those with a high natural soluble solids content: for example, fruits. The soluble solids level would have to be increased for products high in protein or starch. Bone (3) outlines the criteria

for choosing such solutes. At present, glycerol, sodium chloride, sugars and combinations of these seem to be the most useful solutes. (4)

A major problem in choosing a humectant is the flavour it imparts to the product.

Commercially, IMF technology has gained its greatest strides in the pet food industry. The main humectant used is sucrose, in amounts ranging from 20-30%. Dogs do not object to this excessive sweetness, but most humans would not find many dishes palatable. (16) This problem will of course have to be solved before IMF are acceptable for human consumption.

IMF were thought to be remarkably stable during their initial development. Labuza (18) states that both lipid oxidation and non-enzymatic browning reach a maximum between  $A_w$  0.50 and 0.85. Enzymatic activity also increases with increasing  $A_w$  but is easily controlled by blanching or cooking.

Both browning and autoxidation cause off-flavour and odors, textural changes such as increased toughness, loss of water binding capacity, loss of colour or darkening of colour, and decreased nutritional quality. Lipid oxidation is especially serious because of the low threshold levels of the obnoxious products. The  $C_8-C_{12}$  aldehydes are all detectable by smell at levels as low as .001 to .01 ppm. (23)

This research will investigate the development of non-enzymatic browning and lipid oxidation in an intermediate moisture meat product. This study will also investigate the effects of high concentrations of

citric acid (1% to 2%) which reduce the pH and thus possibly retard non-enzymatic browning. As a sequestering agent, citric acid binds trace metals and possibly retards lipid oxidation. Citric acid imparts a pleasant sour taste sensation that can be used to mask the harsh bitter-sweet flavour of glycerol, or the excessive sweetness of glucose and saltiness of sodium chloride.

## LITERATURE REVIEW

Intermediate moisture foods were originally thought to be stable to oxidative rancidity. Brockman (4) observed no significant chemical, physical or sensory changes in military experimental IMF after four months storage at 38°C. Loncin et al. (25) observed no difference in the oxidation rate between Aw 0.11 and 0.75. They did observe a marked increase in the peroxide value at Aw below 0.18. Their work was done on spray dried milk powder.

Lipid oxidation has long been known to be a problem at the low water activities of dehydrated foods. An increase in the moisture from Aw 0 was observed to decrease oxidation. Salwin (33) found the moisture content, with optimum protective effect, to be near the monolayer value for most foods. In a review, Labuza (18) states the main protective mechanisms are:

- 1) Water hydrogen bonds with the hydroperoxides, protecting them from decomposing and initiating more free radicals.
- 2) Water hydrates the metal catalysts of lipid oxidation. Water can also form insoluble metal hydroxides, removing the catalysts completely.
- 3) Water decreases the stability of free radicals, reducing the total number available for initiating lipid oxidation.

Heidelbaugh and Karel (12) concluded from their studies that an increase in Aw from 0.51 to 0.75 increased lipid oxidation. In addition, they found that glycerol added to the model system reduced the antioxidant

effect of water at low water activities. Without glycerol, the optimum  $A_w$  at which lipid oxidation was at a minimum, was 0.51 and with glycerol the  $A_w$  was 0.20. Labuza (18) presents a "stability map" showing increased autoxidation in the intermediate moisture range. He and his co-workers have verified their earlier findings of increased oxidation rate with increased moisture content. (21), (20), (5), (6)

It is interesting that Heiss and Eichner (14) also presented a "stability map" about the same time as Labuza, showing no increase in oxidation with increasing moisture content at high water activities.

The pro-oxidant effect of water in the IM range of  $A_w$  0.6 to 0.8 is probably due to an increased solubility of metal catalysts to reaction sites. (18), (12) Labuza (18) found that the rate of lipid oxidation reached a maximum at high water activities. A decrease in oxidation was observed by increasing  $A_w$  from 0.6 to 0.75. In their model system this increase in  $A_w$  more than doubled the moisture content suggesting a decrease in rate due to a dilution effect. In later work, Chou et al. (5) observed increased lipid oxidation up to  $A_w$  0.89. It is doubtful whether a decrease in oxidation does occur in the high moisture foods except at very high water activities.

Non-enzymatic browning has been observed to reach a maximum rate between  $A_w$  0.3 and 0.7, depending on the type of food product. (13), (18), (25) Labuza (18) accounts for the maximum as follows:

- 1) increased diffusion and solubility of reactants with increased moisture content contributes to the increased browning rate at low water

activities;

2) further increasing the moisture content results in mass dilution causing the observed maximum browning rate, followed by a decrease, at high water activities.

Loncin et al. (25) feel that the decrease in browning at the high water activities is due to an inhibition by water, which is one of the first products of browning. Eichner and Karel (8) found that both mass dilution and product inhibition reduce browning but that the inhibition by water was far greater. Their results also show that in systems of high viscosity, as at low water activities (0.32), browning is decreased. The addition of glycerol to this low water activity system had a plasticizing effect, increasing the rate of browning.

From the above literature it would appear that the rate of autoxidation and non-enzymatic browning could determine the shelf life of an IMF. A means for inhibiting or reducing these reactions would have to be used in order to make such a product successful for human consumption.

Much work has been done on the use of antioxidants. For commercially rendered fats, the best all-round method is a combination of butylated hydroxyanisole (BHA), propyl gallate, and citric acid. (10) BHA appears to be the most useful phenolic antioxidant, with low toxicity ( $LD_{50}$  5000 mg/kg body weight of rats). Butylated hydroxytoluene (BHT) has been observed to increase liver weight and cause abnormal cellular behavior. Propyl gallate is more effective in dried meats than in products with more moisture, such as sausage. (9) Chou and Labuza (6) in their study of antioxidants in IMF found ethylenediaminetetracetic acid (EDTA) to be most effective, reducing

oxidation ten to twenty times over a control. BHA was found to be more effective at the lowest moisture contents studied.  $\alpha$ -tocopherol was found to be a fairly poor antioxidant.

Citric acid has an advantage in being a natural constituent of many fruits and vegetables and would find approval from the general population as a food additive. It is being used in combination with other antioxidants. (9) In low levels it has a synergistic effect. For example, it prevents discoloration caused by the reaction of propyl gallate with iron, and it increases the stability of  $\alpha$ -tocopherol. (9) There is some doubt of the effectiveness of citric acid alone as an antioxidant. Labuza et al. (21) found that citric acid (100 ppm fat basis) was more effective at intermediate moisture levels. Their reason was that chelating agents most likely become bound to protein, reducing their effectiveness. Later, Chou and Labuza (6) found that adding 0.5 moles of sodium citrate per mole of metal reduced lipid oxidation in a model system two times.

Lemon et al. (24) found in systems that are free of trace metals or primary antioxidants, citric acid does not prevent autoxidation. It appears to have only a minor protective effect on animal fats unless there is a high proportion of heavy metals present, causing a low natural stability. (9) Further work is required to determine if citric acid would be more effective in high concentrations.

There are various methods of inhibiting non-enzymatic browning. Moisture control as mentioned above is only practical in very low moisture systems. Browning is negligible at temperatures below  $-20^{\circ}\text{C}$ . Fermentation to remove sugars has been used with spray-dried egg whites. Chemical food

additives are the simplest method. Of the various additives, sulfites in the form of sulphur dioxide or sodium bisulfite are the most effective. The sulphite reacts with the sugar moiety, thus inhibiting the reaction. (35)

Sulfites are limited by a corrosive taste apparent at levels above 500 ppm. (10) It is volatile and tends to disappear from open systems. It also destroys thiamine.

Non-enzymatic browning is base catalyzed. Reynolds (32) observed an increase in reaction rate of 1.6 to 1.7 times going from pH 3.5 to 4.7, and 2.1 to 2.4 times from pH 4.7 to 5.6. Song and Chichester (34) observed in a model system that the rate increased considerably as pH approached the pKa value of the reactive amino acid. They also found that  $\alpha$ -methyl-D-glucoside is far less reactive than glucose and that D-arabinose, which forms less stable hemiacetals, is more reactive, all suggesting that the free aldehydic form is necessary for reaction.

## METHODS AND MATERIALS

Materials

One boneless beef hip from two different animals was used to represent two blocks. Each hip, having the surface adipose fat removed, was ground once in a Hobart meat grinder with a  $\frac{1}{4}$ " hole plate. The ground meat was mixed well by hand for a more homogeneous sample. The above procedure was followed with the adipose tissue.

Citric acid, sodium citrate, sodium chloride and potassium sorbate used in formulating the infusion solutions were all "Fisher" reagent grade. U.S.P. grade glycerol was obtained from a local chemical supplier.

Gelatin and potato starch used in formulating the raw meat patties were "Fisher" reagent grade.

1. Preparation of samples for lipid oxidation experiments

The ground beef was divided into three lots and ground adipose fat was added to each lot estimated to give 10%, 15% and 20% fat contents respectively. 5% gelatin and 5% starch (total wet weight basis) were added to each lot so that these stabilizers would increase the firmness of the cooked product.

Twelve infusion solutions were formulated with 4 different glycerol contents and 3 different citrate contents. Table I summarizes the 36 sample combinations and shows the final fat, citrate and moisture contents. 0.75% potassium sorbate was added to all the infusion solutions

Table I Sample Combinations for Lipid Oxidation Analysis Showing Fat, Citrate, Glycerol, Moisture Contents and Water Activities

	Glycerol:water Ratio of Infusion Solution							
	80:10		65:25		50:40		35:65	
Fat Content raw patty								
Fat Content cooked patty								
Citrate Content cooked patty								
Citrate Content Infusion sol.								
	<u>Water Activity and Moisture Content of Cooked Patty</u>							
	Aw	MC	Aw	MC	Aw	MC	Aw	MC
20% 6.55%	0.654	27.03	0.708	35.47	0.760	42.95	0.811	51.97
±0.26	0.648	28.35	0.699	34.86	0.759	44.19	0.821	51.65
	0.642	26.28	0.698	36.57	0.755	44.65	0.803	54.41
15% 5.19%	0.680	31.55	0.711	37.55	0.772	44.76	0.813	52.75
±0.21	0.675	31.27	0.719	38.98	0.767	45.45	0.826	54.65
	0.648	31.98	0.700	38.64	0.766	45.67	0.827	54.11
10% 3.26%	0.658	28.84	0.717	37.46	0.772	42.63	0.814	53.11
±0.19	0.663	31.19	0.706	38.30	0.757	42.74	0.825	54.41
	0.654	30.02	0.701	37.81	0.762	44.30	0.811	54.40
Average Water Act.*	0.655 ± 0.06		0.707 ± 0.05		0.763 ± 0.04		0.818 ± 0.06	
Average Moisture Cont.*	29.94 ± 0.70		37.61 ± 0.48		45.07 ± 0.47		53.62 ± 0.46	
Average Glycerol Cont.*	42.17 ± 1.30		36.65 ± 1.28		30.03 ± 0.90		21.70 ± 0.53	

\*Values of cooked patty.

to inhibit mold contamination. 7.5% sodium chloride was added as a seasoning and to help reduce the water activity. The infusion solution was adjusted to pH 6.0 using a citric acid:sodium citrate ratio of 1:10.39.

Patties were formed by hand from 50g of the above raw meat mixtures. The dimensions were approximately 8 cm in diameter by 1 cm in thickness. Each 50g patty was placed in a 600 ml glass beaker with 100 g of infusion solution and equilibrated at 40°C. After 18 to 24 hours of equilibration, the patty-infusion solution combination in each beaker was heated in a hot water bath (95°-100°C) for 20 minutes, then cooled and stored at 40°C for approximately another 20 to 24 hours. The beakers were slightly heated in hot water to liquefy the excess gelled infusion solution. The patties were then drained of excess solution and packaged in sealable pouches. The pouches were produced by Seaforth Plastics Ltd., Burnaby, British Columbia (code name LDS2). They are a laminate of nylon (1 mil thickness) on the outside surface and polyethylene (3 mil thickness) on the inside surface. Oxygen permeability is 1.2 cc/100 in<sup>2</sup> per day at 1 atm pressure and 72°F. Water permeability is 0.4g/100 in<sup>2</sup> per day at 100°F and 92% relative humidity. The patties were stored in the pouches for various times at 22°-24°C prior to analysis. The maximum storage time was 6 months.

## 2. Preparation of samples for non-enzymatic browning experiments

The raw patty mixture was prepared in the same manner as above, except that no extra fat was added.

The infusion solution contained 4 different glycerol contents, 2 different citrate contents and 3 different citric acid:sodium citrate

Table II Sample Combinations for Non-Enzymatic Browning Analysis Showing Fat, Citrate, Moisture, Glycerol Contents and Water Activities

	Glycerol:Water Ratio of Infusion Solution										
	80:10	65:25	50:40	35:65							
Acid:Salt Ratio											
Infusion Solution											
pH Cooked Patty											
Citrate Content											
Infusion Solution											
Citrate Content											
Cooked Patty											
Water Activity and Moisture Content of Cooked Patty											
	Aw	MC	Aw	MC	Aw	MC					
1:1.174	4.96	1.5%	1.0%	0.651	30.80	0.694	38.07	0.755	43.55	0.819	52.74
	±0.008	5.0%	3.3%	0.657	32.14	0.688	34.79	0.719	38.99	0.822	48.47
1:2.547	5.38	1.5%	1.0%	0.644	31.82	0.699	39.26	0.772	44.52	0.817	53.25
	±0.07	5.0%	3.3%	0.655	29.71	0.700	35.60	0.734	38.26	0.805	48.77
1:10.381	6.01	1.5%	1.0%	0.552	32.75	0.697	38.29	0.764	44.76	0.821	53.49
	±0.04	5.0%	3.3%	0.650	29.54	0.699	33.43	0.716	38.68	0.821	50.99
Av. Water Act.*	0.653 ± 0.006			0.696 ± 0.007				0.741 ± 0.009		0.816 ± 0.008	
Av. % Moisture Cont.*	30.92 ± 0.77			36.88 ± 0.67				41.60 ± 0.76		51.30 ± 0.55	
Av. % Glycerol Cont.*	47.22 ± 0.83			37.85 ± 0.93				30.03 ± 0.90		21.70 ± 0.53	

\*Average values of cooked patty.

ratios. The 24 sample combinations are outlined in Table II showing final citrate contents, glycerol contents, moisture contents and pH.

Patties used for water soluble pigment extraction were the same size as those used in the lipid oxidation experiments. Patties used for Hunter colour parameter determinations were formed by hand from 200g of raw meat mixture and to a size of approximately 14 cm in diameter and 2 cm thick. These large patties were necessary to cover the optical port of the Hunter Colour Meter. The 200g patties were placed in 4000 ml beakers with 400 g of infusion solution. The equilibration, cooking and packaging procedure is the same as for the lipid oxidation experiments.

#### Peroxide Value Determination

Whole patties were removed from their pouches, comminuted in a Waring Blendor for 10-30 sec and placed in screw cap jars.

Duplicates of approximately 15g of each sample were freeze-dried in cellulose extraction thimbles for 30 hours, then extracted with petroleum ether using a Goldfish Extractor for 12 hours. The peroxide value was determined by the AOAC method (2) on the 1-2g of fat extracted. Moisture contents were calculated from the weight loss during drying.

#### Water Soluble Pigment Determination

Whole patties were removed from their pouches and cut in half. One half of each patty was comminuted in a Waring Blendor and then placed in individual screw cap jars. The remaining half patties were replaced in their pouches which were then resealed and stored for a future determination.

Duplicates of approximately 10g of each sample were freeze-dried in aluminum drying dishes of 50 mm diameter and 15 mm deep. The dried samples were steeped in distilled water and filtered through a Milipore filter system (1.2 $\mu$  pore size) by the method of Karel and Nickerson (17). The O.D. of the filtrate was measured on a Beckman DB Spectrophotometer. Grinding of the dry sample to pass through a 2 mm screen was omitted because glycerol caused the dry particles to stick together and plug the 2 mm screen of a Wiley Mill. Wet samples were pureed in a Waring Blendor to compensate for this omission.

#### Hunter Lab Value Determinations

Colour parameters of whole patties in their pouches were determined using a Hunter Lab Colour Difference Meter (Model D25). Values were determined twice on each side of the pouch. The instrument was standardized against the white colour standard.

#### Water Activity Determinations

Water activity was determined on the 10 to 20g of samples remaining in the screw cap jars after some of the sample had been removed for peroxide determinations or water soluble pigment determinations. A two-holed rubber stopper was fitted to the top of the jars and air was recirculated over the sample and through a sensor of an EG&G Dew Point Hydrometer (Model 880) for 1½ hours at 20°C. 1½ hours was found to be long enough to equilibrate the highest moisture content samples. One determination was made on each sample.

### pH Determination

The sample pH was determined on 10g of sample after the water activity determinations. The 10g sample was blended with 100 ml of distilled water for 30 seconds in a Waring Blendor. (7) One determination was made on each sample.

### Glycerol Determinations

Glycerol content was determined on 5g of random sample for peroxide determinations or water soluble pigment determinations. The test procedure used is an AOAC (1) tentative method. Wet samples were extracted with acetone for 18 hours to remove the glycerol. The dried extract was dissolved in water and oxidized with an excess of potassium dichromate. The excess dichromate was determined by titrating the oxidized mixture with ferrous ammonium sulfate, with diphenylamine as the indicator. All determinations were made at the end of the test period.

### Crude Fat Determinations

The fat content was determined from 5g of random samples for peroxide value or water soluble pigment determinations. The standard method of the AOAC (2) was applied using petroleum ether for extracting the lipid material. Anhydrous ether was found to partially extract glycerol as well as fat; therefore, it was not used. All determinations were made at the end of the test period.

## RESULTS AND DISCUSSION

The peroxide value (P.V.) increased in all samples during the first months of observations. Samples with an  $A_w$  of 0.765 reach a maximum peroxide value between 120-160 days, and samples with an  $A_w$  of 0.818 reach a maximum P.V. in 60-120 days, as shown in Figure 1. Peroxides are unstable intermediates of oxidation. The P.V. at any one time is a function of the rate of formation minus the rate of decomposition. A maximum P.V. is reached when the rate of decomposition equals the rate of formation; and when the peroxides decompose faster than they are formed, the P.V. decreases with time. (28)

The peroxide value has been widely used for studying lipid oxidation. A peroxide value of 20 meq. peroxide/Kg fat has been associated with the threshold detection of rancidity. (37) A problem of using the P.V. for indicating the actual degree of rancidity is that peroxides do not have a flavour or odour. Some fish products have very high peroxide values without an apparent off-odour. (23) Detailed odour analysis was not used in this study, but rancid odours were apparent in the meat samples during the third and fourth month of storage, when samples reached peroxide values of 30 to 50 meq. peroxide/Kg fat. The odour was very objectionable in most samples but particularly in samples with water activities of 0.818. Labuza et al. (21) reported similar findings where rancid odours were detectable in intermediate moisture chicken samples at a P.V. of 40-50 meq. peroxide/Kg fat.

All the three variables tested, fat, citrate and moisture

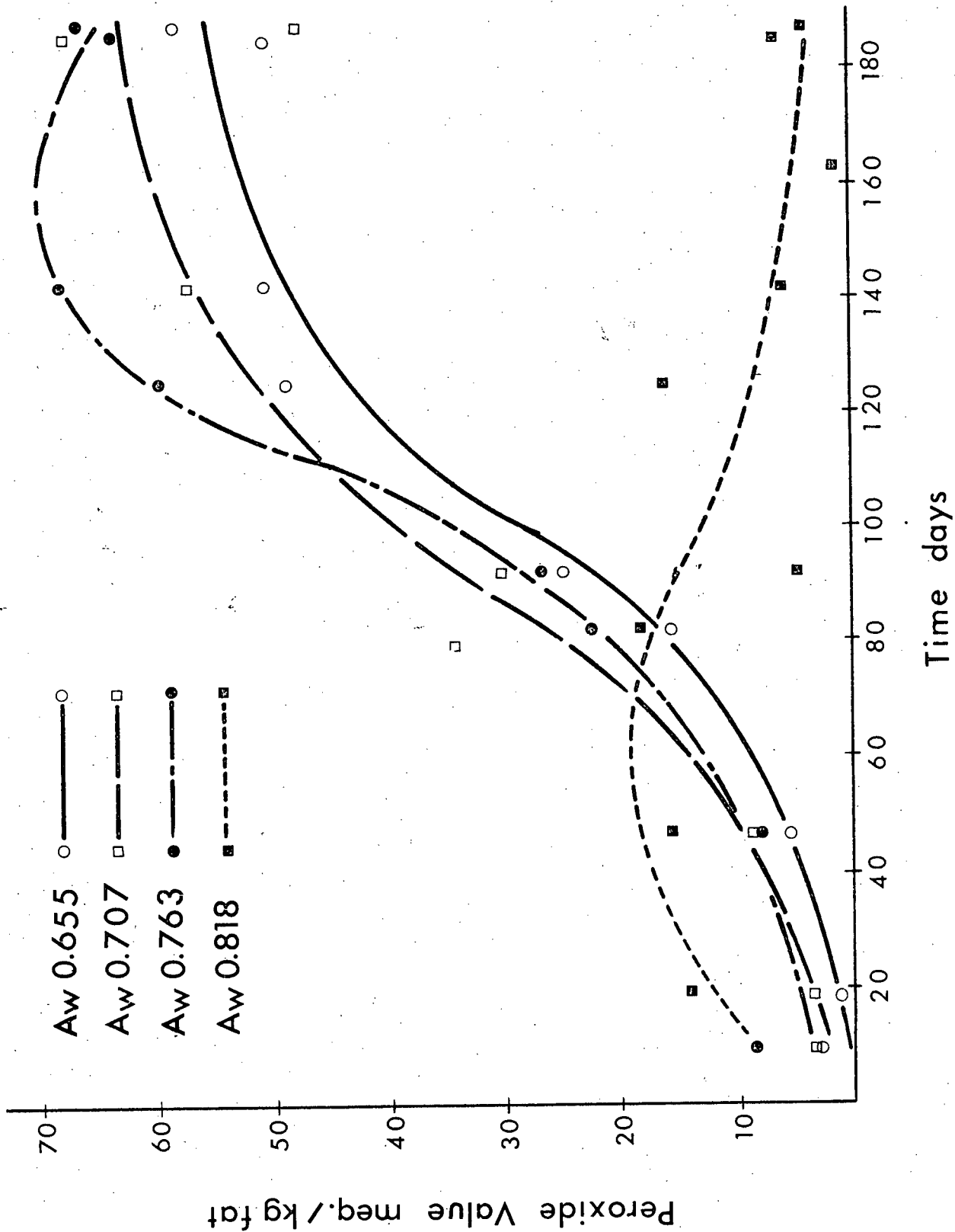


Figure 1. Change in Peroxide Value at Different Water Activities (0% Citrate)

content, had a significant effect on the peroxide value (Table III). Changes in water activity had the greatest effect. Increasing  $A_w$  from 0.655 to 0.763 significantly increased the average P.V. (Table IV). In samples with water activities of 0.818, a decrease in the average P.V. was observed after approximately 110 days.

Reaction rates were calculated using the kinetic equation derived by Labuza et al. (19) for a methyl linoleate model system.

$$1. \quad \frac{d \text{ROOH}}{dt} = K_m \text{ROOH}^{\frac{1}{2}}$$

$K_m$  = monomolecular rate constant

ROOH = peroxide value.

They state the equation is valid if the substrate concentration does not change significantly. Observations past the maximum P.V. were excluded from the calculations for  $K_m$ . The maximum P.V. occurs when the amount of available oxidizable fat is so depleted that the rate of peroxide decomposition equals, then exceeds the rate of formation. (28) The coefficient of determination to the rate equation was variable, ranging from  $r^2 = 0.66$  to 0.99 (Table V). There was a better correlation of the data to equation 1 by eliminating peroxide values that appeared to level off with time. A second reason that some correlations were low is that the two blocks were assumed to oxidize at the same rate, so that the composite data could be used in the calculating of  $K_m$ . This assumption was not true, as will be explained later. It was, of course, assumed that the rate equation 1 does apply to the meat system under study so the experimental results could be compared more easily than by comparing P.V. alone.

Table III Analysis of Variance-Peroxide Values

Source of Variance	d.f.	F value
Replicate	1	54.9
Time	4	182.1
Fat	2	13.8
Citrate	2	20.4
Moisture	3	12.9

All F values significant at  $\alpha = 0.01$ .

Table IV Duncans Multiple Range TestEffects of Different Water Activities on Peroxide Values

Aw	mean P.V. <sup>1</sup>	mean P.V. <sup>2</sup>
0.655	14.21 a	22.43 a
0.707	18.31 b	27.70 b
0.763	27.50 c	36.90 c
0.818	30.46 c	27.46 b

Means within each column with different subscripts are significant at  $\alpha = 0.05$ .

1. First three observations.
2. Total observations.

Table V Monomolecular Rate Constants (Showing Coefficient of Determination) to Equation 1

Water Activity	Citrate Content	Fat Content								
		3.26%			5.19%			6.55%		
		K <sub>m</sub> * x 10 <sup>-2</sup>	N**	r <sup>2</sup>	K <sub>m</sub> * x 10 <sup>-2</sup>	N**	r <sup>2</sup>	K <sub>m</sub> * x 10 <sup>-2</sup>	N**	r <sup>2</sup>
0.655	0%	2.83	18	0.669	3.93	18	0.795	3.43	18	0.922
	1.0%	2.00	20	0.665	2.65	17	0.729	2.66	17	0.911
	2.2%	2.00	20	0.742	2.32	15	0.713	1.94	19	0.720
0.707	0%	2.97	19	0.762	3.30	18	0.789	2.14	17	0.765
	1.0%	4.69	9	0.749	3.09	18	0.793	1.95	19	0.747
	2.2%	3.44	11	0.945	3.14	17	0.851	2.10	20	0.710
0.763	0%	4.24	14	0.823	4.49	18	0.899	2.56	18	0.870
	1.0%	3.76	17	0.909	3.07	13	0.972	3.21	13	0.884
	2.2%	3.93	7	0.939	3.79	17	0.800	3.66	8	0.820
0.818	0%	4.17	8	0.902				10.11	6	0.854
	1.0%	8.80	6	0.987	8.96	5	0.924	5.36	10	0.956
	2.2%	5.01	15	0.944	3.87	12	0.870	4.13	14	0.805

\* monomolecular rate constant (meq. perox./Kg fat)<sup>1/2</sup>/day

\*\* number of observations used in calculation

∂r r<sup>2</sup> = coefficient of determination

The lipid oxidation rate was observed to increase with increasing water activity and a sharp increase in rate was noticed between  $A_w$  0.763 and 0.818 (Figure 2). There was no apparent decrease in the rate over the water activity range studied, as found by Labuza (18) in model systems.

Cooked meats have been noted to deteriorate rapidly due to lipid oxidation, with off-flavours detected as early as two hours after cooking. (36) Most cooked meats have a water activity in the range of 0.70 to 0.80. (20) Lipid oxidation also occurs in fresh meats ( $A_w = 0.99$ ) at refrigerated temperatures. (11) Using these high water activities would not be a practical approach for controlling lipid oxidation because of resultant increases in yeast and bacterial growth.

The effects of citric acid-sodium citrate on the average P.V. at different water activities was not consistent. There was definitely no effect at  $A_w$  0.707 and 0.763 (Table VI). Increasing citrate levels in the infusate slightly reduced the six month average P.V. at  $A_w$  0.655, but markedly increased the P.V. at  $A_w$  0.818. It was observed that samples at  $A_w$  0.818 and 0% citrate did not reach very high maximum peroxide values compared to the samples with citrate (Figure 3). It would appear that citric acid has some effect on the breakdown of peroxides. It would be difficult from this study to predict how citric acid produces a high P.V. Citric acid could be increasing the stability of the hydrogen peroxide or its free radical. It could also reduce the rate of peroxide decomposition by sequestering the trace metal catalysts involved in the reaction or by some other method. This protective effect has also been observed for milk

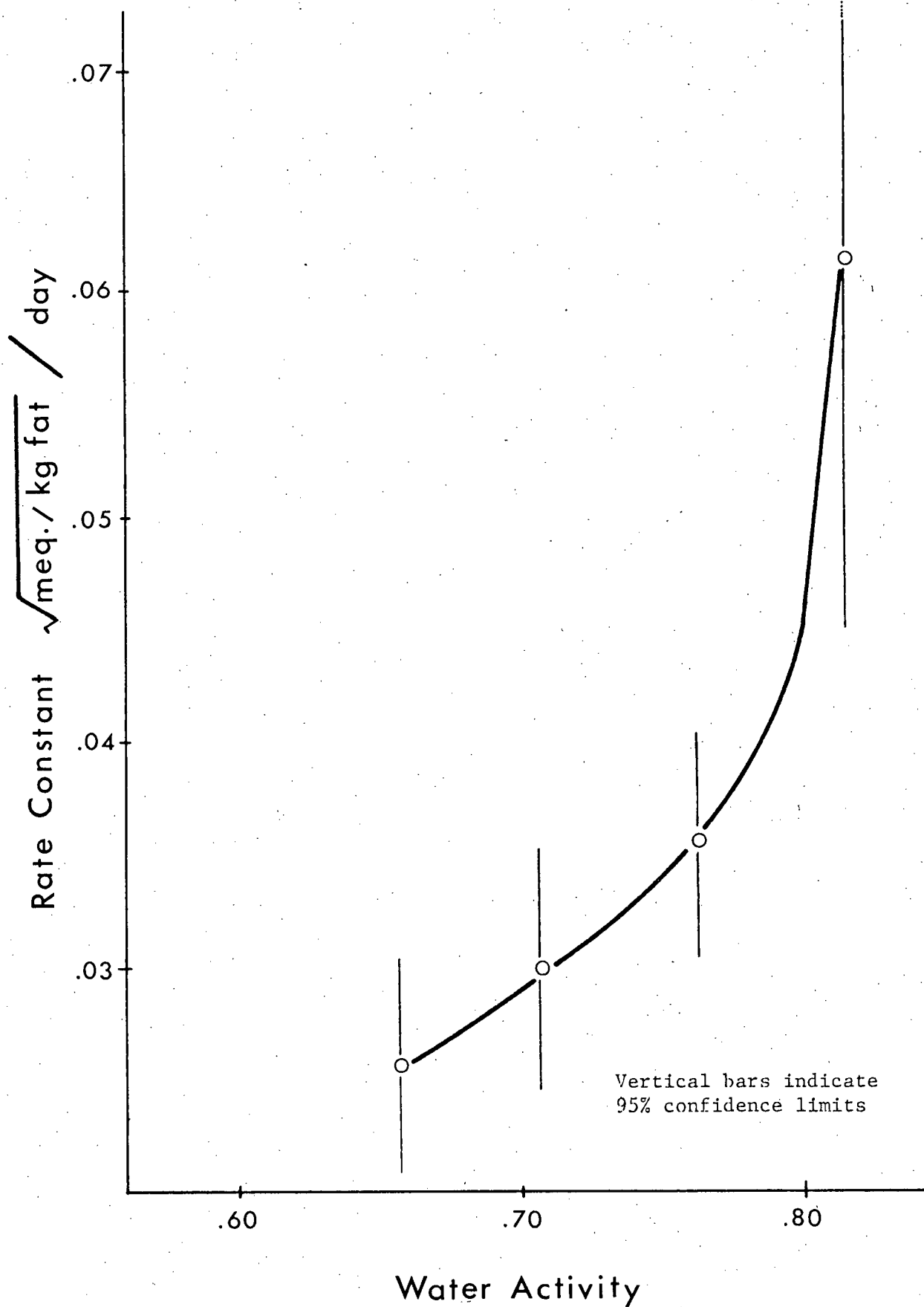


Figure 2. Rate of Lipid Oxidation at Different Water Activities

Table VI     Duncans Multiple Range Test  
 Effects of Different Citrate Levels and  $A_w$  on  
 Peroxide Values

Citrate Level	Mean Peroxide Value			
	Water Activity			
	0.655	0.707	0.763	0.818
%				
0.0	25.84 a	28.07 c	36.81 d	10.16 e
1.0	22.80 ab	28.28 c	36.52 d	24.63 ac
2.2	18.72 b	26.73 ac	37.37 d	47.10 f

means with different subscripts are significant at  $\alpha = 0.05$

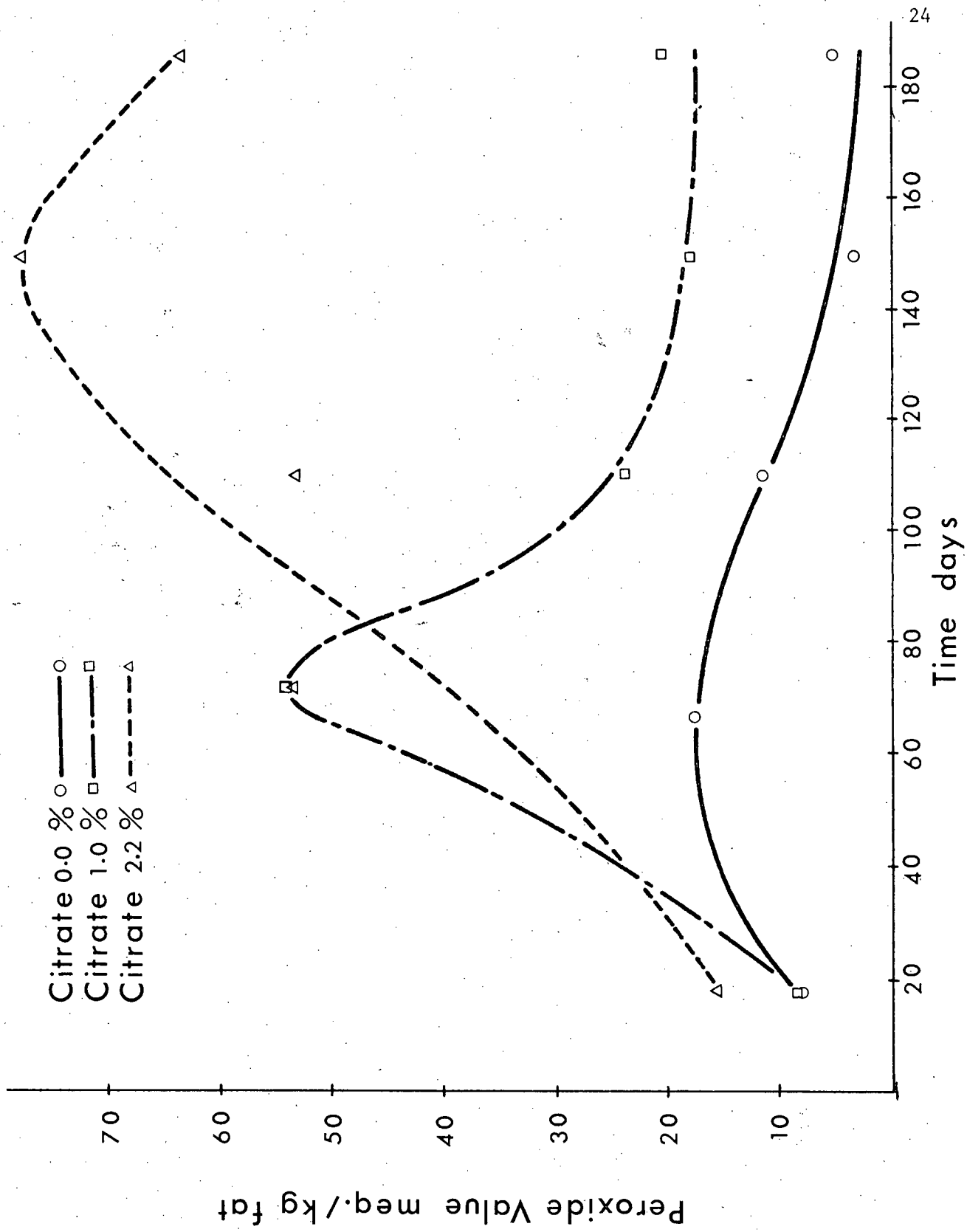


Figure 3. Change in Peroxide Value at Different Citrate Levels (Aw 0.818)

peroxides. (15) The mode of action was not explained. Citrate had no apparent effects on the peroxide value at the lower water activities studied (Figure 4). Sodium citrate crystalizes out of solutions at water activities below  $A_w$  0.75. (6) It is probable that citrates would not be mobile to reach reaction sites to protect peroxides at lower water activities than  $A_w$  0.763 studied.

It is doubtful that citric acid has any effect on the rate of peroxide formation. There was little change in the rate constant ( $K_m$ ) at  $A_w$  0.707 and 0.763, and a slight decrease at  $A_w$  0.655 (Figure 5). There appeared to be a slight increase in  $K_m$  at  $A_w$  0.818 by increasing the citrate level in the infusate. Samples at this water activity reached maximum P.V. very rapidly, limiting the number of observations useful for calculating rate constants (Table V). As a result, the confidence limits ( $\alpha = 0.05$ ) are very large.

In summary, citric acid was not effective at preventing peroxide formation in the water activity range studied, but it did protect peroxides from decomposing. In cooked meats, ferric hemochromogen produced from heat-denatured myoglobin and hemoglobin is possibly the major catalyst in peroxide formation and decomposition. (27) If citric acid is not an effective sequesterant for this hemo-bound iron, citrate would have no effect on peroxide formation. If this theory is valid, citrate would have to protect peroxides from decomposing by increasing the stability of the peroxide radical rather than by decreasing the catalytic effect of the ferrous hemochromogen.

Varying the fat content had the least effect on peroxide values

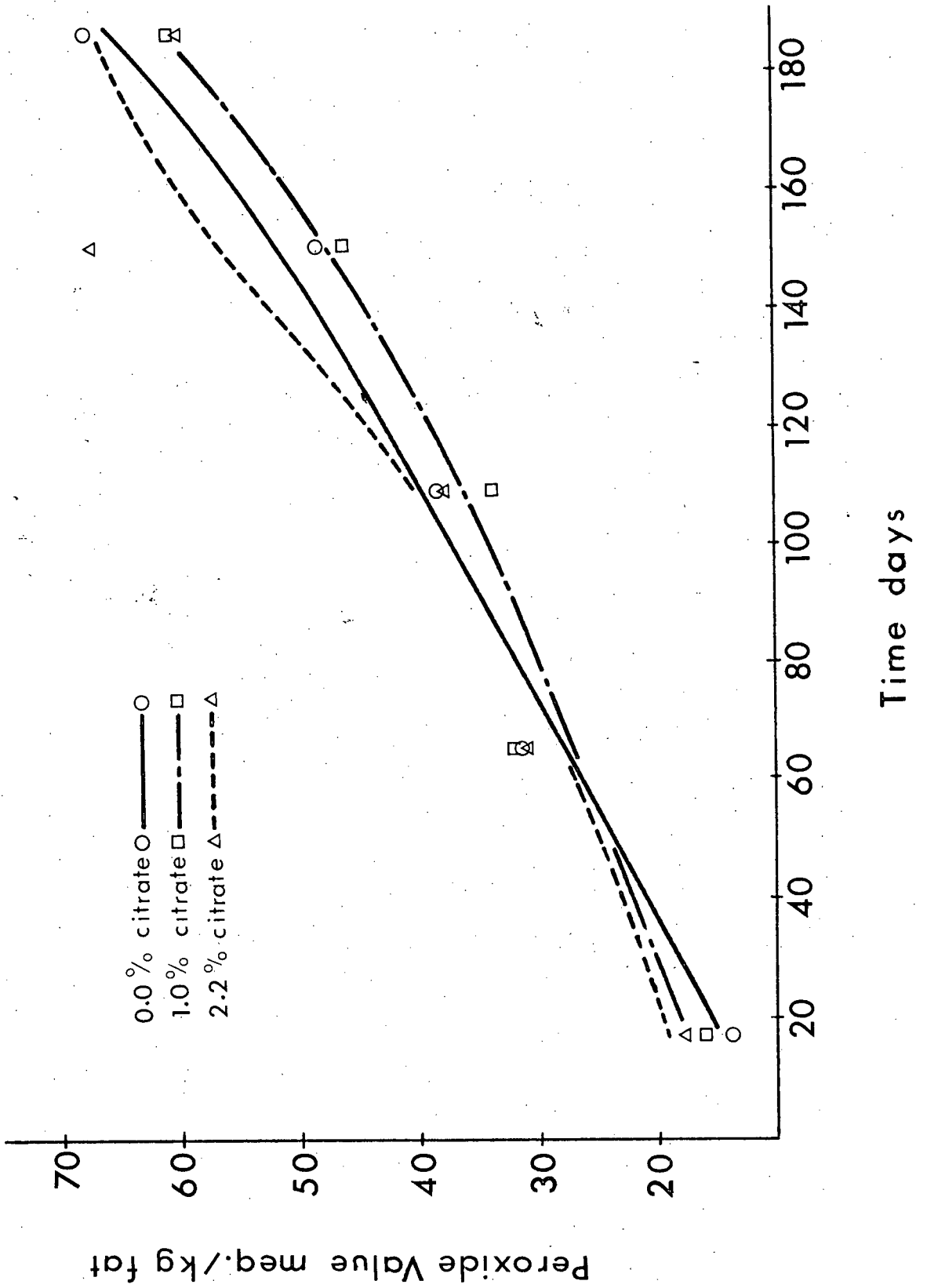


Figure 4. Change in Peroxide Value at Different Citrate Levels (Δw 0.707)

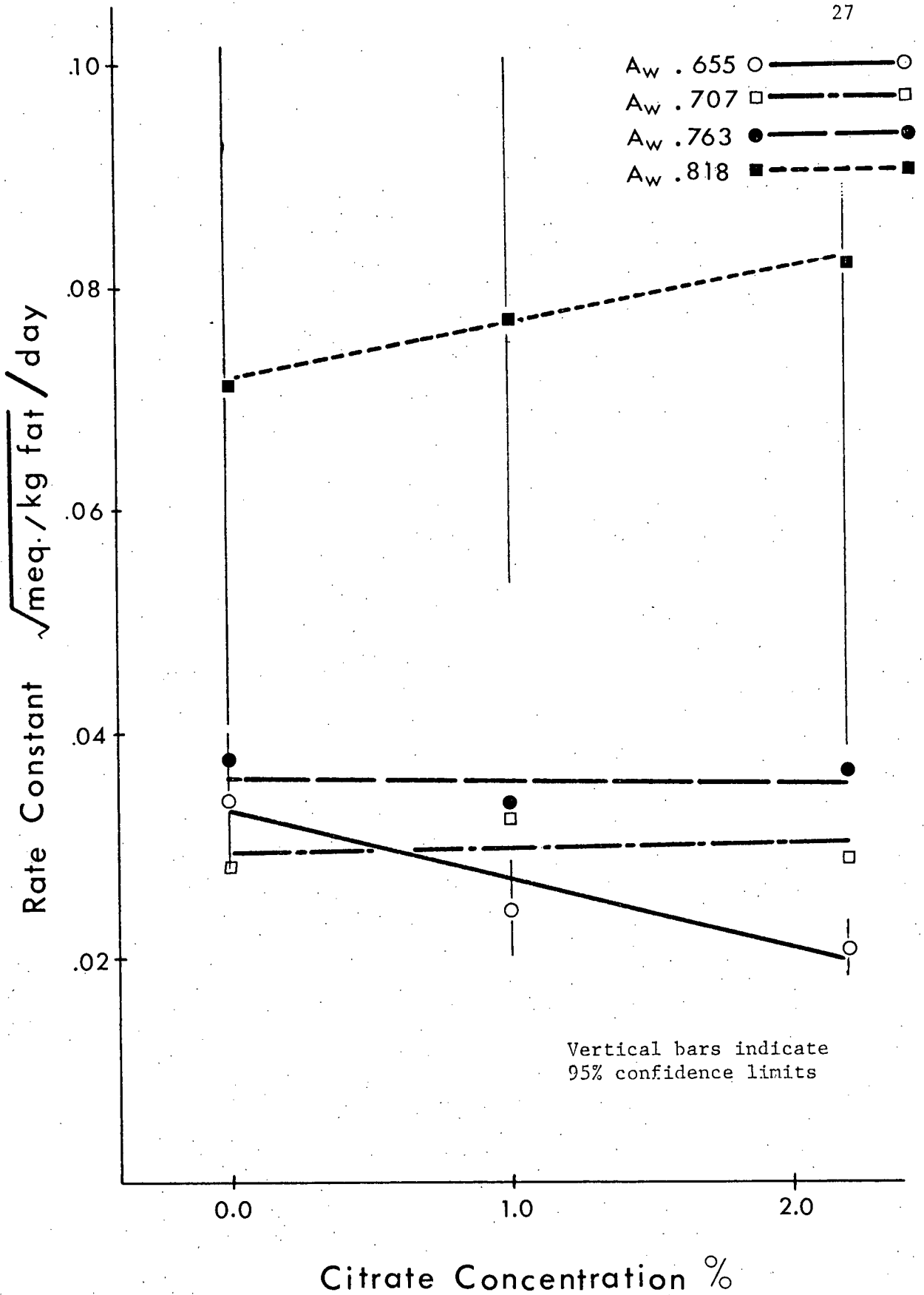


Figure 5. Rate of Lipid Oxidation at Different Citrate Concentrations

(Table III). A higher average P.V. was observed at 5.2% fat than at 3.3% or at 6.6% (Table VII). There appeared to be little consistency as to whether increased fat content increased or decreased oxidation. It is possible that the amount of fat is not an important factor in lipid oxidation. Watts (38) found no relation between the amount of fat in cooked beef to TBA values. For some unknown reason, samples with 5.2% fat also had a significantly higher moisture content than 3.3% and 6.6% fat contents. The higher moisture content could explain the higher P.V. at 5.2% fat.

A significant decrease in pH was observed through the time period (Table VIII). The pH change in samples at Aw 0.657, 0.707, 0.763 was small, but samples at Aw 0.818 decreased by about 0.3 pH units over the test period (Figure 6). The pH appeared to decrease at a steady rate between 30 and 190 days, suggesting peroxide decomposition is at a steady rate in this period. Aldehydes formed from the scission of peroxides are oxidized to the corresponding carboxylic acids. (30) It has also been postulated that aldehydes can be decarboxylated to produce formic acid and its esters. (26) Formic acid has a lower dissociation constant than other carboxylic acids, and therefore, decreases the pH more.

Samples for non-enzymatic browning analysis developed very low absorbencies in water extracts over the test period. The optical density (O.D.) increased from an average of 0.004 to 0.007 units in 185 days ( $\lambda = 420 \text{ m}\mu$ ). This suggests that a very little amount of water soluble melanoidens were formed. If non-enzymatic browning was occurring to any extent in the test samples the increase in O.D. would be much higher. (17)

Table VII Duncans Multiple Range Test  
Effects of Fat Levels and Aw on Peroxide Values

Fat Level	Peroxide Value				Av.P.V.
	Water Activity				
%	0.655	0.707	0.763	0.818	
3.3	18.79 a	23.59 ab	37.73 de	32.92 c	28.30 f
5.2	25.88 b	35.66 cdc	39.36 e	26.09 b	31.75 g
6.6	22.57 ab	23.84 b	33.62 cd	23.36 ab	25.85 h

Means with different subscripts are significant at  $\alpha = 0.05$ .

Table VIII Duncans Multiple Range Test  
Change in pH with Time

Time Day	pH
30	6.12 a
97	6.03 b
150	6.01 bc
180	5.99 c
193	5.95 d

Means with different subscripts are significant at  $\alpha = 0.05$ .

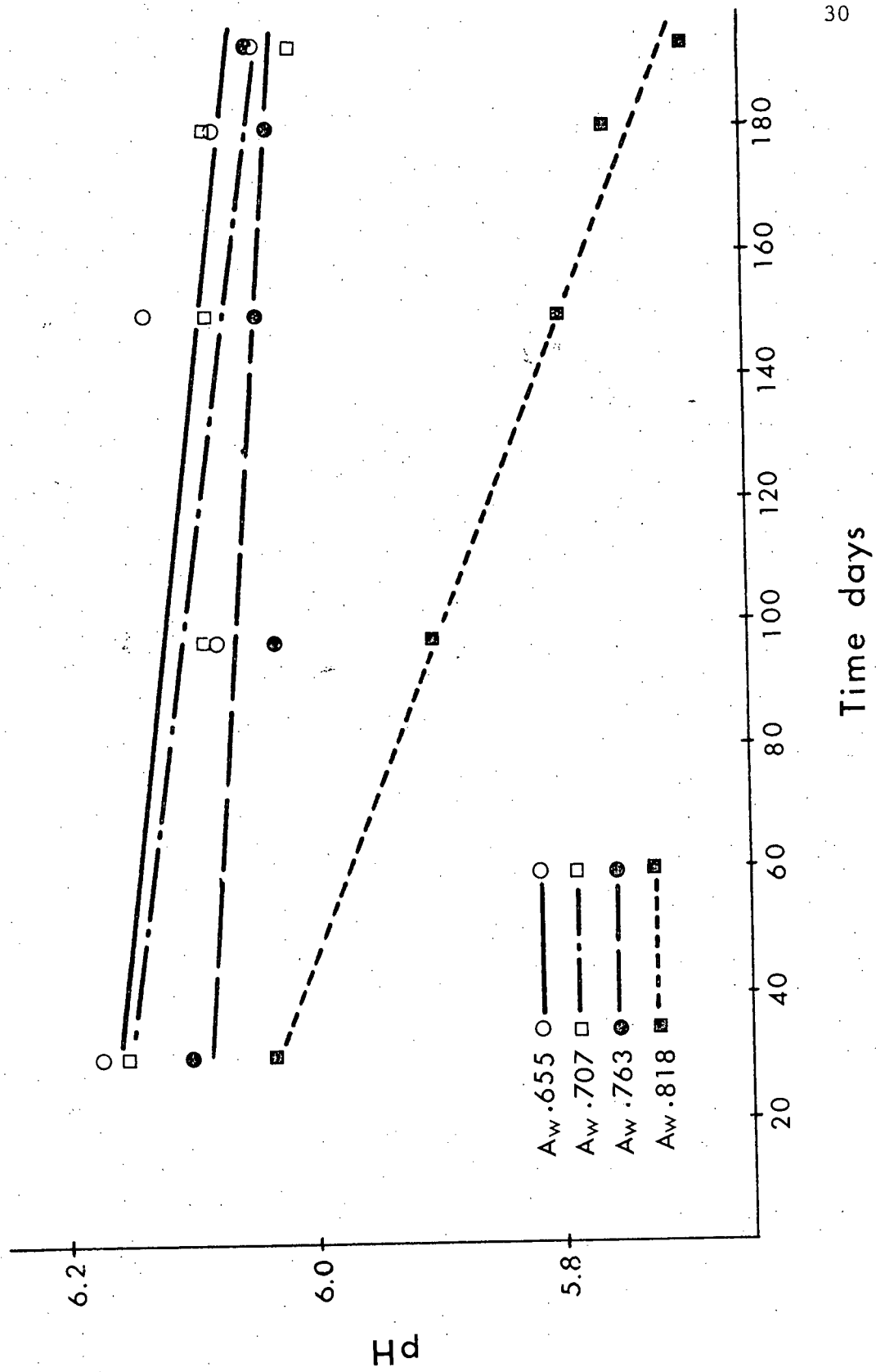


Figure 6. Change in pH with Time at Different Water Activities

The test variables did not affect the O.D. in a way indicative of browning. For example, the pH had no significant effect on the O.D. Increasing pH should increase the O.D. (32) Decreasing water activity appeared to reduce slightly the O.D. This is the opposite to results expected from non-enzymatic browning. (18) Samples at Aw 0.657 showed the highest O.D., but did not always produce clear filtrates (1.2 $\mu$  filter pore size).

Hunter L and b values increased significantly with time (Table X). Hunter a values did not change appreciably.

An increase in L values is indicative of increasing lightness, and in this case, a fading of the brown "meat" colour. This loss of colour suggests that the cooked meat pigments are being oxidized. Watts (37) found that myoglobin, as well as being a catalyst for lipid oxidation, is also oxidized, resulting in colour fading. Heat denatured myoglobin would be the primary meat pigment in this study. There would be very little melanoiden formation because of the low cooking temperatures used. Melanoidens and the heme group of myoglobin are very unsaturated molecules, therefore, would be susceptible to oxidation. Loss of colour would result from the destruction of the molecule. The loss of the brown "meat" colour was visually apparent during the test period.

Increasing b values indicate that the intensity of the yellow colour of the samples is increasing. One of the products of lipid oxidation is the formation of polymers. They are brown in colour and in low concentrations could account for increasing Hunter b values. The increase in yellowness was not noticeable visually.

Table IX Analysis of Variance - Hunter L and b Values

Source of Variance	d.f.	Hunter L Values	Hunter b Values
		F score	F score
Blocks	1	25.9	14.1
Time	4	52.1	210.9
pH	2	140.5	151.6
Citrate cont.	1	52.3	12.1
Water activity	3	213.8	309.0

All F scores are significant at  $\alpha = 0.01$ .

Table X Duncans Multiple Range TestChanges in Hunter L a b Values with Time

Time Day	L Values	a Values	b Values
10	29.7 a	2.7 a	5.1 a
68	32.6 b	2.1 b	7.0 b
111	33.9 c	2.4 b	8.0 c
170	34.6 cd	2.2 b	9.1 d
213	35.3 d	2.2 b	10.0 c

Means within each column with different subscripts are significant at  $\alpha = 0.05$ .

Changes in water activity had the greatest effect on Hunter L and b values (Table IX). The Hunter colour values increased rapidly at the beginning of the test period (Figure 7). The rate of change decreased with time to a possible maximum value or a slow constant rate of increase. Hunter L values would be expected to reach a maximum because the total amount of denatured myoglobin that can be oxidized is constant.

Samples at Aw 0.653 were visibly much darker in colour than the samples at higher water activities. The dark brown colour of the lowest moisture samples closely resembled that of a meat patty if it had been cooked by frying. Initial Hunter L and b values were increased by water activity (Table XI). It is probable that the rapid change in colour in the first ten days accounts for some but not all of this initial difference between the water activities. Increasing the glycerol content affected the colour of the cooked meat patties. Although texture was not assessed experimentally, it was observed that patties at low water activities were much harder to break up than were those with higher water activities. The higher glycerol concentrations also increased the stiffness of the gelled infusate after cooking. It is possible that glycerol, by removing "free" water surrounding the meat proteins, gelatin and starch, allows closer intermolecular contact and interaction upon cooking, causing increased toughness and the darker colours.

Figure 8 shows the difference between Hunter colour values at the first observation and each subsequent observation. There was a slight difference in the amount of Hunter L, and b increased in value at different water activities (Figure 8). Higher water activities tended to show a greater increase in yellowness and colour fading than a lower

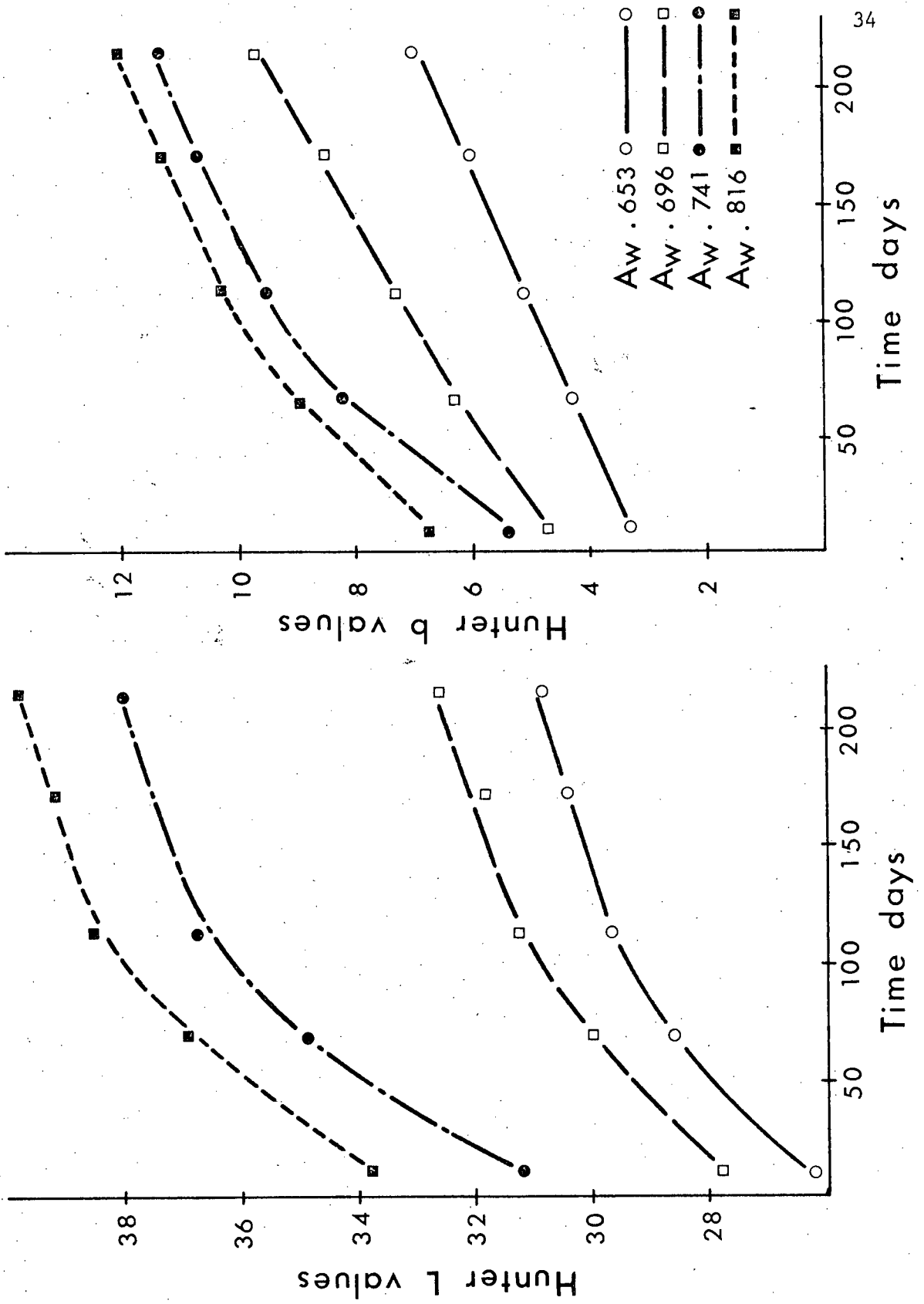


Figure 7. Change in Hunter L and b Values at Different Water Activities

Table XI     Duncans Multiple Range Test  
Initial Hunter L and b Values at Different Water  
Activities

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Water Activity	L Values	b Values
0.653	26.1 a	3.3 a
0.696	27.8 a	4.7 b
0.741	31.2 b	5.4 b
0.816	33.8 c	6.8 c

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Means within each column with different subscripts are significant at  $\alpha = 0.05$ .

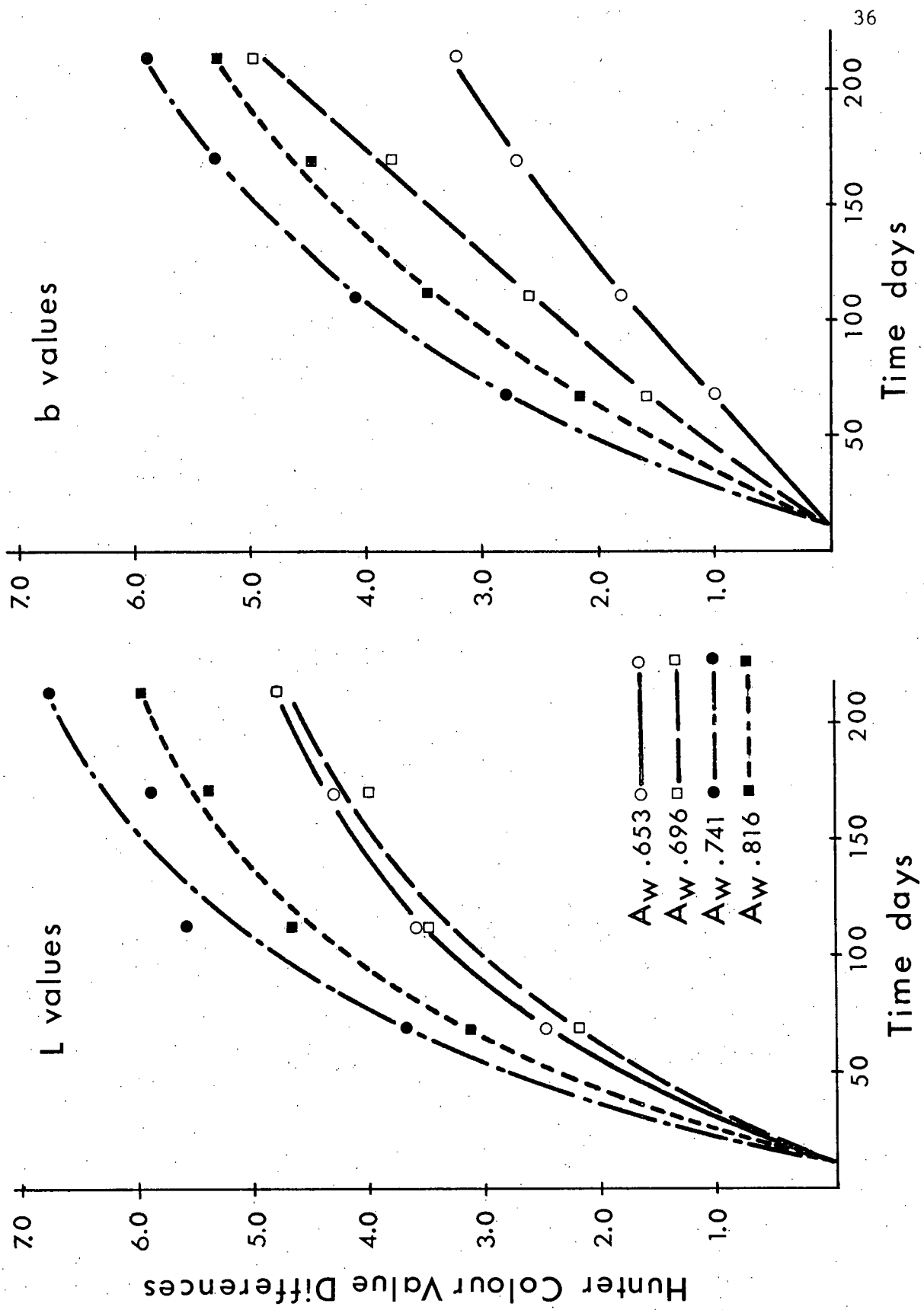


Figure 8. Differences in Hunter Colourmeter Values Between the Initial Observation at 10 Days and Each Subsequent Observation at Different Water Activities

water activity. This is consistent with the observation that increases in peroxide values are greater at high water activities. Colour changes were not as dependent on changes in water activity as was the P.V. The greatest significant difference was between samples at  $A_w$  0.696 and 0.741.

The sample pH had very little significant effect on initial Hunter L and b values (Table XII). The greatest difference was the effect the pH had on the change in values with time (Figure 9). Samples at pH 4.96 and at 5.38 increased in yellowness and the colour faded more than in samples at pH 6.01. A pH dependency was found in fresh meats where low pH increased peroxide values greater than did high pH. (37) This dependency disappeared in cooked meats where the P.V. did not increase appreciably. Later Tims and Watts (36) found that cooked meats increased TBA values faster than fresh meats, but the effect of pH was not documented. Green (11) found that if fresh meat was kept at high pH (6.2) it remained red under aerobic conditions and rancid odours did not occur. The results of this study suggest that the oxidation of cooked meat pigments is dependent on pH.

Block 2 was observed to fade more and develop higher peroxide values than block 1 (Table XIII). The greatest significant difference between these blocks was the pH. The average pH of block 2 was lower than that of block 1 in both lipid oxidation test samples and water soluble pigment extraction samples. This is a further indication that low pH increases lipid oxidation in intermediate moisture cooked meats.

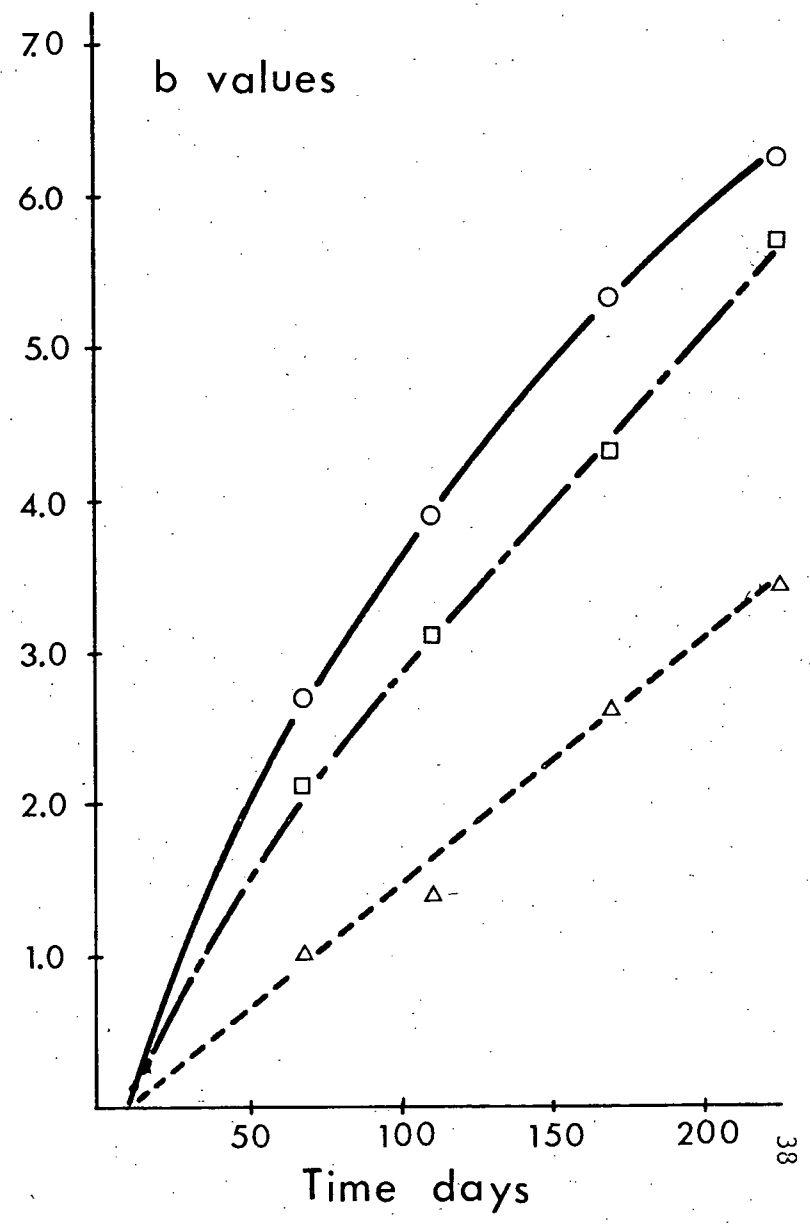
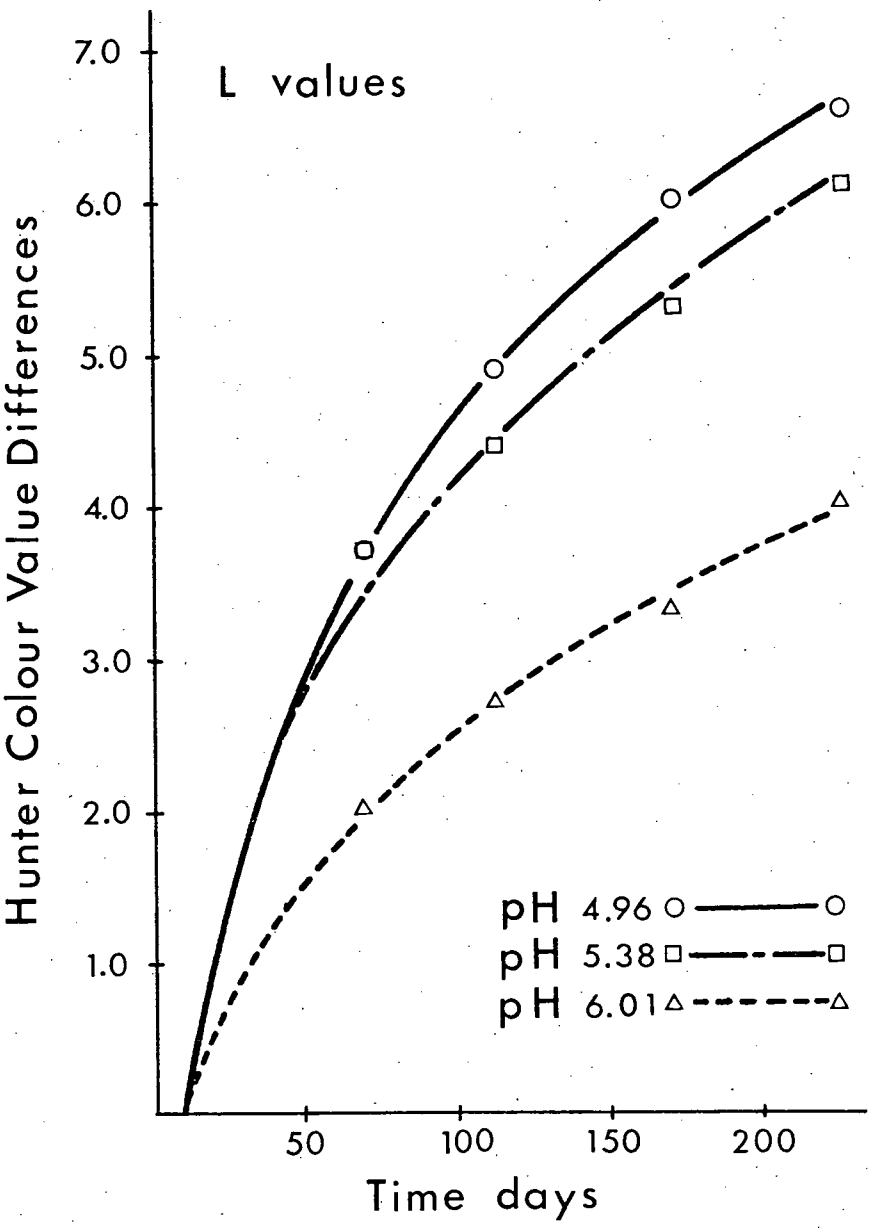


Figure 9. Differences in Hunter Colourmeter Values Between the Initial Observation and Each Subsequent Observation at Different pH Levels

Table XII Duncans Multiple Range Test  
Initial Hunter L and b Values at Different pH

pH	L Values	b Values
4.96	30.9 a	5.2 a
5.40	30.8 a	5.3 a
6.01	27.6 b	4.7 a

Means within each column with different subscripts are significant at  $\alpha = 0.05$ .

Table XIII Analysis of Variance Block Differences

Lipid Oxidation Test Samples			
Mean Values	Block 1	Block 2	F score
peroxide value	25.22	32.05	182.1*
pH	6.24	5.82	1530.8*
moisture cont.	41.66	40.59	12.9*
water activity	0.735	0.735	0.2 N.S

Non-enzymatic Browning Test Samples			
Mean Values	Block 1	Block 2	F score
Hunter L	32.5	33.9	52.1*
Hunter b	7.6	8.1	14.1*
pH	5.54	5.37	142.0*
moisture cont.	29.22	40.91	33.1*
water activity	0.733	0.722	16.5*

\*significant at  $\alpha = 0.01$

The pH was observed to decrease during lipid oxidation (Figure 6) and low pH was found to catalyse pigment oxidation (Figure 9). It is interesting that the acid by-products of lipid oxidation accelerate further oxidation.

In Figure 9 and Figure 8 increases in Hunter L values correspond to the increases in Hunter b values. The reaction profiles are quite similar. The increase in Hunter b values would not necessarily be expected to follow the same shape of curve as increases in Hunter L values if the b values were the result of polymer formation. Colour development (Hunter b values) from polymerization would be expected to have a longer lag phase in relation to myoglobin oxidation (Hunter L values), polymerization being an end product of lipid oxidation. Figure 9 suggests that a loss in the brown "meat" colour corresponds to an increase in yellow by-products. Lawrie (22) reports that during high temperature storage (35°C) of dehydrated meat, myoglobin is converted to bile pigments causing the meat to pale and yellow. It is possible that the increase in Hunter b values is due to bile pigments and not initial polymerization products as previously mentioned.

This study suggests that lipid oxidation is a problem to be considered when formulating an intermediate moisture meat product. Samples prepared for this study were limited to three or four months' shelf life by rancid odours. Citric acid, the only antioxidant tested, did not control the development of peroxides. Much investigation will have to be done to prolong the shelf life of these meat products to meet public approval.

### Conclusion

1. Lipid oxidation was the prominent deteriorating factor of the intermediate moisture meat patties produced, limiting their shelf life to three or four months.
2. This study confirms that as water activity increases between  $A_w$  0.65 and 0.82, lipid oxidation increases.
3. Citric acid has possibly very little effect on the rate of peroxide formation but does protect peroxide breakdown.
4. The pH decreased during lipid oxidation.
5. Pigment oxidation in the meat patties resulted in their colour fading and yellowing.
6. Low pH increased the rate of pigment oxidation.

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