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THE COLOUR OF COOKED CURED PORK.

I.—Estimation of the Nitric oxide–Haem Pigments

By H. C. HORNSEY

A simple and rapid method is described for extracting and measuring the nitric oxide–haem pigments present in cooked cured meat.

Selective extraction as a nitric oxide–haem–acetone complex is achieved by the use of an acetone/water solvent. Other meat pigments are not extracted under the conditions used.

The acetone/water ratio is shown to be critical, maximum extraction being obtained with a ratio of 4 : 1, due allowance being made for the moisture present in the meat. After filtration, the optical density is measured spectrophotometrically.

With the inclusion of hydrochloric acid in the solvent, the method can be adapted to measure the *total* pigments present.

Introduction

The concentration and the stability of the cured meat pigments nitroso-myoglobin and nitroso-haemoglobin are of great importance to all concerned with cured meat products, and particularly is this so in the case of cooked cured pork products.

Generally, the assessment of the depth and stability of colour is determined visually, and comparisons made at the same time by an expert can provide useful information. However, in order to determine the factors affecting the cause and rapidity of fading, an objective measurement became necessary. The rate of fading of cooked meat pigments can only be studied if a fairly rapid estimation is used, in which no further oxidative changes take place during the determination.

Anderton & Locke[1] have recently published a note on the extraction of these pigments by first wetting the meat with acetone, and then extracting with ether. In these laboratories, a method of extracting the pigment by means of 75% acetone in water has been in use for several years, chiefly to assess the degree of conversion of fresh meat pigments to those of the cured meat. This method is simple and reasonably accurate, and its use has been adopted for the study of the distribution and fading of colour in cooked gammons which will form the subject of subsequent papers.

Experimental

Extraction of colour

During the routine examination of imported cooked meat products for the presence of synthetic colouring materials, interference was frequently found when the natural nitric-oxide pigments were present. These colours appeared to be extracted when acetone was used as a solvent, and also to some extent when alcohol was used. This observation led to a closer examination of the possibility of using acetone for estimating the amounts of cured meat pigments present.

When the minced lean portion of cooked cured meat was triturated with acetone, it was found that some extraction of colour was achieved. This did not appear to be complete, however, and some colour still remained in the tissues. Further, the degree of extraction did not appear to be constant, as variable results were obtained when different ratios of acetone to meat were used. Exhaustive extraction was tried, and was also found to give incomplete and variable results. It appeared, however, when smaller amounts of acetone were used, that less residual colour was left in the tissues. Lean meat contains 65–70% of water, and it was therefore thought that this effect was due to different acetone/water ratios. This point was therefore investigated, due allowance being made for the moisture present in the sample.

Experiment I.—Minced lean meat was first mixed to a smooth paste with approximately 10 ml. of the solvent. The remainder of the solvent was then added, and after 5 minutes, with intermittent mixing, and then filtering, the intensities of the colours of the resulting solutions were measured in a 1-cm. cell at a wavelength of 540 mμ using a Unicam S.P. 600 spectrophotometer. The results are shown in Table I and graphically in Fig. 1.

Table I

Optical densities of extracts from lean meat using different concentrations of acetone

Lean meat		Solvent		Acetone concentration, %	Optical density
Wt., g.	Water present, ml.	Acetone, ml.	Water, ml.		
5	3·5	96·5	0	96·5	(× 4) 0·324
5	3·5	46·5	0	93	(× 2) 0·330
10	7	43	0	86	0·360
10	7	40	3	80	0·370
10	7	37·5	5·5	75	0·360
10	7	35	8	70	0·308
10	7	32·5	10·5	65	0·270
10	7	30	13	60	0·225

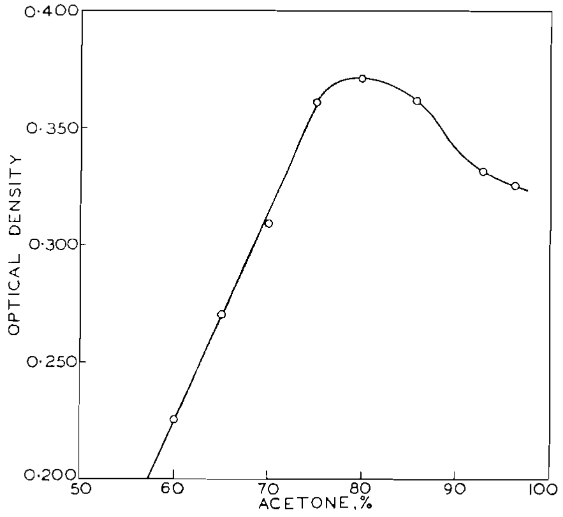

FIG. 1.—*The effect of varying the strength of an aqueous acetone solution, on the extraction of colour from cooked cured pork gammon*

From these results (Table I, Fig. 1) it can be seen that there is a fairly critical acetone/water ratio for maximum extraction of the colour, i.e., between 75 and 85% acetone. Exhaustive extraction with this strength of acetone gave a colour-free residue with those lean meats which showed complete conversion of pigment, i.e., which did not contain any denatured haemoglobin/myoglobin which was uncombined.

Experiment II.—To confirm that only the nitroso derivative of the blood and muscle pigments was being extracted, and that the other forms of the pigments, viz., reduced haemoglobin, oxyhaemoglobin, and methaemoglobin, were not contributing some colour also. Solutions of these were obtained in the following manner.

Equal aliquot parts of a haemolysed blood solution were treated with sodium hydrosulphite, air, ferricyanide, and hydrosulphite with sodium nitrite, respectively, and half of each were denatured by boiling for 1 min. All were then diluted to 50 ml. with acetone and water to give a final concentration of 80% acetone. After filtering, the optical densities were measured at 540 mμ in a 1-cm. cell.

Table II shows that little or no interference occurred with these pigments. Methaemoglobin gave a trace of haematin in solution, but the optical density at 540 mμ was $<2\%$ of that of the nitrosohaemochrome.

Table II

Optical densities of extracts of blood pigments

Pigment	Optical density of acetone extract
Reduced haemoglobin	0·003
,, ,, (denatured)	0·005
Oxyhaemoglobin	0·003
,, (denatured)	0·005
Methaemoglobin	0·010
,, (denatured)	0·010
Nitroso-haemoglobin	0·302
,, (denatured)	0·600

Experiment III.—Comparison of this method of extraction by 80% acetone with the procedure outlined by Anderson & Locke,[1] who do not recommend any specific amounts of acetone and ether, gave the results shown in Table III.

Table III

Optical densities of extracts of 10 g. of lean meat containing 7 ml. water prepared with acetone and with acetone + ether

Lean meat	Solvent	Acetone : total water ratio	Optical density at 540 mμ in 1-cm. cell
	40 ml. acetone + 3 ml. water	4 : 1	0·360
	8 ml. acetone + 35 ml. ether	1·14 : 1	0·215
	14 ml. acetone + 35 ml. ether	2 : 1	(× 1·12) = 0·291
	28 ml. acetone + 35 ml. ether	4 : 1	(× 1·4) = 0·350

The above values, which are corrected for the extra dilution introduced by the ether, show that this procedure gives variable and lower extraction unless the ratio of acetone used to the water present (in the meat) reaches 4 : 1, i.e., the same critical value which was found necessary in the absence of the ether. The other ratios give values which lie on the same curve as in Fig. 1. This suggests that the ether is only acting as a diluent and does not affect the extractions.

Stability and nature of extracted colour

The bright red acetone extracts were stable for at least an hour, then gradually faded to a yellow-brown colour. This process was accelerated in very strong light, and in the presence of air, but was never rapid enough (when using cooked meats) to cause interference with the determination. Ample time, therefore, is available for making the measurements, before any fading commences.

The typical wavelength/absorption curve for the extracted colour before fading is shown in Fig. 2 together with that of the same extract after standing 24 hours in the laboratory under normal lighting conditions.

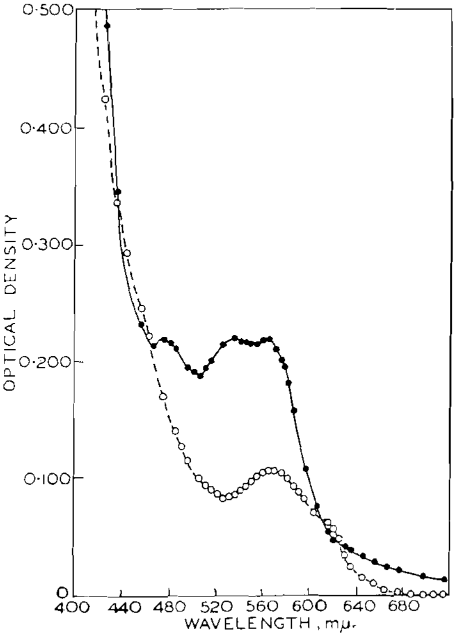

FIG. 2.—*Optical densities of*

●—●—●—● (*a*) *An extract of cooked cured pork gammon (nitroso-haem–acetone complex) with* 80% *acetone (on acetone + total water present)*

○- -○- -○- -○ (*b*) *The same extract after standing for* 24 *hours under normal lighting conditions in the laboratory*

The curve for the extracted pigment showed absorption maxima at 476, 535 and 563 mμ. This is somewhat similar to the curve of aqueous nitroso-haemoglobin but has the double peaks rather flattened and at slightly shorter wavelengths, with an additional peak at 476 mμ. The minimum at approximately 500 mμ tends to be flattened and moved slightly to longer wavelengths by this additional peak of the acetone complex.

A similar curve to that of the 80% acetone extract was obtained when haematin was reduced with sodium hydrosulphite, a trace of sodium nitrite added, and diluted with four parts of acetone.

The curve in Fig. 2 of the yellow-brown pigment left in solution after exposure of the acetone extract had an absorption maximum at 566 mμ and a minimum at 525 mμ. A solution of alkaline haematin in 80% acetone was found to give an identical absorption curve. Hunter's data[2] give a somewhat similar curve for an alcoholic solution of alkaline haematin, which is of identical shape but moved 30 mμ towards the longer wavelengths.

Hamsik[3] has described the acetone and alcohol complexes of haem, and the evidence, which is also confirmed by Anderton & Locke,[1] indicates that the extracted pigment is the acetone complex of nitric oxide haem. In acetone solution the decomposition product appears to be alkaline haematin, as distinct from the acid haematin which Anderton & Locke[1] have found in their ether/acetone extracts, and which we also have been able to confirm.

It had been noticed that *uncooked* cured meat which contains the undenatured nitroso pigments, although readily yielding its colour in 80% acetone, gave an extract which was much more susceptible to rapid fading. This rate varied with each individual sample of uncooked meat examined. Some samples gave almost as good a stability as is found with the extracts of cooked meats, whereas others showed some signs of fading after a few minutes. Again, when experiments were attempted using solutions prepared from pure (thrice recrystallized) haemoglobin and myoglobin, almost instantaneous fading to haematin occurred on dilution of the aqueous solutions with acetone.

These results indicate that reducing substances present to a varying degree in the uncooked meats, and to a greater degree in the cooked meats, are also extracted into the 80% acetone, and confer stability for some time on the acetone–nitroso-haem complex by acting as a ' buffer ' against the light-catalysed air oxidation.

Consideration of the possible substances present indicate that free SH compounds such as cysteine and reduced glutathione are the most likely compounds to produce this effect. Both are capable of reducing the ferric haem pigments to the corresponding ferrous compounds.[4] With pure pigments they would be absent, and in uncooked meats would probably exist partly in the oxidized and partly in the reduced forms, depending on the conditions within the meat, whereas in cooked meats, at the low oxidation-reduction potentials and low oxygen tensions of denatured tissue, it appears likely that they would be present entirely in the reduced form.

If this is indeed the explanation, then the addition of cysteine to solutions of pure pigments should result in stabilization. Accordingly, 1 ml. of a fresh 0·5% solution of neutralized cysteine hydrochloride was added to 9 ml. of a solution of nitroso-myoglobin prepared from crystalline myoglobin. On dilution of this with 40 ml. of acetone, no fading of the red colour occurred for several hours, whereas in the absence of the cysteine, oxidation to haematin was almost instantaneous.

The addition of cysteine, in the extraction process, although not necessary when examining cooked meats, may, however, be advantageous when uncooked meat is being investigated.

This effect of cysteine and glutathione is also of importance in the stability of the pigments within the meat, and this aspect is further considered in work on the fading of hams, which will be published later.

Method of measurement in cooked meats

The lean meat, after trimming off the fatty tissue, is minced, mixed and then repassed through the mincer. This operation should be carried out in a darkened room, and with the minimum of delay, as it will be shown in a later paper that even a short exposure to light will lead to a slow reaction with air afterwards, even if it is then stored in the dark.

Ten g. of the minced sample, in a tall beaker to prevent undue evaporation, are first mixed to a smooth paste with approximately 10 ml. of a mixture containing 40 ml. of acetone and 3 ml. of water. The remainder of the acetone solution is then added, and after five minutes with intermittent mixing, the solution is filtered. The light absorption of the filtrate is measured at a wavelength of 540 mμ using a 1-cm. cell, with an 80% acetone/water solution as a blank. The values so obtained may be used directly as a comparative measure of the pigment concentration.

The addition of a known volume of liquid, i.e., 40 ml. of acetone + 3 ml. of water + 7 ml. of water derived from 10 g. of meat, was adopted in preference to dilution to 50 ml. in a graduated flask, for the following reasons :

1. Correction for the volume of the insoluble meat tissues was avoided.
2. Calculation of the required proportions of acetone and water was simpler.
3. Transference from one vessel to another, leading to increased aeration of the extract, was avoided.

Adaptation to measurement of total pigments

Replacement of 1 ml. of water by 1 ml. of concentrated hydrochloric acid in the solvent used, and keeping for 1 hour before filtering, gave a solution of acid haematin in the 80% acetone. This is composed of haematin derived from any uncombined pigments present, together with that resulting from the oxidation of the nitric oxide pigments. The optical density of this filtrate at 640 mμ is then a measure of the *total* haem pigments present in the meat.

Standardization

Conversion to units of the concentration of pigment involves the preliminary standardization of a nitroso-myoglobin solution. This is not a simple procedure, and as comparisons and not absolute values were of primary importance for the future work envisaged, the following method was adopted :

The absorption at 540 mμ of an 80% acetone extract of nitroso-haemoglobin (derived from whole blood) was measured. To this solution was added one drop of concentrated hydrochloric acid, and after setting aside for 2 hours to complete the oxidation, the absorption was again measured at the peak wavelengths of acid haematin in 80% acetone, i.e., 640 mμ and 512 mμ. Measurement of the absorption of a standard acid haematin solution in 80% acetone (Fig. 3) then enabled both the total pigments and the extracted nitric oxide pigments to be expressed in terms of parts per million of haematin.

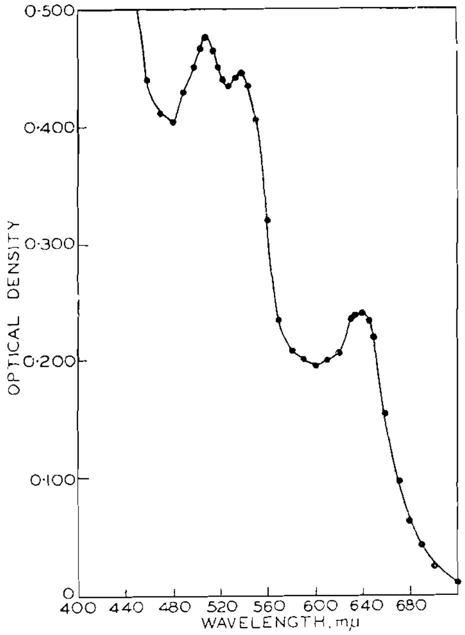

FIG. 3.—*Optical density of a 0·05mM-solution of haematin hydrochloride in 80% acetone containing 2% hydrochloric acid*

Triplicate standardization of haematin hydrochloride (Fe 8·46%) dissolved in 0·1N-sodium hydroxide, then diluting to 0·05M with acetone, conc. hydrochloric acid and water to give a final concentration of 80%, 2%, 18%, respectively, gave the following results:

$$E^{\text{mM}}_{512} \text{ of acid haematin in } 80\% \text{ acetone} = 9{\cdot}52$$
$$E^{\text{mM}}_{640} \text{ of acid haematin in } 80\% \text{ acetone} = 4{\cdot}80$$

From this it can be calculated that when using 10 g. of meat and a total fluid volume of 50 ml., the absorption at 640 mμ in a 1-cm. cell, multiplied by the factor 680 gives the concentration of total pigments in the meat as p.p.m. of haematin.

Standardization of the nitroso-haem extracts derived from blood, cooked cured pork, and cooked cured beef, by the above method, all gave identical results, i.e.,

$$\begin{aligned} E^{\text{mM}}_{540} \text{ of acetone–nitroso-haem in acetone } 80\%, \text{ water } 20\% &= 11{\cdot}3 \text{ (blood)} \\ &= 11{\cdot}3 \text{ (pork)} \\ &= 11{\cdot}3 \text{ (beef)} \end{aligned}$$

Under the recommended conditions, therefore, using 10 g. of meat, and a total fluid volume of 50 ml., the absorption of the acetone–nitroso-haem at 540 mμ in a 1-cm. cell multiplied by the factor 290 gives the concentration of nitroso pigments in the meat as p.p.m. of haematin.

In the estimation of total pigments, readings at both 512 mμ and 640 mμ should be made, and the ratio should not be greater than 2·0 if oxidation of the nitroso-haem to haematin is complete, as the following data shows:

	Acid haematin in 80% acetone	Nitroso-haem–acetone in 80% acetone
Ratio $\frac{E_{512}}{E_{640}}$	1·9	>5·0

Solutions in 80% acetone of both acid haematin and acetone–nitroso-haem were found to conform with Beer's Law, straight lines passing through the origin being obtained in both cases.

Conclusions

In cooked lean meat, both the total and nitroso pigments, expressed as parts per million of haematin, can be quickly assessed, and the amount of uncombined pigment obtained by difference. The degree of conversion of the original pigments to the nitric-oxide derivatives can also be obtained.

The proposed method for measuring nitroso-haemochromogen is rapid (<10 minutes after weighing), and is therefore very suitable for following the sequence of events in fading experiments, allowing samples of exposed meats to be withdrawn for analysis at short time intervals.

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SOME EFFECTS OF A SOIL CONDITIONER ON A HEAVY AND A LIGHT SOIL IN ABERDEENSHIRE

By JOYCE PRINGLE and W. T. H. WILLIAMSON

The remarkable effect of a soil conditioner on the germination of wheat in pot experiments with a heavy soil of the Cruden Bay Association is described. A field experiment was also carried out resulting in no effect on the crop but in a significant increase in the amount of water-stable aggregates in the soil. On a light soil, improvement in growth of carrots was observed.

Introduction

Numerous experiments with soil conditioners have been carried out in widely separated localities with very varied results. For instance, the use of a soil conditioner has been reported to give no increase of yield with potatoes in Hertfordshire,[1] with field beans in New South Wales[2] and with ground nuts in Jamaica.[3] On the other hand, marked improvements in crop yield have been found with vegetables such as radishes,[4] tomatoes[5] and carrots.[6] In the course of studies on soil structure in this department, one of the soils concerned, an extremely intractable one varying in texture from a silty clay loam to a sandy clay loam, seemed eminently suitable for testing the effects of a soil conditioner. Pot experiments to test the effect on germination were carried out during winter under artificial daylight in the laboratory. These were followed by field trials laid down in the following spring.

Experimental

The soil used is derived from Old Red Sandstone drift and is situated at Cruden Bay on the east coast of Aberdeenshire. After a period in arable cultivation it becomes exceedingly difficult

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CURED COLOR DEVELOPMENT DURING FERMENTED SAUSAGE PROCESSING

ABSTRACT

Color development of fermented sausages was determined by pigment analyses and Gardner color values. Sausages were studied during the fermentation phase, after heat processing to 60°C (internal) and after dehydration for 8 and 16 days. The percent conversion of total pigments to the cured nitric oxide heme pigment form significantly ($P < 0.05$) increased during a 21 hr fermentation phase. Differences in the cumulative heat input to sausages during fermentation at 38°C was noted as the factor responsible for the initial development of cured meat color. Maximum pigment conversion obtained on heat processing appeared dependent on the extent of prior color development during fermentation. Color of sausages as determined by Gardner color values was in agreement with pigment analysis data. Color development found at the heat process phase was also dependent on the extent of sausage fermentation. Dehydrated sausages had variable losses of cured pigment content although color values did not show significant changes from those values found after heat processing.

INTRODUCTION

DEVELOPMENT of cured meat color associated with the nitric oxide heme pigment has been delineated with respect to the mechanisms and conditions of the series of reactions involving myoglobin, sodium nitrite and nitrate, and reductants (Siedler and Schweigert, 1959; Fox and Thomson, 1963; Fox, 1966; Kelly and Watts, 1957, Fox and Ackerman, 1968). Changes in color characteristics of some cured meat products such as ham and frankfurters have been followed using analyses for the cured pigment (Fox et al., 1967; Pate et al., 1971; Mandigo and Kunert, 1973; Simon et al., 1973). Townsend (1973) examined several extractant combinations for determining the amount of nitric oxide heme pigments in various dry sausages differing in moisture content. Color measurements have been reported for a semidry, fermented summer style sausage (von Elbe et al., 1974). Townsend and Davis (1972), Lu and Townsend (1973) and Townsend et al. (1975) have scored dry sausage appearance and color by use of panel evaluations.

The fermentation phase of sausage preparation involves a slow but substantial heat input for proper lactic acid bacterial growth and activity. Heat processing to 60–66°C produces essentially the completed color production found at the process phase (Fox et al., 1967). Although not studied in cured meats, Landrock and Wallace (1955) reported that in fresh meats, dehydration increased the concentration of meat pigments at the meat surface. Townsend (1973) found greater conversion of the total heme pigments to the nitric oxide heme pigment form in sausage products containing 45–60% moisture compared to sausage containing 25–30% moisture.

This study was conducted to determine cured color development during the fermentation and heat processing phases of fermented sausage manufacture and to further determine color stability during subsequent dehydration. Products were studied at several pH levels by terminating the sausage fermentation at selected pH values in the pH range of 5.4–4.8. This pH range is generally found in fermented sausages (Kramlich, 1971).

Table 1—Sausage ingredients

Ingredients	Quantity per kg beef[a]
Curing agents:	
sodium nitrite	0.078g
sodium nitrate	0.156g
sodium erythorbate	0.47g
salt	30.000g
Seasonings:	
ground black pepper	2.50g
ground white pepper	1.87g
mustard powder	2.50g
garlic powder	0.62g
sucrose	2.50g
Starter materials:	
P. acidilactici (LACTACEL)[b]	5.05 ml
dextrose	7.51g
Added water	25.00 ml

[a] Boneless beef prepared at 22–24% fat content
[b] A frozen concentrate starter culture produced by Merck & Co., Inc. Rahway, NJ. A culture suspension is prepared for inoculation by diluting 6 oz concentrate with 18 oz water.

EXPERIMENTAL

Meat source and sausage preparation

Fresh boneless beef and beef fat were obtained from the state inspected Meats Laboratory of the Animal Science Department at Clemson University. The lean and fat were separately ground through a 9 mm plate, packaged in approximately 1.14 kg units and frozen at −20°C for 1–3 months. Thawing was done at ambient temperature for 4–6 hr, followed by 14–16 hr storage at 2°C. From proximate analyses performed on samples collected prior to freezing, the lean and fat quantities were combined in portions necessary to yield a final mix of 22–25% fat. Combined portions were reground once through a 9 mm plate prior to sausage preparation.

Sausage mixes (Table 1) were prepared in a Hobart H-600 mixer. The $NaNO_2$ and $NaNO_3$ were blended with the meat, followed immediately by sodium erythorbate, seasonings, dextrose, and 0°C water. After 2 min of mixing, the salt (NaCl) was added and mixed in for 30 sec. The starter culture, *Pediococcus acidilactici* (LACTACEL, Merck & Co.), was then added to an approximate level of 2×10^7 cells/g sausage. Total mixing time was approximately 4 min. Initial mix temperatures were approximately 2°C and increased to approximately 8°C during blending. Two replicate sausage batches were prepared in different weeks using the same lot of beef and beef fat.

Sausage fermentations and processing

Prepared mixes were stuffed in 52 mm diam dry sausage fibrous casings (Union Carbide) to approximately 454g. The sausage chubs were placed in an air-conditioned smokehouse (Vortron) and fermented at 38°C and 95% relative humidity. The pH was periodically monitored by examining randomly selected chubs. As the sausage pH decreased, a set of 10 chubs was removed, immediately placed in plastic bags, chilled in an ice water bath, and stored at 0°C until heat processed.

Fermentation was terminated as close as possible at the following pH values: 5.3, 5.1, 4.9 and 4.8. The initial unfermented (0 hr) sausage had a pH near 5.9. It should be noted that the approximate time requirements to reach these pH values are given in Table 2. The average pH on two replications was used to assign sausages within pH groups.

Table 2—Percent of total heme pigments converted to the nitric oxide heme pigment at several process phases for fermented sausage

		Percent conversion[a]			
				Dehydrated	
Sample pH	Fermentation period, hr	Fermenting mix	Heat processed	8 days	16 days
5.93	0.0	3.0a	54.3a	50.0a	40.2a
5.32	9.5	18.0b	72.3b	71.8b	66.8b
5.15	12.0	32.4c	79.4bc	78.6c	67.4b
4.94	15.0	49.4d	83.4c	77.0c	81.0c
4.80	21.0	64.4e	83.8c	79.4c	70.0b

[a] Any two means within a column having one of the same letters are not significantly different at $P < 0.05$.

Table 3—Gardner color values of fermented sausage at several process phases

Process phase	Sausage pH	Gardner L value	Gardner a_L value	Gardner b_L value
Fermenting mix	5.93	47.2	5.4	10.4
	5.32	46.4	6.9	9.4
	5.15	46.2	8.1	9.0
	4.94	44.0	9.6	8.3
	4.80	42.6	12.6	7.5
Heat processed	5.93	41.2	10.0	6.9
	5.32	43.8	12.0	6.8
	5.15	41.2	12.0	6.8
	4.94	42.0	12.0	6.7
	4.80	43.0	12.6	6.8
Dehydrated, 8 days	5.93	41.4	10.4	6.2
	5.32	41.8	10.0	5.9
	5.15	42.9	11.6	5.8
	4.94	40.6	12.2	6.2
	4.80	40.9	13.3	6.6
Dehydrated, 16 days	5.93	40.1	10.6	6.5
	5.32	42.2	11.6	6.9
	5.15	43.3	11.8	6.8
	4.94	42.3	12.1	6.0
	4.80	43.4	11.6	6.2

Table 4—Composition of fermented sausage at several process phases[a]

			Dehydrated	
Component	Fermenting mix	Heat processed	8 days	16 days
Moisture, %	55.68	54.80	46.58	34.95
Protein, %	17.42	18.01	20.91	25.33
Fat, %	22.52	22.99	27.36	32.10
Ash, %	3.41	3.56	4.18	5.14

[a] Averages (n = 10) from replicate sausage batches with samples of each sausage pH group

After fermentation, all sausages were initially heated at 71°C for 45 min, increased to 77°C for 45 min, and finally heated at 82°C until an internal temperature of 60°C was obtained (2.0–2.5 hr total). The sausage chubs were cooled to 20°C with a cold water spray and placed in a 12 ± 2°C drying room having 20–25 air changes/hr. The air relative humidity ranged from 80 to 84%. Sausage chubs were randomly selected for analysis at 8 and 16 days of drying.

Sampling and compositional analyses

Fermented and nonfermented sausages were collected for compositional and color analyses at the following process phases: (a) on completion of mixing; (b) after fermentation (no sample for nonfermented); (c) after heat processing to 60°C internal; (d) after 8 days drying; and (e) after 16 days drying.

All samples were analyzed for moisture, fat, ash and protein (Kjehdahl N × 6.25) following AOAC (1975) procedures. Sausage pH was determined as described by Keller et al. (1974).

Separate sausages, in chub form, were collected for heme pigment analyses and color measurements.

Heme pigment analysis

The casings on sausages were removed and the exterior 5–6 mm of the sausage trimmed off. The inner core was ground twice through a 5 mm plate. The preparation was conducted as closely as possible in the absence of light. Ground samples were placed in foil-covered and capped glass containers and stored at 0°C. Pigments were extracted within 24 hr.

The procedures for determining the content of nitric oxide heme pigments and total heme pigments were as described by Hornsey (1956) with a modification in extraction technique. Modification was required due to a problem of obtaining adequate dispersion of the ground sample in the extractant. A similar problem was encountered by Fox et al. (1967) with frankfurter samples.

For nitric oxide heme pigments, the acetone-to-water ratio for extraction was maintained at 4-to-1 with allowance for moisture present in the sausage sample. A Tri-R homogenizer with teflon pestle was used to homogenize 4g of sample with an acetone-water volume calculated (including sample moisture) to yield 80% acetone in 20 ml total extractant. Samples were homogenized for 2 min, allowed to sit in the dark for 8 min and then filtered through Whatman #4 paper into 1-cm spectrophotometer tubes. The filtrates were collected and read immediately at 540 nm. The concentration of nitric oxide heme pigments was calculated using the 540 nm absorption coefficient given by Hornsey (1956).

Total heme pigments were determined using the above procedure except that 1 ml of conc HCl was substituted for 1 ml of water in the acetone-water extracting solution (Hornsey, 1956). After homogenization, samples were allowed to sit for 60 min prior to filtering. The total heme pigment concentration was calculated from the absorption at 640 nm using the absorption coefficient of Hornsey (1956). The results, reported as percent conversion, are the percent of total heme pigments converted to the nitric oxide heme pigment.

Color measurements

Color values were determined with a Gardner Color Difference Meter, Model C4 (Gardner Laboratory, Inc., Bethesda, MD). The instrument was standardized with a pink standard plate (No. CG-6632; L = 52.9, a_L = 31.9, b_L = 11.4). Results were expressed as Gardner L, a_L and b_L values.

Due to the coarse-cut fat and lean particle dispersion typical to this class of sausages, direct surfaces could not be measured without considerable variation between samples of the same sausage chub. Thus core samples of 50g were mixed with 50g distilled water and blended in an Osterizer for 30 sec. Values obtained were in agreement with the previous findings of von Elbe et al. (1974).

RESULTS & DISCUSSION

Pigment analyses

The percent of total heme pigments existing as the nitric oxide heme pigment is given in Table 2 for several phases in the processing of a fermented sausage. During fermentation of the raw sausage mix, there was a significant ($P < 0.05$) increase in the percent of conversion to the cured pigment form. The increase in the cured pigment content of the fermenting sausages was the result of the heat input over successive fermentation time intervals at 38°C. Fox et al. (1967) reported that the time-temperature relationship during cooking of frankfurters was critical to the rate and percent of nitric oxide pigment formed. In their study, cured color development occurred mainly as the temperature increased from 49°C to 60°C. In addition, holding the raw frankfurter mix at 38°C showed 50% conversion of the total heme pigment to the nitric oxide heme pigment in 20–25 min. In the current study, maximum conversion of 64.4% on fermentation was found after 21 hr at 38°C. Analysis of the initial unfermented (pH 5.93) sample held for several days in the dark at 0–2°C showed little change over the 3.0% reported in Table 2.

The total amount of heat input during heat processing of the sausages was assumed to be equivalent for each group since all sausages were heated from 0°C to 60°C (internal) at one time. The percent pigment conversion (Table 2) on heating appeared dependent on the extent of prior color development during fermentation. The nonfermented (pH 5.93) sausage showed significantly ($P > 0.05$) less conversion (54%) than the fermented sausages (72–83%). Maximum conversion of approximately 83% occurred in sausages fermented below pH 5.0. In practice, fermented sausages range in pH from 5.4–4.4, with most sausage types having a pH near 5.0 (Acton and Dick, 1976).

The maximum pigment conversion attained at the heat processing stage may be due to the quantity of the nitrite plus nitrate mixture used in the sausage formulation (Table 1). Although nitrate is slowly converted to nitrite, Dethmers et al. (1975) reported the appearance of small amounts of nitrite in nitrate containing thuringer processed without nitrite. They suggested generation of nitrite during fermentation and cooking from the inherent nitrate content of meat and added nitrate in processing. A combination of 78 ppm nitrite and 156 ppm nitrate was used in the current study. The recommended level of nitrite for sausages employing frozen concentrate starter cultures of *P. acidilactici* is 78 ppm nitrite and 156 ppm nitrate (Anon., 1969). *P. acidilactici* does not reduce nitrate to nitrite (Buchanan and Gibbons, 1974) although part of the indigenous flora may possess some activity. Using 78 ppm nitrite in frankfurter formulations, Simon et al. (1973) reported 71–76% pigment conversion and 83–88% conversion when 156 ppm nitrite was utilized. Fox et al. (1967) found a conversion estimated at 80–85% for frankfurters heated to 60–66°C and processed with a combined cure of 154 ppm nitrite and 1248 ppm nitrate.

Since the extent of pigment conversion ranged from 54–83% at the end of heat processing, it is probable that the combined effects of increasing acidity and incremental increases in heat input during fermentation determined the efficiency of total nitrite utilization in the heated sausage.

On dehydration there was a small decrease in the percent of converted pigment as found after heat processing (Table 2). At the end of 8 days, only slight losses were noted. Losses at 16 days of drying ranged from a maximum of an approximate 25% decline in value for the nonfermented (pH 5.93) sausage group to a miminum of an approximate 3% decline for sausages fermented to pH 4.94. No consistent relationship with sausage pH was established.

Townsend (1973) compared the percent conversion of several fermented and dried sausages and found greater conversion in sausages containing 45–60% moisture than in those containing 25–30% moisture. The results in Table 2 are in agreement with Townsend's (1973) findings if comparisons of conversion percentages are made with sausage moisture content on heat processing and drying (Table 4). Further study of the pigment system in cured, dehydrated meats varying in pH value is needed.

Color determinations

Color values of the sausages at various process phases are given in Table 3. With the fermenting sausage mixes, L values decreased as the pH of the meat decreased and appeared darker. The a_L and b_L values were also affected by sausage pH. The a_L value increased, indicating an increase in redness while the b_L value decreased, indicating a decrease in yellowness. The change of color values corresponded well with the pigment conversion data discussed above.

Heat processed sausages and those dried for 8 days showed similar L, a_L and b_L values and did not appear to be related to sample pH. Excellent comparison was found between the sausage color values in this study and those reported for a nondried, fermented summer sausage by von Elbe et al. (1974). Their sausage prepared with 156 ppm nitrite and stored 7 days without light had an L value of 48.6, a_L of 11.7, and b_L of 7.8. The lower L range of 41.2–43.8 and b_L value range of 6.7–6.9 for heat processed sausages in the current study (Table 3) is probably related to the lower fat content (Table 4) of 22.9% as compared to 33.1% in the study of von Elbe et al. (1974). It must be noted that the color values reported are from core samples which exclude the darker and drier outer surface of the sausage chub.

Compositional analyses

Data on the composition of the sausages is given in Table 4. The increase in percentages of protein, fat, and ash constituents is the result of moisture loss on heat processing and drying and is in agreement with previous studies (Keller et al., 1974; Acton and Keller, 1974). From a color analysis viewpoint, the quantity of fat versus lean, and type of muscle tissue will affect both pigment (myoglobin) levels and lightness-to-darkness characteristic of prepared meat products. The fermented sausages of 8 days had weight losses averaging 18.9%. The average weight loss increased to 32.4% at 16 days of drying. Based on the classification system proposed by Acton and Dick (1976), the 8-day product was "semidry" and the 16-day product was "medium dry."

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Turkey Ham Properties on Processing and Cured Color Formation[1]

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ABSTRACT Turkey hams were prepared from boneless thigh meat and evaluated for yields, texture, and color development when processed in the smokehouse to internal temperatures from 37.8 C to 71.1 C.

Meat yields at various smokehouse temperatures significantly ($P<.05$) decreased from 96.5% at 37.8 C to 87.3% at 71.1 C. Shear values also significantly ($P<.05$) decreased as the product temperature increased, indicating tenderness development. Microwave reheating/cooking of hams removed from the smokehouse at 48.9, 60.0, and 71.1 C gave final yields of approximately 85%, irrespective of previous smokehouse cooking temperature. Taste panelists scored the ham at 7.2 on a nine-point preference scale for microwave reheated, cooked hams; these scores were not affected by previous smokehouse temperature.

Heme pigment conversion to the nitric oxide heme pigment form showed a dependence on the internal temperature of the product during heating, typical of cured meat products. The Gardner a/b ratio showed the largest zone of color development between 43 C and 49 C. Gardner +a values (for redness) were significantly ($P<.05$) correlated with the level of nitric oxide heme pigment produced on heating the turkey hams.

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INTRODUCTION

Binding of small meat pieces and chunks to form larger, uniform quality poultry products has been investigated by several researchers (Maesso *et al.*, 1970a, 1970b; Schnell *et al.*, 1970, 1973; Acton, 1972a, 1972b; Wardlaw *et al.*, 1973; Uebersax and Dawson, 1976; Acton and Keller, 1976). Many of the factors involved in meat binding were reviewed by Vadehra and Baker (1970).

The majority of these formed products, such as poultry rolls, loaves, and roasts do not involve curing procedures. However, curing methods have been reported for whole poultry carcasses (Besley and Marsden, 1941; Hindman *et al.*, 1963; Chatterjee *et al.*, 1971; Hale *et al.*, 1977) and curing ingredients are essential to the production of poultry frankfurters (Baker *et al.*, 1968; Froning *et al.*, 1971; Baker and Darfler, 1974) and other poultry sausages (Keller and Acton, 1974; Dhillon and Maurer, 1975; McMahon and Dawson, 1976).

The functions of sodium or potassium nitrite in meat curing are to provide antimicrobial properties, to develop characteristic flavor and color, and to exert antioxidant qualities in the prepared products (Lechowich *et al.*, 1978; CAST, 1978). The development of cured meat color is dependent on the reaction and reaction conditions involving myoglobin, sodium nitrite, and reductants (Siedler and Schweigert, 1959; Fox, 1966; Fox and Ackerman, 1968). Fox *et al.* (1967) reported that the time-temperature relationship during cooking (in frankfurters) is critical to the rate and amount of nitric oxide heme pigment formed. Heat processing to 60 to 66 C internally produces essentially the maximum amount of cured pigment found in most cured products (Fox *et al.*, 1967; Monagle *et al.*, 1974; Acton and Dick, 1977). The USDA requires a minimum internal temperature of 68.3 C for cured, cooked poultry products (USDA, 1973).

In this study a formed product, turkey ham, was evaluated for product yields, texture development, and color formation during heat processing from 37.8 C to 71.1 C. Cured pigment analysis and tristimulus colorimetry were used to assess the relationship between chemical and visual occurrences in color formation. Products removed from the smokehouse at internal temperatures of 48.9 to 71.1 C were further evaluated for yields and flavor scores after refrigerated storage and microwave reheating/cooking to 71.1 C.

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EXPERIMENTAL PROCEDURE

Product Preparation and Processing. Frozen boneless turkey thigh meat in 18.1 kg blocks was obtained from a regional plant operating under USDA inspection. The meat blocks had been frozen at −20 C for approximately 60 days. Meat blocks were band-sawed into 2.54 cm thick slabs and thawed at 12 C for 24 hr, then held at 2 C for 16 hr.

Replicate batches of a turkey ham product were prepared using a Hobart H-600 mixer. Ingredients used in each batch and their quantities per kg of meat were as follows: .156 g $NaNO_2$, .546 g of sodium erythorbate, 10.0 g of sucrose and 30.0 g NaCl. The nitrite and erythorbate were added and blended into the meat for 1 min, followed by the salt and sugar. Total mixing time was 6 min for each batch. The initial mix temperature was approximately 2 C and increased to 8 C at the end of mixing. Each batch was then held at 2 C for 90 min.

The ham mixes were stuffed into 84 mm diameter easy peel fibrous casings (Union Carbide) to form approximately 2.2 kg chubs. The chubs were positioned in a flat ham press (Koch), secured by springs, and placed in an air-conditioned smokehouse (Vortron).

The chubs of each batch were initially heated at 65.5 C for 45 min, increased to 76.6 C for 45 min, and finally heated at 85 C until an internal product temperature of 71.1 C was attained (4.5–5.0 hr total). The smokehouse humidity was maintained at 40% relative humidity over the process period. During this heating schedule, two chubs at random were removed as the internal product temperature changed by approximately 5.6 C, starting at 37.8 C (internal) and ending at 71.1 C (internal). The chubs were immediately cooled in an ice-water bath, then held at 2 C for analysis.

Microwave Final Cook and/or Reheating. Sets of two ham chubs removed from the smokehouse at internal temperatures of 48.9, 60.0, and 71.1 C were stored at 2 C for 6 days and then reheated and/or cooked to 71.1 C internally in an Astro-Chef 747 Microwave Oven (Dyna-Tronics). The chubs were individually placed in the oven operating at 2450 MHZ on the "Bake" cycle (15 sec pulses). The chubs required an average of 45 min to attain 71.1 C and the internal temperature taken 15 min after heating ranged from 73 to 76 C. Microwave heated chubs were then held 1 day at 2 C before analysis.

Compositional Analyses, Product Yields, and Shear Values. The ham mix and chubs collected during the heating intervals or after smokehouse and microwave cooking were analyzed for composition, color, percent weight loss, and shear values. Replicate samples were analyzed in duplicate for moisture, fat, ash, and protein (Kjeldahl N × 6.25) following AOAC (1975) procedures. Product yields at each internal temperature were based on the weight change from the initial raw chub weight.

For shear measurements, a Food Technology Corporation Press equipped with a 136 kg load cell was used with a 30 sec downstroke at range 300. Shearing force for 4 mm slices of the ham chubs was calculated as kg force/g-cm^2 of surface area exposed to the shear blades.

All data was analyzed by analysis of variance and the significance of means tested by Duncan's method (Steel and Torrie, 1960).

Heme Pigment Analyses. The methods for nitric oxide heme pigments and total pigment were those described by Hornsey (1956) with the extraction procedure modifications outlined by Acton and Dick (1977). The results, reported as percent conversion, are the percent of total heme pigment converted to the nitric oxide heme pigment (wet sample basis).

Color Measurements. Color values were measured with a Gardner Color Difference Meter, Model C4 (Gardner Laboratory, Inc., Bethesda, MD). The instrument was standardized with a pink standard plate (No. CG-6632; L = 52.9; a = 31.9; b = 11.4). Results were expressed as the Gardner a/b ratio. Sample preparation for color values followed the procedure of Acton and Dick (1977) used for sausages having a coarse-cut fat and lean particle dispersion.

Panel Evaluations. Taste preference panels of 21 untrained members were conducted using slices of the microwave heated samples. Slices were served in randomized order and at room temperature (22 C). Panelists scored their preference ratings on a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely).

RESULTS AND DISCUSSION

Turkey ham yields significantly ($P<.05$) decreased with increasing internal temperatures while heating in the smokehouse (Table 1). The yields reflect the loss of moisture content on heating, as seen in the general compositional analyses of Table 2. The lowest yield, 87.3% at an internal temperature of 71.7 C, is in agreement with the expected yields of boneless pork

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TABLE 1. *Characteristics of turkey ham on initial heat processing and after microwave reheating*

Internal temperature (C)	Yield at smokehouse (%)	Shear value (kg/g-cm^2)	Microwave reheated to 71.1 C Yield (%)	Microwave reheated to 71.1 C Preference score^1
37.8	96.5^{a}	. . .	. . .	. . .
48.9	94.3^{ab}	.42^{a}	85.4^{a}	7.2^{a}
60.0	92.2^{b}	.27^{b}	85.0^{a}	7.2^{a}
71.1	87.3^{c}	.18^{c}	85.1^{a}	7.2^{a}

[a,b,c] Any two means within a column having the same letter are not significantly different (P>.05).

[1] Panel preference scale: 9 = like extremely; 1 = dislike extremely.

hams at that temperature (Schmidt, 1977). The turkey hams of internal temperatures lower than 71.1 C (Table 1) would not be considered acceptable as cured-cooked products since the USDA poultry regulations require a minimum final internal temperature of 68.3 C (USDA, 1973). However, final cooking of the lower internal temperature turkey hams by the microwave oven showed that the earlier higher yields at the smokehouse decreased to equivalent yields of the 71.1 C cooked hams after microwave reheating (Table 1). Irrespective of previous heating from 48.9 C to 71.1 C, product yields after microwave reheating/cooking were not significantly (P<.05) different. There is no final yield advantage to be gained from use of smokehouse temperatures lower than 71.1 C if the product is to be reheated to 71.1 C in a microwave oven, as may occur in the foodservice industry.

No significant (P<.05) differences in panel preference scores occurred among the various microwave cooked or reheated products (Table 1). The microwave treatment of samples was included since the product may be served as heated, thick-sliced ham steaks.

Shear values (Table 1) measured during the smokehouse heating phase show that increased tenderness, reflected by decreasing shear values, were found as the product was heated. No determinations were conducted on microwave heated products.

The percent of total heme pigment converted to the nitric oxide heme pigment form is shown in Figure 1. With increasing internal temperature of the turkey hams, significantly (P<.05) greater quantities of cured pigment were formed. Development of the cured pigment was favored by two occurrences: a) the heat accumulation from increments of thermal energy input on heat processing to 71.1 C, and b) the presence of sodium erythorbate as a reaction accelerator. The temperature-pigment conversion relationship shown in Figure 1 agrees with that reported for frankfurters (Fox *et al.*, 1967). The total heating period for the

TABLE 2. *Proximate composition of turkey ham*^{a}

Constituent	Raw mix	Smokehouse product (71.1 C)	Microwave reheated/cooked product (71.1 C)
Moisture (%)	72.2 ± .9	68.8 ± .6	64.8 ± .1
Fat (%)	3.7 ± .6	4.9 ± 1.2	6.0 ± .2
Protein (%) (N X 6.25)	19.4 ± .4	21.2 ± .9	24.2 ± .1
Ash (%)	3.1 ± .4	3.4 ± .7	3.0 ± .1

[a] Means ± standard deviations.

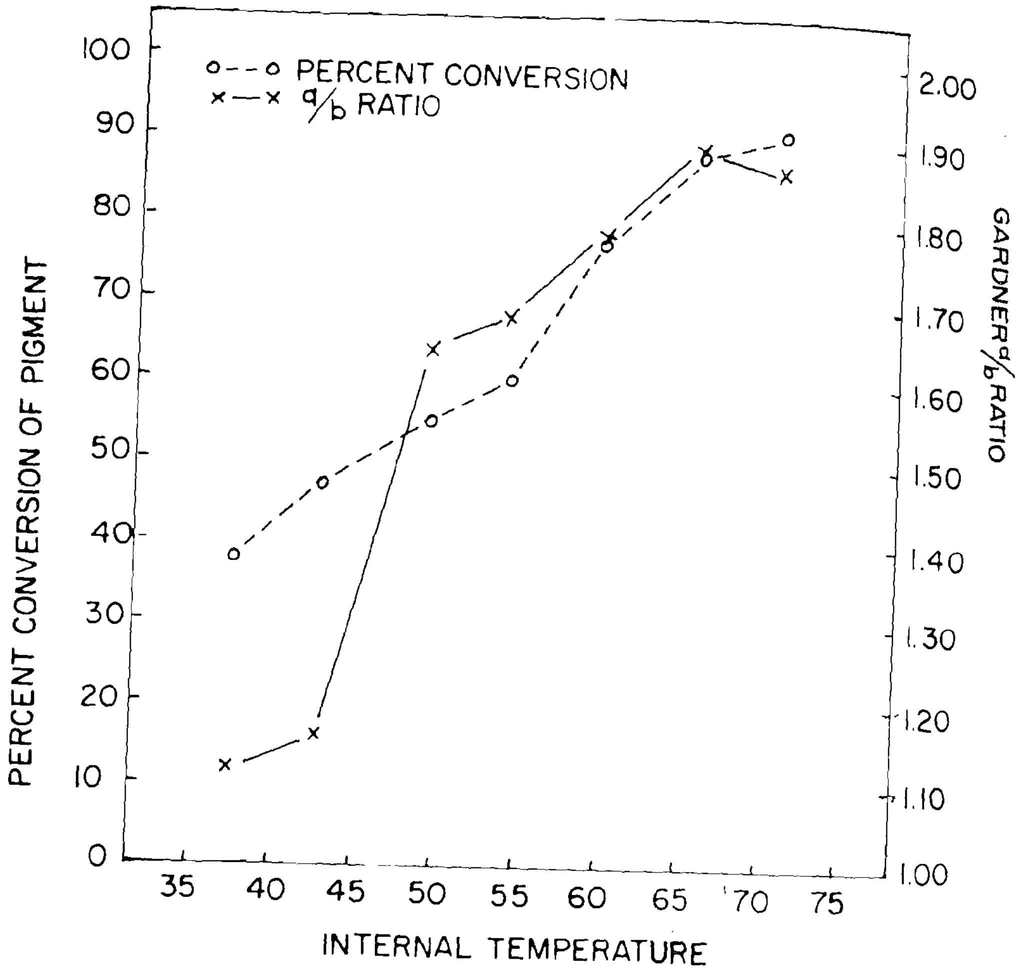

FIG. 1. Percent conversion of total heme pigment to nitric oxide heme pigment and Gardner a/b ratio during heat processing of turkey ham.

turkey hams was longer (4.5 to 5.0 hr) than for frankfurters (1 to 1.5 hr), primarily due to differences in product diameter.

The maximum pigment conversion of 91.3% at 71.1 C was attained with 156 ppm ingoing nitrite. Maximum conversions of 83 to 88% have been reported for frankfurters, dry sausages, and boneless pork hams (Simon *et al.*, 1973; Acton and Dick, 1977; Terlizzi *et al.*, 1978).

Gardner a/b ratios show that the zone of greatest color formation occurred between 43 C and 49 C (Figure 1). The a/b ratio provides an index of color change, reducing two color parameters, +a (redness) and +b (yellowness), to one (Francis and Clydesdale, 1975). From 49 C to 66 C redness development increased as the internal temperature of the turkey hams increased during heating. A slight decrease in the a/b ratio occurred from 66 C to the final product temperature of 71.1 C. It must be noted that the correlation coefficient (r) between Gardner +a values and percent conversion of total heme pigments was .80. The correlation coefficient was significant ($P<.05$), indicating the value of relating actual cured pigment to the main characteristic color value.

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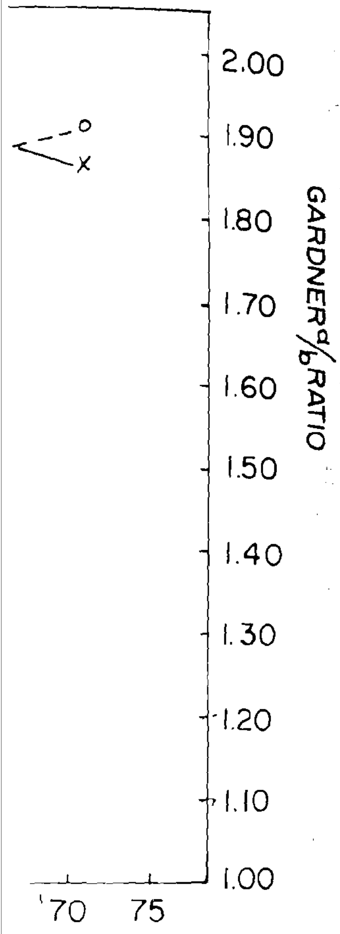

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