Relation of Free Sulfhydryl Groups to Cured Meat Color

BETTY M. WATTS,
ANNA MARIE ERDMAN,
and JANE WENTWORTH

Department of Food and Nutrition,
The Florida State University,
Tallahassee, Fla.

Experiments were carried out to determine the role of free sulfhydryl groups from denatured protein in the development of the color of cured meat. Typical cured meats were examined for the presence of free sulfhydryl groups, and the role of these groups and of added nitrite in fading of cured meat was investigated. Cured meats differed greatly in content of free sulfhydryl groups. Dipping meats with a positive nitroprusside test in 2% nitrite gave protection against fading upon irradiation with strong light or restored color that had faded. Dipping in 0.2% nitrite gave protection against irradiation with dim light. In the absence of free sulfhydryl groups, nitrite accelerated oxidation of cured meat pigment.

THAT PROTEIN DENATURATION and oxidation of the heme pigments of meat are related has been pointed out (*8*). The denaturation of oxyhemoglobin or oxymyoglobin of fresh meat brings about oxidation of the heme iron to give the brown methemoglobin or metmyoglobin, a reaction that has been clearly demonstrated by much early work. In contrast, various treatments which bring about protein denaturation, the most common of which is heat, accelerate the curing process and in fact appear to be essential to the development of good cured meat color under most conditions. The formation of the cured meat pigments, in turn, involves a reduction of both metmyoglobin and nitrite, or of some complex of the two.

If the denaturation itself is responsible for the reduction involved in the curing process. it might be inferred that free sulfhydryl groups on the protein are responsible, as these are the strongest reducing groups known to be released by the denaturation process. These groups are normally tied up in many native proteins in intramolecular linkages. As the protein molecule unfolds during denaturation, these linkages are broken and active sulfhydryl groups appear (*1*, *6*). If the denaturation is followed by coagulation, the sulfhydryl groups are progressively tied up again, this time in intermolecular linkages. Thus free sulfhydryl groups go through a maximum during the heat coagulation of many native proteins, including myosin and egg albumin.

One object of the present research was to seek evidence supporting the hypothesis that the necessary reductions in the formation of the cured meat pigments may be effected entirely independently of the enzymatic reducing systems of muscle tissue, by sulfhydryl groups freed during the denaturation of a native protein. Another object was the examination of a number of typical cured meats for the presence of free sulfhydryl groups and investigation of the role of such groups in the subsequent fading of cured meats.

Experiment I

Denatured Egg Albumin as Source of Sulfhydryl Groups for Reduction of Methemoglobin and Nitrite. In order to eliminate the enzymatic reducing systems of muscle tissue as a factor in the formation of nitric oxide hemochromogen, an attempt was made to induce the formation of the cured meat pigment by mixing hemoglobin and nitrite with egg white and then heating the mixture to free sulfhydryl groups from the egg albumin.

Hemoglobin was prepared from hog corpuscles. The red cells were washed three times with at least 4 volumes of 0.9% sodium chloride, laked with distilled water, and freed from stroma material by adjusting the pH to 5.8 and filtering. Fresh egg white was whirled for a few seconds in a Waring Blendor to break up the thick white and render it homogeneous. Ascorbic acid stock solutions were freshly prepared. All solutions were adjusted to pH 5.8 before mixing and a phosphate buffer of the same pH was used.

The final concentration of all ingredients in the experimental mixture (A) was as follows:

Egg white	50%
Hemoglobin	0.4%
Nitrite	0.1%
Phosphate buffer, pH 5.8	0.02*M*

Aliquots of the same mixture were also prepared containing, in addition to the above, (B) 0.2% ascorbic acid as a positive control to obtain the maximum pink color of the cured meat pigment, and (C) 0.1*M* iodoacetic acid, which is known to combine with sulfhydryl groups and so eliminate their reducing activity (*2*). A negative control (D) was also prepared, from which the nitrite was omitted and 0.1% potassium ferricyanide substituted as an oxidizing agent, thus giving the brown color of the ferric heme.

Aliquots of each of the experimental mixtures were transferred to beakers and heated in a water bath with constant stirring for designated times. All samples were rated for pink color on a scale of 0 to 10. The ferricyanide sample was designated zero throughout and the bright pink obtained by the ascorbic acid—treated sample after mild heating was designated 10.

A small portion of each sample was removed to a spot plate and tested qualitatively for the presence of free sulfhydryl groups by the nitroprusside test (*2*). The test was carried out by adding to the surface of the denatured protein a drop of concentrated ammonium hydroxide, followed by a drop of 5% nitroprusside. A deep rose color develops in the presence of free sulfhydryl groups. The color is stable for only a few minutes, then fades. The intensity of the color was indicated roughly by + marks—i.e., ++ = bright rose, ± = very slight test.

The results of the nitroprusside test for sulfhydryl groups during heating of the mixtures are shown in Table I. The original egg white preparation, in the absence of hemoglobin, gave a very faint nitroprusside test This was greatly intensified during the first 5 minutes of heating as the temperature rose from 70° to 85° C., but fell off after longer heating, as coagulation progressed. In the samples containing either iodoacetic acid or ferricyanide, no free sulfhydryl groups were detectable after the first 5 minutes of heating. Ferricyanide oxidizes sulfhydryl groups as well as the heme iron. The iodoacetic acid, on the other hand,

Table I. Appearance of Sulfhydryl Groups during Heating of Egg White Mixtures

Time of Heating, Min.	Temp. Attained, °C.	Nitroprusside Test A	B	C	D
0	25	±[a]			
2	70	±[a]			
5	85	++	++	+	±
10	89	++	++	−	−
30	89	±	+	−	−

[a] Difficulty was experienced in applying the nitroprusside test to the liquid mixtures containing hemoglobin. The results at 0 and 2 minutes of heating were obtained on an identical preparation from which the hemoglobin was omitted.

is not an oxidizing agent, and reacts specifically with sulfhydryl groups, so that any effect on the color of the hemoglobin is presumably exerted indirectly by eliminating the reducing action of sulfhydryl groups.

Color changes during heating are shown in Figure 1. Immediately after mixing, all samples have a brownish color, due to the reaction of nitrite with oxyhemoglobin to give methemoglobin (*3*). In the sample containing ascorbic acid, partial reduction occurred during the 60 minutes of standing at room temperature before color comparisons were made with all heated samples. A very bright pink color developed during the first 5 minutes of heating, which probably represents complete conversion of the methemoglobin to nitric oxide hemochromogen. This color was only slightly less intense after 30 minutes of heating.

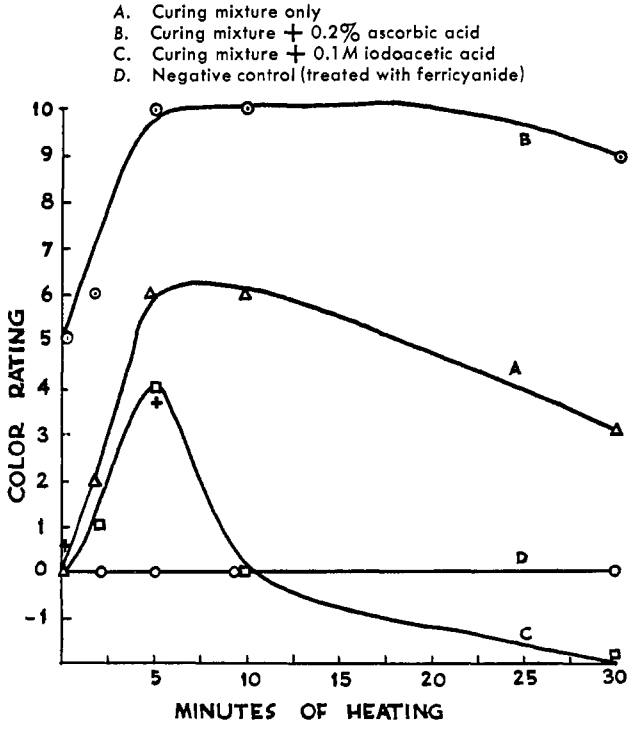
In the absence of ascorbic acid, formation of the pink cured meat pigment parallels the appearance of free sulfhydryl groups. The maximum color developed does not represent complete reduction of the ferric heme under the conditions of this experiment. The concentration of egg albumin in the experimental solutions is approximately 6% and the total sulhydryl groups liberated on denaturation by different agents range from 0.9 to 1.3% expressed as cysteine equivalents (*7*). In lean pork, on the other hand, protein comprises 15 to 20% of the meat and sulfhydryl groups equivalent to 1.15% cysteine may be liberated from the chief protein, myosin, in denaturation (*4*). Slower heating for a longer time and with less exposure to oxygen might be expected to result in more effective utilization of the reduction activity of the protein sulfhydryl groups.

Experiment II

Free Sulfhydryl Groups in Cured Meats. The preceding experiment furnished presumptive evidence that sulfhydryl groups freed by protein denaturation can bring about a reduction of ferric hemes and nitrite resulting in the cured meat pigment. The next step was to determine whether free sulfhydryl groups exist in cured meats and, if so, whether they bear any relationship to the stability of cured meat color. The wide variety of processing times and temperatures, salt concentrations, etc., to which meats are subjected during the curing process might be expected to result in differences in the extent of protein denaturation and of coagulation following denaturation and this in turn should lead to differences in free sulfhydryl groups present in the cured product.

A number of typical cured meats from six different packing houses were obtained from local markets. All were of excellent color typical of the cured product. With the exception of the bacon, which could be obtained only in sliced form, all meats were bought in bulk and a freshly exposed surface was used for testing purposes. The qualitative nitroprusside test, as described in the preceding experiment, was used. The comparative strength of the nitroprusside reaction on the different meats (Table II) is a very rough indication of the free sulfhydryl groups present. Varying amounts of fat and red pigments originally present in the meat influence the test; nevertheless it is clear that wide differences exist in sulfhydryl groups present in cured meats.

Figure 1. Development of cured meat pigment in egg albumin solutions



Experiment III

Light Fading of Cured Meats as Influenced by Sulfhydryl Groups. In the following experiments on light fading, the meat surfaces were covered with polyethylene and exposed to white light of designated intensities. The color at any given time of exposure was rated on a scale of 0 to 10. The positive standard, designated 10, consisted of a freshly cut surface of the meat in question. The negative standard (0) was obtained by soaking a piece of the same meat in a 1% solution of potassium ferricyanide for 5 minutes, then rinsing off excess ferricyanide with distilled water. This treatment gives the brownish color of the ferric denatured globin hemochromogen.

In the normal process of light fading, the poorest color obtained is that of the ferricyanide-treated controls, designated 0. This color is assumed to represent complete oxidation of the heme iron to give ferric hemochromogen. However, further oxidative attack on the porphyrin ring may result in complete loss of the porphyrin color, giving a putty gray surface (*8*). This final stage of porphyrin decomposition may be illustrated by treating the meat surface with hydrogen peroxide. Samples so treated were rated −10 for comparative purposes.

It was first established that the rate of fading of the cured meats tested was not correlated with the intensity of the nitroprusside reaction. Most of the samples tested faded at approximately the same rate with a given light intensity. With a light intensity of 125 foot candles complete oxidation to the color of the ferricyanide-treated control generally took place in 1.5 to 3 hours. With a light intensity of 20 foot candles, most samples were approximately half faded in 6 hours. In each experiment, however, some samples faded less rapidly than the majority, and these differences

were unrelated to the number of free sulfhydryl groups present.

Experiment IV

Influence of Added Nitrite on Recovery from Light Fading. Published studies on the mechanism of the light fading of cured meat pigments are lacking. From analogy with the well established behavior of carbon monoxide hemoglobin and from recent unpublished reports (*5*), it may be inferred that visible light causes a dissociation of the nitric oxide from the heme. Such a dissociation would be expected to result in immediate oxidation of the heme iron, as the ferrous hemochromogens are known to be very labile to oxidation by atmospheric oxygen.

Table II. Free Sulfhydryl Groups in Cured Meats

	Description of Meat	Nitroprusside Reaction
1.	Ham, tenderized	++++
2.	Ham, ready to eat	++++
3.	Ham, cooked, pressed —i.e., "boiled"	+++
4.	Salami, large	+++
5.	Chopped, pressed ham	++
6.	Comminuted canned ham	++
7.	Liver sausage	+
8.	Bologna, sample 1	±
9.	Bologna, sample 2	−
10.	Frankfurters	−
11.	Bacon, sliced	−

If this mechanism is correct, and if free sulfhydryl groups are capable of reducing ferric hemochromogens and nitrite, then it might be predicted that if sufficient nitrite were made available, meats that were rich in sulfhydryl groups would recover from the light fading when stored in the dark, whereas samples lacking in sulfhydryl groups would show no such recovery.

Accordingly, several meats, representing a range of nitroprusside reactions, were first oxidized by exposure to light, then divided into two portions, one of which was kept as a control and the other dipped for 20 seconds in a solution containing a large excess (2%) of nitrite. Both pieces were covered with polyethylene and stored in the refrigerator at 7° C. (45° F.) in the dark. Color ratings were made at appropriate intervals and changes in the nitroprusside reaction were also followed.

The results are shown in Figure 2. As previously stated, the rate of oxidation during the period of illumination was not related to available sulfhydryl groups. All untreated samples eventually faded to the color of the negative control and thereafter showed little or no change until the onset of bacterial spoilage. On the other hand, samples dipped in nitrite showed definite color changes in storage, which were closely associated with the nitroprusside reaction. Almost full recovery of the original bright pink color was obtained with boiled ham, which gave a strong nitroprusside test. At the other extreme, bologna showing an original negative nitroprusside reaction was not only not improved in color by the nitrite treatment, but was in fact faded beyond the color of the negative control, indicating probable attack on the porphyrin ring by the nitrite.

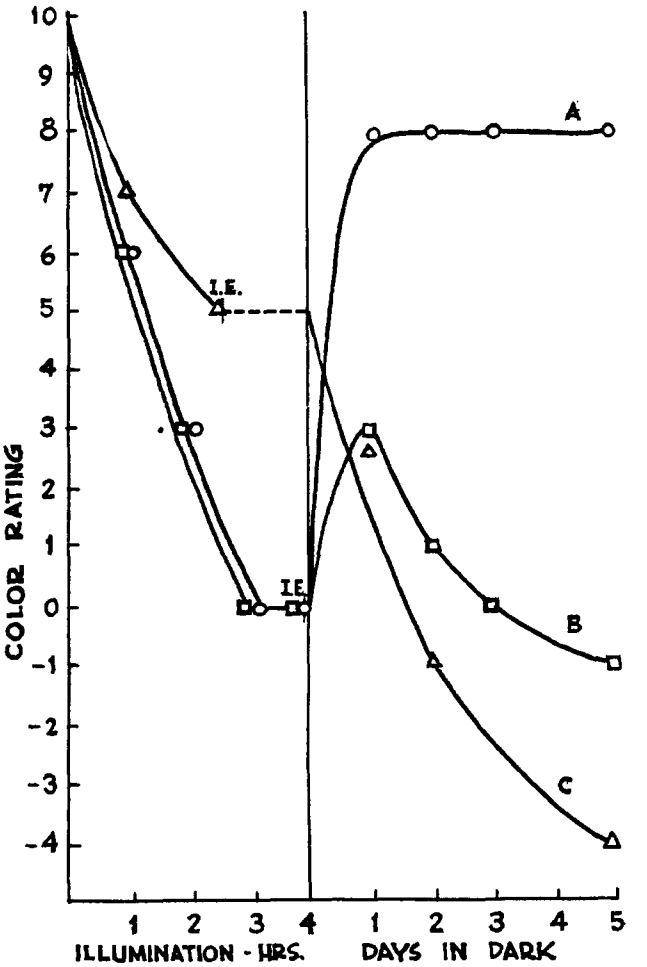


Figure 2. Effect of dipping in strong nitrite on color of light-faded meats of varying sulfhydryl content

A. Boiled ham NP test, +++
B. Bologna 1 NP test, ±
C. Bologna 2 NP test, −
I.E. Illumination ended, samples covered and stored in refrigerator

Surface sulfhydryl groups on meats could be eliminated by treatment with iodoacetic acid. Thus, a sample of canned comminuted ham, which originally gave a ++ nitroprusside test, gave a negative test when dipped for 20 minutes in 0.1*M* iodoacetic acid (previously adjusted to the pH of the meat). The original untreated product showed marked recovery from light fading when dipped in the nitrite solution, similar to that shown by the boiled ham in Figure 2. On the other hand, the iodoacetic acid–treated sample rapidly faded on subsequent treatment with nitrite.

Experiment V

Effect of Nitrite Concentration and Intensity of Illumination on Color Changes in Canned Comminuted Ham. A commercial comminuted ham product was removed from the can and sliced uniformly. Some of the slices were untreated, others were dipped in 2 or 0.2% concentrations of nitrite. Some slices from each treatment were then illuminated with an intense light (125 foot candles), others were illuminated with a dim light (15 foot candles), and still others were stored in the dark, all at room temperature. At the end of this 4-hour period of illumination, all samples were covered and stored in a refrigerator at 7° C. (45° F.) for several weeks. Color ratings and nitroprusside tests were made at intervals throughout the lighting and storage periods.

The results are shown in Figure 3. Untreated samples did not fade appreciably during 4 hours in the dark at room temperature, but gradually oxidized to the color of the negative controls during a 21-day stay in the refrigerator. With mild illumination, they faded considerably during exposure and the fading was continued in the refrigerator. With intense illumination fading was complete after 1.5 hours. In all cases the nitroprusside test became progressively weaker during the course of the experiment but was still faintly evident at the end of 21 days.

Dipping the meat in the less concentrated nitrite (0.2%) did not affect the rate of disappearance of sulfhydryl groups. This treatment was sufficient to inhibit completely the surface oxidation which occurs in the dark. With dim lighting, the fading was partially inhibited and the meat regained its initial color upon subsequent storage in the dark. With very intense illumination the protection achieved was negligible and return of the color in the dark was partial and delayed.

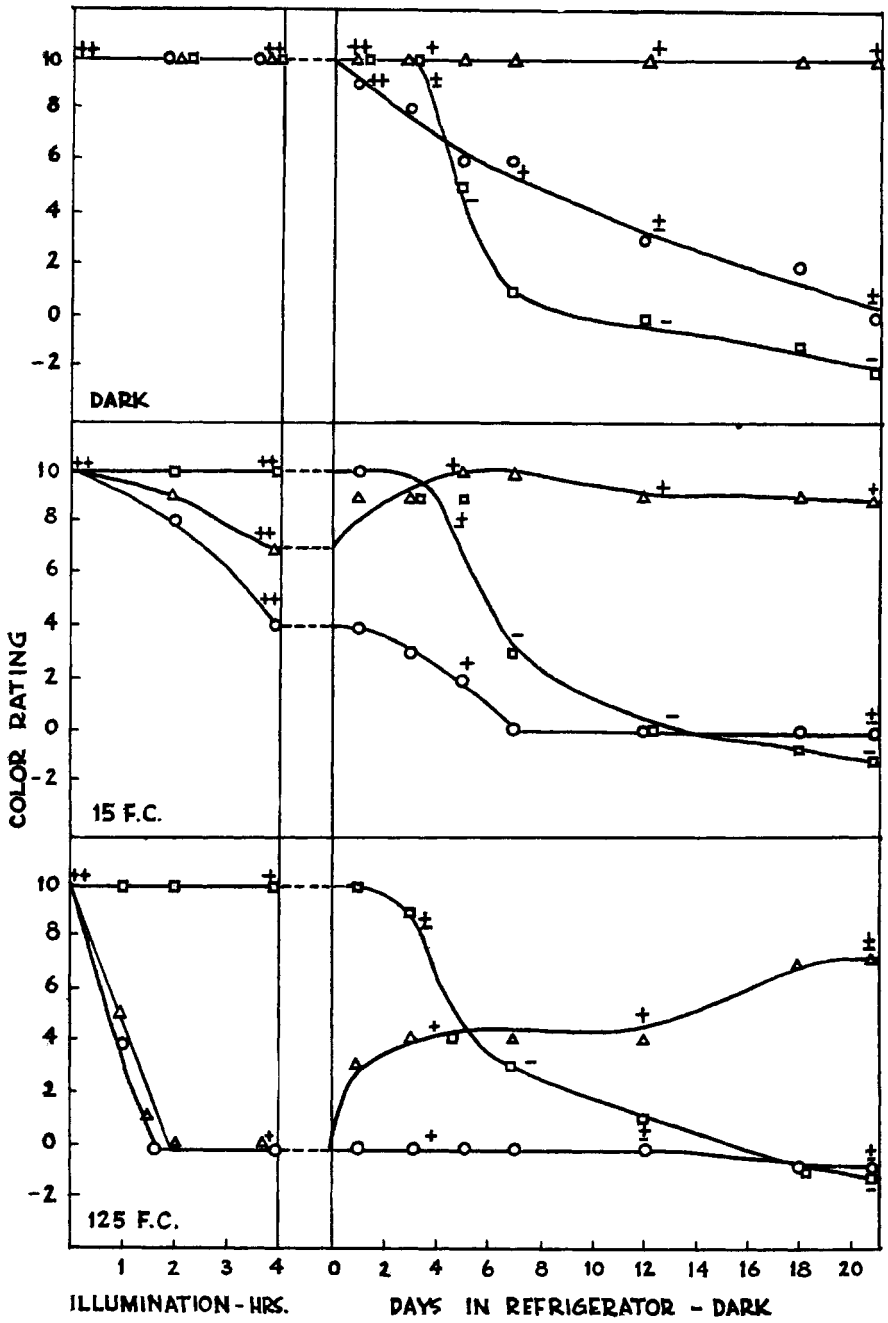


Figure 3. Effect of dipping in nitrite solutions on color changes in canned cured meat product exposed to several intensities of light

○ Control, no added nitrite
△ Dipped in 0.2% nitrite for 20 seconds
□ Dipped in 2.0% nitrite for 20 seconds
+ and — marks accompanying each curve indicate strength of nitroprusside reaction

Exposure of the surface to a high concentration of nitrite completely inhibited all fading by light, even with intense lighting. Other experiments in which light intensity was increased to 250 foot candles gave similar results. However, this concentration of nitrite caused more rapid loss of sulfhydryl groups. The nitroprusside test became negative within 3 to 5 days' storage and this coincided with a rapid fading of color to negative values, which are believed to indicate attack of the porphyrin ring as well as oxidation of the iron.

It is clear from this and the preceding experiment that in heat-sterilized canned meats, where there can be no question of enzymatic activity, the reduction of both nitrite and the ferric hemochromogen may be brought about by protein sulfhydryl groups, even at meat surfaces fully exposed to oxygen. As long as the meat gives a strong nitroprusside test, the limiting factor in preventing the light dissociation and subsequent oxidation is the nitrite concentration. Under these conditions, the addition of another reducing agent (ascorbic acid) either alone or with the nitrite has little effect on the light fading as compared with controls treated with nitrite alone.

This experiment was designed primarily to shed light on the role of nitrite and reducing groups on the fading of cured meats rather than to devise practical protective measures; nevertheless, the possibility of practical benefits should not be overlooked. The concentrations of nitrite present in the dipping solutions were 10 and 100 times, respectively, the concentration of nitrite allowed in meat. However, the meat was exposed to the solutions for only a few seconds, so that no more than a thin surface layer would be expected to show an increased nitrite content.

The very significant color protection achieved in the absence of strong illumination with the lower of the two nitrite concentrations was accomplished without significant destruction of sulfhydryl groups. However, the deleterious effect of added nitrite on color when reducing groups are not present is obvious from the above experiment and from the experience of the meat packing industry. Further experiments are in progress on changes in sulfhydryl content of cured meats under various conditions, including freezer storage, in order to establish a basis for selection of appropriate measures for control of color fading.

Summary and Conclusions

The reduction of metmyoglobin and nitrite necessary for the formation of the cured meat pigment, nitric oxide hemochromogen, may be brought about by sulfhydryl groups freed during denaturation of muscle proteins rather than by reducing enzyme systems.

The role which sulfhydryl groups remaining in the meat after curing play in stabilizing the cured meat pigment has also been investigated. The fading of the cured meat pigment is presumed to be due to dissociation of the nitric oxide hemochromogen followed by oxidation of the heme iron to give the brown ferric hemochromogen. The dissociation and subsequent oxidation are greatly accelerated by light.

When applied to a number of typical cured meat products, the nitroprusside test for free sulfhydryl groups varies from a strong positive reaction in hams to negative in certain types of bologna and frankfurters. Sulfhydryl groups protect the color, if sufficient nitrite is present. Dipping meats which give a positive nitroprusside test in strong nitrite for a few seconds prevents the fading in fresh samples and restores the pink color to surfaces that have already been faded by light; a nitrite concentration of 0.2% in the dipping solution is sufficient to prevent oxidative fading indefinitely in the dark and to retard fading with dim light. With very in-

tense illumination, much stronger nitrite solutions (2.0%) must be employed for protection during the lighting period. This concentration of nitrite accelerates oxidation of sulfhydryl groups present.

Whereas added nitrite protects the cured meat pigment from oxidation in the presence of protein sulfhydryl groups, it accelerates oxidation in their absence. This is true in meats such as bologna, which originally gave a negative nitroprusside test, as well as in meats in which the free sulfhydryl groups have been removed by exposure to high concentrations of nitrite for several days or to iodoacetic acid for a few minutes. Under these conditions, nitrite causes greater fading than the limiting value produced by long exposure to light or by treatment with an oxidizing agent (ferricyanide). This is interpreted to mean that the nitrite attacks the porphyrin ring as well as oxidizing the heme iron.

Literature Cited

(1) Anson, M. L., *Advances in Protein Chem.*, **2**, 361 (1945).
(2) Chinard, F. P., and Hellerman, L., "Methods of Biochemical Analysis," D. Glock, ed., Vol. 1, p. 1, Interscience Publishers, New York, 1954.
(3) Greenberg, L. A., Lester, D., and Haggard, H. W., *J. Biol. Chem.*, **151**, 655 (1943).
(4) Greenstein, J. P., and Edsall, J. T., *Ibid.*, **133**, 397 (1940).
(5) Kampschmidt, R. F., Abstracts of Papers, Division of Agricultural and Food Chemistry, 126th Meeting, Am. Chem. Soc., New York, 1954.
(6) Mirsky, A. E., and Pauling, L., *Proc. Natl. Acad. Sci. U. S.*, **22**, 439 (1936).
(7) Putnam, F. W., in "The Proteins," H. Neurath and K. Bailey, eds., Vol. 1, (B), p. 807, Academic Press, New York, 1953.
(8) Watts, B. M., *Advances in Food Research*, **5**, 1 (1954).

Received for review September 24, 1954. Accepted November 29, 1954. Work financed in part by a grant from Swift & Co. to the Florida State University.

SWEET POTATO DEHYDRATION

Effects of Conditions of Storage of Raw Materials on Chemical Properties of Dehydrated Products

JETT C. ARTHUR, Jr., and T. A. McLEMORE

Southern Regional Research Laboratory, New Orleans, La.

JULIAN C. MILLER, LLOYD G. JONES, and WILLIAM A. SISTRUNK

Louisiana Agricultural Experiment Station, Baton Rouge, La.

The changes in chemical composition of raw sweet potatoes during storage at 50°, 60°, and 70° to 75° F. and the properties of the derived dehydrated products indicate that a storage temperature of 60° F. is superior for maintenance of raw materials. The sugar content of sweet potatoes stored at 50° F. increased more than that of potatoes stored at 60° or 70° to 75° F. The shrinkage (loss in fresh weight) of materials stored at 70° to 75° F. is much greater than that of materials stored at 50° and 60° F. Sweet potatoes of the Unit I Porto Rico variety stored at 60° F. for not more than 4 to 6 months yield dehydrated products having a bright yellow-orange color, a carotene content of 225 to 250 p.p.m., an ascorbic acid content of 35 to 40 mg. per 100 grams, and a rehydration of about 100%.

Sweet potatoes are grown in more than 22 states and are one of the leading vegetable crops in the United States, with a production in 1953 of about 30,000,000 bushels, concentrated principally in the lower Atlantic and south central states (*24*). Sweet potatoes are an acceptable and nutritious food having a carotene content of 4 to 7 mg. %, an ascorbic acid content of 17 to 33 mg. %, and a high caloric value (*7*).

During World War II large quantities of dehydrated foods were procured and used by the Armed Services. Dehydrated sweet potatoes made an important contribution to this supply. However, in 1946 commercial production of dehydrated sweet potatoes for food ceased, because of termination of procurement by the Armed Services and the lack of interest in the product by the civilian market.

In 1951 interest in the possible reactivation of the sweet potato dehydration industry developed. The lack of scientific data to guide in drafting specifications and activating the industry was indicated by the Quartermaster Food and Container Institute for the Armed Forces. One phase of the problem was to determine the effects of temperature and time of storage of sweet potatoes on their properties and on the properties of derived dehydrated products. Changes in the properties of sweet potatoes during storage under different conditions have been reported by numerous investigators (*1, 4–6, 8, 11–15, 17, 19, 20, 22, 23, 26, 27*). Wagley investigated the effect of storage of sweet potatoes in relation to canning and observed that changes in chemical composition resulted in loss of texture, development of off-flavors and from the canner's standpoint, economic losses (*25*). Hopkins and Phillips reported that, after harvesting, a marked and regular change in the amount of sugars in sweet potatoes occurs (*10*). When the potatoes were cured and then stored at different constant temperatures, the content of sugars increased at 50° and 55° F. but decreased at 60°, 65°, and 70° F., indicating that a critical temperature range exists for the storage of sweet potatoes.

The purpose of this investigation was, therefore, to extend these studies and to determine the effects of storage of raw materials on their shrinkage, moisture, and contents of carotene, ascorbic acid, and sugars; on the color, contents of carotene and ascorbic acid, and rehydration characteristics of derived dehyrated products; and on the losses of raw materials during processing.

Methods

Moisture. A 2-gram ground sample, passing through 20-mesh screen but held on 40-mesh screen, was weighed into tared, dry, aluminum weighing dishes (approximately 2 inches in diameter and 3/4 inch in depth) with tightly fitting covers. The dishes, with cocked lids,