Table III. Recovery of ETM from Various Commodities

ETM added, ppm	ETM recovered, % Spinach	Tomato	Lettuce	Bean
0.05	97.9	96.4	90.1	98.1
0.10	98.1	93.0	83.2	98.1
0.50	96.4	94.7	93.0	94.7
1.00	85.4	89.1	85.6	92.2

The recoveries obtained from other commodities fortified with ETM are given in Table III and are similar to those found for apple. The overall recovery is comparable to that reported for a polarographic method (Engst and Schnaak, 1970).

LITERATURE CITED

Alvarez, M., Creekmore, R. W., Rosen, R. T., *Tetrahedron Lett.*, 939 (1973).
Beer, R. J. S., Naylor, A., *Tetrahedron Lett.*, 2989 (1973).
Engst, R., Schnaak, W., *Z. Lebensm.-Unter.-Forsch.* **144**, 81 (1970).
Hylin, J. W., *Bull. Environ. Contam. Toxicol.* **10**, 227 (1973).
Ludwig, R. A., Thorn, G. D., Miller, D. M., *Can. J. Bot.* **32**, 48 (1954).
Ludwig, R. A., Thorn, G. D., Unwin, C. H., *Can. J. Bot.* **33**, 42 (1955).
Pluijgers, C. W., Vonk, J. W., Thorn, G. D., *Tetrahedron Lett.*, 1317 (1971).
Thorn, G. D., Ludwig, R. A., *Can. J. Chem.* **32**, 872 (1954).

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Sulfhydryl Group Content of Chicken Breast Muscle during Post-Mortem Aging

Total and nonprotein sulfhydryl content of chicken breast muscle was measured during post-mortem aging. Excised muscle portions were aged in an ice bath for 0, 15, 30, 45, 60, 120, 240, or 360 min post-mortem and then frozen in a Dry Ice-methanol bath. Three different methods were used to homogenize the muscle tissue for analysis: (1) pulverized, frozen tissue was homogenized with 0.02 *M* ethylenediaminetetraacetic acid (EDTA) in a blender; (2) pulverized, freeze-dried tissue was digested with acidified pepsin; and (3) pulverized, frozen tissue was sonicated in a diluent of 8 *M* urea. Homogenates were assayed for sulfhydryl group content using Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)] and for nitrogen content by a micro-Kjeldahl method. Analysis of variance showed that sulfhydryl concentration did not change significantly with aging time within any of the homogenate preparation methods. Digesting the tissue with acidified pepsin produced a significantly higher sulfhydryl content than homogenizing the tissue with a blender in 0.02 *M* EDTA or with sonic oscillation in 8 *M* urea. Total sulfhydryl content of chicken breast muscle by the three procedures ranged from 0.414 to 0.564 μmol of SH/mg of nitrogen.

Among the many chemical processes taking place during the post-mortem aging of muscle, a decrease in sulfhydryl group content has been considered as a possible mechanism for the development of rigor and subsequent post-rigor tenderization by workers in this laboratory (Chajuss and Spencer, 1962; Gawronski *et al.*, 1967). Both groups of workers reported a decrease in sulfhydryl content in excised chicken breast muscle during the first 2 hr of post-mortem aging. Chajuss and Spencer (1962) proposed that disulfide–sulfhydryl exchange reactions were involved in formation and relaxation of a strained three-dimensional network of protein within the muscle. The results of studies in another laboratory (Caldwell and Lineweaver, 1969), however, have indicated that no change in the sulfhydryl content of chicken breast muscle occurs during post-mortem aging. More recently, Hay *et al.* (1972) reported an increase in sulfhydryl content in actomyosin extracted from chicken breast muscle at 3 hr post-mortem. However, they found no significant change in the sulfhydryl content of actomyosin extracted from chicken leg muscle at 0, 3, 24, and 168 hr post-mortem. Wu and Sayre (1971) observed that the number of sulfhydryl groups in myosin extracted from chicken red and white muscle is essentially the same at 30 min and 24 hr post-mortem.

This study was undertaken to provide additional information to the data concerning sulfhydryl content in chicken breast muscle reported by Chajuss and Spencer (1962), Gawronski *et al.* (1967), and Caldwell and Lineweaver (1969). A substantial point of difference in the assay procedures of these workers is the method of tissue preparation for sulfhydryl analysis. In order to determine whether tissue preparation was responsible for the variation in results, a more recent method of sulfhydryl assay (Sedlak and Lindsay, 1968) was chosen to measure tissue sulfhydryl concentration in homogenates prepared according to the preparation procedure described by each of the above three groups of workers. According to Sedlak and Lindsay (1968), their method of sulfhydryl assay was found to yield highly reproducible results.

MATERIALS AND METHODS

Source of Muscle Samples. Three mature White Leghorn fowl were slaughtered for use with each of the three homogenate preparation methods. All birds received exactly the same treatment, except for the method of preparing the homogenates. The birds were dispatched as needed by severing the jugular vein, esophagus, and trachea with a knife. They were allowed to bleed for 2–3 min. The wings and legs were restrained during bleeding. The right and left pectoralis major muscles were excised and four portions were removed from each muscle. All zero-time samples were frozen within 3–5 min after the birds were dispatched. The remaining seven portions were then placed in polyethylene bags in an ice bath and allowed to age for 15, 30, 45, 60, 120, 240, or 360 min before freezing in a Dry Ice bath. Frozen samples were stored in polyethylene bags surrounded by Dry Ice until the appropriate

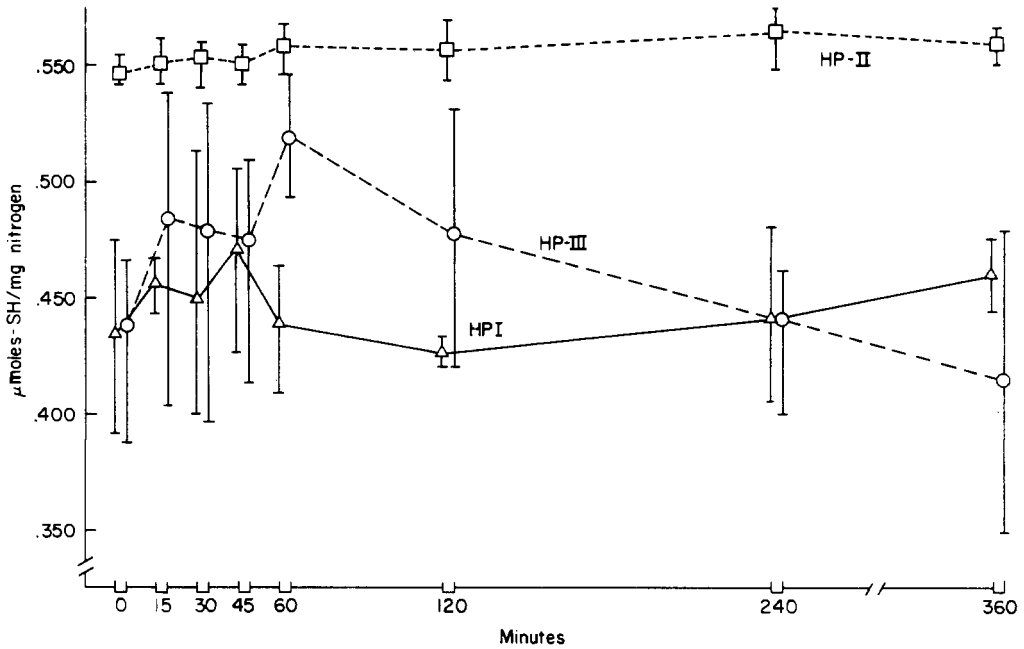

Figure 1. Average and ranges of total sulfhydryl concentration at various aging times from each of the three homogenate preparation (HP) methods, using mature White Leghorn hen pectoralis major muscle.

analysis was made. Storage time was always less than 5 days.

Homogenate Preparation Method I (HP-I). This muscle homogenate was prepared with slight modifications according to the method of Caldwell and Lineweaver (1969). The frozen muscle sample was placed in a precooled Waring Blendor and pulverized with several pieces of Dry Ice. That portion of pulverized muscle and Dry Ice which passed through a precooled 20-mesh sieve was used for assay. The Dry Ice was allowed to sublime at −10° for 15 hr within loosely covered petri plates. Approximately 5 g of frozen pulverized muscle was added to 50 ml of cold (0–5°) 0.02 *M* EDTA (ethylenediaminetetraacetic acid disodium) in a 50-ml Sorvall blender cup. [An EDTA solution instead of water was used to prepare the homogenates to prevent possible enzymatic alteration of sulfhydryl groups in the homogenates (Sedlak and Lindsay, 1968)]. Specially prepared fittings allowed nitrogen gas to be passed by way of the shaft from the lid assembly to the blade of the blender and into the solution. Nitrogen was bubbled into the muscle–EDTA mixture in the blender cup for 2–3 min to prevent possible air oxidation of sulfhydryl groups. The fittings were then removed and the solution homogenized at speed 10 for 1 min, shaken 10 times, and homogenized one additional minute. During these operations and the time prior to analysis the homogenate was kept cold in an ice bath.

Homogenate Preparation Method II (HP-II). The method of Gawronski *et al.* (1967) was used to prepare this homogenate. Frozen muscle samples were freeze-dried and stored in an evacuated desiccator over $CaSO_4$ until analysis could be carried out. Freeze-dried samples were ground to a fine powder in a mortar. Approximately 200 mg of ground sample was added to 20 ml of a 0.010% pepsin (1:60,000; 2× crystallized and lyophilized) solution which had been prepared with a 0.05 *M* HCl solution previously flushed with nitrogen. Nitrogen was bubbled through the tissue acidified pepsin solution and it was allowed to stand at room temperature for 2–3 hr.

Homogenate Preparation Method III (HP-III). The procedure of Chajuss and Spencer (1962) was used to prepare the homogenates for the third method. A preweighed frozen sample was ground in a precooled mortar with 3–5 vol of Dry Ice and the mixture quantitatively transferred to a 10 kHz Raytheon sonic oscillator chamber. Twenty milliliters of 8 *M* urea (freshly prepared from recrystallized urea) was added and the air in the chamber replaced with nitrogen. The chamber was sealed and the tissue sonicated for 20 min at maximum power output. During oscillation the chamber was continuously cooled with water from an ice–water bath.

Sulfhydryl Analysis. Assay for nonprotein and total sulfhydryl groups was carried out following the method of Sedlak and Lindsay (1968) which used Ellman's reagent. The procedure was the same for all homogenate preparation methods except for the pH and molarity of the buffers, which were varied among the homogenate preparation methods to achieve a final pH of 8.1–8.2. HP-I and HP-III were analyzed for nonprotein and total sulfhydryl content. HP-II was analyzed for total SH only, because digestion with acidified pepsin disrupted the proteins so thoroughly that there was little difference between total and nonprotein SH values in these homogenates.

Nitrogen Analysis. Nitrogen content of the homogenates was measured using a micro-Kjeldahl method. For HP-III, muscle tissue samples were blended with 20 ml of distilled water, in place of urea, to measure nitrogen content.

Calculations. Absorbance readings for both nonprotein and total SH were converted to moles of SH per liter by reading directly from a standard curve prepared with glutathione. Absorbance was linear with gluthionone concentrations over a range of $1\text{–}10 \times 10^{-5}$ *M*. Using 8 *M* urea instead of 0.02 *M* EDTA as the solvent for the standard did not change the absorbance readings.

Values of SH in moles per liter for HP-I and HP-II were converted to micromoles of SH per milligram of nitrogen by multiplying by the appropriate dilution factor and dividing by the amount of nitrogen in each sample. HP-III values were first expressed in micromoles of SH per gram of tissue, and then converted to micromoles of SH per milligram of nitrogen using the average milligrams nitrogen per gram of tissue experimentally determined for each bird.

RESULTS AND DISCUSSION

The concentration of total sulfhydryl groups in chicken breast muscle at various aging times is presented in Figure 1. Only the total sulfhydryl content of the muscle is reported, nonprotein sulfhydryl content being so small

that it was considered to be negligible. Average nonprotein sulfhydryl content in homogenates was 0.019 μmol of SH/mg of nitrogen.

Analysis of variance showed that sulfhydryl concentration did not change significantly with time of aging within a preparation method ($P = 0.05$). Duncan's Multiple Range test showed HP-II to have significantly higher values than HP-I or HP-III for all aging periods, while HP-I and HP-III were not significantly different from each other ($P = 0.05$).

The greater range of values observed for HP-I and HP-III at each aging time, as shown in Figure 1, indicates that homogenization using a Sorvall blender or sonic oscillator may cause variability in masceration of the tissue and therefore affect the efficiency of exposure of sulfhydryl groups in some way to give a wider range of readings than when tissue is digested with acidified pepsin (HP-II). In triplicate analyses of a particular homogenate, the experimental values were nearly identical indicating the homogenate itself was uniform. In addition, recovery of internal standards was essentially 100%, indicating the method of analyzing for sulfhydryl groups is reproducible and accurate.

The total SH concentration in chicken breast muscle agreed closely with those values reported by Chajuss and Spencer (1962) and was 0.200–0.300 μmol of SH/mg of nitrogen lower than those reported by Gawronski *et al.* (1967). Total sulfhydryl concentration reported by Caldwell and Lineweaver (1969) was about 2 to 3 times higher than that reported here, and nonprotein sulfhydryl concentration was about 30 times higher than the results reported in this study. This difference may be due to the method of analysis used to measure thiol concentration. Those workers used pH values of 6.8 and 7.6 to measure nonprotein and total sulfhydryl content, respectively, by the procedure of Jocelyn (1962), while a pH of 8.1–8.2 was used in this work. Sedlak and Lindsay (1968) have found Jocelyn's method to have poor reproducibility and recovery and reported that color production between protein sulfhydryls and DTNB at a pH of 6.8 or lower would seriously affect the accuracy of total and nonprotein sulfhydryl determination by Jocelyn's method. Another source of variation in results may be due to the use of mature White Leghorn hens as experimental material by these workers and the use of broilers by Caldwell and Lineweaver. Caldwell and Lineweaver used electric stun in dispatch of the birds, while these workers restrained the birds during bleeding.

CONCLUSION

The results of measuring sulfhydryl groups during post-mortem aging reported here show no statistically significant change in sulfhydryl concentration with time. No attempt was made to follow the time course of rigor during post-mortem aging, and it is possible that any meaningful changes in sulfhydryl concentration may have been canceled by bird differences attributable to muscles entering rigor at different times. The influence of variation in the time pattern of rigor could be minimized by measuring pH of the excised muscle sample to follow the post-mortem muscular changes (Marsh, 1954) and adjusting sampling times accordingly. This precaution could be recommended for subsequent studies.

It appears that the method of tissue homogenate preparation does not fully account for the differences in results reported by Chajuss and Spencer (1962) and Gawronski *et al.* (1967) and those reported by Caldwell and Lineweaver (1969). It is possible that the decrease in sulfhydryl concentration during the first 2 hr of post-mortem aging reported by Chajuss and Spencer (1962) and Gawronski *et al.* (1967) was due to the methods of assaying for sulfhydryl content. A comparison of the nitroprusside method, amperometric titration, and the Ellman's reagent method of Sedlak and Lindsay (1968) of assaying sulfhydryl content in muscle tissue at defined stages of rigor would further contribute to determining the reason for conflicting results.

LITERATURE CITED

Caldwell, K. A., Lineweaver, H., *J. Food Sci.* **34**, 290 (1969).
Chajuss, D., Spencer, J. V., *J. Food Sci.* **27**, 411 (1962).
Gawronski, T. H., Spencer, J. V., Pubols, M. H., *J. Agr. Food Chem.* **15**, 781 (1967).
Hay, J. D., Currie, R. W., Wolfe, F. H., *J. Food Sci.* **37**, 346 (1972).
Jocelyn, P. C., *Biochem. J.* **85**, 480 (1962).
Marsh, B. B., *J. Sci. Food Agr.* **5**, 70 (1954).
Sedlak, J., Lindsay, R. H., *Anal. Biochem.* **25**, 192 (1968).
Wu, C.-S. C., Sayre, R. N., *J. Food Sci.* **36**, 133 (1971).

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Phytosterols of *Cannabis* Smoke

The 3-β-hydroxysterols present in American-grown (MS-13) *Cannabis* were identified and quantitated in its smoke. The free sterol fraction of the smoke contained campesterol, stigmasterol, and β-sitosterol in essentially the same ratio as that found in the plant material.

The 3-β-hydroxysterols campesterol, stigmasterol, and β-sitosterol have recently been identified (Doorenbos *et al.*, 1971) and quantitated (Foote and Jones, 1974) in extracts of *Cannabis*. The phytosterols have also been isolated and quantitated in tobacco and its smoke (Stedman, 1968). It was therefore of interest to determine their presence in marihuana smoke. Previously it has been demonstrated that the phytosterols are precursors of carcinogenic hydrocarbons in smoke (Wynder *et al.*, 1959).

Quantitative analyses of sterol content were performed on the smoke condensate of *Cannabis* cultivated at the University of Mississippi (MS-13 Mexican male) by a standard method (Doorenbos *et al.*, 1971). Seventy-millimeter cigarettes were hand rolled and smoked on a smoking machine taking a 40-ml puff of 2-sec duration every minute (Adams and Jones, 1973). The average weight per cigarette was 0.78 g. The smoke condensate was trapped in Celite at Dry Ice–isopropyl alcohol temperature and removed from the trap by washing with methylene chloride. The smoke condensate obtained from smoking 390 g of