flour mostly as pyridoxine, 75 and 71%, respectively. Over 90% of the vitamin B_6 values of lean beef and milk solids were pyridoxal and pyridoxamine—40% pyridoxal and 55% pyridoxamine in the lean beef, and 62% pyridoxal and 36% pyridoxamine in the milk solids. The Texas laboratory also assayed these samples microbiologically using the procedures, including fractionation, developed at Beltsville. Results for the total vitamin B_6 values were as follows: dried lean beef, 14.91 μg. per gram; dried Lima beans, 6.99; nonfat dry milk solids, 3.68; whole wheat flour, 3.51. Agreement between the data obtained by two laboratories working independently was strong evidence that the chromatographic fractionations of extracts and microbiological procedures were reproducible and should be applicable routinely for the determination of vitamin B_6 in foods.

From these data, it was concluded that the bioassay measures free and combined forms of vitamin B_6 and that the bioassay values agree with the values obtained microbiologically on chromatographed, fractionated extracts of these samples.

Acknowledgment

The authors gratefully acknowledge the assistance in these studies of E. W. Murphy, R. M. Stewart, and R. M. Vegors of the Food Composition Laboratory, Beltsville, Md., and J. Godwin and M. Bayliss of the Texas Agricultural Experiment Station, College Station, Texas.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 9th ed., 1960.
(2) Bliss, C. I., *Biometrics Bull.* **1,** 57 (1945).
(3) MacArthur, M. J., Lehmann, J., *J. Assoc. Offic. Agr. Chemists* **42,** 619 (1959).
(4) Parrish, W. P., Loy, H. W., Jr., Kline, O. L., *Ibid.*, **38,** 506 (1955).
(5) Richardson, L. R., Wilkes, S., Ritchey, S. J., *J. Nutr.* **73,** 363 (1961).
(6) Toepfer, E. W., Lehmann, J., *J. Assoc. Offic. Agr. Chemists* **44,** 426 (1961).
(7) Toepfer, E. W., MacArthur, M. J., Lehmann, J., *Ibid.*, **43,** 59 (1960).
(8) Toepfer, E. W., Zook, E. G., Orr, M. L., Richardson, L. R., *U. S. Dept. Agr., Agr. Handbook* **29** (1951).
(9) Woodring, M. J., Storvick, C. A., *J. Assoc. Offic. Agr. Chemists* **43,** 63 (1960).

Received for review August 3, 1962. Accepted November 2, 1962.

STORAGE EFFECTS ON BEEF

The Effect of Three Years of Freezer Storage on the Thiamine, Riboflavin, and Niacin Content of Ripened and Unripened Beef

BERNADINE MEYER, MARY MYSINGER, and RUTH BUCKLEY

Agricultural Experiment Station and College of Home Economics, University of Tennessee, Knoxville, Tenn.

The change in thiamine, riboflavin, and niacin content of longissimus dorsi and semimembranosus muscles of eight Hereford steers was determined after 3 years of freezer storage. Samples were ground and frozen on the day of slaughter and after 21 days of ripening at 34° F. During 3 years of storage at 0° F., thiamine increased significantly in unripened beef but was unchanged in ripened beef. Riboflavin increased slightly in both ripened and unripened beef. Niacin decreased significantly in unripened beef but was unchanged in ripened meat. Although all changes were statistically significant, none was of such magnitude as to be important nutritionally.

THEORETICALLY, vitamins of the B-complex should be stable to freezer storage, especially in foods packaged to minimize oxidative changes and treated to reduce enzyme activity. However, in raw meat there is the possibility of enzymatic changes occurring during freezer storage. Several reports as to the stability of thiamine, riboflavin, and niacin in animal tissues during freezer storage have been published (*2, 4–8, 11–13*). Detailed comparison of results of these studies is complicated by variations in the storage times and temperatures employed, as well as by variations in the amount of aging of the meat prior to freezing.

Generally, reported losses of thiamine were slight (*7, 8*) and nonsignificant (*5, 6, 13*) in red meats and poultry (*2, 11*). Results of tests for the stability of riboflavin were less consistent. Reported changes ranged from a decrease of 31% (*7*) to an increase of 22 to 42% (*13*) in pork loins, and decreases up to 46% were reported for beef rib steaks (*5*). Kotschevar (*4*) obtained greater losses of thiamine and riboflavin in sliced calf liver than in ground calf liver stored in an atmosphere of carbon dioxide, suggesting that oxidation during freezer storage could be a factor in losses of these two vitamins.

The amount of aging prior to freezing was reported to have an effect on niacin retention in pork (*12*). Niacin decreased 18% in 32 weeks in loins aged for 1 day before freezing, but no loss occurred when loins were aged for 3 or 7 days. Others have reported nonsignificant changes in the niacin content of beef rib steaks (*5*), pork chops (*6, 7*). and beef liver (*4*) during freezer storage.

Beef may be ripened (aged) for several days or weeks prior to freezing. No data were found on how this practice affects the retention of B vitamins during subsequent freezer storage. Therefore, the present study was designed to investigate the effects of ripening prior to freezing and of long-time storage on the thiamine, riboflavin, and niacin content of beef.

Experimental Procedure

Meat for this study was procured from four pairs of Hereford steers. Each pair consisted of a grain-finished and grass-finished animal. Thiamine, riboflavin,

Table I. Thiamine Content of Ripened and Unripened Beef before and after 3 Years of Freezer Storage[a]

Amount of Ripening before Freezing	Moist Basis: Before freezing, (mg./100 grams)	Moist Basis: After 3 years at 0° F. (mg./100 grams)	Moist Basis: Change due to freezer storage, %	Fat-Free, Dry Basis: Before freezing, (mg./100 grams)	Fat-Free, Dry Basis: After 3 years at 0° F. (mg./100 grams)	Fat-Free, Dry Basis: Change due to freezer storage, %
None						
Longissimus dorsi	0.058 ± 0.002	0.079 ± 0.002	+36[b]	0.254 ± 0.009	0.334 ± 0.007	+32[b]
Semimembranosus	0.071 ± 0.004	0.099 ± 0.006	+38[b]	0.308 ± 0.015	0.418 ± 0.022	+36[b]
Av.	0.065	0.089	+37[b]	0.281	0.376	+34[b]
21 Days						
Longissimus dorsi	0.064 ± 0.002	0.063 ± 0.003	−2	0.278 ± 0.008	0.268 ± 0.012	−4
Semimembranosus	0.079 ± 0.003	0.079 ± 0.003	−1	0.336 ± 0.011	0.326 ± 0.011	−3
Av.	0.072	0.071	−1	0.307	0.297	−3

[a] Values are averages of 8 samples ± standard error of mean. [b] Significant ($P \leq 0.01$).

Table II. Riboflavin Content of Ripened and Unripened Beef before and after 3 Years of Freezer Storage[a]

Amount of Ripening before Freezing	Moist Basis: Before freezing (mg./100 grams)	Moist Basis: After 3 years at 0° F. (mg./100 grams)	Moist Basis: Change due to freezer storage,[b] %	Fat-Free, Dry Basis: Before freezing (mg./100 grams)	Fat-Free, Dry Basis: After 3 years at 0° F. (mg./100 grams)	Fat-Free, Dry Basis: Change due to freezer storage,[b] %
None						
Longissimus dorsi	0.18 ± 0.004	0.20 ± 0.007	+12	0.77 ± 0.022	0.84 ± 0.035	+9
Semimembranosus	0.21 ± 0.010	0.24 ± 0.013	+11	0.93 ± 0.040	1.01 ± 0.056	+9
Av.	0.20	0.22	+11	0.85	0.93	+9
21 Days						
Longissimus dorsi	0.17 ± 0.008	0.19 ± 0.007	+12	0.74 ± 0.043	0.81 ± 0.032	+9
Semimembranosus	0.20 ± 0.010	0.22 ± 0.008	+10	0.86 ± 0.044	0.92 ± 0.035	+8
Av.	0.19	0.21	+11	0.80	0.86	+8

[a] Values are averages of 8 samples ± standard error of mean. [b] Freezer storage effects significant ($P \leq 0.01$).

Table III. Niacin Content of Ripened and Unripened Beef before and after 3 Years of Freezer Storage[a]

Amount of Ripening before Freezing	Moist Basis: Before freezing (mg./100 grams)	Moist Basis: After 3 years at 0° F. (mg./100 grams)	Moist Basis: Change due to freezer storage, %	Fat-Free, Dry Basis: Before freezing (mg./100 grams)	Fat-Free, Dry Basis: After 3 years at 0° F. (mg./100 grams)	Fat-Free, Dry Basis: Change due to freezer storage, %
None						
Longissimus dorsi	8.0 ± 0.3	6.6 ± 0.2	−17[b]	34.8 ± 1.4	28.0 ± 1.0	−20[b]
Semimembranosus	8.1 ± 0.3	6.7 ± 0.2	−18[b]	35.3 ± 1.5	28.4 ± 1.0	−20[b]
Av.	8.1	6.7	−17[b]	35.0	28.2	−20[b]
21 Days						
Longissimus dorsi	5.3 ± 0.3	5.4 ± 0.2	+2	23.0 ± 1.4	22.9 ± 0.8	0
Semimembranosus	5.3 ± 0.2	5.6 ± 0.2	+5	22.5 ± 0.7	23.2 ± 0.7	+3
Av.	5.3	5.5	+4	22.8	23.0	+1

[a] Values are averages of 8 samples ± standard error of mean. [b] Significant ($P \leq 0.01$).

Table IV. Mean Squares for Thiamine, Riboflavin, and Niacin (Mg./100 Grams)

Source	df	Thiamine: Moist bosis	Thiamine: Fat-free, dry basis	Riboflavin: Moist basis	Riboflavin: Fat-free, dry basis	Niacin: Moist bosis	Niacin: Fat-free, dry basis
Steers	7	0.00032[a]	0.00502[a]	0.00274[a]	0.05922[a]	2.053[a]	36.665[a]
Freezer storage (FS)	1	0.00215[a]	0.02822[a]	0.00722[a]	0.07903[a]	5.899[a]	171.610[a]
Ripening (R)	1	0.00046[a]	0.01087[a]	0.00181[b]	0.04923[a]	60.665[a]	1212.781[a]
Muscles (M)	1	0.00411[a]	0.06490[a]	0.01925[a]	0.29309[a]	0.217	0.276
FS × R	1	0.00248[a]	0.04410[a]	0.00003	0.00048	10.152[a]	201.640[a]
FS × M	1	0.00005	0.00084	0.00001	0.00006	0.008	0.360
R × M	1	0.00000	0.00056	0.00018	0.00983	0.001	1.381
FS × R × M	1	0.00004	0.00087	0.00000	0.00017	0.079	1.102
Error	49	0.00004	0.00079	0.00030	0.00583	0.326	6.377

[a] Significant ($P \leq 0.01$). [b] Significant ($P \leq 0.05$).

and niacin content of the semimembranosus and longissimus dorsi muscles of these animals before freezing, as well as details of the feed management and ripening have been reported by Meyer *et al.* (*9*, *10*).

Freezing and Storing Meat. Approximately 0.5-pound samples of the freshly ground longissimus dorsi and semimembranosus muscles, prepared as previously described (*9*), were placed in freezer storage. Samples were prepared and frozen on the day of slaughter and after 21 days of ripening at 34° F. The meat was wrapped in laminated locker paper, frozen in a blast freezer at −15° F., and stored 3 for years in a 0° F. room. On removal from freezer storage, the meat was sampled for vitamin assays as soon as it had thawed sufficiently to permit thorough mixing. Any drip that developed on thawing was reincorporated into the ground sample.

Vitamin Methods. The methods

used for determining the vitamin content after 3 years of freezer storage were essentially the same as those used for the fresh meat. Niacin and riboflavin were determined by microbiological assay, and thiamine by oxidation to thiochrome. Procedures, with slight modifications, were those recommended by the Association of Vitamin Chemists (*1*), using enzymatic digestion for the simultaneous release of B-complex vitamins. Standard recoveries of thiamine, riboflavin, and niacin, respectively, averaged 93, 89, and 101% with fresh meat, and 87, 95, and 98% with frozen meat.

Analysis of Data. No significant differences in thiamine, riboflavin, or niacin content of these two muscles of beef were associated with feed management before freezing (*9*). Therefore, the analysis of variance of the vitamin determinations was done only with respect to the effects of freezer storage, ripening, and muscles. Thus, data from the four pairs of animals constituted eight replications of tests of 3 years of storage on beef frozen unripened and after 21 days of ripening. When significant interactions between ripening and freezer storage effects were observed, tests of significance among ripening-freezer storage means were made according to Duncan (*3*).

Results

Thiamine. A highly significant interaction between ripening effects and freezer storage effects was observed for thiamine (Table IV). The thiamine content of both the longissimus dorsi and semimembranosus muscles increased significantly during 3 years of freezer storage (Table I) in unripened beef but was unchanged in ripened beef. It has been shown (*9*) that ripening for 21 days caused an increase ($p = 0.001$) in thiamine in these muscles from both grain-finished and grass-finished beef. If the factor(s) responsible for the thiamine increases during the ripening of the fresh meat were operative during freezer storage, it is not clear why the ripened meat failed to increase in thiamine content during storage also. There was more thiamine in unripened beef after 3 years of freezer storage than in fresh beef ripened for 21 days. The magnitude of the increase was approximately the same whether calculated on a moist or fat-free dry basis; therefore, the increase does not appear to be due to concentration from moisture loss during freezer storage. Since the unripened meat was sampled for assay of fresh meat and placed in freezer storage within 2 hours of slaughter, it seems possible that some postmortem increase in thiamine may be confounded with freezer storage effects.

Riboflavin. Riboflavin increased very slightly but significantly during 3 years of freezer storage (Table II) in both the ripened and unripened beef. While this increase was fairly consistent whether calculated on a moist or fat-free dry basis, it is of no importance nutritionally. In general, higher standard recoveries of riboflavin were obtained on the frozen meat than on fresh meat. This could be a factor contributing to the apparent increase in riboflavin.

Niacin. When beef was frozen unripened, the niacin content was decreased significantly after 3 years of storage at 0° F., but in the ripened beef it was essentially unchanged (Table III). Similar findings have been reported for pork (*12*). This interaction between ripening and freezer storage effects was highly significant statistically (Table IV) whether calculated on a moist or fat-free dry basis. The average loss of about 17% niacin in the unripened beef during 3 years at 0° F. was considerably less than the 33% loss that occurred during 3 weeks of ripening at 34° F. The slower rate of disappearance at freezing temperature points to the possibility that niacin losses during ripening and freezing may be due to enzymatic breakdown.

Discussion

This study was designed to determine the stability of thiamine, riboflavin, and niacin in ripened and unripened beef during long-time freezer storage. It was not intended to measure either the nutritive value or the acceptability of the meat. Nevertheless, a few samples were prepared as beef patties and the flavor was scored by an informal panel. The average score of all samples tasted was 5.6 out of a possible 9 points, indicating that the flavor of the meat was still fairly acceptable after 3 years in 0° F. storage.

It should be emphasized that the increases in thiamine and riboflavin, even though statistically significant, have no nutritional importance. It is doubtful also whether the loss of 17% niacin is important in American diets.

Acknowledgment

The authors thank J. W. Cole, Department of Animal Husbandry and Veterinary Science, for procuring, slaughtering, and aging the beef; R. J. Cooper for help with statistical analyses; and Kathleen McCook and Juanita Thomas for technical assistance with vitamin determinations.

Literature Cited

(1) Association of Vitamin Chemists, Inc., "Methods of Vitamin Assay," 2nd ed., Interscience, New York, 1951.
(2) Cook, B. B., Morgan, A. F., Smith, M. B., *Food Res.* **14,** 449 (1949).
(3) Duncan, D. B., *Biometrics* **11,** 1 (1955).
(4) Kotschevar, L. H., *J. Am. Dietet. Assoc.* **31,** 589 (1955).
(5) Lee, F. A., Brooks, R. F., Pearson, A. M., Miller, J. I., Volz, F., *Food Res.* **15,** 8 (1950).
(6) Lee, F. A., Brooks, R. F., Pearson, A. M., Miller, J. I., Wanderstock, J. J., *J. Am. Dietet. Assoc.* **30,** 351 (1954).
(7) Lehrer, W. P., Jr., Wiese, A. C., Harvey, W. R., Moore, P. R., *Food Res.* **16,** 485 (1951).
(8) *Ibid.*, **17,** 24 (1952).
(9) Meyer, B., Thomas, J., Buckley, R., *Food Technol.* **14,** 190 (1960).
(10) Meyer, B., Thomas, J., Buckley, R., Cole, J. W., *Ibid.*, **14,** 4 (1960).
(11) Morgan, A. F., Kidder, L. E., Hunner, M., Sharokh, B. K., Chesbro, R. M., *Food Res.* **14,** 439 (1949).
(12) Westerman, B. D., Oliver, B., Mackintosh, D. L., J. Agr. Food Chem. **3,** 603 (1955).
(13) Westerman, B. D., Vail, G. E., Kalen, J., Stone, M., Mackintosh, D. L., *J. Am. Dietet. Assoc.* **28,** 49 (1952).

Received for review August 29, 1962. Accepted January 22, 1963. Published with the approval of the Director of the Tennessee Agricultural Experiment Station.