HPLC Determination of Riboflavin, Niacin, and Thiamin in Beef, Pork, and Lamb after Alternate Heat-Processing Methods

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A relatively simple and sensitive method for the quantification of riboflavin, niacin, and thiamin by reversed-phase HPLC was developed. The HPLC methods enabled a rapid and reliable determination of vitamins B_1 and B_2 and niacin content. The most accurate results were obtained by a direct analysis of riboflavin and niacin simultaneously, using UV and fluorescence spectroscopy. However, extraction of the converted thiamin to thiochrome was necessary to allow for the removal of interfering fluorescence compounds prior to analysis in the fluorescence spectrophotometer. Riboflavin and niacin levels ($P < 0.05$) were stable in all of the heat-processing methods and meat cuts except beef shortloin steaks. Thiamin was stable only in the lamb loin chops. Heat-processing methods for both pork and beef had significant differences in the levels of thiamin when compared to the fresh samples. Analysis of these vitamins by HPLC was less time consuming than the standard AOAC methods.

High-performance liquid chromatography (HPLC) is used extensively in the pharmaceutical industry for a rapid method of vitamin analysis. HPLC methods have been developed for the pure, high-concentration levels of vitamins found in these products. HPLC methods to determine B vitamins in foods have been investigated to provide an accurate, rapid method of analysis. Reversed-phase ion-paired HPLC was effective when Toma and Tabekhia (1979) analyzed vitamins B_1 and B_2 and niacin in rice. Ang and Moseley (1980) converted riboflavin to lumiflavin, and thiamin to thiochrome, for fluormetric detection of these vitamins in meat products. In comparison to UV, fluorescence detection is more selective and detects fewer interfering compounds that are prevalent in muscle foods (Ang and Moseley, 1980). Fellman et al. (1982) used fluormetric detection for B vitamins in various foods. Following oxidation, these samples were concentrated to accomplish simultaneous determination of riboflavin and thiamin. Skurray (1981) used paired-ion chromatography combined with UV and fluorescent spectroscopy to detect vitamins B_1 and B_2 and niacin in various foods. Skurray (1981), Toma and Tabekhia (1979), and Fellman et al. (1982) ran standard Association of Official Analytical Chemists (AOAC) methods concurrently with the HPLC methods with excellent results.

The 166 meat products sampled and used in this study were unique in that one of the methods of heat processing involved a rotating hot-air system (Rair), described by Ibrahim and Unklesbay (1986). With this process, meat products are produced that are lower in calories and more tender than other methods of heat processing. These advantages have caused considerable interest in the Rair system. However, the effect of such processing on important nutrients in meat products is unknown. This study explored heat-processing effects, by using HPLC to determine vitamins B_1 and B_2 and niacin contents in beef, pork, and lamb products produced with both the Rair system and conventional methods.

MATERIALS AND METHODS

Meat Procurement. Seven beef carcasses with a mean weight of 304 kg, from the same kill day and of the USDA choice grade quality (3, carcasses had a modest degree of marbling; 4, carcasses had a small degree of marbling) and the USDA Yield Grade 3, were purchased. They were stored for 7 days at 2–3 °C to simulate the period of time that elapses between slaughter and when consumers most likely would consume the product. Paired flank steaks (rectus abdominous muscle) were removed from the carcasses and trimmed of excess fat deposits. Foreshanks were removed and cut into two cross-cut shanks. Shortloin steaks, IMPS No. 179 (*Meat Buyers Guide*, 1976) were cut to a thickness of 2.54 cm, and subcutaneous fat in excess of 1.27 cm was removed.

Seven pork loins were obtained from barrow carcasses with a selected quality score of 3, based on the Iowa State Pork Quality Standards (*Standards for Pork Color, Firmness and Marbling*, 1969). The mean carcass mass was 79 kg. The loins were stored for 7 days at 2–3 °C. Pork loin chops, IMPS No. 1412 (*Meat Buyers Guide*, 1976), were cut to a thickness of 2.54 cm, and subcutaneous fat in excess of 0.6 cm was removed. Half of the chops were breaded. Chops were individually weighed (Toledo Model No. 4032Y) before they were breaded. By volume, one part batter (Golden Dipt Co., St. Louis, MO) was mixed with two parts water. After a chop was removed from the batter, it was coated with crumbs (Golden Dipt Co., St. Louis, MO) and reweighed. The total breaded weight was no more than 20% of the weight of the original chop.

Eighteen lamb loins, IMPS No. 232 (*Meat Buyers Guide*, 1976), of USDA choice grade quality and of USDA Yield Grade 3 were purchased. All loins were from the same kill day. The mean carcass weight was 26 kg. The loins were vacuum packaged and stored for 7 days at 2–3 °C, as discussed for beef products. Lamb loin chops, IMPS No. 1232 (*Meat Buyers Guide*, 1969), were cut to a thickness of 2.54 cm, and the subcutaneous fat in excess of 1 cm was removed.

All products were individually packaged in laminated freezer paper and stored at −23 °C for 48 h and −17 °C for no longer than 14 days. Products were tempered at 4 °C for 24 h prior to heat processing.

Heat-Processing Methods. *Moist Heating.* The beef flank steaks with a mean mass of 601 g; and beef cross-cut shanks, 704 g, were placed in a casserole dish (Microwave Cookware with Silverstone Coating, Regal Ware, Inc., Kewaskum, WI) and covered with either 175 or 750 mL of tap water, respectively. Covered dishes were placed in a preheated (163 °C) electric conventional oven (Whirlpool, Model No. RYE3960A-2). The beef flank steaks were heat

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processed for 1.25 h; and the beef cross-cut shanks, 2.5 h. The internal muscle lean tissue temperature of both cuts reached 99 °C after 50 min of heat processing.

Charbroiling. The beef shortloin steaks, nonbreaded pork loin chops, and lamb loin chops were heat processed on a preheated (260 °C) electric charbroiler (Toastmaster, Model No. 4224). One thermocouple (Type K, 28 gauge, Kapton coating) attached to a digital thermometer (Omega Model No. 2176A) was placed into the lean tissue at the approximate geometric center of each product. The beef shortloin steaks were removed when rare (57 °C), medium (68 °C), and well (74 °C) stages of doneness were attained. Pork loin chops were removed at well (74 °C); lamb loin chops, at medium (68 °C) and at well (74 °C) degrees of doneness.

Rotating Hot Air. A rotating hot-air oven (Rair, Model No. VL 21-20) was preheated to 287 °C. Water (60 mL) was added to the oven after the product was placed in the oven. The door was closed immediately. The beef, breaded and unbreaded pork, and lamb products were heat processed to the same internal temperatures as previously described.

Deep-Fat Frying. An electric deep fat fryer (Hobart Model No. AK20) was used. Partially hydrogenated soybean oil (Supreme Liquid Frying Shortening, The Miami Margarine Co., Cincinnati, OH) was preheated to 177 °C. Breaded pork chops were heat processed to a 74 °C internal temperature.

Sample Preparation. Following heat processing, all steaks and chops were trimmed of visible subcutaneous and intermuscular fat. The breading was removed from the pork chops and discarded. Lean tissue was stripped from the bone. These portions were ground in a food grinder (Hobart, Model No. 45K) equipped with a 32-mm plate. Ground samples were portioned into polyethylene bags, sealed, and stored at −20 °C for no longer than 30 days until thawed for extraction. Samples were protected from light.

Vitamin Extraction. With slight modifications meat samples were prepared and extracted as outlined by the AOAC (1984) and Ang and Moseley (1980). A 5-g sample of ground meat was hydrolyzed with 60 mL of 0.1 N HCl plus 2 mL of 6 N HCl at 121 °C (776 Torr) for 30 min. The mixture was cooled to room temperature and adjusted to pH 4.0–4.5 with 2 M sodium acetate (ca. 5 mL). Four-milliliter portions of each enzyme (5% takadiastase and 10% papain) were added and the samples incubated (42–45 °C) for 2.5–3.0 h. Proteins were precipitated by adding 2 mL of 50% trichloroacetic acid. Samples were heated in 100 °C water bath for 10 min. Cooled samples were brought to volume (100 mL) and filtered through Whatman No. 40 filters. Samples were stored in amber vials at −20 °C. The total procedure was performed under subdued light; sufficient illumination was only available to enable safe handling of samples and instrumentation. Recovery samples were prepared by adding a known amount of reference thiamin, riboflavin, and niacin prior to the extraction process.

Apparatus and Conditions. Riboflavin and niacin were determined simultaneously. Niacin was monitored on a Perkin-Elmer (LC-75 autocontrol spectrophotometric detector) UV, wavelength 254 nm. Riboflavin was determined by a fluorescence spectrophotometer, excitation 464 nm, emission 540 nm (Perkin-Elmer, 650-10S). All samples were plotted against standards for peak height versus concentration. Standard curves were all linear. Data were recorded on a Shimadza R-12 strip chart recorder. Separation of 10–20-μL samples was accomplished on an Alltech C_{10} 10-μm column in line with a Perkin-Elmer Series 3B HPLC. The mobile phase was 0.02 M phosphate buffer/30% MeOH (pH 7.0) with a 1.0 mL/min flow rate. Thiamin was converted to thiochrome and extracted. Conversion was completed by combining 10 mL of filtered sample with 5 mL of freshly prepared 1% potassium fericyamide in 15% NaOH. The thiochrome was extracted with 10 mL of isobutyl alcohol in a separation funnel. Mobile phase remained the same as for riboflavin and niacin, but the detection wavelengths were excitation at 378 nm and emission at 430 nm. Portions of 10–20 mL were injected, and samples were plotted against standard linear curves.

Table I. Mean Thiamin, Riboflavin, and Niacin Contents of Beef Flank Steak and Beef Cross-Cut Shanks before and after Moist-Heat Processing[a–c]

nutrient, mg/100 g	flank steak raw	flank steak heat processed[d]	foreshank raw	foreshank heat processed[d]
thiamin	$0.11^B \pm 0.01$	$0.14^A \pm 0.01$	$0.10^B \pm 0.01$	$0.14^A \pm 0.01$
riboflavin	$0.16^A \pm 0.01$	$0.19^A \pm 0.02$	$0.22^A \pm 0.01$	$0.21^A \pm 0.02$
niacin	$4.72^A \pm 0.26$	$4.60^A \pm 0.21$	$5.74^A \pm 0.34$	$5.89^A \pm 0.38$

[a] $N = 7$. [b] Mean values ± standard error of the mean. [c] Where superscripts (A, B) differ horizontally within a cut of beef, mean values differ significantly ($P < 0.05$) from each other. [d] Heat processed to an internal temperature of 99 °C.

Statistical Analysis. Analysis of variance procedures were used to evaluate raw and heat-processed values for beef flank steaks and cross-cut shanks. For beef shortloin steaks and pork loin chops, randomized complete block designs with 2 × 3 and 2 × 2 factorial arrangements for heat processing and doneness, respectively, were used. Carcasses were the replication. For lamb loin chops, the four treatments (2 × 2 factorial arrangement of heat-processing methods and doneness) were evaluated by three repetitions of a balanced incomplete block with three replicates and six incomplete blocks for repetition. Chops from a lamb carcass formed an incomplete block. Appropriate analysis of variance for repetition of a type V-B1B (Cochran and Cox, 1950) was used. Analysis for all experiments was as described by Snedecor and Cochran (1980). Computerized procedures for analysis of variance (ANOVA) and general linear models (GLM) were used (SAS, 1982).

RESULTS AND DISCUSSION

Figure 1 shows an example of typical chromatograms. The detection levels were 1.0 ng for niacin and 0.25 ng for riboflavin and thiamin. All standard curves were linear and independent of injection volume.

Significant differences ($P < 0.05$) among raw and heat-processed values for thiamin were revealed for all cuts of meat except lamb loin chops (Tables I–IV). With moist-heat methods for beef flank steaks and beef cross-cut shanks, levels of detectable thiamin increased with heat processing. When rotating hot air was used with beef shortloin steaks, thiamin levels for the three degrees of doneness did not differ significantly ($P > 0.05$) from raw values. However, when beef steaks were charbroiled to either 68 or 74 °C, thiamin levels were significantly reduced from the raw products. Thiamin losses for pork (Table III) were significantly less for charbroiling than for the other methods. A recent IFT status report of the effects of food processing on nutritive values (1986) stated that thiamin was unstable in the presence of heat. Given these findings, interrelationships among the conditions within the internal structure of the meat tissue and the thermal

Table II. Mean Thiamin, Riboflavin, and Niacin Contents of Beef Shortloin Steaks before and after Alternate Heat Processing[a–c]

nutrient, mg/100 g	raw	heat processed: rotating hot air 57 °C	68 °C	74 °C	charbroiling 57 °C	68 °C	74 °C
thiamin	$0.11^{A} \pm 0.02$	$0.09^{AB} \pm 0.01$	$0.10^{A} \pm 0.02$	$0.10^{A} \pm 0.01$	$0.09^{AB} \pm 0.01$	$0.06^{C} \pm 0.01$	$0.07^{BC} \pm 0.01$
riboflavin	$0.10^{A} \pm 0.02$	$0.08^{B} \pm 0.01$	$0.08^{B} \pm 0.01$	$0.09^{AB} \pm 0.01$	$0.07^{B} \pm 0.01$	$0.06^{B} \pm 0.01$	$0.07^{B} \pm 0.01$
niacin	$3.51^{A} \pm 0.32$	$3.62^{AB} \pm 0.42$	$4.05^{B} \pm 0.60$	$3.86^{AB} \pm 0.39$	$3.59^{AB} \pm 0.37$	$3.65^{AB} \pm 0.41$	$3.75^{AB} \pm 0.31$

[a] $N = 7$. [b] Mean values ± standard error of the mean. [c] Where superscripts (A, B, C) are similar horizontally, mean values do not differ significantly ($P > 0.05$) from each other.

Table III. Mean Thiamin, Riboflavin, and Niacin Contents of Pork Loin Chops before and after Alternate Heat Processing[a–c]

nutrient, mg/100 mg	raw	rotating hot air[d]: no breading	rotating hot air[d]: breading	charbroiling:[d] no breading	deep-fat frying: breading
thiamin	$0.81^{A} \pm 0.04$	$0.68^{B} \pm 0.04$	$0.68^{B} \pm 0.08$	$0.76^{AB} \pm 0.05$	$0.69^{B} \pm 0.04$
riboflavin	$0.14^{A} \pm 0.02$	$0.20^{A} \pm 0.04$	$0.18^{A} \pm 0.03$	$0.19^{A} \pm 0.03$	$0.19^{A} \pm 0.03$
niacin	$4.69^{A} \pm 0.81$	$3.88^{A} \pm 0.28$	$3.79^{A} \pm 0.17$	$3.87^{A} \pm 0.33$	$3.92^{A} \pm 0.24$

[a] $N = 7$. [b] Mean value ± standard error of the mean. [c] Where superscripts (A, B, C) differ horizontally, mean values differ significantly ($P < 0.05$) from each other. [d] Pork chops were heat processed to an internal temperature of 74 °C.

Table IV. Mean Thiamin, Riboflavin, and Niacin Contents of Lamb Loin Chops before and after Alternate Heat Processing[a,b]

nutrient, mg/100 g	raw	heat processed: rotating hot air 68 °C	74 °C	charbroiling 68 °C	74 °C
thiamin	0.17 ± 0.01	0.16 ± 0.62	0.16 ± 0.02	0.18 ± 0.02	0.17 ± 0.02
riboflavin	0.13 ± 0.01	0.16 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.01
niacin	4.56 ± 0.23	4.60 ± 0.62	4.76 ± 0.46	4.29 ± 0.44	4.57 ± 0.22

[a] $N = 18$ for raw and $N = 9$ for heat-processed values. [b] Mean values ± standard error of the mean.

Table V. Mean Percent Recovery of Thiamin, Niacin, and Riboflavin[a]

nutrient	meat: beef	pork	lamb
thiamin	98.1	95.2	90.4
niacin	97.5	89.8	85.1
riboflavin	96.3	95.2	97.7

[a] $N = 3$.

environmental conditions of the heating systems need further study for their effects upon thiamin content.

Riboflavin levels were found to be stable in all heat-processing methods and meat products studied, except for beef shortloin steaks (Table II). Compared to the raw values, all heat-processing methods except for rotating hot air (74 °C) significantly reduced ($P < 0.05$) riboflavin values for beef. However, among the six treatments studied, no differences ($P > 0.05$) were found. Thus, each one could be equally recommended.

With niacin, significant differences ($P < 0.05$) were only revealed among the raw and heat-processed samples for beef shortloin steaks (Table II), the highest occurring with a rotating hot-air oven and a medium degree of doneness. These findings confirmed the heat stability of niacin (IFT, 1986).

HPLC analysis of vitamins B_1 and B_2 and niacin of the various meat samples was very reproducible. This is evident in the low standard errors of the mean. The method of extraction provided high levels of recovery (Table V). HPLC analysis of these vitamins is considerably less time consuming than the standard AOAC methods. Other researchers, Fellman et al. (1982) and Skurray (1981), found comparable results using HPLC and AOAC methods. The reduced handling of the samples with HPLC analysis increases its accuracy and decreases sample analysis time.

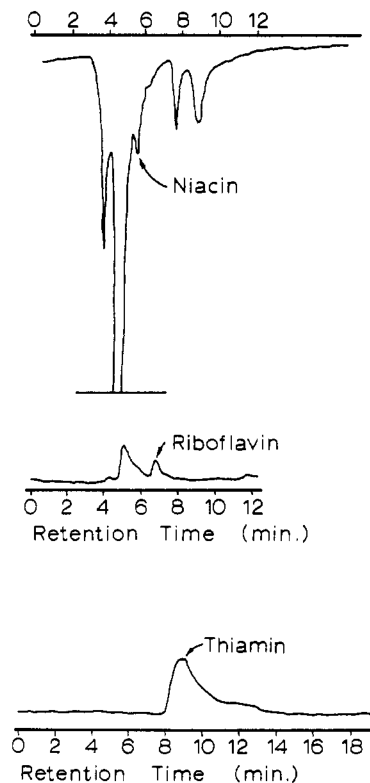

Figure 1. Simultaneous HPLC chromatogram of 0.25 ng of riboflavin and 1.0 ng of niacin and chromatogram of 0.25 ng of thiamin.

With the use of an automatic sampler and data processing system this process would be even more efficient.

Registry No. Riboflavin, 83-88-5; niacin, 59-67-6; thiamin, 59-43-8.

LITERATURE CITED

Ang, C. Y. W.; Moseley, F. A. "Determination of Thiamin and Riboflavin in Meat and Meat Products by High-Pressure Liquid Chromatography". *J. Agric. Food Chem.* **1980**, *28*, 483-486.

Association of Official Analytical Chemists. *Official Methods of Analysis*, 14 ed.; AOAC: Washington, DC, 1984.

Cochran, W. G.; Cox, G. M. *Experimental Design*, 2nd ed.; Wiley: New York, 1950.

Fellman, J. K.; Artz, W. E.; Tassinari, P. D.; Cole, C. L.; Augustin, J. "Simultaneous Determination of Thiamin and Riboflavin in Selected Foods by High-Performance Liquid Chromatography". *J. Food Sci.* **1982**, *47*, 2048-2050, 2067.

Ibrahim, N.; Unklesbay, N. F. "Comparison of Heat Processing by Rotating Hot Air and Deep Fat Frying: Effect on Selected Nutrient Content and Protein Quality of Catfish". *J. Can. Dietet. Assoc.* **1986**, *47*(1), 26-31.

Institute of Food Technologists. "Effects of Food Processing on Nutritive Values, A Scientific Status Summary". *Food Technol.* **1986**, *49*(12), 109-116.

Meat Buyers Guide; National Association of Meat Purveyors: Tucson, AZ, 1976.

SAS. *SAS User's Guide: Statistics*; SAS Institute: Cary, NC, 1982.

Skurray, G. R. "A Rapid Method for Selectively Determining Small Amounts of Niacin, Riboflavin, and Thiamin in Foods". *Food Chem.* **1981**, *7*, 77-80.

Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 7th ed.; Iowa State University: Ames, IA, 1980.

Standards for Pork Color, Firmness and Marbling; Iowa State University: Ames, IA, 1969; PM-452.

Toma, R. B.; Tabekhia, M. M. "High Performance Liquid Chromatographic Analysis of B-Vitamins in Rice and Rice Products". *J. Food Sci.* **1979**, *44*, 263-265, 268.

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Peanut Hull Flavonoids: Their Relationship with Peanut Maturity

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As peanuts mature, the color of the mesocarp layer of the peanut shell changes from yellow to orange to brown and finally to black. On the assumption that the flavonoid content of the shells could be associated with these color changes, a study of the 5,7-dihydroxychromone, eriodictyol, and luteolin contents of peanut shells was initiated. Total flavonoid content increased as the mesocarp color changed from yellow to black. Relative concentrations of the three flavonoids also changed with maturity. Eriodictyol was the predominant flavonoid in the shells from immature peanuts whereas luteolin was the predominant flavonoid in shells from mature peanuts.

Because of the indeterminate fruiting characteristics of peanuts, seeds of varying maturity are on the plant at harvest time. The most appropriate time to harvest is when the largest number of sound mature kernels is present. Mature peanuts produce better products, and a high percentage of mature kernels increases the value of the peanut crop. Over the years subjective and objective methods for determination of maturity have been developed and used by the peanut industry (Sanders et al., 1982). Probably the oldest method for estimating maturity is based on the color of the internal surface of the hull. As the peanut matures, this internal hull color changes from white to dark brown (Schenk, 1961). Pattee et al. (1974) used seed coat and internal pericarp characteristics, including color, to develop the Physiological Maturity Index. In 1979, Drexler and Williams devised a nondestructive method for predicting harvest date and determining maturity of fresh Florunner cv. pods. The method, which involved scraping off a small portion of the hull exocarp to expose the color of the mesocarp, is described in detail by Williams and Drexler (1981). It is referred to as the Pod Maturity Profile or the Hull-Scrape Method and segregates peanuts into six maturity classes based on mesocarp color. Flavonoids are compounds that could be responsible for or contribute to the color of the mesocarp.

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Using column chromatography Pendse et al. (1973) isolated three flavonoids—5,7-dihydroxychromone, eriodictyol, and luteolin—from peanut hulls. Daigle et al. (1983) used high-performance liquid chromatography (HPLC) to separate and identify flavonoids in peanut flour and testa. This paper extends the use of HPLC to the flavonoid content of peanut hulls and demonstrates how the amounts of these compounds vary in peanuts of increasing maturity.

MATERIALS AND METHODS

Peanut Hull Samples. In mid-May 1984, Florunner, cv. peanuts, were planted in a research plot in Tifton, GA. To obtain peanuts at increasing stages of maturity, plants were harvested at 79, 93, 107, 121, and 135 days after planting (DAP). Each time, plants were hand-dug, hand-picked, packed in dry ice, and shipped to the Southern Regional Research Center, USDA—ARS. Peanut hulls were removed by hand, washed, and then freeze-dried.

In 1986, Florunner cv. peanuts planted in early May were grown in three closely monitored research plots (6.1 m × 12.3 m), one at ambient temperature (mean 25.6 °C), one at 2-3 °C above ambient, and the third maintained at 2-3 °C below ambient. The mean temperatures were determined by averaging the 8:00 a.m. and 4:00 p.m. temperature from 28 DAP (start of treatment) until harvest at 148-155 DAP. At harvesting time, the fields were hand-dug and all pods of harvestable size were hand-picked and then washed. Approximately 10 kg of peanuts from random field and plot locations was used in each sample. By the method developed by Williams and Monroe (1986), the exocarp of the peanut hulls was scraped off by gentle abrasion in a slurry of small glass beads in water. Removal

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