EFFECT OF FROZEN STORAGE AND PACKAGING MATERIALS ON SELECTED NUTRIENTS IN GROUND PORK

by

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B.S., University of Madras, 1985

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1989

Approved by:

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INTRODUCTION

In industrialized countries such as the U.S meats provide significant amounts of nutrients. In the American diet meat supplies 26% of the intake of thiamin and 27% of that of iron (Jensen,1981). Pork is one of the major meat sources in the U.S diet. Pork is an excellent source of the B-vitamins, especially thiamin, and a good source of protein and inorganic elements(Marchello et al.,1985). The thiamin content of beef and veal is considerably lower than that in pork.

Most of the pork eaten today has spent some time in a freezer, either in a grocery store or in homes of consumers. This is especially true in rural areas where freezing of home-produced products is common; and fairly large amounts, especially of pork, are preserved this way. An average storage period of four months is estimated (Jul,1984).

Some frozen meat also is used after thawing for preparation of retail packaged frozen meats or frozen meat dishes. Similarly, some products which are sold fresh have been prepared from frozen materials and may be frozen again in home freezers. Thus, a certain amount of repeated freezing will take place and should be taken into account

when the total effect of freezing on nutrient intake is considered (Jul,1984).

The ability of meat to retain its natural water content is termed its water-holding capacity. During freezing, ice-crystallisation and ionic shifts result, causing decreases in water-holding capacity and increases in thaw drip. The drip that occurs during thawing may result in the loss of water-soluble vitamins and minerals.

In this study we were concerned with the losses of thiamin, iron, and zinc. The objectives of this study were to determine if the losses in these nutrients in ground pork - due to drip or other destructive processes - increased with increased length of storage, and if these losses were affected by the packaging material.

REVIEW OF LITERATURE

Changes in structure and water holding capacity

The advantages of temperatures below the freezing point in prolonging the useful storage life of meat, and in discouraging microbial and chemical changes, tend to be offset by the exudation of fluid ("drip") on thawing. Proteins, peptides, amino acids, lactic acid, purines, vitamins of the B-complex, and various salts are among the many constituents of drip fluid. The extent of drip is determined by factors of two kinds. In one category are factors which determine the extent to which the fluid once formed will drain from the meat. Among these are the size and shape of the pieces of meat (in particular the ratio of cut surface to volume), the orientation of the cut surface with respect to muscle fiber axis, the prevalence of large blood vessels,and the relative tendency for evaporation or condensation to occur during thawing of meat (Lawrie,1974).

Anon and Calvelo (1980) concluded that up to a freezing time of 17 minutes, crystal formation is mainly intracellular ; longer than that it is assumed to be mainly extracellular. Crystals grow by the adherence of water molecules to already formed crystals. Thus, these

crystals will grow only where free water molecules are present and may penetrate cell walls and, thus, puncture them and cause drip.

Offer and Trinick (1983) reported that the unifying hypothesis for this phenomenon is that gains or losses of water in meat are due simply to swelling or shrinking of the myofibrils caused by expansion or shrinking of the filament lattice. The main structural components of meat are the myofibrils which occupy about 70% of the volume of lean meat. Myofibrils contain about 20% protein, the remainder being water. Thus, most of the water in meat is present within the myofibrils, in the spaces between the thick and thin filaments. Moreover, the interfilament spacing is not constant but varies with pH, sarcomere length, ionic strength, osmotic pressure, and whether the muscle is relaxed or in rigor. Therefore, changes in the water holding capacity of meat possibly are caused by changes in the volume of the myofibrils resulting from changes in the interfilament spacing.

Thiamin

Thiamin, or vitamin B_1, a water soluble vitamin, is one of the least stable vitamins. Since it is water soluble, it could be leached out in the drip; and if the drip is not consumed, there might be a serious loss of

this vitamin.

Thiamin consists of a substituted pyrimidine linked to a substituted thiazole by a methylene group. Thiamin degradation is considered to be a nucleophilic displacement reaction on the methylene group in which OH- is the displacing base (Archer and Tannenbaum,1979).

The stability of thiamin also is determined by pH, temperature, ionic strength, buffer type, and other reacting species. Thiamin is inactivated by nitrite, possibly via reaction with the amino group on the pyrimidine ring (Tannenbaum, 1980).

Thiamin plays a key role as a coenzyme in the intermediary metabolism of a-keto acids and carbohydrates. Thiamin can exist in meat in a number of forms, including free thiamin, the pyrophosphoric acid ester (cocarboxylase), and bound to the respective apoenzyme (Tannenbaum,1980) .

Thiamin studies

Moss et al.(1983) reported that the cuts from the front section of the pork carcass (arm picnic, shoulder blade steaks, and blade loin roast) generally were lower in B-vitamins and higher in inorganic nutients than cuts from the rear of the carcass (sirloin roast, ham butt, and ham shank). They also reported that grade significantly

affected the fat and B_1 contents of the lean. Vitamin B_1 ranged from 0.85-1.0mg/100gm across all grades (mean =0.95, C.V=22%).

Egi et al.(1986) reported an extremely high content of thiamin triphosphate in pig skeletal muscle; 62.2% of the total thiamin content was detected as the triphosphate in these tissues.

Nestorov and Kozhukhorova (1975) used Bulgarian white pigs to study changes in thiamin and retinol concentration in liver, Longissimus dorsi, and Gluteus medius muscle tissue during freezing and freezer storage. Samples were analysed immediately postmortem and after frozen storage for < 3 months at -20 C. Mean thiamin concentration immediately postmortem and after 3 months storage was (mg%) 0.54 and 0.49, 0.76 and 0.70 , and 0.84 and 0.78, respectively.

Nestorov and Khozukhorova (1975) also studied livers of 10 Bulgarian brown calves (12-18 months of age at slaughter) for changes in the thiamin content during storage at -4 C for two days; at 0 C for two or four days; or at -5 or -15 C for two, 20, or 40 days. The fresh liver contained 0.36mg thiamin/100gm. Loss of thiamin tended to increase with increasing storage temperature; the highest loss (14.87%) was recorded for the sample

stored at 0 C for four days. Thiamin content of the sample stored at -5 C increased slightly between the 20th and 40th days of storage; the authors suggested that this was due to thiamin-forming micro-organisms.

Rogowski (1987) reported that thiamin content of frozen roast pork decreased from 0.58 to 0.42 mg/100gm after 3-12 months storage, although the losses were greater during heat steriliza tion than during freezing.

Hilker (1976) studied meat from tuna, beef, and pork for thiamin-modifying activity and detected a thermostable factor. He suggested that this thermostable factor was hemin , which reacts with the thiamin to form a derivative which gives a negative reaction for thiamin in the thiochrome test. Formation of the hemin /thiamin derivative is dependent on pH, temperature, light, and oxygen. He also suggested that the hemin /thiamin reaction product was active as thiamin, or that thiamin was regenerated during digestion.

Early Studies

Cook et al. (1949) studied the effects of frozen storage on thiamin in various turkey tissues stored 3 to 9 months at -23 C and noted no significant loss of thiamin, except in one lot of breast and leg muscle. Meyer et al. (1963) found that thiamin increased significantly in

unripened but remained unchanged in ripened beef stored for three years at 0 F. Bowers et al. (1979) noted that unfrozen pork hams contained more thiamin than frozen-stored pork hams.

Iron and Zinc

Iron and zinc are known to have biological functions in man and are considered essential by the Food and Nutrition Board of the National Research Council. Recommended dietary allowances have been established for zinc and iron by the National Research Council (NRC,1974). Consumer groups and regulatory agencies are demanding an accurate description of the nutrient content of foods.

Moss et al.(1983) attempted to find general trends within nutrient classes of seperable lean of pork. Their data did not verify the value for iron reported in the standard reference tables published by USDA (Watt and Merrill,1963). Iron values for raw pork in Agriculture Handbook No. 8 (Item #1684 value = 2.9mg/100g) were calculated as 15 mg of iron for each 100 gm of protein and were not based on actual analysis. The iron values obtained from the study of Moss et al.(1983) (0.92mg/100gm) verified the value 0.9mg/100g of Paul and Southgate (1978). Moss et al.,(1983) also concluded that although the levels of zinc, copper, and manganese were

not significantly affected by grade or region, the iron values appeared to be slightly influenced by region.

Marchello et al. (1984) showed that that individual beef muscles varied in the amount of specific minerals . They indicated that shoulder muscles had a greater quantity of iron and zinc present in their muscles than did leg or loin. Schricker et al.(1982) determined the total iron in Biceps femorus, Gluteus medius, Longissimus dorsi and Triceps brachii and found these contained 9.9, 9.5, 7.6, and 13.1 ug/g, respectively, on a wet weight basis.

Murray et al. (1981) studied the relationship between mineral content and tenderness of meat. Calkins et al. (1981) had suggested that tenderness was related to the oxidative capacity of the muscle. Since several minerals vary with muscle color and function (Cassens and Cooper,1971), Murray et al.(1981) proposed that it was not unreasonable to suggest that muscle mineral content may be related to tenderness. McClain and Mullins (1969) noted that more tender bovine Longissimus dorsi was higher in water solubles, total pigment, and total iron content. Vavak et al. (1976) reported that variation in the levels of the minerals iron, cobalt, zinc, and copper accounted for almost all of the variation in the tenderness of the

cooked bovine Semimembranosus muscle and that the mineral levels of fresh raw meat could successfully predict cooked meat tenderness. Murray et al.(1981) concluded that although the concentration of minerals was not useful for the prediction of shear values, Ca and Fe alone accounted for 10-20% of the variation in shear values.

Most of the soluble iron present in meat is in the form of hemoglobin or myoglobin. Pork is considered to have 40% heme iron. Hemoglobin and myoglobin are sarcoplasmic proteins, which are readily extractable by water (Lawrie,1974). This may result in the appearance of these compounds in the drip and, hence, loss of heme iron. The importance of protein and heme iron in iron absorbtion cannot be overemphasized.

Meat is an unique source of iron due to its iron-absorption enhancing factor, possibly digested protein, and the presence of heme (Lee and Shimoaka,1984). Heme and non-heme iron are absorbed by different mechanisms (Halberg,1981). Also, the bioavailability of heme iron is not affected by dietary factors that enhance or inhibit the availability of the non-heme iron. The absorbtion of non-heme iron is subject to enhancement by ascorbic acid or meat extract and to inhibition by tea and chelating

agents; absorbtion of heme iron is not influenced by these factors.

Martinez-Torres et al.(1981) reported proteins from animal tissues such as those present in beef, lamb, chicken, fish, pork and liver increase the iron absorption. Several authors have suggested mechanisms by which proteins from animal tissue enhance iron absorbtion. Martinez-Torres et al.(1981) concluded that in the case of heme iron, the globin degradation products from hemoglobin may enhance iron absorption by preventing polymerization of heme. Cysteine also was reported to increase the iron absorption by preventing iron polymerization, an effect applicable to non-heme as well as heme iron.

Studies employing single food items tagged biosynthetically with radio iron have shown that absorption from vegetable or cereal foods is usually less than 5% as compared with 15-20% absorption from animal sources such as beef, liver, and fish (Cook and Monsen,1979). Lee and Shimaoka (1984) studied the effect of both nitrite and erythorbate on the chemical forms of iron in meat, and the changes which may occur in cured meats during frozen storage, refrigeration, and cooking.They found that sausages with erythorbate (only) contained higher ionic and ferrous iron, and the

sausages containing both nitrite and erythorbate had higher ferrous and less ferric iron levels (ferrous iron is more available than ferric iron). They concluded that erythorbate may form soluble chelates with iron as well as reduce the iron to a more available ferrous form.During 160 days of frozen storage, no changes were detected in the forms of iron, residual nitrite, erythorbate, or malonaldehyde levels.

Zenoble and Bowers (1977) reported that the iron content of cooked breast and thigh muscles of turkey was significantly lower than in raw samples. They also reported that when these muscles were placed in an ice slush before processing, inorganic constituents leached into the ice water and an appreciable portion of these constituents were minerals.

Cassens et al. (1963) reported that zinc content in various porcine muscles varied with color and myoglobin concentration and that dark muscles had greater concentrations of zinc than did light. They also found an increased concentration of zinc in muscles that were used most. Rasanen et al. (1972) found game birds to have a higher concentration of iron in their muscles than did chickens, attributed to a higher percentage of dark muscles in game birds than in domestic fowl, but the

muscles of the two species had similar amounts of zinc and copper.

Zenoble and Bowers (1977) measured zinc in raw breast of turkey to be 0.9mg/100gm, which was lower than the 1.8mg/100gm reported by Gormican (1970). Their values for iron were 0.84mg/100gm for raw breast and 1.6 mg/100gm for raw thigh.

Schricker et al . (1982b) determined the zinc concentration in four muscles (Biceps femorus, Gluteus medius, Longissimus dorsi, and Triceps brachii) each from beef, pork, and lamb and observed that the concentration of zinc in the four muscles varied among species. Within each species only the Triceps brachii varied from the other muscles studied. Jorgensen and Wegger (1976) found the zinc content in skeletal muscles of pigs differed with the type of muscle. Zinc concentrations were highest in typical red muscles and lowest in white muscles. Cassens et al. (1963) reported that zinc deficiency in pigs did not decrease the level of zinc in the muscles. Schricker et al. (1982b) found zinc concentration for pork Biceps femorus, Gluteus medius, Longissimus dorsi, and Triceps brachii were 16.2, 15.5, 14.9 and 26.3 ug/g, respectively,on a wet-weight basis.

Doornenbal and Murray (1981) studied the effects of age,

breed, and sex on mineral contents of certain muscles of cattle. They found that sex and breed did not explain much of the variation although Fe, Ca , Zn, Mg, and Na were affected by age. The diaphragm muscle had higher levels of Fe and Zn than did the other two muscles studied, Longissimus dorsi and Semimembranosus. These differences in mineral content between muscles are similar to those reported by Kuhne(1976) and Kotula and Lusby(1975). Those authors suggested that the different demands made on muscles was the reason for a variable distribution of certain elements. Kuhne (1976) found this to be especially true for zinc, which was found in higher concentration in those muscles involved in movement.

Hazell (1982) purchased pork (loin chops) in the frozen condition and determined the iron and zinc content of the samples after they had thawed. No information is available, however, as to how long the chops had been stored in the frozen state. Hazell (1982) found an average iron value of 6.9ug/g (values ranged from 5.5-8.3ug/g).

Packaging

The kind of packaging is also an important factor for the quality of frozen foods. New methods of packaging are being investigted by the industry for protecting pork cuts. Although not a good storage film, PVC film has been

investigated as a means for extending the storage life of beef (Smith and Carpenter,1973), (Berry et al. 1971), and other authors (Rea et al. 1972; Smith and Carpenter,1973) concluded that PVC film was effective in reducing shrinkage and in maintaing the desirability of beef for periods approximating 7 days. Vacuum packaging has been shown to extend the shelf life of fresh pork by reducing shrinkage, surface discoloration,and bacteria count (Smith et al. 1974). The effects of packaging on the nutritive value of meat has not been widely studied, and very little information on this subject could be found.

When choosing a type of packaging material for storing meat the following considerations are important: permeability to water vapor and gases such as carbon-dioxide, nitrogen and oxygen, and the density and thickness of the film. The quality of meat depends on moisture content, growth of bacteria and molds, enzymic hydrolysis , fat oxidation and browning. Different foods require varying water vapor and gas permeability, and the effect of temperature on this permeability varies (Kropf,1980).

Rosset (1978) in his study on controlled atmosphere storage of meat reported that there were three packaging methods which are classified according to techniques

used, desired storage life, and destination: (1) packaging in a water vapor-tight but gas permeable (oxygen, carbon-dioxide) film; internal atmosphere similar to air; no change in bright red surface color(oxymyoglobin), bacterial growth slowed by refrigeration; limited shelf life (a few days); direct sale to customers; (2) packaging in water vapor-and-gas tight film; normal atmosphere with reduced volume and pressure (vacuum packaging); internal changes during storage viz. reduced oxygen, increased carbon dioxide; darkening of meat surface color; arrested microbial growth, except for micro-aerophilic and acidophilic (especially lactic acid) bacteria; shelf life up to eight weeks; color regained after opening (oxygenation); mostly used in the wholesale trade; (3) packaging in water vapor-and-gas tight film; internal atmosphere with high oxygen and high carbon-dioxide (gas injection); no change in bright red color; arrested growth of pathogenic and spoilage flora; shelf life 12-15 days; sold directly to consumers.

The gas permeability of PVC is relatively high which offers good protection against rancidity. The water vapor permeability is high and is useful in preventing condensation on the inside of the film. Thin plasticed PVC film is widely used in supermarkets for the shrink

wrapping of trays containing cuts of fresh meat. It must be tough, able to withstand low temperatures, be shrinkable, and have good clarity and gloss (Schwartz and Goodman,1982).

The name "Saran" represents a series of vinylidene chloride copolymers with vinyl chloride or acrylonitrile. Saran film, with its moisture proofing and vapor barrier properties, plus its high degree of transparency, has become a very popular packaging material. The film maintains serviceability to sub-zero temperatures (Schwartz and Goodman,1982).

Bogh-Sorensen (1982) studied the effects of packaging on quality in pork tenderloins and hamburgers with added soy proteins. The two packaging treatments were polyethylene pouches and vacuum packaging. The samples were stored at -10 , -18 or -24 C and the taste, texture, and juiciness of tenderloins and taste, texture, and overall quality of hamburgers were evaluated at monthly intervals. Although the difference in the quality of tenderloins was not greatly affected, for hamburgers vacuum packaging improved quality retention during frozen storage.

Methods of Analysis

Chemical Assay for Thiamin

The most sensitive chemical technique for the measurement of thiamine involves its extraction from the sample after dilute acid hydrolysis and enzymatic digestion. The pH of the acid extract ensures that the thiamin is very stable, even when heated. The enzyme solution used contains phosphatase which hydrolyzes any phosphate esters of thiamin present, converting bound thiamin to its free form. The next step involves the oxidation of the extracted thiamin to thiochrome followed by fluorimetric quantitation. Oxidation can be achieved with alkaline potassium hexacyanoferrate (III), cyanogen bromide, or mercury II chloride. The oxidation of thiamin using the above reagents is not specific; non-thiamin compounds that are fluorescent are formed and will give an erroneous higher reading for thiamin (Kennedy and McCleary,1981).

The enzyme Thiaminase I was used by Kennedy and McCleary (1981). Thiaminase I is very specific for thiamin and produces a thiamin-free blank. It is used to detect non-thiamin compounds that give off a flourescence. The activity of Thiaminase I is enhanced by the addition of the co-substrate base, pyridine. This modified procedure

allows the direct measurement of thiamin in crude extracts and, thus, is much simpler and more rapid than other procedures that involve ion-exchange purification of the thiamin.

Hofmann (1983) developed a modified procedure to determine the thiamin content in meat samples by blocking the SH- groups using N-ethylmaleimide and then releasing the bound thiamin by hydrolysis with hydrochloric acid and clarase treatment. An amberlite ion-exchange column was used to separate the thiamin, after which it was eluted using hydrochloric acid. This was followed by a ring opening reaction at pH 11 and reaction with Ellmans reagent at pH 8, after which the extinction value was determined at 412 nm. Results agreed well with those by the conventional thiochrome method (mean difference ranging from -5.8 to + 4.6%). This method is less sensitive than the thiochrome method although it is considerably simpler.

To avoid the use of resin, various chemical techniques have been devised to destroy thiamin to produce " thiamin -free" blanks and, thus,to obtain some estimate of the level of non-thiochrome flourescence in the sample extract. However, these chemical techniques are non-specific (Kennedy and McCleary,1981).

Microbiological Assay for thiamin

Although the thiochrome method is the most sensitive chemical method, it is less sensitive than the microbiological method which requires less equipment and material for assay. However, microbiological methods can suffer from poor reproducibility with slight variation in procedure or if non-chemically defined media are used (Lamden, 1972).

Microbiological methods for the analysis of vitamins are based on the observation that certain microorganisms require specific vitamins for growth. When the samples containing the vitamin are added to a nutrient medium and innoculated with a specific bacteria, growth over a specified incubation time will be directly proportional to the amount of vitamin present. Growth is measured photometrically, and the sample solution can be compared accurately (Voight, 1978).

Keller (1985) measured thiamin content of corn arepas by both the chemical and microbiobiological methods and found these values to be significantly different. The microbiological method showed an increased amount of thiamin in the Mexican and Kansan arepas but showed a decrease in thiamin amount for Venezuelan arepas.

Determination of thiamin by High Performance Liquid Chromatography (HPLC)

Determination of thiamin by the HPLC method involves the extraction of the vitamin from the sample after acid hydrolyses. Treatment with papain removes interference from proteins, and diastase liberates the vitamin bound in the phosphate form. The clear filtrate is then applied either manually or by means of a fully automated system to a HPLC column. Thiamin is eluted by a mixture of disodium hydrogen phosphate dihydrate, potassium dihydrogen phosphate, and ethanol. Thiamin is then oxidised by alkaline potassium ferricyanide to thiochrome and determined fluorimetrically (Osborne and Voogt,1978).

Measurement of Iron and Zinc

The technique most favored for the determination of trace elements in foods and beverages is atomic absorption spectrometry, the method reported in over 70% of the papers reviewed. There is,however, a growing interest in ICP-AES (Inductively Coupled Plasmas-Atomic Emission Spectrometry), although this technique only accounted for some 15-20% of the literature (Brown et al, 1987).

The 'ash' of meat products may be defined as the inorganic residue remaining after the combustion of the sample in the presence of air at atmospheric pressure. It

is composed of the mineral constituents of the sample in the form of oxides, sulphates, and chlorides, the proportions of which depend on the initial composition of the sample and the ashing conditions (Perez and Andujar,1981). Ashing temperature plays an essential role in this determination. Temperatures above 600 C cause volatilization losses of various minerals (Joslyn, 1970).

The two most common sample preparation procedures used to solubilize biological and food samples are wet ashing and dry ashing. Protasowicki (1986) compared dry ashing to wet ashing in fish tissue and found that dry-ashing procedures which involved mineralisation with nitric acid, produced acceptable accuracy and recovery of standard additions. Wet ashing involves digestion with concentrated nitric acid and 70% perchloric acid.

Various authors have suggested other methods of preparing the samples. Ahlgren et al. (1987) used a carbon-dioxide laser to decompose of organic materials. This method is fast in comparison to wet-oxidation. Contents of iron, zinc,and other minerals were determined in certified reference materials following laser ashing and wet ashing. Values determined by laser-ashing were in good relation with certified values (and with those determined after wet ashing).

After the sample is digested and brought up to volume with distilled deionized water, it is aspirated into the flame of an atomic absorption spectrophotometer. This is the atomization stage in which free analyte atoms are generated within a confined zone coincident with the spectrometer's optical path. These free atoms absorb light and the wavelength at which light is absorbed is specific for a particular chemical element. The amount of light absorbed is proportional to the concentration of absorbing atoms. A calibration graph is obtained by measuring responses of the analytical blank and those of the standards and plotting the responses against known concentrations. The analyte concentration of the sample is found by interpolation on the curve (Bennett and Rothery, 1983).

MATERIALS AND METHODS

Materials

All combinations of 5 packaging treatments and 2 freezer storage times were used. The packaging materials used were a combination of oxygen permeable, oxygen - impermeable, and light-impermeable materials. They were vacuum packaging, the control (Cryovac® bags, 7/12"), Saran Wrap® (Dow chemicals), PVC, Reynolds Wrap Heavy Duty Aluminum Foil® (Reynolds Metals Company), and Saran Wrap®

overwrapped with aluminum foil (Table 1).

Pork (boneless picnic) was obtained from a commercial source (Flint Hills Foods, Alma, Kansas). The samples were prepared by grinding the pork using a Hobart grinder (model#4732), first through a 3/16" and then through a 1/8" plate. A Hobart fat tester (model#F101), was used to estimate the fat content to approximately 20%.

A portion of the ground pork was analyzed for thiamin, iron,and zinc; and the rest was weighed into 0.5 lb samples and packaged with the various packaging materials. The samples were held at -17 C for 13 or 26 weeks in an upright Hotpoint freezer. The four replications prepared with an interval of one week between them allowed for ease of analysis. After the designated storage period of 13 or 26, weeks the samples were taken out of frozen storage, thawed in the packaging at ambient temperature (approximately 22 C), and analyzed for thiamin, iron, and zinc contents using standard AOAC (1984) methods. Since some packaging materials did not produce sufficient drip, the drip was not analysed. The drip was not reconstituted into the sample.

Methods

Thiamin by the Thiochrome method

Each sample was prepared by extracting 10gm of ground tissue with 0.1N hydrochloric acid and an enzyme solution (diastase and sodium acetate). The thiamin in the sample was converted to thiochrome by alkaline potassium ferricyanide. The fluorescence of the thiochrome was determined using a photofluorometer (Sequoia-Turner, model # 450). The photofluorometer was checked between readings with a working quinine solution. All the solutions were at room temperature. Galvanometer deflections for the standard, standard blank, 5ml assay solution, and the assay solution blank were read and mcg of thiamin per gm of sample were calculated. Duplicate samples were simultaneously carried through the assay procedure. Prelimnary determinations gave low blanks and hence, the decalso procedure was not necessary and was omitted from the procedure. The thiamin content was calculated on a wet-weight basis.

Thiamin content of the sample in mcg/g=

U - UB/S - SB x 1/5 x 100/Wt of Sample

Deflections of unknown = U

Deflections of unknown blank = UB

Deflections of the standard = S

Deflections of the standard blank = SB

The factor 1/5 converts the reading to mcg/ml instead of mcg/5ml.

Iron and Zinc by Atomic Absorption Spectrophotometry

The sample was dried for eight hours at 100 C and then dry ashed overnight at 550 C in order to remove the organic material. The residue was dissolved in dilute acid and heated for at least 20 minutes. This solution was made up to 50ml with distilled deionized water and stored in sample bottles before being sprayed into the flame of an atomic absorption apparatus.

Determinations were carried out using a Video 11 Instrumentation laboratory AA/AE spectrophotometer with an oxidising air-acetylene flame using a wavelength of 248.3nm, a bandwidth of 0.3nm, and a lamp current of 8mA for iron and a wavelength of 213.9nm, a bandwidth of 1.0nm, and a lamp current of 5.0mA for zinc.

The instrument was calibrated with known standards for each series of determinations on each element. The standard solution was diluted using hydrochloric acid to concentrations that fell within the working range.

The calibration solutions and the reagent blank solution were measured. While the samples were being run, the calibration values were checked periodically to see if

they remained constant. A calibration curve was obtained by plotting the absorption values against the respective metal concentrations in ug/ml. The metal concentration (ug/ml) that corresponded to the absorption value of the sample and the blank was read from the graph.

Methodological precision and accuracy were evaluated by concurrent analysis of National Bureau of Standards Bovine Liver Standard.

Calculations

Metal content (mg/100g)	=[(a-b) x V]/10W
or (mg/1000g)	=[(a-b) x V]/W
Weight (g) of samples	= W
Volume (ml) of extract	= V
Concentration (ug/ml) of sample solution	= a
Concentration (ug/ml) of blank solution	= b

Statistical Aanalysis

Data for thiamin, iron and zinc content in fresh pork and pork samples that had been frozen stored in five different packaging materials over two time periods (13 and 26 weeks) were analysed by analysis of variance (ANOVA) for a randomized complete block design (RCBD) (Snedecor and Cochran,1980). LSD was used to determine significant differences between means. SAS program was

used to run the analysis of variance and to compute the LSD's.

RESULTS AND DISCUSSION

Thiamin

The thiamin content of fresh, ground pork varied from 0.58mg/100g to 0.78mg/100g among the replications (Appendix A,p 35) Since thiamin content is dependent on % fat (the four replications varied slightly in % fat), it is not surprising that there was a slight variation in thiamin content. Moss et al.(1983) reported a mean value for thiamin of 0.81mg/100g (fat=9 g/100g seperable lean), which is higher than the values observed in the present study.

The mean thiamin values before and after storage for 13 or 26 weeks revealed no significant differences (Tables 1 and 4). There were no differences in thiamin values of pork packaged in different wrapping materials and no significant interaction between packaging and time. The F-value for differences in mean thiamin values between the two time periods, for the various packaging materials also revealed non-significant differences. These results verify those of Lee et al. (1954), who found the thiamin retention for frozen stored pork Longissimus dorsi after six months storage at -18 C to be 101% Meyer et al.(1963) found no loss as thiamin retention in ground beef

Longissimus dorsi and Semimembranosus, after three years storage at -18 C, was 134%. However, Lehrer et al. (1951) reported a thiamin retention of 68% for frozen stored pork Longissimus dorsi stored for six months at -18 C. Although the F-values were not significant, there were small differences in mean thiamin values among the various packaging materials over the two time periods (Appendix A, p.54). Vacuum packaging retained more thiamin than any other packaging material after 13 weeks. PVC tended to show the lowest retention (Fig.1). Percent retention after 13 weeks, averaged across all packaging materials, was 98% (Appendix A, p.54). After 26 weeks Reynolds Wrap® Heavy Duty Aluminum Foil retained more thiamin than any other wrapping material; vacuum packaging tended to show the least (Fig.2). Percent retention, averaged across all packaging materials, was 96% (Appendix A, p.54)

Iron and Zinc

The iron content of fresh, ground pork ranged from 9.2 ug/g to 11.9ug/g among the replications (Appendix B,p.55), compared to 9.0ug/g (Paul and Southgate ,1978), and 9.2 ug/g (Moss et al, 1983). According to Moss et al. (1983), the cuts from the front section of the carcass were higher in inorganic nutrients, and loin chops were

the lowest. Shricker et al. (1982) determined the total iron found in Biceps femorus, Gluteus medius, Longissimus dors i, and Triceps brachii and found that these contained 9.9, 9.5 , 7.6, and 13.1ug/g, respectively, on a wet-weight basis. Data in the present study compare favorably with those of Shricker et al. (1982).

The zinc values varied from 16.7 to 25.0 ug/g (Appendix C, p.56). Large differences exist in zinc content of various porcine muscles. Shricker et al.(1982) found the concentration of zinc in Biceps femoris, Gluteus medius, Longissimus dorsi and Triceps brachii to be 16.2, 15.5, 14.9 and 26.3ug/g , respectively, on a wet-weight basis. Other authors have reported their data on a fat-free dry basis; so direct comparison with such data may not be appropriate.

The mean values for iron before and after frozen storage revealed significant differences ($p<0.001$) (Table 2). However, there were no differences in effects of the packaging materials or their interaction between packaging and time. There were significant differences between iron contents of fresh ground pork and pork that had been stored for 13 or 26 weeks (Table 4). The mean values for iron averaged from all the packaging materials for 13 and 26 weeks are 5.84 and 6.11ug/g, respectively (Table 4).

Hazell (1982) found an average value of 6.9ug/g iron in frozen pork chops.

Although the effect of packaging materials was not significant, there were minor differences. Samples wrapped in Reynolds Wrap® Heavy Duty Aluminum Foil and Saran Wrap® overwrapped with aluminum foil gave slightly better retention, after 13 weeks, than the other packages. Saran Wrap® tended to show the lowest retention. (Fig. 1)

Minor differences were also observed after 26 weeks. Saran Wrap® and Saran Wrap® overwrapped with aluminum foil showed slightly higher retention than the other wrapping materials. PVC tended to show the lowest retention. (Fig. 2)

Table 3 shows that for zinc there were no differences in packaging and that there was no interaction between packaging and time. There were significant differences between zinc contents of fresh ground pork and pork that had been stored for 13 and 26 weeks. The mean value of zinc averaged across the packaging materials for 13 and 26 weeks are 17.41 and 17.09ug/g, respectively (Table 4). Hazell (1982) found an average value of 14.8ug/g with a range of 13.0-16.5ug/g.

Fig. 3 shows the minor differences between the packaging materials in zinc retention after 13 weeks.

Reynolds Wrap® Heavy Duty Aluminum Foil® gave better retention than any other packaging material; Saran Wrap® showed the lowest. After 26 weeks samples packaged by vacuum packaging retained slightly more zinc than samples packaged in other wrapping materials, and PVC showed the lowest. (Fig. 4).

Personal Observations

During the thawing of samples at ambient temperature, the author noticed that samples that were packaged by vacuum packaging thawed faster and had slightly increased drip. This may be attributed to a larger proportion of the surface of the sample being exposed to the thawing conditions, since the vacuum packaged samples were packaged in thinner layers and spread over a larger area as compared to the rest of the samples.

Recommendations

Little is known as to the forms of iron and zinc lost in the drip. Further research would help to determine the percentage of heme and non-heme iron that was present in the initial product and percentage loss of these forms of iron after frozen storage. This will help in understanding the importance of adding drip when cooking the meat.

SUMMARY

Fresh ground pork was first analysed for thiamin, iron, and zinc content. The pork was then packaged by vacuum packaging (control), Saran Wrap®, Reynolds Wrap® Heavy Duty Aluminum Foil®, PVC, or Saran Wrap® overwrapped with heavy duty aluminum foil. The samples were stored at 17 C for 13 or 26 weeks after which they were thawed and again analysed for thiamin, iron and zinc content to determine losses due to frozen storage. The drip that resulted during the thawing of the samples was not reincorporated into the sample.

The data were analysed using a randomized complete block design (RCBD). LSD was used to determine significant differences in means.

The thiamin content did not change significantly over time or with the packaging materials. There was no interaction effect of time and packaging material. The content of iron decreased significantly between the initial and 13 weeks, but did not show an appreciable loss between 13 and 26 weeks. Iron content was not significantly affected by the packaging material. Zinc content decreased significantly over the first 13 weeks but did not change between 13 and 26 weeks. The packaging

materials had no significant effect on zinc content. Since the drip was not reincorporated, it is likely that these nutrients were lost in the drip.

CONCLUSIONS

Under the conditions of this study,

1. The packaging materials did not effect the thiamin, iron and zinc content of frozen stored ground pork.
2. Pork samples freezer stored for 13 or 26 weeks experienced a loss in iron and zinc (but not in thiamin) ($P < 0.05$), likely due to drip loss, since drip was not reincorporated with the meat.
3. Frozen storage for 26 weeks compared to 13 weeks did not produce any significant changes in any of the parameters measured.

Table 1. Packaging characteristics

Type of packaging	Oxygen barrier	Light blocker
Vacuum packaging	yes	no
Saran Wrap®	yes	no
Aluminum Foil	yes	yes
PVC film	no	no
Saran Wrap® overwrapped with aluminum foil	yes	yes

Table 2.F-values for variables among sources for thiamin

Sources of Variation	F-value[a]
Packaging	0.49
Time[b]	1.44
Pack*Time	1.16
Initial vs 13wks	1.36
Initial vs 26wks	0.22
Pack vs 13wks	0.59
Pack vs 26wks	1.07

[a] P>0.05 for all

[b] 13 vs 26wks

Table 3.F-values for variables among sources for iron

Sources of Variation	F-value
Packaging	1.27 NS
Time	0.25 NS
Pack * Time	0.87 NS
Initial vs 13wks	33.98***
Initial vs 26wks	30.73***
Pack vs 13wks	0.28 NS
Pack vs 26wks	1.86 NS

*** P<0.001, NS P>0.05

Table 4.F-values for variables among sources for zinc

Sources of Variation	F-value
Packaging	0.31 NS
Time	0.25 NS
Pack*Time	0.19 NS
Initial vs 13wks	7.59**
Initial vs 26wks	9.28**
Pack vs 13wks	0.20 NS
Pack vs 26wks	0.30 NS

** P<0.01, NS P>0.05

Table 5. Mean[1] values for thiamin, iron and zinc

Time	Thiamin	Iron	Zinc
Initial	0.65[a]	11.39[a]	20.45[a]
13weeks	0.62[a]	5.84[b]	17.41[b]
26weeks	0.64[a]	6.11[b]	17.09[b]
LSD for comparing initial to 13 or 26 weeks	0 .048	1.942	2.254

[1] Mean of four replications, averaged across all wrapping materials over time

[ab] Means with different letters are different ($p<0.05$)

Figure 1. Thiamin content[1] after 13 weeks for the various packaging materials.

[1] Mean of four replications

VP = Vacuum packaging

SW = Saran Wrap®

AF = Reynolds Wrap® Heavy Duty Aluminum Foil

PVC= Polyvinyl chloride film

SWA= Saran Wrap® overwrapped with aluminum foil

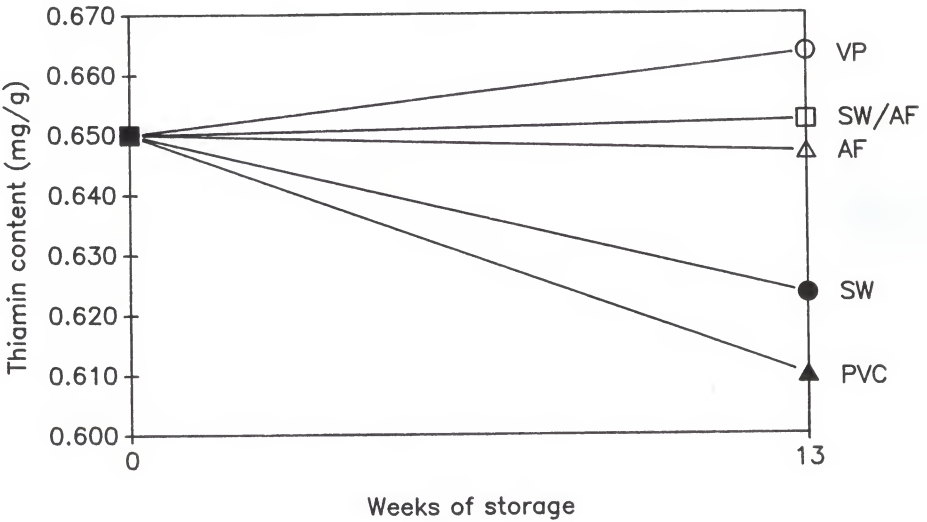

0.670
0.660
0.650
0.640
0.630
0.620
0.610
0.600
Thiamin content (mg/g)
0
13
Weeks of storage
VP
SW/AF
AF
SW
PVC

Figure 2. Thiamin content[1] after 26 weeks for the various packaging materials.

[1] Mean of four replications

VP = Vacuum packaging

SW = Saran Wrap®

AF = Reynolds Wrap® Heavy Duty Aluminum Foil

PVC= Polyvinyl chloride film

SWA= Saran Wrap® overwrapped with aluminum foil

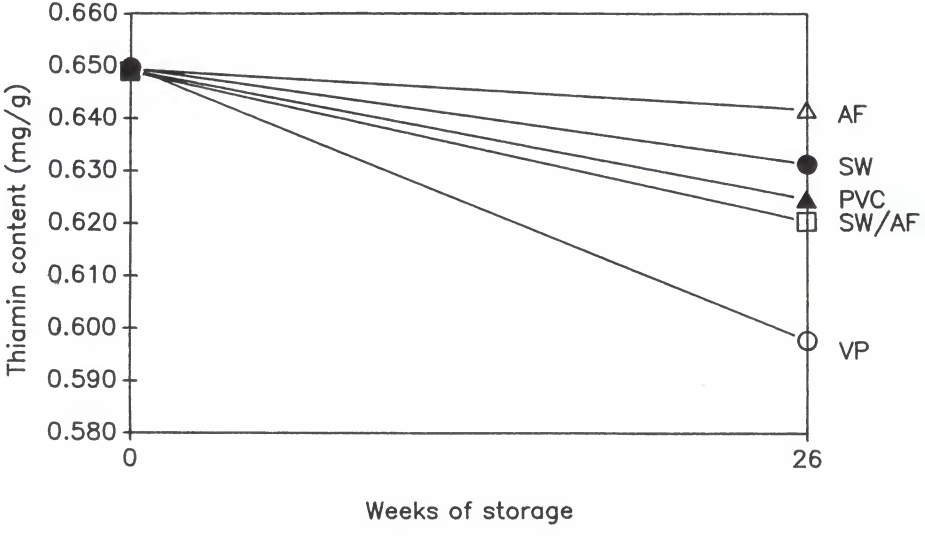

0.660
0.650
0.640
0.630
0.620
0.610
0.600
0.590
0.580
Thiamin content (mg/g)
0
26
Weeks of storage
AF
SW
PVC
SW/AF
VP

Figure 3. Iron content[1] after 13 weeks for the various packaging materials.

[1] Mean of four replications

VP = Vacuum packaging

SW = Saran Wrap®

AF = Reynolds Wrap® Heavy Duty Aluminum Foil

PVC= Polyvinyl chloride film

SWA= Saran Wrap® overwrapped with aluminum foil

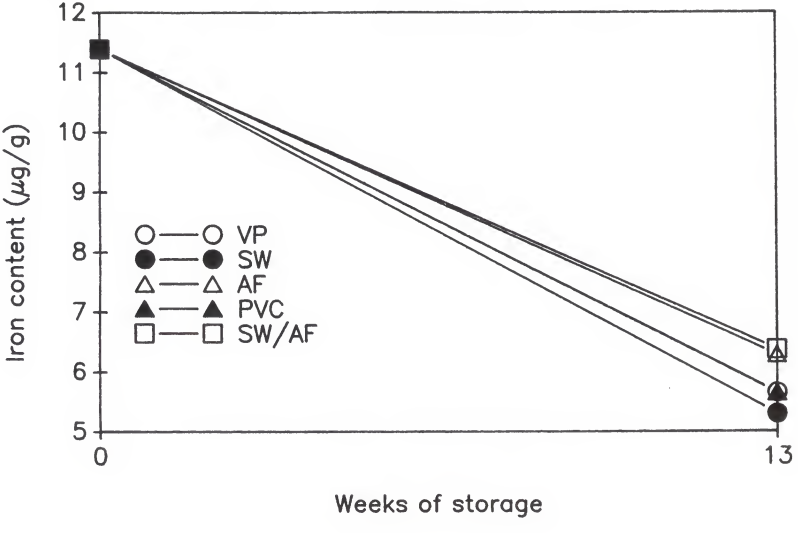

12
11
10
9
8
7
6
5
Iron content (μg/g)
VP
SW
AF
PVC
SW/AF
0
13
Weeks of storage

Figure 4. Iron content[1] after 26 weeks for the various packaging materials.

[1] Mean of four replications

VP = Vacuum packaging

SW = Saran Wrap®

AF = Reynolds Wrap® Heavy Duty Aluminum Foil

PVC= Polyvinyl chloride film

SWA= Saran Wrap® overwrapped with aluminum foil

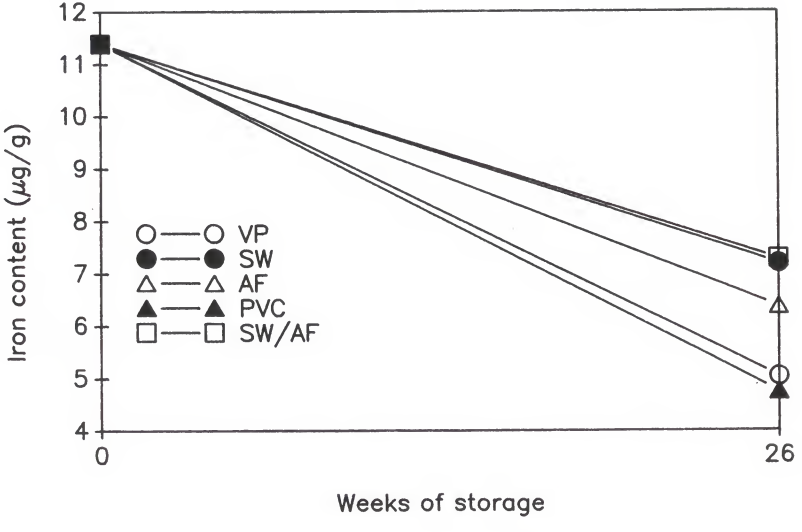

12
11
10
9
8
7
6
5
4
Iron content (μg/g)
VP
SW
AF
PVC
SW/AF
0
26
Weeks of storage

Figure 5. Zinc content[1] after 13 weeks for the various packaging materials.

[1] Mean of four replications

VP = Vacuum packaging

SW = Saran Wrap®

AF = Reynolds Wrap® Heavy Duty Aluminum Foil

PVC= Polyvinyl chloride film

SWA= Saran Wrap® overwrapped with aluminum foil

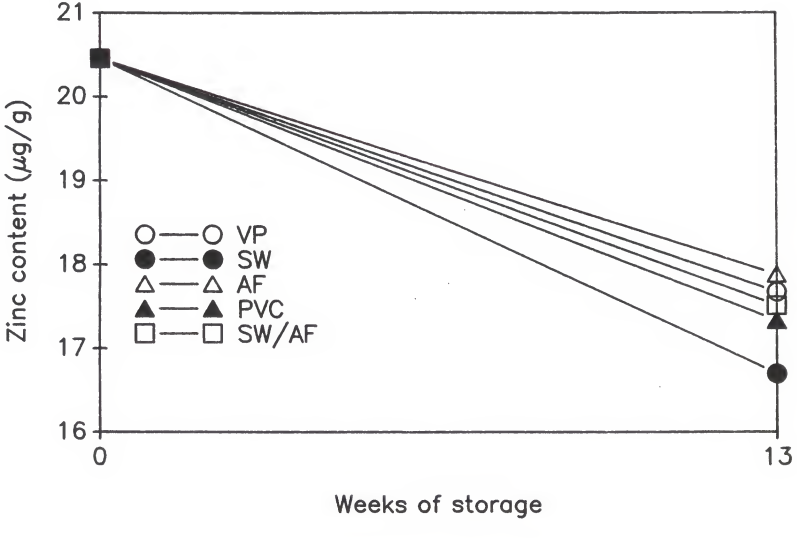

21
20
19
18
17
16
Zinc content (μg/g)
VP
SW
AF
PVC
SW/AF
0
13
Weeks of storage

Figure 6. Zinc content[1] after 26 weeks for the various packaging materials.

[1] Mean of four replications

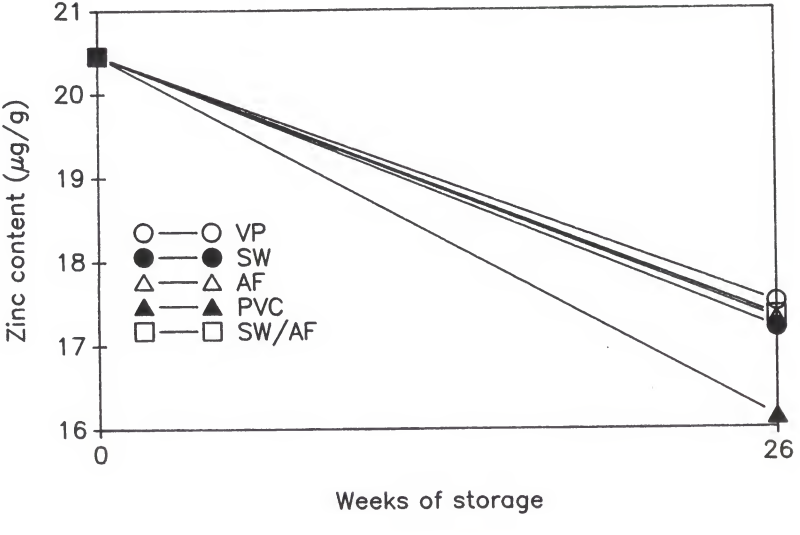
VP = Vacuum packaging

SW = Saran Wrap®

AF = Reynolds Wrap® Heavy Duty Aluminum Foil

PVC= Polyvinyl chloride film

SWA= Saran Wrap® overwrapped with aluminum foil


21
20
19
18
17
16
Zinc content (μg/g)
VP
SW
AF
PVC
SW/AF
0
26
Weeks of storage

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ACKNOWLEDGEMENTS

The author wishes to extend sincere appreciation to Dr. Carole A. Z. Harbers, Associate Professor of Foods and Nutrition and Major Professor, for advice and guidance through the entire program. The author would also like to thank Dr. Donald Kropf, Professor, Department of Animal Sciences and Industry and Dr. Meredith Smith, Associate Professor of Foods and Nutrition, for serving on the author's committee and also preparing at short notices.

Sincere gratitude is also extended to Mrs. Jeanett Gelroth, (AIB) for helping with the mineral analysis and to Dr. Dallas Johnson, Professor, Department of Statistics for help with the statistical analysis.

The author also wishes to thank Mrs. and Mr. Reddy for all the encouragement and love extended. I want to thank my parents for always doing the best for my future, regardless of how it effected them. They provided me with the most positive atmosphere I could possibly have. I just wish I had obeyed them more often.

The author also wishes to thank Dr. Joseph Zayas, Professor of Foods and Nutrition who made it possible for me to enter the Graduate Program. The author extends gratitude to Dr. Jane Bowers, Head of Department of Foods and Nutrition for trusting my capabilities and for not

throwing me out when I did not perform upto the mark.

Thanks are also extended to Mrs. Jean Craig for helping me with the thiamin analysis and providing a constant stock of delightful cookies!

The author finally wishes to thank Mr. Kulvinder Singh Gill for all the wholesome and healthy criticism and for providing love and encouragement.

Ofcourse I wish I had listened to you all more often!

APPENDIX

APPENDIX A

Raw data for thiamin mg/100gm (wet-weight):

Thiamin content of fresh ground pork for the replications:

#1	#2	#3	#4	Average
0.77	0.66	0.57	0.58	0.65

Thiamin content[1] after frozen storage for 13 or 26 weeks:

Packaging materials	13wks	26wks
Vacuum packaging	0.66	0.60
SaranWrap®	0.62	0.63
Aluminum Foil	0.65	0.64
PVC	0.61	0.63
SaranWrap® overwrapped with aluminum foil	0.65	0.62
% Retention	98.2	96.0

[1] Mean of four replications

APPENDIX B

Raw data for iron ug/g (wet-weight):

Iron content of fresh ground pork for the replications:

#1	#2	#3	#4	Average
9.18	12.78	11.93	11.68	11.39

Iron content[1] after frozen storage for 13 or 26 weeks:

Packaging materials	13wks	26wks
Vacuum packaging	5.65	5.03
SaranWrap[R]	5.30	7.19
Aluminum Foil	6.28	6.36
PVC	5.64	4.74
SaranWrap[R] overwrapped with aluminum foil	6.37	7.27
%Retention	51.3	53.7

[1] Mean of four replications

APPENDIX C

Raw data for zinc ug/g (wet-weight):

Zinc content of fresh ground pork for the replications:

#1	#2	#3	#4	Average
19.05	21.02	25.03	16.73	20.45

Zinc content[1] after frozen storage for 13 or 26 weeks:

Packaging materials	13wks	26wks
Vacuum packaging	17.66	17.49
SaranWrap[R]	16.69	17.19
Aluminum Foil	17.87	17.30
PVC	17.32	16.13
SaranWrap[R] overwrapped with aluminum foil	17.51	17.34
%Retention	85.1	83.6

[1] Mean of four replications

EFFECT OF FROZEN STORAGE AND PACKAGING MATERIALS ON SELECT NUTRIENTS IN GROUND PORK

by

INDIRA REDDY

B.S., University of Madras, 1985

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1989

ABSTRACT

During frozen storage of ground pork, chemical and physical changes cause decreased water holding capacity and increased thaw drip. Thaw drip may result in the loss of water-soluble vitamins and minerals. The purpose of this study was to investigate the effectiveness of selected packaging materials for protecting ground pork, for a freezer storage time of 13 and 26 weeks, in conditions similar to home storage, with regards to the nutritive value.

Ground pork with a 20% fat content was analyzed for thiamin, zinc, and iron content. The ground pork was then packaged in 0.5 lb quantities in the following wrapping materials : Vacuum Package (Cryovac® bags), PVC (Polyvinyl chloride), Saran Wrap®, Reynolds Wrap® Heavy Duty aluminum foil®, and Saran Wrap® overwrapped with aluminum foil. The packaged pork was held at -17 C for 13 and 26 weeks in an upright home-style freezer. After the designated storage times, the packages of ground pork were thawed at ambient temperature and then analyzed for thiamin, zinc and iron. Thiamin content was determined using the thiochrome procedure, and zinc, and iron were analysed by atomic absorption.

Results were analysed by analysis of variance using a

randomized complete block design (RCBD).

The wrapping materials did not differ for any of the parameters measured. The thiamin content of ground pork, packaged in various materials, and frozen stored for 13 or 26 weeks was not significantly different from the mean value before freezing.

The iron varied significantly between the mean initial values and values determined after 13 and 26 weeks. The mean iron content after 13 weeks was not significantly different from mean iron content after 26 weeks. The zinc content also varied significantly between the initial values and mean zinc values determined after freezer storage of 13 and 26 weeks. The mean zinc content after 13 weeks was not significantly different from mean zinc content after 26 weeks.