

SENSORY, CHEMICAL AND INSTRUMENTAL ANALYSES
OF FLAVOR, TEXTURE AND COLOR OF PRECOOKED BEEF
FROZEN AND STORED IN MODIFIED ATMOSPHERE PACKAGING

by

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INTRODUCTION

Food companies are aware of the consumer demand for precooked, ready-to-eat products with high eating quality and little preparation time. The Product Development Manager of Hormel Company, Roger J. Sinz, expressed their future goal as, "We've got to have everything ultra-convenient for the consumer of the 1990's" and Dr. Denton of Campbell Soup said "We want a wide variety of good quality, ultra-convenient foods" (Duxbury, 1988). This demand for convenience has resulted because of increased numbers of single-member families, single parents, senior citizens, and working mothers in the society.

Stale, warmed-over flavor/aroma, first recognized in 1958 by Dr. Betty Watts and her students at Florida State University, Tallahassee (Tims and Watts, 1958), has been one of the major drawbacks in marketing precooked, ready-to-eat meat products. The increasing prevalence of distribution and consumption of meat products by airline feeding, fast food restaurants, delicatessens and specialty service restaurants as well as increased marketing of prepared, precooked frozen meat entrees in retail outlets, provide an even greater potential for the development of warmed-over flavor.

A nation-wide survey, including 72 university and college food service managers, and a consumer study were conducted to determine the impact of warmed-over flavor in public feeding systems (Cross et al., 1987). The food

service industry believed that very little if any warmed-over flavor problem existed; however, the consumers of the precooked meat items appeared to substantiate the claim that a prominent warmed-over flavor problem exists.

According to the Food Processing's Annual Top 100 Food Company Survey in 1988 to track R & D efforts, concerns and trends of the food industry, refrigerated/extended life products received high interest from 42% of the companies, and packaging changes received moderate interest from 39% of the respondents-unchanged from last year. Modified atmosphere packaging captured moderate interest from more than 50% of the survey group - up from 35% in 1987 (Swientek, 1988). Food processors should be knowledgeable about warmed-over flavor to aid the processing of packaged, extended-life products. Accomplishment of a short-term goal to improve the processing procedures that eliminate or reduce the development of these flavor/aroma components would aid the marketing of precooked meat products.

The objectives of this study were to 1) identify and compare the difference in sensory flavor, aroma and texture of precooked beef packaged in different modified atmospheres before and after frozen storage, 2) measure the extent of lipid oxidation, the change in texture and color of precooked beef after frozen storage, and 3) relate sensory scores to TBA values and aldehyde contents.

REVIEW OF LITERATURE

Lipid Oxidation of Meat

Mechanism and products

Oxygen, which is ubiquitous in food systems during fresh-state storage, cooking, processing, and post-processing storage, leads to deterioration of food quality (Richardson and Finley, 1985) and thus changes appearance, texture, flavor and odor of food. Many of these changes in foods result from autoxidation (Simic and Karel, 1980).

Autoxidation usually proceeds through free radical reactions (Farmer et al., 1943; Bolland and Koch, 1945; Halliwell and Gutteridge, 1985) comprising initiation, propagation and termination step (Simic and Taylor, 1987). The rate and degree of autoxidative degradation of lipids is related directly to the amount of unsaturation (Love and Pearson, 1971). Hydroperoxides, the initial reaction products, are very unstable and degrade to produce several classes of secondary reaction products (Keeney, 1962; Herz and Chang, 1970). Many of these compounds, including alcohols, ketones, acids and lactones, are major contributors to the oxidized or warmed-over flavor in foods (Lea and Swobada, 1958).

Sensory characteristics

Tims and Watts (1958) first called the flavors developed during storage of precooked meat "warmed-over", "rancid" or "stale" and defined the process as the rapid onset of

rancidity in cooked meat during refrigerated storage. Warmed-over flavor was also used by Sato and Hegarty (1971) to describe the flavor of oxidized cooked meat. Pearson et al. (1977) and Igene et al. (1979a, 1980) contrasted warmed-over flavor, a rapid occurring phenomenon, to the rancidity that develops slowly in frozen raw meat, fatty tissues and rendered fat or lard.

The general pattern of warmed-over flavor development in stored meat: reduction in intensity of cooked meat flavor/aromatics and appearance of stale, cardboardy notes and lower overall sensory quality, was found in turkey (Cipra and Bowers, 1970; Cremer and Richman, 1987), chicken (Harris and Lindsay, 1972), pork (Poste et al., 1986), and beef (Johnson and Civille, 1986; Love, 1988). Poste et al. (1986) noted three phases of flavor changes discerned by a sensory panel: fresh-cooked, warmed-over (an intermediate stage, not yet rancid), and rancid (extremely oxidized flavor) on stored precooked pork.

Johnson and Civille (1986) reported that warmed-over flavor was equally identifiable in meat from different species (beef, pork, turkey and chicken) or different heat treatments (grilling, steaming, baking, etc.) within a species, although the samples varied in intensity. Stored meat, either raw or cooked, undergoes a characteristic series of flavor changes. Several descriptors to follow these changes include warmed-over, stale, cardboardy,

oxidized, painty and rancid.

Method of assessing oxidation

TBA-reactive substances

Unsaturated fatty acids are especially vulnerable to oxidation being readily cleaved at the double bonds to produce various oxidation products. One such product is malonaldehyde (MA) which has been extensively measured by reacting it with 2-thiobarbituric acid (TBA) to give the so-called "TBA value" (Turner et al., 1954; Yu and Sinnhuber, 1957; Sinnhuber and Yu, 1958; Tarladgis et al., 1960; 1962; Zipser et al., 1964; Wilson et al., 1976). This colorimetric analysis also has been accepted as a measure of warmed-over flavor (Tims and Watts, 1958; Tarladgis et al., 1960; Sato and Hegarty, 1971; Love and Pearson, 1974; Rhee, 1978).

The pink pigment obtained in the reaction results from the condensation of two moles of TBA with one mole of MA (Sinnhuber and Yu, 1958). The intensity of color originally was believed to be a measure of MA concentration (Tarladgis et al., 1960; 1964) and has been related to panelists scores for rancidity (Zipser et al., 1964) and warmed-over flavor notes (Igene and Pearson 1979; Igene et al., 1979b).

Whereas the TBA value has been used quite extensively as a measure of food quality, including warmed-over flavor, there are reports (Witte et al., 1970; Pearson et al., 1977; Rhee, 1978; Melton, 1983) that question the validity of the method because of the value of TBA reactive substances

(TBARS). The TBA reaction lacks specificity in that other products of lipid oxidation, such as alka-2,4-dienals, also react with TBA to form a pink complex with the same absorption maximum (532 nm) as the MA-TBA complex (Jacobson et al., 1964; Marcuse and Johansson, 1973; Fujimoto et al., 1984). By using the high performance liquid chromatographic (HPLC) technique to quantify MA, Csallany et al. (1984) suggested that the results of the TBA test should be referred to only as "TBA reactive substances" and should not be equated to free MA.

An additional drawback of the TBA method is the possibility of interfering reactions which can alter the pink color formation (Tarladgis et al., 1960). The net result is that TBA values may decrease over time, while lipid oxidation is known to continue. Malonaldehyde has been shown to react with the primary amino groups of lipids, protein (Tarladgis et al., 1960; Kwon et al., 1965), amino acids (Dillard and Tappel, 1973) and also with itself (Buttkus, 1975) to form Schiff base fluorescent products.

Other limitations of TBA test were listed by Melton (1983): the data are not translatable from species to species (Pearson et al., 1977), data are grossly erratic when frozen samples are analyzed (Witte et al., 1970), and data are quite variable depending on the method of analysis (Rhee, 1978).

Recently, however, Igene et al. (1985) reported that the

major TBA-reactive substance in the distillate of cooked chicken is MA and concluded that its level is indicative of warmed-over flavor. They showed a high correlation ($r=0.87$) between TBA values and panel scores for warmed-over flavor in cooked chicken.

TBA reactive compounds of clear distillates were further analyzed by St. Angelo et al. (1987) using direct gas chromatograph/mass spectrometer methodology. Compounds present in the highest concentration in the distillate were hexanal, 2,3-octanedione, 2,4-decadienal and pentanal.

Melton (1985) reviewed methods for following lipid oxidation such as changes in fatty acids composition, peroxide value, TBA value, carbonyl compounds, aldehydes by GC analyses, oxygen absorption, pentane production and fluorescent products. She concluded that the measurement of TBA reactive substances with all of its problems still was the method of choice for following lipid oxidation in meats because of the knowledge available in the literature concerning its advantages and disadvantages.

Aldehydes by gas chromatography

Compounds associated with lipid oxidation could be used as markers to follow the development of warmed-over flavor. A measure of aldehydes and ketones appears to be a suitable index of warmed-over flavor (Dupuy et al., 1987). Earlier work by Pippen and Nonaka (1963) concluded that n-hexanal, n-2,4-decadienal and other similar compounds contributed to

the rancid flavor in chicken. Hexanal also greatly increased during storage of freeze-dried beef (El-Gharbawi and Dugan, 1965).

Hexanal and pentanal were identified in cooked, uncured pork (Cross and Ziegler, 1965) and two aldehydes, heptanal and n-nona-3,6-dienal have been associated with warmed-over flavor development in cooked turkey (Ruenger et al., 1978). Love and Pearson (1971) reported that hexanal is one of the principle products associated with lipid oxidation and is implicated as a component of warmed-over flavor.

Bailey et al. (1980) reported that the volatile compounds of roasted beef that increased most rapidly during storage at 4° C were hexanal and 2-pentyl furan; pentanal also increased. Carbonyls increased as off-flavor developed in precooked -refrigerated turkey and were detected within hours after cooking from a headspace gas chromatographic profile (Wu and Sheldon, 1988).

The total volatiles of ground roasted beef increased appreciably within hours after storage at 4° C; hexanal increased at the most rapid rate. Increases in other compounds were also observed, e.g., propanal, pentanal, 2,3-octanedione, nonanal, and 2-pentylfuran. Any of these compounds could make excellent markers to follow the development of warmed-over flavor and hexanal should be a useful primary marker of warmed-over flavor development (Dupuy et al., 1987; St. Angelo et al., 1987).

The correlation coefficients between sensory evaluation data and TBA values or marker volatile compounds - two compounds (hexanal and 2,3-octanedione) plus total volatile content - corresponding to warmed-over flavor formation in precooked stored beef ranged from 0.80 to 0.84. Correlation coefficients between TBA and instrumental values ranged from 0.84 to 0.92 (St. Angelo et al., 1987).

The additional advantage of measuring aldehydes by GC methods is that individual oxidation products from meat can be quantified. This method of assessment should be applicable for any type of meat sample -- ground, chunked, sliced, or minced and raw or cooked (fresh or stored).

Headspace gas sampling followed by gas chromatography analysis of hexanal has been used for other products and correlated with the development of rancidity in cereals and as a measure of lipid oxidation prior to onset of rancid odors (Fritsch and Gale, 1977). The headspace gas analysis of pentanal and hexanal also appears to be a good indicator of flavor quality of potato chips (Jeon and Bassette, 1984). High correlation coefficients ($r=0.99$) between hexanal and oxidative degradation of unsaturated fatty acid was found in cooked brown rice (Shin et al., 1986) and particularly in oils with coefficients higher than 0.9 (Dupuy et al., 1978).

Heat process and size reduction

Early research on frozen precooked or partially cooked meat was reported by Watts et al. (1948). Cooked meat is

more susceptible to lipid oxidation than uncooked meat and rapidly develops objectionable flavor and odor referred to as warmed-over flavor (Tims and Watts, 1958; Dugan, 1961; Younathan and Watts, 1960). Cooking would certainly denature the membranes and release phospholipids, which are believed to be the major lipid compounds contributing to warmed-over flavor development in cooked meat (Watts, 1962; Wilson et al., 1976; Igene and Pearson, 1979; Pearson and Gray, 1983; Willemot et al., 1985).

Studies suggesting that the increased rate of lipid oxidation in cooked red meat, compared to uncooked meat, is due to release of nonheme iron during cooking, which acts as the active catalyst for lipid oxidation (Igene et al., 1979a; Chen et al., 1984). The heme iron (activated MetMb) may initiate lipid oxidation in cooked meat and uncooked meat, but nonheme iron plays a greater role in acceleration of warmed-over flavor development, i.e. lipid oxidation in cooked meat (Rhee, 1988; Sato and Hegarty, 1971; Love and Pearson, 1974; Igene et al., 1979a).

The increased nonheme iron content in cooked meat could contribute to a greater susceptibility of cooked meat toward lipid oxidation, but cooking also disrupts meat tissues, bringing the lipid substrates and catalysts in closer proximity and resulting in an increased rate of lipid oxidation in cooked tissues (Rhee, 1988).

Reduction in size of meat by grinding, chopping,

slicing, or emulsification all disrupt membranes and lead to releases of phospholipids and incorporation of air and oxygen into the tissue. Both of these actions increase tissue susceptibility to oxidation and hasten development of oxidative rancidity and warmed-over flavor (Greene, 1969; Sato and Hegarty, 1971; Pearson et al., 1983).

Frozen storage and water activity

Keller and Kinsella (1973) observed increases in TBA values on cooking of ground beef and further increases when samples were stored for 36 days at -18° C. Freezing some foods to just below the freezing point increases degradation rates by chemical reactions because not all the water is frozen out and the chemicals are concentrated in the remaining water (Labuza, 1982).

Protein denaturation occurs in meat and fish during extended storage and makes these products very prone to oxidation, flavor absorption and color changes during extended storage. All these deterioration changes can be prevented, or at least reduced, by proper packaging (Mannhelim and Passy, 1985). Smith (1987) reported that frozen storage (1 day to 26 weeks at -20° C) caused protein insolubilization, increased lipid oxidation, and decreased gel strength of deboned turkey meat.

Karel (1980) stated that water may influence lipid oxidation by influencing the concentrations of initiating radicals, the degree of contact and mobility of reactants,

and the relative importance of radical transfer versus recombination reactions. Labuza et al. (1972) established that lipid oxidation in strained meat from chicken and pork at Aw values in the range 0.75-0.84, proceeds much more rapidly if the samples are adjusted to the desired Aw by desorption rather than by resorption.

Control methods

Several approaches to extending the shelf life of meat products have been investigated, including product formulation (addition of antioxidative additives), irradiation, and packaging. The most frequently used additive for preventing warmed-over flavor is sodium nitrite (Sato and Hegarty, 1971; Fooladi, 1971; Igene et al., 1980; Zipser et al., 1964). Other ingredients added to control the development of this off-flavor on fresh meat are polyphosphates, sodium ascorbate, tocopherols (Igene et al., 1976), flavonoids (Watts, 1962; Pratts, 1972), BHA, BHT, TBHQ (Shelton, 1959; Shahidi et al., 1987), and various Maillard-type browning products such as maltol and reductic acid (Sato and Hegarty, 1971; Roozen, 1987).

However, many of these compounds have peculiar flavor characteristics. For meat, an ideal method would be one that could inhibit the development of warmed-over flavor while not imparting any of its own flavor character. There is a need for technological advances. In light of recent safety concerns and consumer demands, the more favorable approach

may be packaging.

Modified Atmosphere Packaging

The modified atmosphere packaging (MAP) concept for packaged food consists of modifying the atmosphere by vacuum, gas flushing or controlled permeability of the film - thus controlling the biochemical, enzymatic and microbial actions so as to avoid or to decrease the main degradation that might occur. Modified atmosphere packaging has been applied successfully for over 25 years in Europe and in the U.S. Approximately 87% of the 23 billion pounds of meat are packaged in shrinkable barrier materials for industrial, restaurant and institutional use (Lioutas, 1988; Young et al., 1988).

The effectiveness of oxygen-excluding atmosphere (vacuum, carbon dioxide, and nitrogen) in inhibiting spoilage bacteria was documented for pastrami (Laleye et al., 1984), beef (Hanna et al., 1981; Savell et al., 1981), fish (Brown et al., 1980; Lannelonge et al., 1982; Parkin and Brown, 1983), ham (Kemp et al., 1983), veal (Lee et al., 1983), pork (Christopher et al., 1980; Spahl et al., 1981), and poultry (Elliott et al., 1985; Gray et al., 1984).

Ample evidence indicates that MAP can extend the shelf life of many perishable products including meat, fish, poultry, and some forms of produce by 50-400% (Hotchkiss, 1988). In the U.S., MAP is used extensively for raw poultry and pork, but its widest use is for precooked products which

require only minimal reheating such as breaded and fried chicken and prepared entree items.

Vacuum

Vacuum packaging inhibits the growth of aerobic spoilage bacteria such as Pseudomonads, the cold-tolerant bacteria which produce off-flavor, slime, rancidity and various discolorations.

The resulting conditions in the vacuum package favor the growth of facultative anaerobic microorganisms such as lactic acid producing bacteria which result in a sour flavor and a milkiness in the container liquid (Igram, 1962; Seideman et al., 1979) but their growth is inhibited by refrigeration.

Gas filling

Three main gases: oxygen, nitrogen and carbon dioxide and their combinations are commonly used as gas compositions for gas filling packaging. Gas filling has several advantages over vacuum for sliced products. Meat slices are easy to separate, South American Corn Beef being a prime example, and moisture is not exuded resulting in better pack appearance (Noyes, 1986).

The specific product should be considered along with its particular atmosphere needs and temperature requirements. The choices of gas combinations are mainly dependent on previous experiences. For example, several excellent gas combinations are 80% CO₂ and 20% N₂ for cooked thin-sliced

meat handled at 0-2° C; 30% O₂, 30% CO₂ and 40% N₂ for cooked red meat; and 30% CO₂ and 70% N₂ for cooked chicken (Lioutas, 1988). Hotchkiss (1988) found the most beneficial gas mixture for many nonrespiring products is 75% CO₂, 15% N₂, and 10% O₂. A gas mixture used commonly in the U.K. and Ireland for cooked red meat is 25-30% CO₂ and 70-75% N₂ (Johns, 1986).

Carbon dioxide

Carbon dioxide is an active gas and has a bacteriostatic effect which is due to mass-action inhibition of certain bacterial decarboxylating enzymes such as isocitric and malate dehydrogenase (Huffman et al., 1975). It involves the extension of the lag phase, a reduction in the growth rate, and extended shelf-life (Clark and Lentz, 1973; Wolfe et al., 1976). Killefer (1930) found that carbon dioxide at room temperature inhibited the growth of *Streptococcus*, *Proteus*, and *Micrococcus*. Other researchers also found that 25% CO₂ remarkably inhibited the growth of *Pseudomonas*, *Achromobacter*, and *Flavobacterium*.

High carbon dioxide concentrations can cause severe browning of fresh (raw) meat surfaces (Wolfe et al., 1976). The browning reactions may be due to anoxia (Marriott et al., 1977a), or a decrease in pH (Huffman et al., 1975; Marriott et al., 1977b) or by formation of carbonic acid (Taylor and MacDougall, 1973).

Nitrogen

Nitrogen has been used as a diluent or a filler gas (Taylor, 1971) to overcome some exudation and meat distortion caused by some vacuum-packaging techniques (Adams and Huffman, 1972). Nitrogen fill packaging has all the benefits of the vacuum package for product protection and has the added advantage of creating a gaseous cushion for thinly sliced or fragile products. However, there was aerobic bacterial growth and an unpleasant slippery gray or purple outer surface of raw meat when packaged with 100% nitrogen (Smith et al., 1977).

Oxygen

Oxygen has the function of keeping color quality of raw red meat by maintaining the oxygenated form of myoglobin and preventing the formation of brown metmyoglobin (Clark and Lentz, 1973; Taylor, 1971) as well as inhibiting growth of certain anaerobic bacteria such as *Pseudomonas* strains. It is frequently used as a component in gas combinations for meat packaging.

Application for precooked meat

For modified atmosphere packaging, foods can be divided into respiring (fresh produce-live systems) and nonrespiring (cooked meals, pasta, etc.) based on whether there is any biochemical metabolic activity present. This distinction is necessary for various critical parameters (sensory and microbiological quality of raw material, temperature,

packaging material, gas mixture, consumer handling between sale and consumption) and system optimization (Lioutas, 1988).

The real determinant about whether packaged products are acceptable is the sensory quality of the product. Consumers are the final judges of product quality and make decisions on quality based on the appearance, flavor and texture of the product. Any judgment of shelf-life, therefore, must include sensory evaluation of the stored product.

Hotchkiss et al. (1985) tested the quality of cooked poultry stored with 80% CO₂ (the balance being air) at 36° F by a trained sensory panel and found no difference between the stored and fresh samples in overall acceptability after 4 weeks of storage. The major difference was in tenderness, the stored samples were somewhat tougher than fresh ones after only 14 days. Vacuum packaged precooked turkey and pork were more meaty and less oxidized and rancid in flavor and aroma than 100% N₂ and 100% CO₂ packaging treatments for both short-term refrigerated storage and long-term frozen storage (Nolan, 1987).

Vacuum packaged precooked pork roasts were more tender, juicy, and had lower cooking losses and lower microbial counts than those wrapped in polyvinyl chloride film for both refrigerated (4° C) and frozen storage (-20° C) (Jones et al., 1987). Vacuum cook-in-bag was found to increase shelf-life for pork (Prabhu et al., 1988) with only slight

physical and chemical effects on turkey (Smith and Alvarez, 1988). Nitrogen fill packaging improved the shelf life of nitrite-free bacon-like products at 8, 12, and 26° C, retarding discoloration and minimizing exudate. Meat maintained an acceptable appearance longer but odor became unacceptable earlier than in samples vacuum-packaged (Post et al., 1988).

In cooked cured meat loaf, nitrogen packaging improved appearance by retarding greenish discoloration (Lee et al., 1984a). Less surface greening and less exudate loss also were observed in refrigerated pastrami (Laleye et al., 1984) and veal chunks (Lee et al., 1983) when using 100% nitrogen packaging. In a study by McDaniel et al. (1984), beef roasts were precooked and packaged by one of the three methods: vacuum, 100% CO₂, and a gas combination of 15% CO₂, 30% O₂ and 55% N₂. All samples were stored at 4° C for up to 21 days. Vacuum-packaged cooked roasts were preferred by panelists and generally suffered less quality loss. Hsieh and Baldwin (1984) found that both sensory scores for warmed-over flavor/aroma and TBA values were less for sliced reheated precooked beef stored in a vacuum package than in a casserole at 4° C for 2-7 days. They also found that lipid oxidation and the development of warmed-over flavor/aroma occurred mainly during storage of precooked meat and did not increase during reheating.

In the commercial meat production lines in the U.S.,

there is an application and potential future demand for MAP. Pilgrim's Pride, a chicken product producer for both retail and food service markets, uses a resealable "zipper pouch" system for form/fill/seal vacuum of cooked, sliced meat (Hanser and Rice, 1988). "PERDUE DONE IT", a microwave-ready product of precooked chicken parts by Perdue Farms, which earned a "Dupont packaging award" in 1988, utilizes a form/fill/vacuum/gas flush/seal packaging for microwave-ready chicken (Rice, 1988).

Bilmar Foods is supplying vacuum-packaged, precooked turkey breasts to Kroger. According to Kroger's vice president of meat merchandising, William Packer, the poultry case may contain 50% precooked products in the next 5 to 10 years. According to the Dept. of Commerce, processed poultry will account for 25% of the total product mix by the earlier 1990's. The biggest gains will come in the food-service area, precooked refrigerated products and new value-added concepts (Swientek, 1988).

Determination of Freezable Water by Thermal Analysis

Water is the predominant component in meat and most foods since they usually have greater than 50% moisture. The freezing process causes a change of phase in the water, so the properties of the frozen product are influenced significantly by the differences in the properties of water in the frozen and unfrozen states (Heldman, 1982).

Differential scanning calorimetry (DSC) has been used to

determine the thermophysical properties of water in foods to establish the relevant processing parameters (Roos, 1986; 1987; Lovric et al., 1987; Fernandez-Martin and Sanz, 1979; Simatos et al., 1975; Ramaswamy and Tung, 1981). Calorimetry is a favored method for the study of water in biological systems, because information on the physical state and properties of water can be deduced from its pattern of solidification or vaporization (Simatos et al., 1975). Enthalpy is used for calculating the total heat to be removed during freezing because crystallization of water in food products occurs over a wide range of temperature. Not only areas under the peaks of DSC curves directly relate to the quantity of freezable water and can be used in calculation of enthalpy, but the shape of curves also varied depending on the fraction of dry matter, and its chemical composition (Bechtel et al., 1971, Lovric et al., 1987). The lower the moisture content, the lower the melting point and the broader the melting peak (Roos, 1986; Parducci and Duckworth, 1972). With increasing proportion of dry matter in systems, the onset temperature and the peak temperature of DSC peaks decreased and the peaks are less symmetrical (Lovric et al., 1987).

Unfreezable water of biological materials was found to be a function of moisture; 26.4% in reindeer meat (Roos, 1986), 26% in beef (Riedel, 1957) and 32% in plasma (Simatos et al., 1975). The percentage of unfreezable water in foods

depends on the moisture content but the amount of unfreezable water should be constant when expressed on the dry weight basis (Roos, 1987). Pham (1987) studied the water in frozen lamb loin and suggested that fat content affected the bound water/solids ratio, since fat binds very little water. The nature of the protein (muscle fibers or connective tissue) also affects water binding properties of meat (Hamm, 1985).

MATERIALS AND METHODS

Materials

Six 18-in. vacuum packaged USDA Grade Choice shortcut, boneless beef strip loins (11 to 13 lb., IMPS/NAMP No. 180) were obtained from Dillons Supermarket (Manhattan, KS). Backfat on loins was 1/2 in. or less. Loins were kept refrigerated (4° C) for 3 to 7 days until used.

Sample preparation

Whole boneless loins were cooked fat side up on oven racks in open roasting pans in a preheated rotary hearth oven at 325° F. Temperatures in the center of the Longissimus muscle at posterior, middle and anterior locations of loins were recorded during cooking. When the temperature of the middle position reached 70° C, loins were removed from oven and immediately weighed. The longissimus muscle was removed and immediately sliced to 1 cm thick using a Plexiglas guide to control the uniformity of thickness of slices. Tail length of each loin was about 2 in. after cooking.

Three treatments for packaging were used: vacuum, N₂/CO₂ gas filling (vacuum and then backfill with a 80% N₂ and 20% CO₂ combined atmosphere), or air (heat sealing of bags only). Four beef slices were placed into one high oxygen barrier ethylene vinyl alcohol (EVOH) bag (Cryovac Div., W. R. Grace & Co., SC) and sealed according to the assigned treatment (vacuum, N₂/CO₂, or air) by a Super-Vac

vacuum packaging machine (Smith Manufacturing Co., N.J.). Oxygen levels of N₂/CO₂ packages were verified to be lower than 0.5% using a Mocon oxygen analyzer. Gas volume/meat volume ratio in N₂/CO₂ and air packages was approximately 1:2.

Immediately after packaging, half of the samples from each treatment were analyzed (within one hour after cooking) without further heating. The remaining samples were frozen and held frozen (-20° C) for 11 weeks. After 11-week frozen storage, storage bags were punctured and placed in a Sharp Carousel turnable microwave oven (model R-8200) and reheated 6 minutes: 1.5 min/side on thaw cycle and then 1.5 min/side at full power.

The extent of lipid oxidation was determined by measuring TBA reactive substances and the amount of hexanal and pentanal and by evaluation of flavor and aroma by a trained professional sensory panel. Textural properties were measured by an Instron, and also by the sensory panel. Percentages of moisture and fat, freezable water, pH values and HunterLab color values were determined.

Statistical Analysis and Design

A Latin square design was used with position in loin and loin (replication) as sources of variation. Three one-half loins comprised a square. One-half of a loin was used for initial analyses, the other half was evaluated after 11 weeks storage. Two squares, making 6 replications of each

treatment, comprised the study. Data were analyzed by analysis of variance (ANOVA) to determine differences among treatments and positions. Data for samples without storage and those evaluated after frozen storage were analyzed independently.

Chemical and instrumental analysis

<u>Sources of Variation</u>	<u>Degrees of Freedom</u>
Square	1
Position (square)	4
Loin (replication)	4
Treatment (packaging method)	2
Error	6
Total	17

Sensory analysis

<u>Sources of Variation</u>	<u>Degrees of Freedom</u>
Square	1
Position (square)	4
Loin (replication)	4
Treatment (packaging method)	2
Error A	6
Judge	4
Treatment * Judge	8
Error B	60
Total	89

When F-values were significant, means were compared by method of least significant difference (LSD).

Measurements

Sensory analysis

Five professional sensory panelists, extensively trained in both flavor and texture profile methods at the Kansas State Univ., Sensory Analysis Center evaluated beef loin samples for aroma, flavor, and textural properties. Panelists developed descriptors used for evaluation during training.

Aroma and flavor were evaluated for meaty/beefy, warmed-over, and oxidized/rancid notes. Flavor was also evaluated for cardboard and sour notes. Tenderness (ease of mastication) and juiciness also were scored. Samples were scored by panelists indicating intensity of each attribute on a 5-in. (12.7 cm) line scale anchored at each end (none to intense) and in the center (Appendix, p. 69).

At each evaluation period, three samples coded with random digits were presented to the panel in warm covered glass petri-dishes. A piece of freshly cooked beef loin was served as a reference and "warm-up" sample for the sensory panel during each sensory evaluation period for frozen stored samples.

TBA reactive substances

TBA reactive substances were measured using a modification (Kuntapanit, 1978) of the extraction procedure of Witte et al. (1970). Colorimetric absorbance at 530 nm obtained from a Perkin-Elmer ultraviolet spectrometer was

converted to malonaldehyde and reported as mg malonaldehyde/kg meat. A detailed TBA procedure is presented in the Appendix (p. 83).

Hexanal and pentanal

The concentrations of hexanal and pentanal were determined by gas chromatographic analysis of the vapors from a distillate of a five gram sample. The combination of the head space gas sampling method (Bassette and Ward, 1975; Jeon and Bassette, 1984) with the addition of an internal standard, 4-heptanone (Fritsch and Gale, 1977) was used.

A Tracor 540 Gas Chromatograph (Tracor Instruments Austin, Inc.) equipped with a flame ionization detector (FID), a Supelcowax 10 capillary column (30 m x 0.75 mm ID) and a splitless injection mode were used to separate the volatile components. The oven temperature was programmed at 40 ° C for 4 min., increased from 40 to 135 ° C at the rate of 3 ° C/min, 135 to 195 ° C at 10 ° C/min, then finally held at 195 ° C for 5 min to elute all residues. Tentative identification of hexanal and pentanal was made by comparing retention time of volatiles from meat samples with retention time of reference chemical compounds. Results were expressed as ppm hexanal and pentanal in the samples by internal standard peak quantitation method (Lee et al., 1984b). Detailed procedures are presented in the Appendix (p. 86).

Freezable water

The amount of freezable water in beef before storage

was determined using a Perkin-Elmer Differential Scanning Calorimeter, DSC-4 with System 4 Thermal Analysis Microprocessor Controller, a Perkin-Elmer model 56 recorder and a Flexi-cool FC-60 refrigeration unit. Each sample (10 mg sealed in an aluminum pan) was frozen to approximately -35° C for approximately one hour, then warmed to 25° C at a rate of 5° C/min. The thermograms of the samples as they were heated were scanned in the instrument sensitivity range of 10 mcal/sec, recorder sensitivity range of 10 mV and with speed of 10 mm/min.

After 11 weeks of frozen storage, a Perkin-Elmer Differential Scanning Calorimeter System 2 (DSC-2), with scanning Autozero and Intracooler II was used. Ten milligrams of ground muscle were frozen to approximately 210° K (-63° C), held for 10 min, and then warmed to 300° K (27° C) at a rate of 10° C/min. The melting peaks of samples were scanned in the instrument sensitivity range of 0.5 mcal/sec, then normalized by a TAD computer system to a sensitivity range of 8 mcal/sec.

The melting peaks were integrated to determine the enthalpy (ΔH) of samples and calculate the freezable water content.

Shear force

The textural properties were tested mechanically by an Instron Universal Testing Instrument (model 1122) with a 10-in. strip chart recorder. A 500 kg tension-compression cell

using a spindle to transmit the load to a simulated tooth attachment (Prusa and Bowers, 1982) was used.

A 2 kg full scale load with a crosshead speed of 20 mm/min and recording chart speed of 50 mm/min was used. The force needed to penetrate the meat piece (2 in x 1 in x 1 cm) to 8 mm was calculated from the peak height recorded. Measurements were done in triplicate.

HunterLab color values

A HunterLab D54 Spectrophotometer (HunterLab Associate Lab, Inc., Virginia) was used to measure the color of samples. For initial HunterLab color values, measurements were done directly on Cryovac packaged samples. Color of frozen stored reheated samples was measured in HunterLab sample cups. Both Illuminant A and C, light sources Horizon light and Northern daylight, respectively, were used for measurements. Values for L (lightness), a (redness), and b (yellowness) were recorded for each sample.

pH

pH of samples was determined using a Corning digital pH meter with a surface electrode on the surface of the ground meat samples.

Moisture and fat contents

Percentage moisture of all samples was determined by the official AOAC method (1984). Total lipids were extracted from the ground meat samples by method of Folch et al. (1957) using a 2:1 chloroform:methanol solvent.

RESULTS AND DISCUSSION

Total cooking time for individual loins (4.9-6.3 kg) ranged from 2 hr. 40 min. to 3 hr. 38 min. and averaged 3 hr. 10 min. Cooking time in min/kg averaged 33.4. For temperatures recorded at three locations in the center of the longissimus, those at the anterior and posterior positions varied from 69-81° C, and averaged 72.2° C when the center temperature was 70° C. Cooking losses ranged from 22.7 to 29.5 %. Data from all replications for both unstored and 11-week frozen stored beef are presented in the Appendix (p. 71).

Initial Characteristics

Half of the samples were evaluated immediately after packaging to observe the effect of modified atmosphere packaging on precooked beef samples before storage. Generally, sensory characteristics were unaffected by treatment (Table 1). Flavor of samples packaged in N₂/CO₂ was slightly less warmed-over than that of samples packaged in vacuum and air (P<0.05). However, all of the scores were low (less than 1) and the numerical difference was only 0.2 cm on a 12.7cm-scale. Even though statistically significant, it probably was not a practical difference. Gas filling is more effective in eliminating oxygen than vacuum packaging and this slight difference in warmed-over flavor may have resulted from the lower oxygen residues in bags after packaging and before sensory evaluation. Vacuum obtained

Table 1-Initial aroma,¹ flavor,¹ pH, and fat content for packaged cooked beef slices.²

Measurement	Atmosphere			Level of Significance
	Vacuum	N ₂ /CO ₂	Air	
Aroma:				
Meaty	5.0	5.2	5.1	NS
Warmed-over	0.4	0.4	0.5	NS
Oxidized	0.2	0.2	0.3	NS
Flavor:				
Meaty	5.7	5.9	5.7	NS
Warmed-over	0.6b	0.4a	0.6b	*
Cardboard	0.5	0.4	0.5	NS
Oxidized ^e	0.2	0.2	0.3	NS
Sour	1.3	1.3	1.2	NS
pH	5.87a	6.03b	6.00b	*
Fat, %	5.43	5.39	5.68	NS

¹Intensity scale, 0=none to 12.7=very intense.

²Means of 6 replications, means with different letters are significantly different; NS, not different (P>0.10),

* P<0.05.

^eIndicates position effects (P<0.05).

with the Super-Vac vacuum packaging machine with a "7" setting (the one used in this study) was 29.6 in. or more.

The quantitative descriptive analysis (QDA) plot (Figure 1) presents little difference among samples from the three treatments. Beef samples in air packages were slightly less tender (less easy to masticate) than those packaged in vacuum and N_2/CO_2 ($P<0.10$). Both vacuum and N_2/CO_2 packaged meat underwent a slight physical stress during evacuation of bags. The structure of meat could have been altered and made easier to masticate. Oxidized flavor was affected by position; higher scores ($P<0.05$) were obtained for sample from the mid section of the loin.

Little difference among treatments as determined by both chemical and instrumental analysis was found (Tables 1, 2, and 3). The surface pH of vacuum-packaged unstored beef slices after grinding was slightly lower ($P<0.05$) than for those N_2/CO_2 and air-packaged. Nolan (1987) also found that refrigerated precooked turkey slices had slightly higher pH values when packaged in either 100% N_2 or 100% CO_2 than in vacuum. Laleye et al. (1984) also reported higher pH values for nitrogen than for vacuum-packaged pastrami, but the differences were not always significant throughout the whole storage period and for various temperatures. They attributed decrease in pH to activity of lactobacilli and/or dissolution of CO_2 into meat tissue. A decrease in pH usually frequently is attributable to metabolic activity of

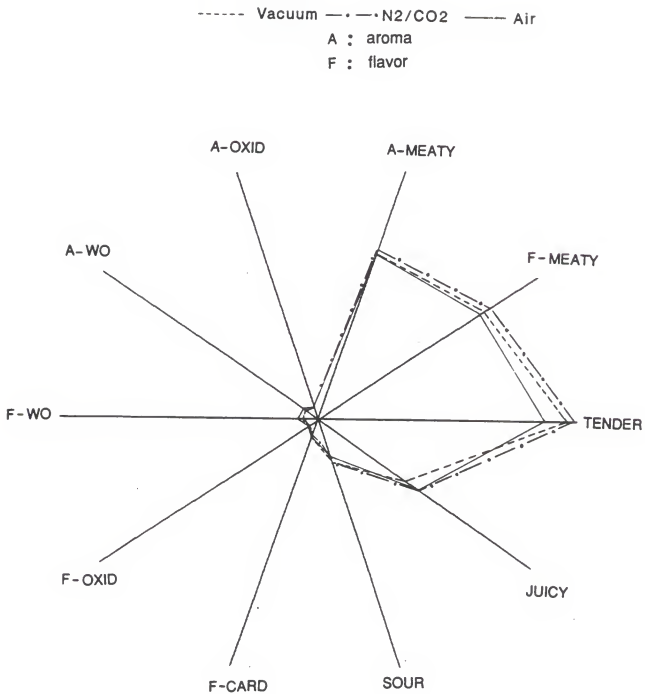


Figure 1 Quantitative descriptive analysis (QDA) plot of initial sensory characteristics for packaged cooked beef slices

Table 2-Initial tenderness,¹ juiciness,¹ total moisture, freezable water and shear force values for packaged cooked beef slices.²

Measurement	Atmosphere			Level of Significance
	Vacuum	N ₂ /CO ₂	Air	
Tenderness (Ease of Mastication)	7.3b	7.5b	6.7a	+
Juiciness	3.2	3.6	3.6	NS
Moisture % ^e	61.8	62.3	62.6	NS
Shear force (Kg)	0.86	0.73	0.83	NS
Enthalpy (cal/g) ³	35.9	36.5	36.8	NS
Freezable water ³ (g/g dry matter)	1.19	1.23	1.25	NS

¹Intensity scale, 0=none to 12.7=very intense.

²Means of 6 replications, means with different letters are significantly different; NS, not different (P>0.10), + P<0.10.

³Means of 5 replications; NS, not different (P>0.10).

^eIndicates position effects (P<0.05).

Table 3-Initial HunterLab color values for packaged cooked beef slices.¹

Measurement	Atmosphere			Level of Significance
	Vacuum	N ₂ /CO ₂	Air	
ILLA				
L	47.79	50.31	49.55	NS
a	8.50	7.89	8.57	NS
b	4.56	4.44	4.71	NS
ILLC				
L	46.73	49.31	48.48	NS
a	3.37	2.95	3.37	NS
b	8.45	8.26	8.69	NS

¹Means of 6 replications; NS, not different (P>0.10).

lactics, whereas the increase in pH is due to activity of proteolytic *Pseudomonas* spp. (Jaye et al., 1962).

Position influenced moisture content of samples in that slices from the outer ends samples (anterior and posterior) contained less moisture ($P < 0.05$) than those from the mid section.

Characteristics after Frozen Storage

After 11 weeks frozen storage, the remaining beef samples were reheated and evaluated. Oxygen levels in N_2/CO_2 packages ranged from 0.13-0.37% and in air packages, 13.7-18.1%. Vacuum and N_2/CO_2 packaged beef slices were similar but both were different from air-packaged beef in aroma, flavor, color and textural properties as measured by sensory, chemical and instrumental methods.

Sensory characteristics

Differences in sensory characteristics among treatments are clearly shown in the quantitative descriptive analysis (QDA) plot (Figure 2). Samples packaged in vacuum and N_2/CO_2 were more meaty, but less warmed-over and less oxidized in aroma ($P < 0.01$) compared with samples packaged in air. Similar results were found for flavor notes in that vacuum and N_2/CO_2 treated samples were more meaty, less warmed-over, cardboardy ($P < 0.001$) and oxidized ($P < 0.01$). Various kinds of modified atmosphere packaging have been reported to improve the sensory quality of precooked turkey (Nolan, 1987), precooked poultry (Hotchkiss et al., 1985), precooked

----- Vacuum -.-.-. N2/CO2 ——— Air

A : aroma

F : flavor

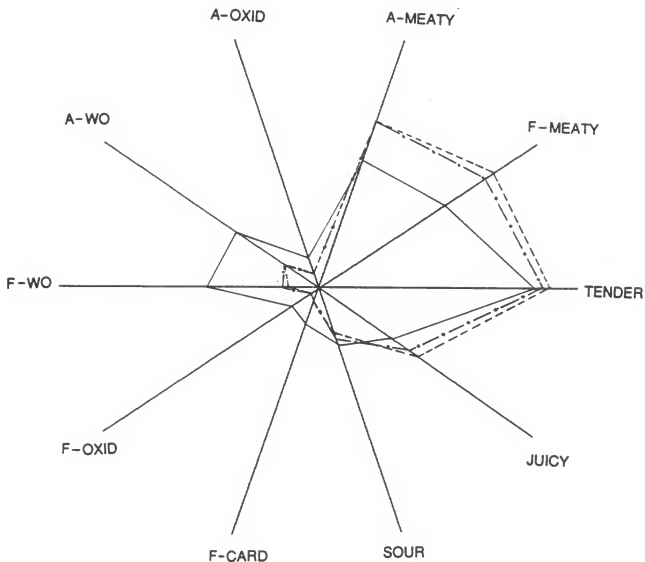


Figure 2 Quantitative descriptive analysis (QDA) plot of sensory characteristics for packaged cooked beef slices after 11-week frozen storage

pork (Nolan, 1987; Jones et al., 1987), and precooked beef (McDaniel, 1984) during refrigerated or frozen storage.

Cardboardy and rancid were the two primary sensory notes associated with stored or reheated off-flavor in meat (Lyon, 1988; St. Angelo et al., 1988). While in this frozen precooked beef study, meat was not rancid, the major off-flavor notes were warmed-over and cardboardy.

Higher oxidized aroma ($P < 0.10$) was found in the middle position of loins and this was consistent with the higher oxidized flavor scores in the middle position found in initial characteristics of beef. Mid position samples reached a temperature of 70°C , while samples from the other positions reached slightly higher temperature. As end point temperature increases, flavor notes such as mouth filling-blend, browned, meaty-roasted can develop and these flavor notes may help to mask warmed-over flavor.

TBA reactive substances

Higher TBA values ($P < 0.01$) were found in air-packaged samples than for other treatments. The overall mean of TBA values of air-packaged beef slices was 4-5 times that of vacuum and N_2/CO_2 packaged beef (Table 4 and Figure 3). TBA values were used as a measurement for development of warmed-over flavor in cooked beef (St. Angelo et al., 1987), cooked turkey (Wu and Shelton, 1988; Nolan, 1987; Smith and Alvarez, 1988) and cooked pork (Nolan, 1987). TBA values of modified atmosphere packaged precooked turkey and pork

Table 4-Aroma,¹ flavor,¹ pH, and TBA, hexanal and pentanal values for packaged cooked beef slices after 11-week frozen storage.²

Measurement	Atmosphere			Level of Significance
	Vacuum	N ₂ /CO ₂	Air	
Aroma:				
Meaty	5.1b	5.1b	3.9a	**
Warmed-over	1.3a	1.3a	2.9b	**
Oxidized ^e	0.4a	0.4a	0.9b	**
Flavor:				
Meaty	6.1b	5.9b	4.4a	***
Warmed-over	0.9a	1.0a	3.3b	***
Cardboard	0.5a	0.5a	1.2b	***
Oxidized	0.3a	0.3a	1.0b	**
Sour	1.4	1.5	1.8	NS
pH	5.96	6.09	6.02	NS
Fat, %	6.06	5.96	6.04	NS
TBA value				
(mg MA/kg meat)	0.04a	0.05a	0.20b	**
Hexanal (ppm)	0.30a	0.29a	0.82b	***
Pentanal (ppm)	0.04a	0.04a	0.10b	*

¹Intensity scale, 0=none to 12.7=very intense.

²Means of 6 replication; means with different letters are significantly different; NS, not different (P>0.10),

* P<0.05, ** P<0.01, *** P<0.001.

^eIndicates position effect (P<0.10).

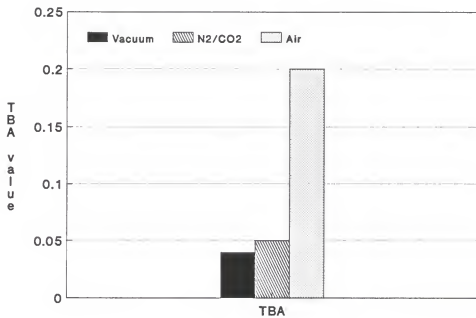


Figure 3 TBA values for packaged cooked beef slices after 11-week frozen storage

during 18-hour refrigerated and 3-month frozen storage were lower than those in air packages (Nolan, 1987).

TBA values were, in general, low in this study. Malonaldehyde is very reactive with other food components and other researchers have reported decreases in the TBA-value with increasing frozen storage of both raw and cooked meat (Chang et al., 1961; Witte et al., 1970; Gokalp et al., 1983). The TBA value is dependent upon the method of determination. The extraction method used in this study was reported to result in values 2-3 times lower than that determined by the distillation method (Williams et al., 1983; Siu and Draper 1978). Beef samples in this study were well packaged and held frozen which should minimize lipid oxidation.

Hexanal and pentanal

The retention time of hexanal was about 11.5 min; pentanal, 7 min, and the internal standard, 4-heptanone, 13.55 min (Figure 5). More hexanal ($P < 0.001$) and pentanal ($P < 0.05$) were found in air-packaged samples indicating further that storage of beef slices in vacuum and N_2/CO_2 inhibited lipid oxidation (Table 4 and Figure 4). The overall content of hexanal in air-packaged beef samples was 2.7-2.8 times that of beef in vacuum and N_2/CO_2 packages; pentanal, 2.5 times.

Low hexanal and pentanal contents accompanied with low TBA values were found to be good indicators for decreased

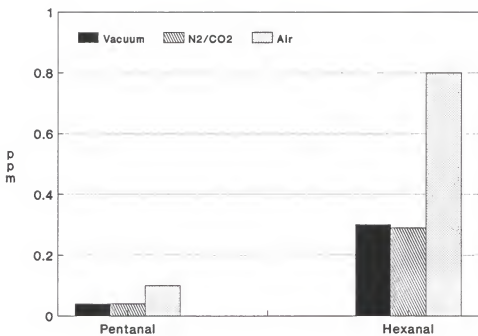


Figure 4 Amount of hexanal and pentanal for packaged cooked beef slices after 11-week frozen storage

IS : internal standard

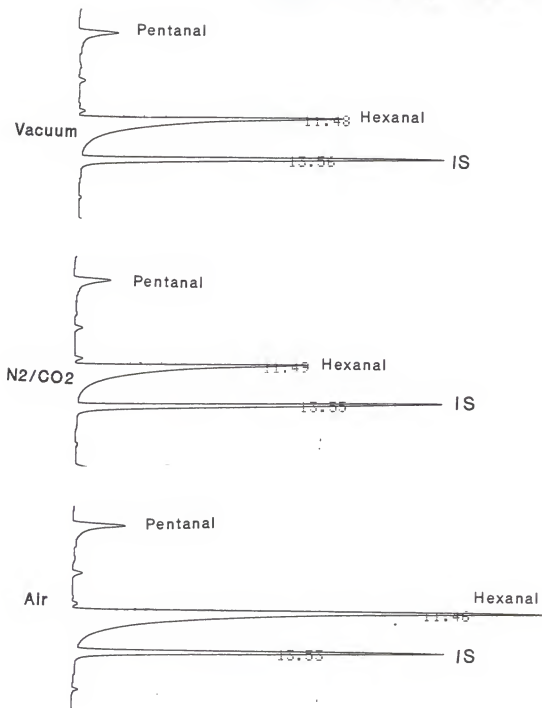


Figure 5 Gas chromatogram of hexanal and pentanal for packaged cooked beef slices after 11-week frozen storage

development of warmed-over flavor in ground beef (St. Angelo et al., 1987) and in this study.

Textural properties

Textural properties of beef were unaffected by packaging treatments as determined by both sensory and instrumental methods (Table 5). The difference in tenderness as noted initially was not present after storage and reheating.

Smith and Alvarez (1988) reported that shear strength decreased in vacuum cook-in-bag turkey rolls during storage. Miller et al. (1985) found that precutting subprimals into beef loin steaks, followed by reassembly and vacuum-package aging tended to decrease retail case life as well as juiciness and tenderness. Refrigerated precooked poultry packaged with 80% CO₂ was found tougher by a sensory panel after 2 weeks storage (Hotchkiss, 1985). Jones et al. (1987) reported that precooking did not increase cooking losses and that the palatability attributes (juiciness, tenderness and pork flavor) of vacuum-packaged precooked pork roasts were as good or better than freshly prepared roasts at both 14 and 28 days refrigerated or frozen storage. However, more research needs to be done to understand the effect of modified atmosphere packaging on textural properties of stored precooked meat.

The Instron shear force for meat slices from the posterior end of the loin was lower ($P < 0.05$) than for other loin positions.

Table 5-Tenderness,¹ juiciness,¹ total moisture, freezable water and shear force values for packaged cooked beef slices after 11-week frozen storage.²

Measurement	Atmosphere			Level of Significance
	Vacuum	N ₂ /CO ₂	Air	
Tenderness (Ease of mastication)	6.8	6.5	6.4	NS
Juiciness	3.6	3.2	2.7	NS
Moisture % ^e	55.6ab	55.0a	56.2b	+
Shear force ^e (Kg)	0.60	0.62	0.64	NS
Enthalpy ³ (cal/g)	28.0	28.3	30.1	NS
Freezable water ³ (g/g dry matter)	0.79	0.77	0.85	NS

¹Intensity scale, 0=none to 12.7=very intense.

²Means of 6 replications, means with different letters are significantly different; NS, not different (P>0.10), + P<0.10.

³Means of 3 replications; NS, not different (P>0.10).

^eIndicates position effect (moisture, P<0.01; shear force, P<0.05).

Total moisture and freezable water content

Percentage moisture of beef samples in air packages were slightly higher ($P < 0.10$) than those in N_2/CO_2 atmosphere, while samples vacuum packaged did not differ from these two extremes. Position also affected moisture content ($P < 0.01$). The posterior loin samples had lower moisture contents than samples from the other positions. The posterior end of loins appeared to be thinner than the anterior end and the internal temperature was higher. More evaporation as a result of higher temperature perhaps made this portion of loin less moist.

Freezable water content of beef slices determined by differential scanning calorimeter was not affected by treatment. Figure 7 in the Appendix (p. 70) shows the peak in DSC thermogram.

pH and fat content

Meat pH from different packaging treatments was not different after storage and reheating. Fat content was not affected by treatment or position.

Color

A minor difference in redness of beef was found when using Illuminant C, light source Northern daylight, but not with Illuminant A, the Horizon light. Beef samples vacuum packaged were redder ($P < 0.10$) than those packaged in N_2/CO_2 and air (Table 6). As in frozen stored precooked turkey, vacuum packaging treatment led to increased redness over

Table 6-HunterLab color values for packaged cooked beef slices after 11-week frozen storage.¹

Measurement	Atmosphere			Level of Significance
	Vacuum	N ₂ /CO ₂	Air	
ILLA				
L	43.33	43.19	42.20	NS
a	5.08	4.99	4.66	NS
b	2.99	3.03	2.80	NS
ILLC				
L	42.68	42.55	41.59	NS
a	1.82b	1.67a	1.61a	+
b	5.58	5.70	5.24	NS

¹Means of 6 replications, means with different letters are significantly different; NS, not different (P>0.10), + P<0.10.

meat packaged with 100% N₂ or 100% CO₂ or air (Nolan, 1987). Smith and Alvarez (1988) found that HunterLab a values of the internal slices of vacuum cooked turkey breast rolls varied inconsistently during 87 days storage at 4° C. Color started to fade to a grayish pink color after 47 days of refrigerated storage. They speculated that the pink color was promoted by reducing conditions and prevented by oxidizing conditions.

When meat heated to 70° C cools, the pigment proteins renature, pigments oxygenate, and the meat turns red due to formation of native protein oxymyoglobin (Fox, 1987). Beef cooked to well done (80° C) would normally have brown color from the denatured globin hemichrome (Fe⁺⁺⁺) pigment, but if meat is cooked or stored in a reducing atmosphere, the cooked pigment will be the denatured globin hemochrome (Fe⁺⁺) which has a strong pink hue (Cornforth et al., 1986; Fox, 1987). Neither of these findings explain the redder color found in frozen-stored vacuum-packaged precooked beef slices than in both N₂/CO₂ and air packages in our study. Ice crystals formed on the surface of precooked beef slices packaged in N₂/CO₂ and air but not on vacuum-packaged samples. This may have affected the color of reheated meat. More work is needed to understand the effect of packaging and storage on color of meat.

Comparison of Measurement Technique for Lipid Oxidation

Correlation coefficients between sensory scores and instrumental or chemical measurements ranged from 0.47 to 0.96. Correlation coefficients between sensory scores and TBA value were high and ranged from 0.73 to 0.96 ($P < 0.001$); sensory scores and hexanal, 0.57-0.89 ($P < 0.05-0.001$); and sensory scores and pentanal, 0.47-0.79 ($P < 0.10-0.001$, Table 7). Of particular importance is the relationship among chemical and instrumental measurement of lipid oxidation in relation to particular flavor notes. Correlation coefficients for TBA value were highest for oxidized flavor ($r = 0.96$, $P < 0.001$) and correlation coefficients for hexanal and pentanal contents were highest for warmed-over flavor (hexanal, $r = 0.89$, $P < 0.001$; pentanal, $r = 0.79$, $P < 0.01$). High correlation was also found between TBA value and hexanal, 0.81 ($P < 0.001$) and pentanal, 0.72 ($P < 0.01$, Table 8).

The TBA method is the most widely used method in measuring extent of lipid oxidation, but trained taste panels were more sensitive in detecting lipid oxidation than TBA method (Nolan, 1987; Lannelongue et al., 1982; Younathan et al., 1980). In this beef study, TBA test, aldehyde measurement by gas chromatograph, and sensory evaluation all effectively measured lipid oxidation. Gas chromatographic techniques are also capable of detecting numerous volatile components which contribute to off-flavor development in their native proportions and thus would more closely

Table 7-Correlation coefficients between sensory scores and TBA, hexanal and pentanal values for packaged cooked beef slices after 11-week frozen storage

	TBA value		Hexanal		Pentanal	
	r	level of significance	r	level of significance	r	level of significance
Aroma:						
Meaty	-.76	***	-.80	***	-.72	**
Warmed-over	.73	***	.60	**	.56	*
Oxidized	.74	***	.57	*	.47	+
Flavor:						
Meaty	-.86	***	-.80	***	-.71	**
Warmed-over	.89	***	.89	***	.79	**
Cardboard	.83	***	.84	***	.75	***
Oxidized	.96	***	.84	***	.75	***

Level of significance of correlation coefficients; + P<0.10, * P<0.05, ** P<0.01, *** P<0.001.

Table 8 Correlation coefficients between TBA values and amount of hexanal and pentanal for packaged cooked beef slices after 11-week frozen storage

	Hexanal		Pentanal	
	r	level of significance	r	level of significance
TBA value	0.81	***	0.72	**

Level of significance of correlation coefficients; ** P<0.01, *** P<0.001.

approximate the human senses than analyzing a group of TBA reactive substances. Sensory quality of food has been and will continue to be more important than any chemical and instrumental results in detecting whether stored packaged precooked products are acceptable by consumers. Chemical and instrumental parameters are meaningless if they are not correlated to sensory attributes such as appearance, flavor and textural properties.

Summary and Conclusion

The effect of modified atmosphere packaging on reducing the development of warmed-over flavor in precooked beef loins during storage was investigated. Three treatments for packaging were used: vacuum, 80% N₂ and 20% CO₂ gas filling and air. Packaged cooked beef slices were evaluated immediately after packaging and after 11 weeks frozen storage for flavor, texture and color by sensory, chemical and instrumental methods. No difference among treatments was found in beef samples without storage. After 11-week frozen storage, samples in vacuum and N₂/CO₂ packages were more meaty, but less warmed-over, oxidized and cardboardy as determined by a sensory panel than those in air-containing packages. Higher TBA values, more hexanal and pentanal were found in air-packaged samples indicating further that storage of beef slices in vacuum and N₂/CO₂ inhibited lipid oxidation and thus, warmed-over flavor. The overall mean of TBA values for air-packaged beef slices was 4-5 times that

of vacuum and N_2/CO_2 packages; hexanal, 2.7-2.8; and pentanal, 2.5. The correlation coefficients between warmed-over flavor sensory attributes and TBA value was 0.87; warmed-over flavor and hexanal, 0.89; and pentanal, 0.79. Textural properties of beef were unaffected by packaging treatments as determined by both sensory and instrumental methods. Vacuum-packaged precooked beef had higher HunterLab a values than those in N_2/CO_2 and air packages after 11 weeks frozen storage.

Exclusion of oxygen by vacuum and N_2/CO_2 packaging prevented formation of unpleasant off-flavors and had little adverse effect on color and textural properties of cooked beef slices. Sensory, instrumental and chemical methods were all effective in detecting the warmed-over flavor of stored packaged precooked beef.

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APPENDIX

PLEASE EVALUATE SAMPLES IN THE FOLLOWING ORDER _____

FORM FOR SENSORY ANALYSIS OF BEEF

NAME _____
DATE _____

INDICATE INTENSITY OF EACH CHARACTERISTIC BY DRAWING A VERTICAL LINE AT THE POINT REPRESENTING THE PERCEIVED INTENSITY.

AROMA:

MEATY/BEEF	NONE	MODERATE	STRONG
WARMED-OVER			
OXIDIZED/RANCID			

FLAVOR:

MEATY/BEEFY				
WARMED-OVER				
CARDBOARD				
OXIDIZED/RANCID				
SOUR ¹				

TENDERNESS:

EASE OF MASTICATION				
---------------------	--	--	--	--

JUICINESS¹

--	--	--	--	--

¹ACIDIC SOUR, NOT SERUMY SOUR. SUSTAINED JUICINESS.

Figure 6 Sensory scorecard
(75% reduction of original size)

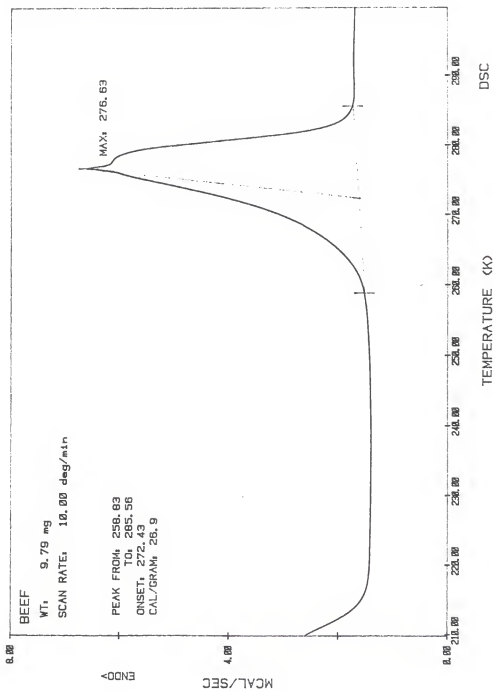


Figure 7 Differential scanning calorimetric thermogram for packaged cooked beef slices after 11-week frozen storage

Table 11 Continued

PK	ACTY	FWG	FCARC	FCXID	FSCUR	TENDER	JUICY
0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9
10	10	10	10	10	10	10	10
11	11	11	11	11	11	11	11
12	12	12	12	12	12	12	12
13	13	13	13	13	13	13	13
14	14	14	14	14	14	14	14
15	15	15	15	15	15	15	15
16	16	16	16	16	16	16	16
17	17	17	17	17	17	17	17
18	18	18	18	18	18	18	18
19	19	19	19	19	19	19	19
20	20	20	20	20	20	20	20
21	21	21	21	21	21	21	21
22	22	22	22	22	22	22	22
23	23	23	23	23	23	23	23
24	24	24	24	24	24	24	24
25	25	25	25	25	25	25	25
26	26	26	26	26	26	26	26
27	27	27	27	27	27	27	27
28	28	28	28	28	28	28	28
29	29	29	29	29	29	29	29
30	30	30	30	30	30	30	30
31	31	31	31	31	31	31	31
32	32	32	32	32	32	32	32
33	33	33	33	33	33	33	33
34	34	34	34	34	34	34	34
35	35	35	35	35	35	35	35
36	36	36	36	36	36	36	36
37	37	37	37	37	37	37	37
38	38	38	38	38	38	38	38
39	39	39	39	39	39	39	39
40	40	40	40	40	40	40	40
41	41	41	41	41	41	41	41
42	42	42	42	42	42	42	42
43	43	43	43	43	43	43	43
44	44	44	44	44	44	44	44
45	45	45	45	45	45	45	45
46	46	46	46	46	46	46	46
47	47	47	47	47	47	47	47
48	48	48	48	48	48	48	48
49	49	49	49	49	49	49	49
50	50	50	50	50	50	50	50
51	51	51	51	51	51	51	51
52	52	52	52	52	52	52	52
53	53	53	53	53	53	53	53
54	54	54	54	54	54	54	54
55	55	55	55	55	55	55	55
56	56	56	56	56	56	56	56
57	57	57	57	57	57	57	57
58	58	58	58	58	58	58	58
59	59	59	59	59	59	59	59
60	60	60	60	60	60	60	60
61	61	61	61	61	61	61	61
62	62	62	62	62	62	62	62
63	63	63	63	63	63	63	63
64	64	64	64	64	64	64	64
65	65	65	65	65	65	65	65
66	66	66	66	66	66	66	66
67	67	67	67	67	67	67	67
68	68	68	68	68	68	68	68
69	69	69	69	69	69	69	69
70	70	70	70	70	70	70	70
71	71	71	71	71	71	71	71
72	72	72	72	72	72	72	72
73	73	73	73	73	73	73	73
74	74	74	74	74	74	74	74
75	75	75	75	75	75	75	75
76	76	76	76	76	76	76	76
77	77	77	77	77	77	77	77
78	78	78	78	78	78	78	78
79	79	79	79	79	79	79	79
80	80	80	80	80	80	80	80
81	81	81	81	81	81	81	81
82	82	82	82	82	82	82	82
83	83	83	83	83	83	83	83
84	84	84	84	84	84	84	84
85	85	85	85	85	85	85	85
86	86	86	86	86	86	86	86
87	87	87	87	87	87	87	87
88	88	88	88	88	88	88	88
89	89	89	89	89	89	89	89
90	90	90	90	90	90	90	90
91	91	91	91	91	91	91	91
92	92	92	92	92	92	92	92
93	93	93	93	93	93	93	93
94	94	94	94	94	94	94	94
95	95	95	95	95	95	95	95
96	96	96	96	96	96	96	96
97	97	97	97	97	97	97	97
98	98	98	98	98	98	98	98
99	99	99	99	99	99	99	99

Table 12 Chemical and instrumental analytical data for packaged cooked beef slices after 11-week frozen storage

SL	FO	REF	TRT	H2O	EAT	PH	FL	FA	J	U	U	U	A	FA	TSR	HEX	HEXAL	PENT	PENTANAL
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Abbreviations used in the Appendix

SQ = square

POS = position

REP = replication

TRT = treatment

VACUUM = vacuum packaging

N₂/CO₂ = 80 % N₂ and 20 % CO₂ gas filling packaging

AIR = heat sealing of packaging bag

JUDGE = panelist

AMEATY = meaty aroma

AWO = warmed-over aroma

AOXID = oxidized aroma

FMEATY = meaty flavor

FWO = warmed-over flavor

FCARD = cardboardy flavor

FOXID = oxidized flavor

FSOUR = sour flavor

TENDER = tenderness

JUICY = juiciness

H₂O = percentage of moisture

FAT = percentage of fat

ILLA_L = HunterLab lightness (L) measured with Northern day
light

ILLA_A = HunterLab red/green (a) measured with Northern day
light

ILLA_B = HunterLab yellow/blue (b) measured with Northern day
light

ILLC_L = HunterLab lightness (L) measured with Horizen light

ILLC_A = HunterLab red/green (a) measured with Horizen light

ILLC_B = HunterLab yellow/blue (b) measured with Horizen
light

Instron = shear force in kg measured with Instron Universal
Testing Instrument

DSC = enthalpy (ΔH) measured with differential scanning
calorimeter

FREEH2O = freezable water content measured with
differential scanning calorimeter

Thiobarbituric Acid (TBA) Test

REAGENTS

1. 9% perchloric acid solution (150 ml 60% perchloric acid/liter). Keep refrigerated.
2. 0.02 M TBA reagent (1.4415 g 2-thiobarbituric acid/500ml).

Dilute to volume with distilled water. Use a hot plate on low heat and a magnetic stirrer to dissolve TBA. Place an inverted beaker over the top of the flask to let steam escape. Refrigerate, after TBA dissolves, until used. Prepare fresh every time before use.

PROCEDURE

1. Accurately weigh 10.0 g ground cooked sample into a Virtis jar.
2. Add 15 ml cold 9% perchloric acid and 20 ml cold, distilled water.
3. Blend in homogenizer at moderate speed for 10 seconds.
4. Filter the blended sample through a Whatman No. 2 filter paper into 50 ml erlenmeyer flasks. Rinse the Virtis jar with 5 ml distilled water and add through the filter paper.
4. Shake filtrate to mix and pipette 5 ml filtrate into a test tube and add 5 ml 0.02 M TBA reagent.
5. Cover, mix and store in the dark for 15 hr. (15-17 hr.).
6. Determine the absorbance on a Perkin Elmer ultraviolet spectrophotometer at 530 nm.

STANDARDS

1. Prepare stock solutions of TEP (1,1,3,3-tetraethoxy propane).

Weigh 0.233 g TEP into a small glass beaker (5 or 10 ml). Quantitatively transfer the TEP to a 1000 ml volumetric flask with several washings of distilled water. This is 1×10^{-6} moles/ml TEP solution. Transfer 1 ml of 1×10^{-6} TEP solution to a 100 ml volumetric flask and dilute to volume with distilled water. This stock solution contains 5×10^{-8} moles TEP/5 ml and is the one used for standards each time. Keep both TEP solutions refrigerated.

2. With each group of meat samples, run TEP standards with concentrations of 0.25, 0.5, 0.75, and 1×10^{-8} moles TEP/5 ml.
3. To 5 ml TEP solution in a test tube, add 5 ml TBA reagent.
4. Cover, mix and store in the dark for 15 hr.
5. Read the absorbance on a spectrophotometer at the same time as meat samples.

CALCULATIONS

1. Using the concentrations and absorbances of the standards, determine the regression equation for the standard curve. The regression equation is: sample concentration + (absorbance - intercept estimate)/concentration estimate. Also check the R-square; if it is low (below 95 or so) something may be wrong with the

standard solutions.

2. From the regression equation, determine the concentration of each sample.
3. Multiple the sample concentration by 0.72 to determine the mg malonaldehyde/ kg meat. The factor of 0.72 was derived from hydrolysis:

(100%) of TEP to malonaldehyde (MA), which weighs 72 g/mole. The 5 ml filtrate analyzed is equivalent to 1 g meat, so X (sample concentration) $\times 10^{-8}$ moles MA can be converted to $X \times 10^{-5}$ moles MA/kg meat. Change moles MA/kg meat to g MA/kg meat yields $72X \times 10^{-5}$ g MA/ kg meat. After converting g to mg, the equation becomes $72X \times 10^{-2}$ mg MA/ kg meat or .72X mg MA/kg meat. TBA values are equivalent to mg MA/kg meat.

Aldehydes by Gas Chromatograph

INTERNAL STANDARD

1. 0.084 ppm 4-heptanone solution: pipette 104 μ l of 97% 4-heptanone (specific gravity = 0.81) into a 100 ml volumetric flask, make it to the volume and mix well. This is the stock solution, keep refrigerated. Dilute the stock solution 10^4 fold into 0.084 ppm 4-heptanone solution before use.

DISTILLATION

1. Transfer 5 grams of blended meat sample into an Aminco micro-Kjeldahl distillation flask, add 10 ml 0.084 ppm 4-heptanone solution.
2. Add 0.5 ml distilled water to a 15-ml graduated conical test tube and place the tube in an ice water bath maintained well above the 5 cm graduation mark on the test tube.
3. Connect a 10 cm tapered glass tube (Pasteur pipette) to the outlet tube of the condenser so its end fits within 3 mm of the bottom of the conical test tube.
4. Distill the sample and collect exactly 5 ml distillate, quickly pipette 2 ml aliquots into a 5-ml serum vial containing 2.2 g anhydrous sodium sulfate, and immediately seal with a serum cap by a manual crimper.
5. Refrigerate the capped vials until they are ready for injection into gas chromatograph.

HEADSPACE INJECTION

1. Place the vial in a 60° C water bath for 2 min, on a Burrell wrist action shaker for 5 min at room temperature and back to the 60° C bath for an additional 8 min.
2. Withdraw 1 ml of vapor from the headspace of the serum vial by inserting the needle of a 1-ml gas tight syringe through the serum cap.
3. Inject the headspace gas into gas chromatograph.

CALCULATION

Area under a peak was integrated by an Altex model C-RIA electronic integrator. Peak size standardization and peak quantitation was done by internal standard method (Lee, et al., 1984). A calibration curve was done using 5 ml solutions containing 0.05, 0.1, 0.5, 1 and 1.5 ppm hexanal or 0.05, 0.1, 0.15 and 0.2 ppm pentanal and 10 ml 0.084 ppm 4-heptanone standard solution. These solutions for the standard curve were carried through the sample pretreatment steps (i.e. distillation) and were then chromatographed. A plot of mass ratio vs. area ratio is then made.

The plot was linear for both hexanal ($r=0.99$) and pentanal ($r=0.98$) in this study, and the response factor was calculated from the slope.

The technique is advantageous because it minimizes quantitative errors due to sample preparation and injection, allows the quantitation of one or more than one component in the sample matrix and requires that chromatographic

resolution only be optimized for the separation of the components of interest and the internal standard.

SENSORY, CHEMICAL AND INSTRUMENTAL ANALYSES
OF FLAVOR, TEXTURE AND COLOR OF PRECOOKED BEEF
FROZEN AND STORED IN MODIFIED ATMOSPHERE PACKAGING

by

SYOU-YU HWANG

B.S., National Taiwan University, 1982

AN ABSTRACT OF A THESIS

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requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

KANSAS STATE UNIVERSITY
Mahattan, Kansas

1988

The effect of modified atmosphere packaging on characteristics of precooked beef slices during storage was investigated. Three treatments for packaging were used: vacuum, 80% N₂ and 20% CO₂ gas flushing and air. Packaged cooked beef slices were evaluated immediately after packaging and after 11 weeks frozen storage (-20° C) for flavor, texture and color by sensory, chemical and instrumental methods.

Little difference among treatments was found in beef samples without storage. After 11-week frozen storage samples in vacuum and N₂/CO₂ packages were more meaty, less warmed-over, less oxidized and less cardboardy as determined by a sensory panel than those in air-containing packages. Higher TBA values, more hexanal and pentanal were found in air-packaged samples indicating further that storage of beef slices in vacuum and N₂/CO₂ inhibited lipid oxidation and thus, warmed-over flavor. The correlation coefficients between warmed-over flavor sensory attributes and TBA value was 0.87; and warmed-over flavor and hexanal, 0.89 and pentanal, 0.79.

After storage and reheating, textural properties of beef were unaffected by packaging treatments as determined by both sensory and instrumental methods. Vacuum-packaged precooked beef had higher HunterLab a values than those in N₂/CO₂ and air packages after 11 weeks frozen storage.