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The effect of post-mortem aging on meat flavor quality in Brangus beef. Correlation of treatments, sensory, instrumental and chemical descriptors

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The flavor of muscle foods is dependent upon factors such as the animal's age, breed, sex, nutritional status and manner of cooking. Most important to the final flavor of the meat is relative age in the post-mortem aging process as it is during this time that many chemical flavor components are formed (Spanier *et al.*, 1990). These components serve either directly as flavor components or as a pool of reactive flavors and intermediates that form many of the characteristic meat flavors after cooking. The post-mortem aging process is identified with enhancement of beef sensory quality due to enhanced tenderization. While this is true for beef texture, it is not true for the overall flavor of meat. Data is shown indicating that, during post-mortem aging, desirable flavors such as beefy, brothy, browned-caramel, and sweet decline while off-flavors such as bitter and sour increase. Published by Elsevier Science Ltd

INTRODUCTION

Several ante mortem factors, e.g. age, breed, sex, nutritional status of the animal, muscle type (Carmack *et al.*, 1995), final end-point cooking temperature (Spanier & Miller, 1996), manner (moist, dry, convection, microwave, etc.) of cooking, and manner (type and duration) of storage, contribute to meat flavor quality (Imafidon & Spanier, 1994; Smulders *et al.*, 1992). However, the flavor and textural changes that occur during the post-mortem aging period and during subsequent cooking and storage (Spanier & Miller, 1996) are among the most important contributors to final meat flavor. Meat shows a significant alteration in the level of numerous chemical components (sugars, organic acids, peptides and free amino acids, and metabolites of adenine nucleotide metabolism such as ATP) during the post-mortem aging period. Many of these changes are due to hydrolytic activity (Dransfield, 1994; Koohmaraie, 1994; Spanier *et al.*, 1990). These compounds serve as a pool of reactive flavor chemicals and intermediates which interact to form additional flavor characteristics during cooking. It is apparent, therefore, that the development of meat flavor quality is a highly dynamic process. It is the objective of this investigation to examine the effect of post-mortem aging on beef sensory characteristics as a function of conditioning-dependent development of flavor precursor compounds and other flavor-producing components.

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MATERIALS AND METHODS

Materials

Matched-grade animals were slaughtered by traditional means, hung, and the inside, top-round (*semimembranosus* muscle) removed within 1 h. Animals were Brangus breed types at 5/8 Angus and 3/8 Brahman. The animals were maintained for 166 days on corn-soybean meal concentrate diet consisting of 79% TDN (total digestible nutrients), 12% CP (crude protein), and 10% F (fiber). Averaged final attributes at slaughter from the duplicate animals were as follows: hot carcass weight, 374.4 kg, USDA grading and certification marbling score range from slight^{55} to small^{20}, USDA quality grade range from select+ to choice−, back fat average 11.5 mm, ribeye area average 76.8 cm^2, and a 3.4 average yield grade.

The *semimembranosus* muscle from the right and left hind quarter of each animal was used. After trimming of all gross fat and connective tissue the muscle was longitudinally sliced into four strips per animal muscle with each slice constituting one 'Rep'. Each of the 16 Reps was cut crosswise into six portions (samples). Each sample was given a numerical notation for tracking. All samples were immediately vacuum-packed in oxygen impermeable bags (to minimize potential lipid oxidation; Spanier *et al.*, 1992*a,b*) and stored in a cold room at 4°C. A sample of each Rep was removed for analysis (see Burger preparation and Sensory analysis sections) at 0 h (+45 min), 4 h, 2 days, 4 days, 7 days, and 14 days. Sample collection within each Rep was randomly determined prior to sampling. Each Rep was examined four separate times/dates by the panel of 12 trained descriptive sensory panelists resulting in data from 16 Reps evaluated during four separate panel presentations/analysis by 12 trained panelists. Temperature and pH of each sample were determined at the times indicated.

Analyses on cooked beef

Ground beef patty preparation

Each sample of each Rep was prepared by grinding the beef with two passes through a 1.0 cm hole grinding disc, followed by two additional passes through a disk with 0.75 cm holes (General™ Slicer/grinder, Model MC-100). The ground meat was portioned into patties weighing 85.0 gram ±0.02. The samples of each Rep were frozen until presentation to the sensory panel. Cooking of each sample was on a Farberware™ grill for 7 min on each side yielding burgers having an appearance of medium to medium/well done.

Thiobarbituric acid reactive substances (TBARS)

TBARS were used to indicate the degree of lipid oxidation and rancidity and were measured by the distillation procedure of Tarladgis *et al.* (1960). The reddish/pinkish chromogen formed by the assay was measured at 532 nm and served as an indication of lipid oxidation and thus indirectly the food flavor quality.

Gas chromatographic (GC) analysis of flavor volatiles

The packed-column GC procedure (Dupuy *et al.*, 1987) was utilized and consisted of a modification of a procedure originally developed by Dupuy *et al.* (1987) for foods other than meat. The column was packed with a Tenax thermostable polymer (2,6-diphenyl-p-phenylene oxide, 60-80 mesh coated with 7% poly-m-phenoxylene). Analysis of the flavor volatiles from the beef patties was performed using an MT-220 gas chromatograph (Tracor, Austin, Texas) with dual independent hydrogen flame detectors; data were collected using an MT22 Westronics recorder and an Hewlett-Packard 3357 automated data system.

Sensory analysis

Descriptive sensory profiles of beef patties were generated by the Spectrum™ method described by Johnsen and Civille (1986). Sensory attributes used included those listed in Table 1 (modified from Johnsen & Civille, 1986). The panel consisted of 12 staff members from the Southern Regional Research Center who were trained in descriptive sensory analysis using a 15 point universal intensity scale similar to that described by Meilgaard *et al.* (1987). All panelists were fully trained and their proficiency statistically tested by Dr Jane Love (Love, 1988) before their data were used in experiments. The majority of the panelists had served on the meat sensory panel for over two years prior to these experiments.

Analyses on raw beef

Sample preparation

Beef extracts (10% w/v) were prepared using the ground raw beef at each time and homogenized with milli-Q water containing 0.02% sodium azide as bacteriostat. The supernatant solution obtained after centrifugation at 30 000 *g* (low-speed beef extract in a Sorvall RC-5B Dupont Inst., Wilmington, DE) was used for electrophoretic analysis.

Electrophoresis

Sodium dodecyl sulfate (SDS) poly-acrylamide gel electrophoresis (PAGE) was performed using a

Table 1. Lexicon of meat flavor descriptors

Salty (STY)	The taste on the tongue associated with sodium ions
Cooked beef (BEF)	The aromatics commonly found associated with matured cooked beef muscle products and found in the broth of boiled beef
Brothy (BRO)	The aromatics associated with the drippings from roasted meat which is characteristic of all meat, i.e. poultry, beef, pork, etc.
Painty (PTY)	The aromatic associated with rancid oil and fat (distinctly like linseed oil)
Serumy (SER)	The aromatic associated with raw beef lean
Browned/caramel (BRC)	The aromatic associated with the outside of grilled or broiled beef (seared but not blackened/burnt)
Cooked liver (CKL)	The aromatic associated with the cooked organ meat liver
Cardboard (CBD)	The aromatic associated with slightly stale beef (refrigerated for a few days only) and associated with wet cardboard and stale oils and fats
Sweet (SWT)	The taste on the tongue associated with sugars
Sour (SOU)	The taste on the tongue associated with acids
Bitter (BTR)	The taste on the tongue associated with bitter agents such as caffeine, quinine, etc.

Pharmacia® PhastSystem electrophoresis unit with precast gradient gels (8–25% with a 4% stacking gel). SDS-PAGE was performed to examine the distribution of proteins in the samples. Electrophoresis of solubilized (Spanier & Bird, 1982) low-speed beef extracts were performed following the manufacturer's directions.

Capillary electrophoresis (CE)

The CE analysis was performed on a BioRad® HPE-100® high performance capillary electrophoresis unit to examine the peptide composition of the low-speed beef extracts. The separating column was a 20 cm×25 μm coated capillary and peaks were detected at 200 nm. Samples were electrokinetically loaded onto the capillary for 8 s at 8 kV. Electrophoresis was run for 10 min at 8 kV (constant voltage) with amperage averaging 13.4 μA and a constant temperature of 24°C. The running buffer was 0.1 M phosphate buffer at pH 2.5. Samples (low-speed beef extracts) were diluted with 0.01 M phosphate buffer. Recording chart speed was set to 1 cm/min, and the sensitivity was set at 0.05 AUFS.

Statistical analysis

The PC version of SAS® (1985) was used to perform all statistical analysis. Panel averages per Rep were analyzed after statistical removal of outliers from the raw data. Principal components solutions were obtained by factor analysis of the relationships among the sensory, chemical and instrumental attributes. Unrotated factor scores from this analysis were then averaged for each experimental combination (defined by the design matrix) and bivariate plots were produced.

Influence of pH on beef homogenates

Homogenates were prepared from 20 g of raw, finely minced, top-round at 45 min *post mortem* placed in 180 mL of 100 mM phosphate buffer containing 137 mM sodium chloride and 2.7 mM potassium chloride. The buffer (before homogenization) and the samples (after homogenization) were adjusted to either pH 4.0, 5.0, 5.5, 6.0, or 7.0. Homogenization was by three, 5 second bursts at full speed using a Tekar Tissuemizer® (Cinncinatti, OH). The homogenized sample was passed through two double layers of cheesecloth to remove any unhomogenized tissue, fat and connective tissue. The resulting filtrate was brought to 200 mL with the phosphate buffered saline at the appropriate pH. Samples at each pH were divided into two groups. To one group the proteolytic inhibitors, pepstatin-A and leupeptin, were added to a final concentration of 2 μM and 10 μM, respectively. The second group was diluted with an equal volume of the same buffer but without the inhibitors. Each sample was placed in cold storage at 4°C and removed at intervals of 0, 2, 4, 7, and 9 days. The pH of each sample was monitored each day and remained stable throughout the 9 days of the experiment. Lack of change in pH also indicated that microbial contamination was minimal. Upon removal from the cold storage, further degradation was avoided by the addition of leupeptin and pepstatin to samples not containing inhibitors. One milliliter of 10% trichloroacetic acid (TCA) was added to 1.0 ml of stored meat homogenate. The TCA-precipitated homogenate was centrifuged for 5 min in a Beckman Microfuge 12 centrifuge at speed setting 6–7. The polypeptides contained in the supernatant solution were assessed in each sample using the bicinchoninic acid (BCA) procedure of Smith *et al.* (1985).

RESULTS

Change in pH and temperature

Two days of post-mortem aging were required for the meat to drop from an initial pH of 6.5 to a uniform pH of 5.4 (Fig. 1A). During the remaining 14 days in cold storage, the pH remained stable at an approximate average of 5.4 pH units. Temperature of round portions, like pH, dropped to its lowest level after 2 days of post-mortem aging and remained at that level until the completion of the experiment (Fig. 1A).

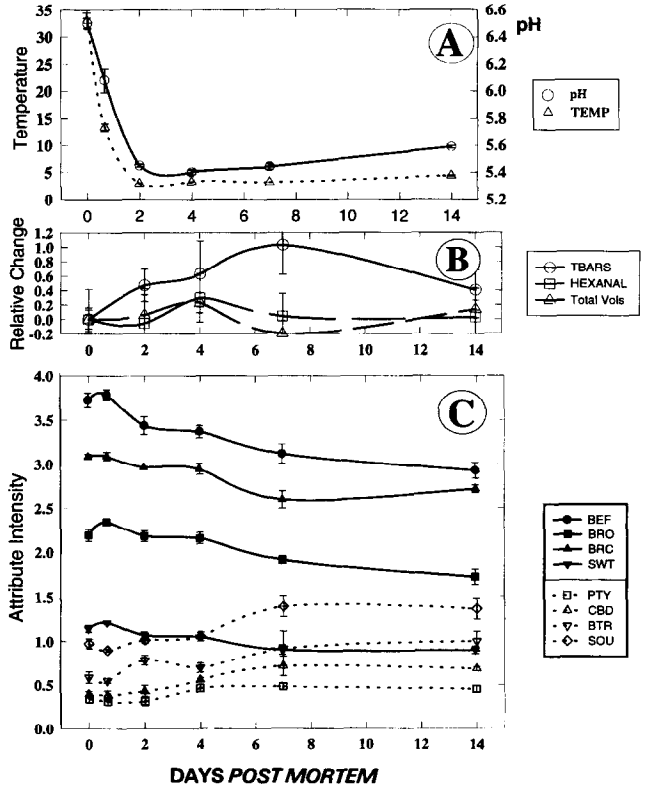

Fig. 1. (**1A**) effect of storage at 4°C on the pH and temperature of aging meat. (**1B**) Effect of post-mortem aging on thiobarbituric acid substances (TBARS), hexanal, and total lipid volatiles ±sem. (**1C**) The effect of post-mortem aging on the sensory characteristics of inside top round ±sem. Storage of the samples was at 4°C for the time indicated. Panelists were trained as described in the text.

Changes in products of lipid oxidation during post-mortem aging

Thiobarbituric acid reactive substances (TBARS), hexanal and total volatiles were used as indicators of lipid oxidation and rancidity development in the meat. No significant change in the products of lipid oxidation was seen (Fig. 1B) based on the overlap of the standard error bars. The lack of appreciable change in these markers of lipid oxidation and rancidity development is not surprising for two reasons. First, the samples were vacuum sealed in oxygen-impermeable bags that would hinder the initiation of oxidation. Second, the model showed negligible lipid oxidation because it used intact meat which exposes less surface area to the environmental oxygen than would a comparable weight of ground beef (Spanier & Miller, 1996).

TBARS and hexanal and total volatiles which are normally strongly correlated with each other in experimental models of warmed-over flavor (WOF) development (St. Angelo *et al.*, 1988), clearly do not show a strong correlation to each other in this post-mortem aging model. Furthermore, these off-flavor/rancidity markers, which normally show strong correlation with the undesirable flavors of PTY and CBD in WOF model systems, are not correlated with any sensory attribute in this non-WOF model.

Changes in sensory attributes with post-mortem aging

Figure 1C shows the change in intensity of several top round sensory attributes as a function of post-mortem storage. During the post-mortem aging there is a gradual decline in the flavor descriptors beefy (BEF), brothy (BRO), browned/caramel (BRC), and sweet (SWT) typically associated with desirable flavor. The intensity (IU) of these descriptors show a second order rate-of-decline of −0.13, −0.05, −0.10, and −0.05 IU/day, respectively. On the other hand, the undesirable aromatic flavor descriptors, painty (PTY) and cardboard (CBD), and the taste descriptors, bitter (BTR) and sour (SOU) show a moderate rate-of-increase in intensity of 0.04, 0.07, 0.06, and 0.08, respectively. Correlation coefficients of linear regression analysis of the rates and of ANOVA analysis of unlike groups were better than 0.9 in all cases.

A correlation matrix (not shown) was prepared to compare the sensory attributes over the range of treatments (days of post-mortem aging). The statistical evaluation indicated a very strong negative correlation between the bitter taste (BTR) and the more desirable flavors beefy (BEF), brothy (BRO), browned/caramel (BRC), and sweet (SWT). On the other hand, the taste of sour (SOU) gave a strong negative correlation only to browned/caramel (BRC). The undesirable aromatic flavor painty (PTY) had a strong negative correlation only with beefy (BEF), while the undesirable aromatic flavor cardboard (CBD) had a strong negative correlation with both beefy (BEF) and browned/caramel (BRC). All four desirable flavor descriptors, i.e. BEF, BRO, BRC, and SWT, had a strong positive correlation with each other. All of the sensory descriptors seem to have a sudden 'hump' or change between 4 and 7 days *post mortem*.

Changes in protein composition with post-mortem aging

SDS-page

Analyses of changes in protein composition of aging beef semimembranosus muscle show several peaks that change during the post-mortem aging process. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) of low speed (30 000 *g*) extracts from beef indicate that a protein of approximately 10 000 Dalton molecular weight reaches peak concentration by 4 days *post mortem* (Fig. 2) and then disappears by 7 days. By 14 days the majority of the material of protein origin found in the post-30 000 *g* extract of beef is of less than 6400 Dalton molecular weight. Since small peptides are not well resolved on PAGE gels, partially due to diffusion out of the gel during post-run fixation, another method, capillary electrophoresis (CE), was chosen.

CE separates small, charged compounds on the basis of the mass to charge ratio. Different protein peaks are distinguished by their distinct electrophoretic mobility and are labeled with a number preceded by the letters, 'CE'. The most significant change is the disappearance of CE-3 along with the appearance of CE peaks 4 and 5 (Fig. 3) and other minor peaks. The area under each electrophoretic peak shows that CE-3 is depleted during the post-mortem aging process (Fig. 4). A major change in protein profile is seen between 4 and 7 days and is temporally related to the change seen in the sensory

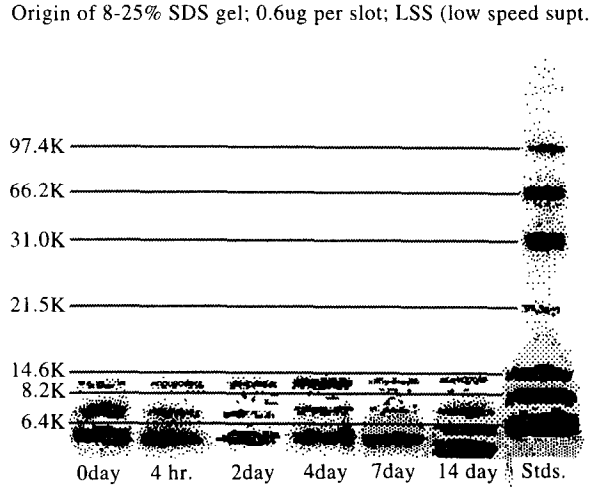

Fig. 2. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) of extracts obtained from the post-30 000 *g* supernatant solution of homogenized beef *semimembranosus* muscle aged for the time indicated on the figure. The gel was an 8–25% acrylamide gradient containing 0.6 μg protein per column (slot). Molecular mass of standards are written on the left in thousands. Gels were stained with coomassie blue.

data (Fig. 1C). Proteolysis of large peptides or proteins could result in a compositional/conformational change as proteases make a series of 'nicks' in CE-3 creating other fragments such as CE-4, CE-6, CE-7, and CE-11 (Figs 3 and 4).

Calibration standards of CE

Comparison of the migration times of the muscle extracts with known calibration standards revealed that CE-3 had a relatively broad retention time of 4 min 27 s to 4 min and 30 s, and the well-characterized flavor-enhancing octapeptide of beef STEP (savory taste enhancing peptide) had an electrophoretic migration time of 5 min 3 s (see 'O' in Fig. 5).

Multivariate factor analysis

Application of multivariate analysis by principal components solutions to the sensory, instrumental, and chemical data and treatments yields a bivariate plot of the variable factors (Fig. 6). This statistical analysis provides an empirical summary of the pattern of inter-correlations among the variables. The data for the flavor attributes are segregated in different regions of the grid. For example, the desirable flavors SWT, BRC, BEF, and BRO cluster in the upper left of the plot, while the undesirable flavors such as CBD, PTY, BTR and SOU cluster in the lower right of the plot. Chemical attributes, such as TBARS, hexanal, and total volatiles, which seem to have no effect or correlation with the

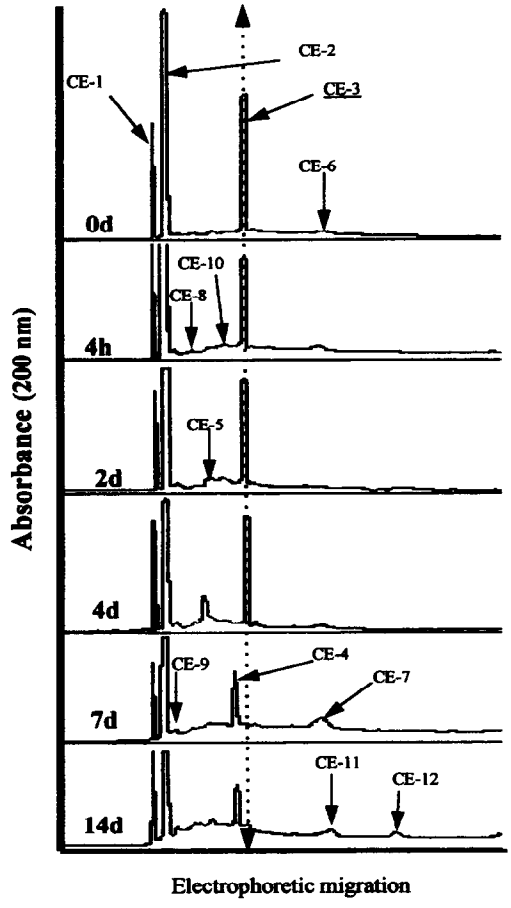

Fig. 3. Capillary electrophoresis (CE) of top round at various times during post-mortem aging. All CE peaks were assigned a number. Run conditionss were as follows: BioRad HPE-100. Preloaded for 8 s at 8 kV. The run was for 10 min at 8 kV constant with the current averaging 13.4 μA. Run buffer and sample buffer were pH 5.0 phosphate buffer at 0.10 mM and 0.01 mM, respectively. Chart speed was 1 cm min^{-1} set at 0.05 AUFS and scanned at 200 nm. The column was a 20 cm×25 μm coated capillary.

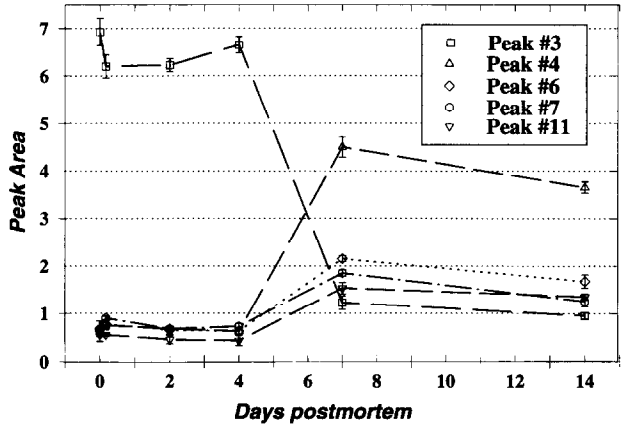

Fig. 4. The area under selected CE peaks (from Fig. 3) are shown here ± sem.

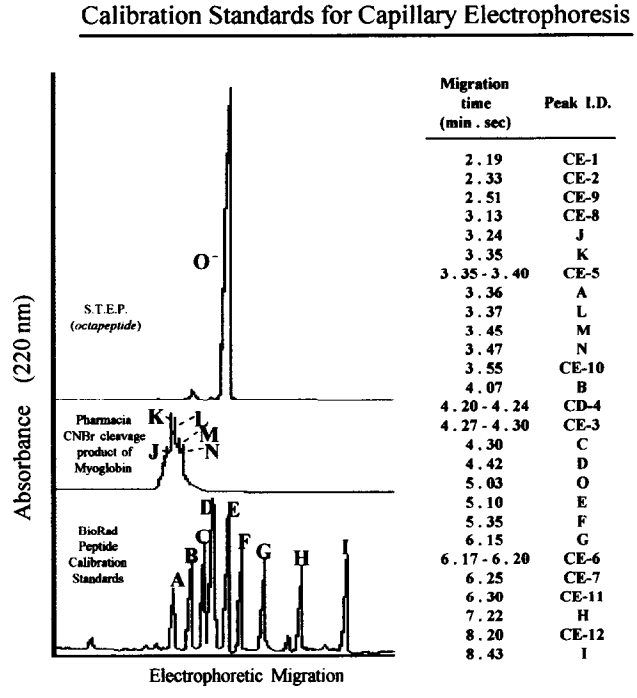

Fig. 5. Retention time of peptide peaks isolated by capillary electrophoresis (CE). CE of STEP (savory taste enhancing peptide), of CNBr cleavage products of myoglobin, and BioRad peptide calibration are shown and their electrophoretic migration times are shown in the table to the right. Runs were conducted as presented in the legend to Fig. 3.

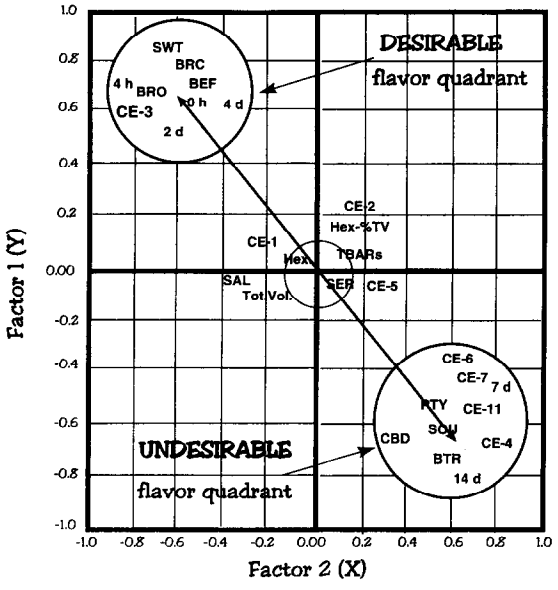

Fig. 6. Bivariate plot of principal components, factor analysis of beef. Comparison of proteins, volatiles, and flavor during post-mortem aging.

sensory attributes, cluster near the center of the grid or at the X and Y intercept. Analysis of the multivariate factors reveals that freshly slaughtered meat (0h) and meat aged 4 h, and 2 and 4 days (4h, 2d, 4d) cluster in the region of the desirable flavors BEF, BRO, BRC, and SWT. On the other hand, meat aged 7 or 14 days (7d, 14d) cluster with the undesirable flavors, PTY, CBD, SOU, and BTR. While these data indicate that a trained sensory panel can accurately assess the flavor of meat (Fig. 1C), it is unlikely that a typical consumer could clearly distinguish these subtle, yet significant, changes. On the other hand, these changes in flavor, no matter how subtle, are important and should be examined for a better understanding of the impact of the different flavor-precursor compounds generated during the post-mortem aging period.

Influence of pH on peptide production in meat

Previous studies by Spanier *et al.* (1990) demonstrated that knowledge of muscle proteinase activity increases our understanding of meat flavor since meat represents a tremendous source of substrates for the proteolytic generation of peptides and amino acids. Amino acids can either act directly as flavor compounds or can react with other meat components such as reducing sugars during storage and/or cooking to produce other flavor components such as the Maillard products (Bailey, 1988; Bailey & Shin-Lee, 1989). The importance of understanding the temporal alterations and kinetics of meat proteinases cannot be overemphasized, both because of the association of peptides and amino acids with desirable and undesirable meat flavor (Tamura *et al.*, 1989) and because of the availability of numerous avenues for proteolytic production of potentially flavorful peptides and amino acids. Numerous peptides are formed by proteolytic enzyme activity during the post-mortem aging and tenderization process (Etherington, 1987; Koohmarie *et al.*, 1988), and these enzymes each have different substrate specificities and pH optima (Bartlett, 1977). Peptides and amino acids are also formed and removed during the processing of cooked and stored meat (Spanier & Miller, 1996).

Experiments designed to examine the effect of pH on the production of proteinaceous material indicated that pH 6.0, 5.5, and 5.0 are the main pH range promoting the production of potential peptidic flavor principles and precursors (Fig. 7). Use of the specific inhibitors pepstatin-A and leupeptin indicate that these alterations with storage/aging of beef homogenates are most likely due to the activity of the endogenous aspartic proteinase, cathepsin D and the thiol-dependent proteinases, cathepsins B, H, and L and calpains (Fig. 7). The combined activity of these proteinases during the post-mortem aging process cannot be overlooked.

DISCUSSION

Food flavor is a combination of several chemical interactions involving proteins, lipids and carbohydrates. Temporal and kinetic studies of meat enzymes and proteins, of meat flavor treatments, etc. indicate the importance of examining more closely the activity of the meat enzymes. Continuing activity of the endogenous hydrolases during the post-mortem aging period constantly changes the flavor components and precursors.

The best overall 'flavor' quality of aged top round meat, i.e. meat with optimum flavor (and possibly texture), was found in meat aged for up to 4 days *post*

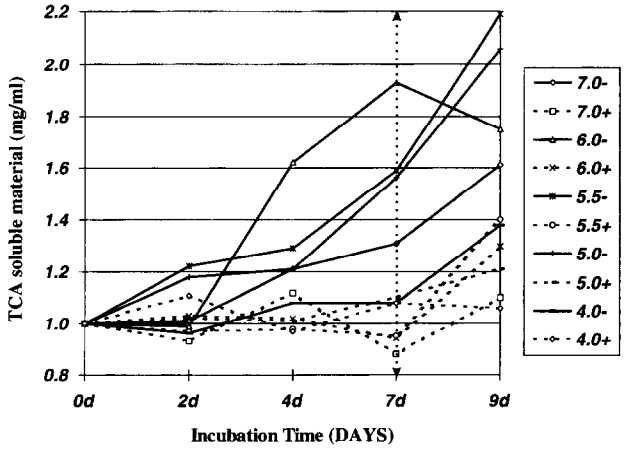

Fig. 7. Influence of pH on the formation of TCA-soluble peptides. The '-' (solid lines) and '+' (dashed lines) denote the absence or presence, respectively, of the protease inhibitors pepstatin-A (2 μM) and leupeptin (10 μM). The pH are depicted via the symbols described in the legend.

mortem. Lipid oxidation which is known to produce free radicals that, in turn, alters flavor through secondary reactions with flavor constituents (e.g. fragmentation of proteins, cross-linking of proteins, peptides, lipids, etc.; Spanier *et al.*, 1992*a*) had little negative impact on flavor in this experiment of post-mortem aging. However, absence of a lipid oxidation effect may be due to vacuum-packing. On the other hand, the activity of various hydrolases, such as the calcium-dependent, calpain proteinases implicated in meat tenderization (Koomaharie *et al.*, 1988), and the cathepsins implicated in the production of flavor peptides (Spanier *et al.*, 1990; Spanier & Miller, 1993, 1996) may play an important role in the temporal generation of flavor in meat during post-mortem aging.

Multivariate analysis is a valuable tool that permits mapping and tracing of flavor development in foods (Spanier *et al.*, 1992*b*, 1992*c*). For example, CE-4, -6, -7, and -11 correlated with undesirable flavor, CE-3 is the first report of the presence of a proteinaceous material that correlates with 'desirable', rather than 'undesirable' flavor components. The CE-3 peptide is not the savory taste-enhancing peptide named STEP (Spanier *et al.*, 1996). CE-3 and STEP have dissimilar electrophoretic mobilities of 4 m 27 s–4 m 30 s and 5 m 03 s, respectively. Whether CE-3 is a flavor-active peptide has yet to be examined. However, based on the strong correlation with desirable flavor, CE-3 is a likely candidate for use as a marker of meat flavor. Antibodies against CE-3 and CE-4 can be used in determining the fate of a beef sample. For example, samples containing high levels of CE-3 with little or no CE-4 can be used for human consumption. Aging meat samples can be monitored for levels of CE-3 and CE-4 to determine their peak time for flavor development. On the other hand, samples with low levels of CE-3 and high titers of CE-4 can be redirected for use in the preparation of other foods, such as restructure products to which other flavorants are added, or to animal foods.

In conclusion, multivariate results may be generated for data systems having several mechanisms, e.g. the cascade of proteolytic enzymes involved in degradation of different proteins during the conversion of muscle to meat (during post-mortem aging). Factor analysis simplifies the relationships that exist in the multivariate data set by isolating and identifying redundancies. Data generated in experiments such as those described above indicate the presence of numerous mechanisms. For example, the initiation of changes in flavor, volatile levels, and protein composition during post-mortem aging is verified by an analysis-of-variance (data not shown) and is demonstrated by the raw data (Figs 1,3 and 4). In this case, the data was distributed, and the factors clustered, in a manner consistent with that of post-mortem aging but not with lipid oxidation. Therefore, factor analysis illustrated that several factors affected the flavor quality of the beef during post-mortem aging.

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