ENZYME-CATALYZED PROTEIN HYDROLYSATES AS PRECURSORS FOR STABILIZING COOKED MEAT FLAVOR DURING REFRIGERATED STORAGE

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SUMMARY

Several protease systems were studied for their effectiveness to produce hydrolysates from beef muscle that would react with other Maillard reaction precursors which when heated to form synthetic meat flavors (SMF) that could prevent oxidation and maintain desirable flavor of cooked chopped beef steaks during storage at 4° C. An Enzyme mixture containing Protease N and Peptidase A ("Protease N") and another containing Alcalase and Fungal Protease ("Alcalase") were superior in their activities for degrading muscle proteins.

Chopped beef steak containing SMF made by heating these hydrolysates with amino acid, sugar and other reagents for the Maillard reaction were grilled (70° C) and analyzed by sensory and chemical methods during storage at 4° C for one week.

An analytical sensory panel obtained data which significantly indicated that steaks prepared with SMF made with these additives had desirable meaty flavor and less warmed-over flavor (WOF) during storage at 4° C for 7 days.

A consumer panel of 55 members found that the cooked chopped beef steaks treated with the SMF were more acceptable than non-treated controls after storage at 4° C for 4 days.

The conclusions made from sensory analyses were confirmed by data obtained from GLC analyses of lipid oxidation volatiles and that for malonaldehyde by TBA methodology.

Introduction.

The high demand for healthy, low fat, pre-cooked meat has expanded the market for fresh cooked meat products. The sale of these items is limited by the rapid oxidation of lipids, the formation of WOF and loss of desirable meat flavor soon after cooking fresh meat. An acceptable method for reducing these undesirable changes in quality is the use of additives made from natural products such as those formed by the Maillard reaction (Bailey, 1988; Bailey et al. 1987; Bailey and Um, 1992). Maillard reaction products (MRP) can minimize lipid oxidation and maintain desirable meaty flavor during refrigeration and frozen storage of cooked meat.

Enzyme-catalyzed animal protein hydrolysates have been used as flavor additives and enhancers (Webster et al. 1982; Weir 1986) because they consist of low molecular weight precursors such as amino acids and peptides. They have a further advantage as meat flavor precursors in that they contain nucleotides and nucleosides and perhaps other ingredients which add to meat flavor and mouthfeel.

Materials and Methods.

Enzyme hydrolysis of muscle proteins. A study was made of the efficiencies of various concentrations of different proteases on the degradation of beef muscle prior to preparation of synthetic meat flavor. The most effective enzyme systems were selected for further study. Endopeptidases used were Protease N (Amano International Enzymes, Troy Va,) and Alcalase (Novo Industry A/S, Bagevaerd, Denmark). Exopeptidases were Peptidase A (Amano International enzymes) and Fungal Protease concentrate (EDC Enzyme Developing, New York, NY.) Other enzyme systems evaluated were less effective.

The final concentrations of the enzymes in the hydrolytic reaction mixture were 0.2, 0.4 and 0.6% (w/w). Reactions for the endopeptidases were carried out at pH 8.0 for 10 hours at 50° C. This was followed by reactions with exopeptidases; Fungal protease at pH 8.0 and Peptidase A at pH 7.0. The reaction period was 10 hours at 50° C.

Preparation of synthetic meat flavor (SMF). The SMF consisted of 50 g of beef muscle hydrosylate prepared with Protease N (0.2%) plus Peptidase A (0.4%) or of Alcalase (0.2%) plus Fungal protease (0.6%) and other Maillard reaction precursors. The other ingredients were 1.5 g ribose, 1.6 g histidine, 1.0 g cysteine, 0.25 g thiamine, 0.5 g IMP and 50 g of beef fat. This mixture was reacted at pH 5.5 (lactic acid) for 45 min. in a convection oven at 127° C. The fat was removed after chilling to room temperature and the mixture was diluted to its original volume with distilled water.

Ground beef steaks treated with SMF. Fresh beef semimembranous muscle consisting of 6% fat was ground to 8 mm and mixed with 2% of one of the two SMF plus 0.4% sodium tripolyphosphate (STP) and 0.5% NaCl. One SMF was prepared from beef muscle hydrosylate digested with Protease N (0.2%) plus 0.4% Peptidase N and labeled "Protease N" flavor and the other SMF was prepared from beef muscle hydrosylate digested with 0.2% Alcalase plus 0.6% Fungal protease and labeled as "Alcalase" flavor. One control group studied contained 2% distilled water plus 0.5% NaCl and another control group of samples contained 2% distilled water, 0.5% NaCl and 0.4% STP.

Each beef steak (100 g) was shaped using a circular hamburger mold and cooked to 70° C internal temperature by grilling for 7 minutes on each side using a Farberware grill. The cooked meat samples were then vacuum packaged and stored up to 7 days at 4° C prior to sensory and chemical analyses.

Sensory analysis. Descriptive analysis was carried out by a trained analytical panel consisting of 7 experienced taste panelists trained to discriminate WOF, juiciness, tenderness and relative fresh meat flavor of beef. Samples were analyzed after 1, 3 and 7 days of storage at 4° C. Judgments were recorded on unstructured 10 cm scales with descriptive terms used as anchors ("non" and "intense" for meaty flavor and WOF and "more" or "less" for juiciness and tenderness). A freshly cooked beef sample was used as a warm-up reference sample at each analysis period.

Consumer panel. Chopped beef steaks evaluated by the consumer panel were prepared with 2% "Alcalase" SMF, 0.5% NaCl and 0.4% STP and the data were compared with that obtained from control samples containing only 0.5% NaCl. The samples were vacuum packaged and stored for 4 days at 4° C prior to analysis. The panel consisting of 55 judges had equal numbers of male and females and equal numbers of students and faculty-staff at the University of Missouri, Columbia. Sensory data evaluation was performed using a paired-preference test (Roessler et al. 1978).

Quantitative chemical analyses. The direct sampling GLC method of Suzuki and Bailey (1985) was used to analyze volatile compounds trapped on Tenax GC and desorbed using a model 490 Dynatherm Analytical Instruments (Kelt, PA) desorber interfaced to a 5% phenylmethylsilicone (SE 54) capillary column in a model 8500 Perkin-Elmer gas chromatograph. The distillation method of Tarladgis et al. (1964) was used for TBA analysis of malonaldehyde.

Results and Discussion.

Enzyme treatments. The most effective enzyme mixtures used to prepare hydrolysates for synthetic beef flavor were Protease N (0.2%) plus Peptidase A (0.4%) and alcalase (0.2%) plus Fungal protease (0.6%) and these enzyme concentrations were used to prepare beef muscle hydrolysates for SMF used to preserve flavor of chopped beef steaks during storage at 4 C following grilling.

Sensory analysis of SMF-treated chopped beef steak by analytical panel. Data in Figure 1 reveal that antioxidant SMF prepared with "Protease" N and "Alcalase" hydrolysates of beef muscle significantly preserved the meaty flavor of cooked chopped steak during storage at 4° C for 7 days. The enzyme hydrolysate SMF-treated samples also maintained better meaty flavor during this period than samples treated only with STP. There were no differences in meaty flavor of samples treated with SMF prepared with the two different enzyme protein hydrolysates. Data in Figure 2 show the effect of the antioxidant SMF treatments on the WOF of vacuum packed cooked-chopped beef steak during storage at 4° C for 7 days. WOF is quite severe in untreated samples, but significantly less in samples treated with STP and with enzyme hydrosylate SMF. The latter samples had significantly less WOF than samples treated with STP alone. These results support those of Bailey (1992) who reported that SMF prepared from MRP and STP reacted synergistically to prevent oxidation and WOF in pork samples during storage for l month at 4° C following cooking, but that treatment

with STP alone did not preserve meaty flavor following cooking and refrigerated storage. The important feature of the use of SMF for both beef and pork appears to be the preservation of the desirable meaty flavor during storage after cooking. The enzyme hydrolysates in the SMF added to the brothy-meaty flavor of these samples which was lost very rapidly in cooked samples treated with only STP and NaCl.

Consumer panel. Pre-cooked chopped beef steaks treated with SMF containing beef muscle protein hydrolyzed with Alcalase and Fungal protease heated with other Maillard reaction ingredients and added along with 0.5% NaCl and 0.4% STP prior to cooking and storage were significantly preferred (Roessler et al., 1978) by members of the consumer panel following storage for 4 days at 4° C.

Lipid oxidation volatiles. Pentanal, hexanal, heptanal, 2,3-octanedione and total volatiles determined by GLC did not increase significantly in cooked chopped beef steak treated with SMF prepared with "Protease N" or with "Alcalase"-hydrosylates during storage for 1 week at 4° C. Their increases in samples treated with only 0.4% STP were insignificant. All of these volatiles increased in concentration during this period in samples treated with 0.5% salt. These volatiles may be good indices of oxidation of lipids in meat samples following cooking (Frankel, 1984; Shin-Lee, 1988), but they do not measure the degree of desirable meaty flavor of cooked meat. Meaty flavor is best measured by sensory analysis procedures as described above.

TBA values of these samples reflected the same degree of oxidation in these cooked beef samples as data from the analysis of individual or total volatiles. TBA values of the untreated control samples were significantly higher than those of the phosphate-treated samples or those treated with SMF prepared with the protease hydrosylates, salt and phosphates.

Conclusions.

Synthetic meat flavor prepared with protease hydrosylate from beef muscle protein and heated with other Maillard reaction ingredients prevent oxidation and WOF, and preserve meaty flavor of cooked-low fat-chopped beef steak during storage at 4° C for 1 week. Sensory analysis is the most appropriate procedure for measuring this protection. Chemical methodology such as analysis of individual oxidation volatiles by GLC or analysis of malonaldehyde by TBA are good indices of lipid oxidation and WOF, but are not good measures of the changes in desirable meaty flavor of cooked chopped beef steak during storage at 4 C for l week.

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