Concentration Profiles of Diamines in Fresh and Aerobically Stored Pork and Beef

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The concentration profiles of putrescine, cadaverine, and to a minor extent histamine were determined in commercial fresh pork and beef stored aerobically at low temperatures. The amines were extracted with perchloric acid, preseparated on a weakly acidic cation exchanger, and quantified by capillary gas chromatography. Chemical analyses were carried out in parallel with determinations of total bacterial numbers and sensory assessments. Meat samples were grouped into three quality classes according to bacterial numbers and sensory quality, and cutoff values for the combined diamine concentration were proposed. With pork, the diamine content made it possible to distinguish between classes 1 and 2 with a maximal disagreement of 14%.

INTRODUCTION

Chemical tests for assessment of the degree of freshness in meats have long been of interest. They should eliminate the disadvantages of the currently used methods for spoilage assessment, such as the measurement of bacterial numbers or sensory judgment. The bacteriological method, which requires at least 48 h of incubation time, is too lengthy for meat processing firms. The sensory judgment as an objective analysis requires a panel of trained persons.

Chemical tests reflect the biochemical changes which occur in meat during storage. Since the majority of the deteriorative changes are caused by bacteria, chemical indicators were frequently sought among decomposition products of bacterial metabolism (e.g., Turner, 1960; Pearson, 1968; Dainty, 1971; Patterson and Edwards, 1975). However, the proposed chemical tests usually did not prove useful, mostly because of the limited concentration changes of the decomposition products.

Recently, however, Mietz and Karmas (1977, 1978) and Karmas (1981) developed a biogenic amine index to classify seafood as acceptable, borderline, or unacceptable. The chemical quality index, which includes putrescine, cadaverine, histamine, spermine, and spermidine concentrations, was reported to be in satisfactory agreement with sensory evaluations. With red meats, a distinct rise of putrescine and/or cadaverine levels was observed in naturally contaminated, putrified pork (Lakritz et al., 1975; Nakamura et al., 1979; Yamamoto et al., 1982). The formation of these diamines in meat is attributed to bacterial enzymatic activity. Typical species of spoilage bacteria, such as pseudomonadaceae and enterobacteriaceae, have been shown to produce significant amounts of putrescine and cadaverine in chill-stored pork and beef (Slemr, 1981).

The observed increase in diamines in deteriorating red meats led us to investigate their concentration profiles in detail. We determined the relationship of putrescine and cadaverine to bacterial numbers and sensory scores throughout aerobic chilled storage of commercial, fresh pork and beef. In addition, changes of histamine concentrations were measured. Meat samples were grouped into three bacteriological (sensory) quality classes and cutoff values of the diamine content between fresh, acceptable, and spoiled meat were determined statistically.

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EXPERIMENTAL SECTION

Storage of Meat Samples. Leg and cutlet of pork and braising steak of beef were obtained on four occasions from retail butcher shops. The meat samples were considered to have been chill stored in accordance with German food laws, which require meats to be held at temperatures below 7 °C until sale. Immediately after purchase, the freshness of the samples was determined by sensory assessment, bacterial count, and chemical analysis. The samples were freed of visible fat and meat slices (ca. 5 × 6 × 1 cm) were placed in sterile Petri dishes to be stored at 5 °C. During storage, samples were removed every day for sensory, bacterial, and chemical analyses. A storage run took about 4–5 days until the meat was obviously spoiled.

Methods. To determine the bacterial density on meat slices the whole sample was homogenized and an aliquote of the homogenate was suspended in 1% Tween 80 solution as described elsewhere (Slemr, 1981). Bacterial counts were determined on Trypton Soya Agar plates, incubated aerobically for 3 days at 30 °C. The overall error of the bacterial count determination was half an order of magnitude.

The sensory quality of meat samples was evaluated by a conventional sensory analysis (e.g., Reuter, 1974). Three trained persons judged odor, appearance of the surface, tenderness, and juiciness of meat. Each property was characterized by six values. A score of 6 indicated the best quality, whereas a score of 1 was associated with badly decomposed meat. The overall score N resulted from the scores of the partial quality features n_i: $N = \sum_i a_i n_i / \sum_i a_i$. The significance of each quality criterion was expressed by the weight coefficient a_i. Odor, the most important organoleptic quality criterion, had the weight coefficient of 5, appearance, tenderness, and juiciness of 2, 1, and 1, respectively.

The analytical procedure for the determination of putrescine, cadaverine, and histamine has been described in detail elsewhere (Slemr and Beyermann, 1984). It consists of extraction with perchloric acid, preseparation of crude extracts on a weakly acidic cation exchanger, and conversion of the amines into their trifluoroacetyl derivatives. The amines were quantified by capillary gas chromatography. The relative standard deviation of the overall analytical procedure was 8% for the sum of putrescine and cadaverine concentrations.

RESULTS

Quality Classes of Meat. Stored meat samples were

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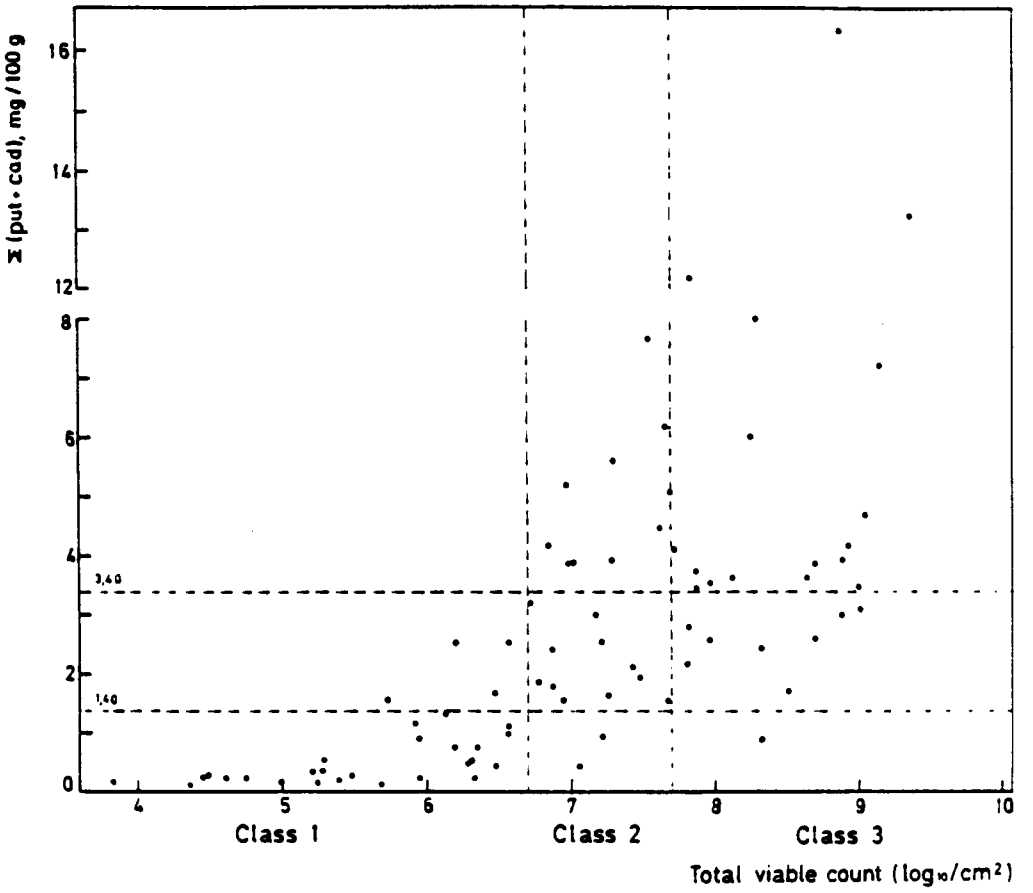

Figure 1. Amine content and bacterial count for naturally contaminated pork. Storage temperature 5 °C.

grouped into three bacteriological quality classes according to their total bacterial numbers (Leistner, 1979).

Class 1: fresh meat, bacterial numbers below $5 \times 10^6/cm^2$

Class 2: acceptable meat, bacterial numbers between $5 \times 10^6/cm^2$ and $5 \times 10^7/cm^2$

Class 3: spoiled meat, bacterial numbers above $5 \times 10^7/cm^2$

Three sensory quality classes corresponded to the bacteriological ones.

Class 1: fresh meat, sensory score $N \epsilon [6,4]$

Class 2: acceptable meat, $N \epsilon [4,3]$

Class 3: spoiled meat, $N \epsilon [3,1]$

Amine Levels in Pork. With pork an increase in the average values of the combined putrescine and cadaverine concentration was noticed as decomposition progressed (Figure 1). In class 1 the mean value of the sum parameter, $\sum$ (put + cad), was 0.67 mg/100 g, with a relative standard deviation (rsd) of 1.00 (n = 30). The mean values of $\sum$ (put + cad) in classes 2 and 3 were 3.25 mg/100 g (rsd = 0.56, n = 23) and 5.63 mg/100 g (rsd = 0.97, n = 27), respectively. The mean basal value of the sum parameter for meat samples with bacterial loads below $5 \times 10^5/cm^2$ was 0.24 mg/100 g (rsd = 0.11, n = 13). Retail samples, which were not as fresh as desirable, had an enhanced level of cadaverine. Cadaverine also caused the increase of the sum parameter during storage in most storage runs.

The cutoff values of the sum parameter between the classes 1, 2, and 3 were determined statistically be means of the minimax principle (Lehman, 1959). Distinction between classes 1 and 2 is associated with a maximal error of 13%, whereas the maximal percentage of disagreement rises to 43% for the differentiation between the classes 2 and 3. There is, however, no need to distinguish between the classes 2 and 3 by means of the sum parameter, since meat spoilage of the latter class is obvious.

The relationship between diamine content and sensory score is analogous to that between diamine content and bacterial number. The mean values of the sum parameter in classes 1, 2, and 3 are 0.76 mg/100 g (rsd = 1.23, n = 28), 2.92 mg/100 g (rsd = 0.67, n = 22), and 5.49 mg/100 g (rsd = 0.94, n = 32), respectively. The cutoff value of the diamine content between the sensory classes 1 and 2 is 1.30 mg/100 g with a maximal error of 14%, and that between classes 2 and 3 is 3.00 mg/100 g with a maximal disagreement of 32%.

Histamine level in pork rose slightly and more slowly. Its mean levels were 0.17, 0.23, and 0.69 mg/100 g in the bacteriological classes 1, 2, and 3. When the histamine concentration was included into the sum parameter, the percentage for correct classification by the amine content was not appreciably altered.

Amine Levels in Beef. The scatter of the diamine levels in beef was throughout all three quality classes too high to enable a satisfactory classification. Distinction between the bacteriological classes 1 and 2 was associated with a maximal error of 45%, and that one between the classes 2 and 3 with a maximal error of 27%. The mean values of the diamine content in the classes 1, 2, and 3 were 3.28 mg/100 g (rsd = 1.87, n = 21), 6.32 mg/100 g (rsd = 1.56, n = 11), and 11.91 mg/100 g (rsd = 1.59, n = 20), respectively.

Histamine levels tended to increase only a minor extent and were not a significant contribution to the sum parameter. The mean histamine concentrations were 0.32, 0.42, and 0.74 mg/100 g in the bacteriological classes 1, 2, and 3.

DISCUSSION

Amine Levels in Fresh Meat. The combined concentration of putrescine and cadaverine was found to be fairly low for fresh *pork*. The average value of 0.24 mg/100 g for samples with bacterial counts below $5 \times 10^5/cm^2$ is very close to diamine concentrations determined by several other authors (Edwards et al., 1983; Yamamoto et al., 1982; Nakamura et al., 1979) in fresh commercial pork. Similarly, the mean histamine concentration of 0.17 mg/100 g in pork of class 1 coincides well with those found by Taylor et al. (1978) in fresh comminuted pork and also with values determined by Spinelli et al. (1974) in fresh pork bellies. The mentioned amine levels can be regarded as representative of fresh pork.

For the diamine concentration in fresh *beef* no comparative values in the literature of the last decade were found. The mean histamine concentration in fresh beef amounted to 0.50 mg/100 g. This value compares favorably with mean histamine levels published by other authors: 0.58 mg/100 g (Taylor et al., 1978) and 0.27 mg/100 g (Rice et al., 1975).

Increase of Amine Levels during Storage. As apparent from Figure 1, the diamine content of pork and its scatter increase markedly at bacterial loads higher than approximately $5 \times 10^5/cm^2$. The variability in amine concentrations among pieces with similar bacterial counts appears to be due mainly to factors influencing the growth and the metabolism of spoilage flora, as errors associated with the determination of the diamine level and the bacterial count are relatively small. These concentration differences can be explained by varying properties of meat substrates from different sources (e.g., pH value) and by microbial floras with different biochemical potentialities for metabolizing amino acids. The breakdown of the diamines was a contributory factor in the scatter of the sum parameter.

As mentioned in the Results, the increase of the diamine concentration was mainly due to cadaverine. During storage this diamine appeared first, reached higher levels than putrescine, and underwent decomposition earlier.

The predominance of cadaverine over putrescine is not consistent with previous investigations about diamine formation in chill-stored red meats (Nakamura et al., 1979; Slemr, 1981; Edwards et al., 1983). Pseudomonas strains, generally known as a dominant element of the chilled meat flora (e.g., Gill and Newton, 1977), produced mainly putrescine, whereas enterobacteriaceae strains formed preferentially cadaverine (Slemr, 1981). Putrescine was the major diamine in meat stored at 4 °C (Nakamura et al., 1979) or at 5 °C (Edwards et al., 1983). On the other hand, cadaverine dominated in pork samples stored in 20 °C (Nakamura et al., 1979) or 25 °C (Yamamoto et al., 1982), where a greater proportion of mesophilic enterobacteriaceae could be expected.

The higher cadaverine levels determined in our experiments indicate that enterobacteiaceae contributed mostly to the diamine formation. The cadaverine predominance leads us to the assumption that the meats had been stored at temperatures higher than the allowed 7 °C prior to sale. This could have caused a shift in bacterial population in favor of enterobacteriaceae.

Histamine formed later and reached appreciably lower levels than either putrescine or cadaverine. Similar amine profiles during chilled storage were observed with comminuted pork and beef (Wortberg and Woller, 1982), and seafood (Mietz and Karma, 1978). The delay in the formation of histamine may be related to the pH values of stored meats. Typical histamine producers, e.g., *Proteus morganii* strains showed maximal activity at pH 6.5 (Eitenmiller et al., 1981). Stored meats reach these pH values at an advanced stage of spoilage, after basic decomposition products had been formed in a sufficient amount. Actually, we observed a significant rise in the histamine level of some extremely spoiled samples of the quality class 3.

The concentration profile of the diamines in *beef* differs from that of pork. Both bacterial counts and diamine levels of freshly bought meat are higher than those of pork. These differences could be explained by the prehistory of beef. Prior to retail, beef is usually left to ripen for 8–14 days at chill temperatures, whereas pork ripens in 2–3 days (Böhme, 1979). The bacterial flora associated with the ripening process can better adapt and will more rapidly reach higher densities with beef than with pork.

The concentration profiles of putrescine and cadaverine indicate that the sum of these diamines could serve as a quality indicator of fresh pork. It seems to apply for meat stored at temperatures slightly above 7 °C, where a fraction of enterobacteriaceae becomes higher than with chilled meat. Under the described experimental conditions the chemical quality indicator permits distinction between fresh and acceptable meat with a maximal disagreement of 14%. To translate these findings into a practical test would, however, require the development of a simplified and fast chemical procedure.

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Registry No. Putrescine, 110-60-1; cadaverine, 462-94-2; histamine, 51-45-6.

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Volatile Compounds from *Pencillium sp.* Contributing Musty–Earthy Notes to Brie and Camembert Cheese Flavors

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Volatile headspace compounds collected on Tenax GC at room temperature (21 °C) from pure cultures of *Penicillium caseicolum* and *Penicillium camemberti* grown on potato dextrose and Czapek's agar were separated by gas chromatography and identified by mass spectrometry, retention indices (I_E), and odor quality. *P. camemberti* and *P. caseicolum* produced similar volatile compound profiles. Mushroom-like, green plant-like aromas were contributed by 1-octen-3-ol, 1,5-octadien-3-ol, 1,5-octadien-3-one, 3-octanol, and 3-octanone. 2-Methylisoborneol contributed strongly to the overall musty/moldy component of aromas of *Penicillium* cultures. 2-Methoxy-3-isopropylpyrazine was found in aged mold cultures (4–6 weeks) only and caused these cultures to exhibit intense earthy/raw potato aromas.

INTRODUCTION

Surface-ripened cheeses involving extensive mold growth during ripening, especially Brie and Camembert, long have been popular in European countries. Although early production of Camembert cheese was accomplished through the use of *Penicillium camemberti* (Thom and Fisk, 1918), current production of both Camembert and Brie cheeses is largely achieved by employing *Penicillium caseicolum* (Kosikowski, 1982). Very limited information exists concerning the biochemical differentiation of these two species of *Penicillium* (Raper and Thom, 1949), but morpologically *P. caseicolum* exhibits a white mycelial mat while *P. camemberti* yields a light tan to greyish mat because of the production of slightly colored conidia.

European consumers generally prefer extensively aged Brie and Camembert cheeses whose flavors reflect not only primary lactic acid fermentations and mold contributions, but also metabolites of secondary surface ripening by yeasts, *Brevibacterium linens*, and some related coryneforms (Olson, 1969; Greenberg and Ledford, 1978; Law, 1982; Rousseau, 1984). Recent interest in these varieties of cheeses by U.S. consumers, however, has stemmed from an acceptance of the less pronounced but nutty, mushroom-like flavors that are found in modestly aged cheeses. Moinas et al. (1973, 1975) and Dumont et al. (1974b, 1976) have identified a substantial number of volatiles in aging Camembert cheeses of French origin, and both groups of researchers concluded that 1-octen-3-ol was a major characterizing compound in the flavor of younger cheeses. A number of alcohol, ester, and sulfur compound metabolites was believed to characterize extensively aged Camembert flavors.

Critical evaluation of the aroma and flavors of both mold cultures and modestly aged Brie and Camembert cheeses revealed that additional musty–earthy notes were present besides the distinctly raw mushroom-like aroma of 1-octen-3-ol. Information concerning the identity of compounds with earthy–mushroom-like quality that are produced by molds and mushrooms is generally limited to saturated or monounsaturated eight-carbon primary and secondary alcohols and corresponding carbonyl compounds (Cronin and Ward, 1971; Kaminski et al., 1972, 1974; Moinas et al., 1973; Dumont et al., 1974b; Halim et al., 1975; Maga, 1981; Tressel et al., 1982). Some claims have been made that certain alkyl benzenes produced by *Penicillium roqueforti* in Blue and Roquefort cheeses contribute musty aromas (Boyd et al., 1965; Day, 1967; Law, 1982), but these claims are largely unsubstantiated and have not been analytically confirmed. Therefore, the objective of this research was to investigate the volatile compounds produced by cultures of *P. camemberti* and *P. caseicolum*, particularly those capable of contributing musty–earthy notes to the flavors of Brie and Camembert cheeses.

MATERIALS AND METHODS

Cultures of *P. caseicolum* no. 874 and *P. camemberti* no. 877 were obtained from the collection of K. B. Raper (Bacteriology Department, University of Wisconsin, Madison, WI) and cultures of *P. caseicolum* ATCC no. 6986 and *P. camemberti* ATCC no. 6985 were obtained from the American Type Culture Collection (Rockville, MD). One culture of *P. caseicolum* was propagated from an isolate obtained recently from a commercial, domestically produced Brie cheese.

Pure cultures of each *Penicillium sp.* were grown on sterile acidified potato dextrose agar slants or Czapek's agar slants (100 mL, Difco Laboratories, Detroit, MI) held in loosely capped 900-mL glass prescription bottles at 17 ± 1 °C. Headspace volatiles produced by young (5–12 days) and mature (30–45 days) cultures were collected during 3- and 24-h periods by purging the headspace of bottles containing the mold cultures at room temperature (21 °C) with a stream of humidified air at a rate of 240 mL/min. Humidified air was produced by bubbling the air stream through 5 mL of sterile water placed in the

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