Determination of Amines in Fresh and Processed Pork

The concentration of a number of amines was determined in fresh, cooked, smoke-cured, and putrefied pork. Analyses were conducted for spermine, spermidine, putrescine, cadaverine, histamine, tyramine, tryptamine, and ethanolamine. The amines were recovered from perchloric acid extracts of the lean meat and derivatized with 1-dimethylaminonaphthalene-5-sulfochloride. The fluorescent derivatives were separated by thin-layer chromatography, extracted, and then quantitated spectrofluorometrically. The concentration per 100 g of fresh tissue ranged from 0.5 mg for tyramine to 189 mg for putrescine. Significant increases in spermine, spermidine, putrescine, and cadaverine occur during putrefaction. Cooking at 71° decreases the concentration of amines.

N-Nitrosamines have been found to be carcinogenic to animals (Magee and Barnes, 1956). These compounds are formed by the reaction of nitrite with secondary amines. Since cured meat products are prepared with nitrite, there is considerable interest in the components of meat, such as amines, that could react with nitrite to form nitrosamines. Crosby *et al.* (1972) detected traces of the following nitrosamines in food: dimethylamine, diethylamine, pyrrolidine, and piperidine. Lijinsky and Epstein (1970) postulated that cadaverine and putrescine upon heating formed piperidine and pyrrolidine, and Bills *et al.* (1973) demonstrated the production of *N*-nitrosopyrrolidine from such polyamines as spermidine and putrescine when heated in the presence of sodium nitrite.

Information concerning the concentrations of free amines in fresh and processed foods has been scant until

recently. Wang (1972) reported on the presence of putrescine, spermidine, and spermine in soybeans. Concentrations of methyl-, dimethyl-, and trimethylamines in salmon and sable were measured by Gruger (1972). Determinations of the levels of volatile amines have been used to assess the effectiveness of cold storage on the keeping of cod by Keay and Hardy (1972), and Florin (1971) measured the same amines, using them as an index of the degree of bacterial action on the breakdown of fish. Takagi *et al.* (1971) confirmed the presence of putrescine, cadaverine, and tyramine in spoiled squid and octopus. A number of amines are known to exert profound physiological changes in the body, and those present in physiological fluids (blood, urine, semen) and special organs (heart, liver, nervous tissue) have been studied extensively (Frazen and Eysell, 1969). However, very few studies have reported on the amine content of skeletal muscle. While much information exists on the interrelationship of meat processing to amino acid composition (Wasserman and Spinelli, 1970; Usborne *et al.* 1968; Piotrowski *et al.* 1970), there is no information on the effect of processing on the concentration of amines. Recently we (Spinelli *et al.*, 1974) studied the effect of processing on the amine content of pork bellies.

We are reporting here the effects of cooking, curing, and putrefaction on the amine content of pork.

EXPERIMENTAL SECTION

Materials. Fresh pork ham-butt portions, purchased over the counter at local supermarkets, were freed of connective and adipose tissue. The meat samples were ground, mixed, and reground four times to ensure complete homogeneity of the muscles in order to exclude the possibility that variations in the amine content were due to inherent differences within the muscle tissue itself. Each ground pork sample was divided into thirds: one-third analyzed without additional processing (fresh), one third cooked to an internal temperature of 71°, and the remaining third stored at 25° for 72 hr prior to analysis to hasten putrefaction. To determine the effect curing may have on altering the amine content, butt portions of commercially cured and smoked hams were analyzed in the same way as the other pork preparations. All samples for analysis were worked up at cold room temperatures of 4°.

Methods. The experimental techniques, similar to those used by Spinelli *et al.* (1974), included extraction and solvent partition procedures for isolation of amines, followed by derivitization of the latter and subsequent chromatographic and spectrophotofluorometric analyses. Amines measured were: spermine, spermidine, putrescine, cadaverine, histamine, tyramine, tryptamine, and ethanolamine.

RESULTS AND DISCUSSION

The eight amines studied in hams can be divided into two groups based on the average concentrations observed. In fresh meat the polyamines (spermine, spermidine, putrescine, and cadaverine) were present in greater quantities than the four monoamines, averages ranging from 17 to 70 mg/100 g of tissue as opposed to the 1 to 2 mg/100 g of tissue for the latter (Table I). There was sufficient variability among the four samples, however, that in two of them spermine and cadaverine concentrations were of the same order of magnitude as the monoamines, as was the concentration of putrescine in a third sample. The variation in the concentration of amines, particularly the polyamines, may be due to the fact that, in addition to normal differences between animals, these hams were purchased at different times in a commercial meat market. The history of the animals from which they came is unknown; butchering and storage treatments are also unknown. Undoubtedly, some degradation, by both bacterial and tissue enzymes, had occurred. Decarboxylation of the amino acids lysine and ornithine is known to yield putrescine and cadaverine, and it has been shown that putrescine is a precursor of spermine and spermidine (Tabor *et al.*, 1958), thus establishing the interrelationship of these polyamines.

Table I. Effect of Cooking and Putrefaction on the Concentration of Some Amines in Pork

Amine	mg/100 g of tissue		
	Fresh	Cooked	Putrefied
Spermine	17.7 (1.2–55.7)	6.1 (2.2–9.7)	806.2 (5.4–2769)
Spermidine	61.9 (13.4–125)	41.9 (9.7–70)	339.9 (20.1–1013)
Putrescine	70.2 (1.7–189.3)	17.0 (2.3–56.5)	149.4 (7.3–351)
Cadaverine	17.1 (1.3–47.9)	9.2 (1.8–24.5)	25.0 (5.5–40.8)
Histamine	1.1 (0.5–2.3)	0.6 (0.4–0.8)	4.5 (1.7–9.2)
Tryptamine	1.9 (1.2–2.9)	1.4 (0.6–2.2)	4.8 (2.5–8.6)
Tyramine	1.1 (0.5–4.1)	0.35 (0.3–0.4)	2.2 (1.3–3.5)
Ethanolamine	1.0 (0.7–1.1)	0.8 (0.4–1.4)	0.8 (0.6–0.9)

Table II. Concentration of Some Amines in Butt Portions of Commercially Cured and Smoked Ham

Amine	Sample, mg/100 g of tissue		
	1	2	3
Spermine	0.6	19.1	79.6
Spermidine	55.4	127.3	15.1
Putrescine	50.4	4.0	1.1
Cadaverine	1.4	63.2	19.4
Histamine	0.4	0.5	0.5
Tryptamine	0.8	6.7	3.5
Tyramine		0.8	0.2
Ethanolamine	0.4	0.4	0.6

Although the data do not necessarily represent the concentration of the amines in freshly slaughtered pigs they may still serve as a base for this study, which was to observe changes in amine concentrations induced by cooking and by spoilage resulting from putrefaction. Heating to an internal temperature of 71° resulted in a substantial decrease in the concentration of the amines, which may be due to volatilization of the amines. Putrefied meat underwent visual and olfactory observable spoilage, and, as expected, there were large increases in the concentrations of the amines, particularly the polyamines. There was considerable variability among the meat samples, however, which may have been the result of differences in the bacterial flora and the activity of the organisms.

Three commercially cured and smoked hams, butt portion, were also examined (Table II). There are no fresh meat controls with which to compare these results, but even in these samples the polyamine concentration is considerably greater, and more variable, than the concentration of the monoamines.

The object of this limited study was to ascertain the influence which processing (cooking, smoke-curing, and putrefaction) has on the levels of some of the free amines present in pork. In retrospect, it appears that some of the

pork ham butts used in the study may have been in the process of undergoing slight bacterial breakdown. This is indicated by the high polyamine content detected in several of the samples. It is, nevertheless, apparent that cooking pork in general decreases the concentration of amines. Putrefaction of pork, on the other hand, causes a significant increase in the concentration of certain amines, in particular spermine, spermidine, putrescine, and cadaverine. Commercial curing and smoking of hams cause no discernible effects on the concentration of the amine content (Table II).

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