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EFFECT OF STORAGE ON THE CONCENTRATION OF PROLINE
 AND OTHER FREE AMINO ACIDS IN PORK BELLIES

4116

ABSTRACT

Proline is a potential precursor in the formation of N-nitrosopyrrolidine, a carcinogen that has been detected in fried bacon. The concentration of free proline and 11 additional amino acids were measured on the first and eighth day after slaughter and storage at 2°C. The free amino acids (proline, alanine, glycine, isoleucine, leucine, methionine, phenylalanine, tyrosine, valine, glutamic acid, cystine, aspartic acid) were determined on separated lean tissue, on the adipose tissue, and on samples of intact green pork bellies. The concentration of most of the amino acids increased with storage. Proline in the intact pork bellies increased approximately 52% after 1 wk of storage. Over the same period, free proline in the lean and in the adipose tissues increased 50 and 90% respectively. Effects of extended storage (28 days) on the concentration of free proline in lean tissue was also determined.

INTRODUCTION

IN 1956, MAGEE AND BARNES reported on the carcinogenicity of N-nitrosodimethylamine in rats. In a few years it became evident that nitrosamines as a class are potent carcinogens in test animals. The possibility exists, although unproven at present, that humans may also be affected by these compounds.

Nitrosamines are formed by the reaction of nitrite with amines, principally secondary amines. Their presence has been reported in numerous foods such as fish, cheeses, and cured meats (Crosby et al., 1972). Of the ten or more possible volatile nitrosamines actively being analyzed for in foods, dimethylnitrosamine, nitrosopyrrolidine, and nitrosopiperidine are the three found most frequently and in the highest concentrations.

While most nitrosamines in cured meat products are not found on a consistent basis, nitrosopyrrolidine has been found consistently in fried bacon (Crosby et al., 1972; Sen et al., 1973; Fazio et al., 1973; Pensabene et al., 1974). Nitrosopyrrolidine, however, has not been detected in uncooked bacon. In the last 2 yr a great deal of effort has been directed toward elucidating the pathway leading to the formation of nitrosopyrrolidine in fried bacon. In 1970, Lijinsky and Epstein suggested that secondary amines could be formed as foods are cooked. They postulated that the cooking of tissue protein could produce free amino acids such as proline and hydroxyproline, and that the secondary amine, pyrrolidine, could be formed by heating the diamine putrescine; which is known to be present in meat and in fish. They also postulated that proline ingested in foods could be nitrosated in the stomach to nitrosoproline, and then enzymatically decarboxylated to nitrosopyrrolidine by bacteria in the duodenum and the small intestine. Bills et al. (1973) demonstrated in model systems that nitrosopyrrolidine could be produced from such precursors as putrescine, proline, and nitrosoproline, when subjected to conditions similar to those encountered on frying bacon. Kushnir et al. (1975) isolated and identified nitrosoproline in uncooked bacon and suggested this is a precursor for nitrosopyrrolidine. Gray and Dugan (1975) recently demonstrated nitrosopyrrolidine formation by heating nitrite with

ham connective tissue or collagen; both types of preparations contain relatively large quantities of bound proline and hydroxyproline. It seems evident that proline, a natural component of meat, could be a precursor of nitrosopyrrolidine.

Several investigators (Bowers, 1969; Osborne et al., 1968) in their research on porcine muscle quality and flavor, have determined the presence of free amino acids. However, the studies were limited to examination of the composition of the longissimus muscle.

The object of the study reported here was to determine the concentration of free proline and other amino acids in the lean and adipose tissues of green pork bellies before processing into bacon and to note the changes that occur during the first week of storage, a reasonable holding period for green bellies prior to curing.

EXPERIMENTAL

Methods and materials

Pork bellies were obtained from a commercial processor approximately 24 hr after slaughter. Six bellies were skinned, quartered, and sliced into small pieces. Random samples were removed, mixed, and 100g used for each analysis. This represented the intact tissue. Other portions of these bellies were separated into lean and adipose tissue; the tissues were cut into small segments and mixed in order to achieve homogeneity. The composition of bellies was approximately 62% adipose and 38% lean tissues. The flow chart of the procedure for extraction of proline is shown in Figure 1. 100g of tissue was macerated

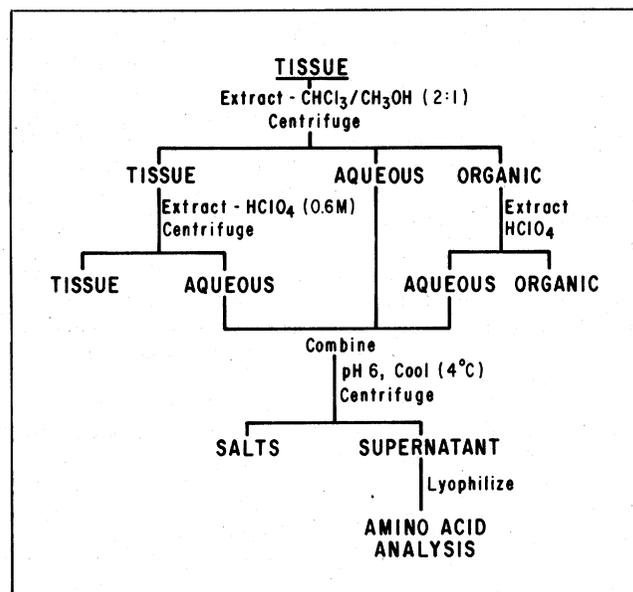


Fig. 1—Flow chart of procedure for extraction of proline.

and extracted in a blender (Waring, Model 91-262) with 100 ml Folch's reagent (2/1 chloroform/methanol) at high speed for 1 min. The slurry was centrifuged and the three layers [tissue, chloroform (organic) layer, and upper aqueous phases] were separated. The tissue residue was reextracted twice with 100 ml Folch's reagent and the supernatants combined. The combined chloroform layers were extracted three times with 100 ml of 0.6M perchloric acid. The perchloric acid extracts were pooled. The residual tissue was extracted in a blender with perchloric acid and centrifuged. The layers were separated and the tissue extracted twice more. All perchloric acid extracts and the initial aqueous phases were combined and adjusted to pH 6 with KOH and cooled. The precipitated potassium perchlorate was removed by centrifugation. The precipitate was washed with ice cold deionized water and the washings were added to the supernatant. The supernatant was concentrated by freeze drying and redissolved in 10 ml of sodium citrate buffer, pH 2.2. The acidic and neutral amino acids were separated by passing the extract through a column packed with Spherix XX8-60-0 resin using an accelerated system developed by Spackman et al. (1958). The instrument used was a Phoenix Precision Instrument Company amino acid analyzer model M-7800.

To study the effect of storage, green bellies were kept in a refrigerator at 2°C for 1 wk and then assayed as described. A separate storage study extended over a 4-wk period was also conducted. Two bellies were used for this study and the determinations done in duplicate.

RESULTS & DISCUSSION

THE CONCENTRATIONS of free proline found in the tissues from six fresh (green) bellies are shown in Table 1. The pork was stored for 8 days and the free amino acids were determined on the first and eighth day. The average proline content of intact tissue and lean tissue (adipose removed) was 14.9 μ M and 23.9 μ M per 100g of wet tissue, respectively. The concentrations of free proline in the intact and lean tissues both increased approximately 50% (51.6% and 47.6%) after storage for 1 wk. Adipose tissue contained less than one-half the concentration of free proline present in the intact tissue. After 1 wk storage, however, free proline in the adipose tissue increased 96%. The increase in free proline due to proteolysis during postmortem storage is expected, but the fact that the rate of increase is twice as great in the adipose tissue as in lean is not expected. No explanation can be offered at this time.

Based on the concentration of proline present in adipose and lean tissues, and with the knowledge of the ratio of these tissues in the whole belly, the concentration of proline in the intact belly can be determined. Table 2 shows that these values approximate the concentrations actually found.

The average concentration of the other acidic and neutral amino acids measured in this study are summarized in Table 3. Alanine, glycine, isoleucine, leucine, methionine, phenylalanine, tyrosine and valine increase in all tissues after a week of storage. The glutamic acid level increased in the intact and lean tissue after 8 days but remained unchanged in the fatty tissue. The concentration of cystine did not appear to change, and aspartic acid showed a decrease in the intact and lean tissue after 1 wk.

The observation that a greater degree of proteolysis takes place in adipose tissue on storage, as is the case with proline, was also noted in nine of the 11 other amino acids measured. Only glycine and glutamic acid demonstrate a greater relative percentage increase in the lean tissue.

The effects of extended storage at 2°C on the concentration of free proline in the lean tissue of green bellies is given in Table 4. Proline concentration was determined over a 4-wk period. The average concentration of proline increased from 26 μ M per 100g on the first day to 80 μ M on the 28th day. After 16 days, spoilage was clearly evident from the odor and color of the meat. Macerating this tissue did not yield higher levels of free proline.

Several studies on the conditions required for formation of nitrosamines from amino acids have been performed, including such parameters as pH, temperature, and nitrite. Bills et al. (1973), tested glutamic acid, glutamine and hydroxyproline in

Table 1—Effect of storage at 2°C on the concentration of free proline in pork bellies—intact, lean, and adipose tissues

Sample	Free proline (μ M/100g of tissue)					
	Intact		Lean		Adipose	
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
1	21.1	30.2	25.8	36.3	5.8	9.4
2	16.4	19.4	14.2	37.2	5.3	15.4
3	12.1	24.9	22.0	30.6	6.7	7.2
4	13.7	14.9	35.3	38.4	5.1	12.1
5	11.7	25.8	18.9	31.6	6.7	10.1
6	14.2	20.4	26.7	37.9	4.4	12.9
Avg	14.9	22.6	23.9	35.3	5.7	11.2

Table 2—Concentration of free proline in intact bellies actually found, as compared to that determined by calculations from lean and adipose tissues

	Free proline (μ M/100g of tissue)			Intact belly ^a
	Adipose	Lean		
Day 1 —	Found	5.7	23.9	14.9
	Calculated ^b			12.6
Day 8 —	Found	11.2	35.3	22.6
	Calculated ^b			20.2

^a Average green belly composition: 62.3% adipose and 37.7% lean

^b Calculated to composition of intact green belly

Table 3—Effect of storage at 2°C on concentration of free amino acids in pork bellies—intact, lean and adipose tissues

Amino acid ^b	Conc of free amino acids (μ M/100g tissue) ^a					
	Day 1			Day 8		
	Intact	Adipose	Lean	Intact	Adipose	Lean
Ala	143	40	244	188	58	320
Gly	109	32	148	132	37	181
Ile	10	3	14	15	9	21
Leu	17	6	23	29	20	35
Met	4	1	6	13	6	21
Phe	8	3	11	14	9	19
Tyr	7	3	11	11	6	14
Val	18	6	28	28	20	33
Glu	27	19	33	50	17	98
Cys	15	5	19	15	7	18
Asp	19	2	55	12	6	17

^a All values are averages of six samples.

^b Ala, Alanine; Gly, Glycine; Ile, Isoleucine; Leu, Leucine; Met, Methionine; Phe, Phenylalanine; Tyr, Tyrosine; Val, Valine; Glu, Glutamic Acid; Cys, Cystine; Asp, Aspartic acid

Table 4—Effect of extended storage at 2°C on the concentration of free proline in the lean tissue of pork bellies^a

Sample	Days postmortem						
	1	2	4	7	14	21	28
A	27.2	40.0	38.8	51.0	40.8	57.0	90.3
B	25.6	22.8	37.2	43.8	40.8	44.0	71.0
Avg	26.4	31.4	38.0	47.4	40.8	50.5	80.7

^a All values are averages of duplicate determinations.

an oil-water system, which at 170°C in the presence of sodium nitrite, did not form nitrosopyrrolidine. Ender and Ceh (1971) found that heating leads to the decarboxylation of amino acids to form corresponding amines, but only proline leads to the formation of nitrosopyrrolidine. Glycine, sarcosine (N-methylglycine), and valine produced mainly dimethylnitrosamine, while alanine formed dimethylnitrosamine and diethylnitrosamine. It appears evident that the major amino acid which may be an in vitro precursor in the formation of nitrosopyrrolidine is proline; and that the concentration of free proline increases in both the lean and adipose tissues of green bellies during storage, but at a faster rate in the latter.

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