Meat Flavor. Method for Rapid Preparation of the Water-Soluble Low Molecular Weight Fraction of Meat Tissue Extracts

Polystyrene resin Amberlite XAD-2 can be used to isolate the water-soluble low molecular weight fraction of meat. Aqueous extract of meat was deproteinized with picric acid and the excess picric acid was removed by adsorption on a column of the resin. The low molecular weight components of meat, which include the precursors of meat aroma, were eluted from the resin column with water. The fraction of meat thus obtained gave on pyrolysis a meat-like odor similar to that obtained from meat extract deproteinized by dialysis. The procedure described is simple, inexpensive, requires a much shorter time for deproteinization of tissue extracts than does dialysis, and yields a sample suitable for organoleptic evaluation and pyrolysis studies. Simultaneously with the removal of picric acid by chromatography on Amberlite XAD-2, fractionation of extract components may be achieved, particularly purine derivatives and some amino acids.

In connection with our studies on meat flavor precursors, we required the preparation of the water-soluble low molecular weight fraction of raw beef muscle, which contained the basic meat aroma precursors. Such a fraction has been obtained by the removal of the water-soluble proteins from an aqueous extract of meat by dialysis (Batzer *et al.*, 1960). Dialysis, however, is a slow process, generally requiring 16 hours or longer and allowing changes to take place in the sample (Stein and Moore, 1954). Thus, for routine handling of a large number of samples it became desirable to seek other means of obtaining the required low molecular weight fraction of meat.

Various protein precipitating agents have been employed for the preparation of protein-free tissue extracts. These range from inorganic salts, acids, and metal oxides to organic compounds such as trichloroacetic acid, ethanol, picric acid, sulfosalicylic acid, and even sulfonated diesel oil (Isacescu and Iancu, 1957). Hamilton and Van Slyke (1943) evaluated a number of protein precipitants and found that 1% aqueous picric acid solution was suitable for the deproteinization of plasma. They showed that picric acid precipitation gave α-amino nitrogen values for plasma comparable to those obtained by dialysis.

Since in our work samples were to be used subsequently for pyrolysis and organoleptic evaluation, it was necessary that the excess deproteinization reagent be completely removed from the extract solution. Thus substances such as acids or bases, capable of reacting with tissue extract components on heating, and protein precipitants possessing an odor, such as trichloroacetic acid, were unsuitable for our purposes unless they could be removed prior to pyrolysis. Picric acid, which has found wide application particularly in amino acid analysis (Stein and Moore, 1954), appeared to be a promising deproteinization reagent. Excess picric acid may be removed by retention on the strong anion exchange resin, Dowex 2 (Cl^-), according to the procedure of Stein and Moore (1954). However, dilute hydrochloric acid solution is used in this procedure to elute the extract components from the resin.

In our work on fractionation of beef diffusates (Zaika *et al.*, 1968) we employed a polystyrene resin crosslinked with divinylbenzene, Amberlite XAD-2, to isolate a fraction containing the precursors of meat aroma. On this resin, which does not have functional groups, nonpolar or aromatic molecules are adsorbed, while polar molecules pass through the column without adsorption (Hopkins, 1967). As a result, the following procedure was developed for the preparation of protein-free tissue extracts: Step 1. precipitation of protein from the tissue extract by picric acid, and Step 2. removal of excess picric acid by chromatography of the solution on a column of Amberlite XAD-2 resin on which the picric acid is strongly adsorbed. By proper selection of the chromatographic conditions, and with water as the eluting agent, the low molecular weight fraction of meat extract may be obtained free from excess deproteinizing agent.

EXPERIMENTAL

Preparation of Extract. Beef muscle (40 grams) was homogenized for 2 minutes in an electric blender with 80 ml. of 1% aqueous picric acid solution precooled to 4° C. The homogenate was transferred to centrifuge tubes with the aid of a small quantity of cold water, and the insoluble matter was removed by centrifugation at 12,000 × G for 30 minutes. A total of 110 ml. of deproteinized beef muscle extract was obtained. The sample preparation was carried out at 4° C.

As an alternate method of preparation of the tissue extract, 50 grams of beef muscle were homogenized with 100 ml. of water at 4° C., and the insoluble matter removed by centrifugation. Solid picric acid (0.9 gram) was added to the supernatant solution, the mixture was stirred for 10 minutes and centrifuged to remove the precipitated protein. The entire operation was carried out at 4° C.

Removal of Picric Acid from Beef Extract by Chromatography on Amberlite XAD-2. Crosslinked polystyrene polymer Amberlite XAD-2 (20- to 50-mesh, Rohm and Haas Co.) was purified by extensive washing with methylene chloride–methanol (1 to 3, v./v.), methanol, and finally water. The resin was suspended in water and poured into a chromatographic column to give a resin bed 2.9 × 34.5 cm. Picric acid extract (55 ml.), equivalent to 20 grams of beef muscle, was applied to the chromatographic column. Elution was carried out with water at a flow rate of 0.9 ml. per minute until the picric acid began to emerge from the column. Fractions (7 ml.) were collected and analyzed to locate the extract components. A flow rate of 2 ml. per minute has in recent experiments been found satisfactory, making it possible to complete the elution in *ca.* 6 hours.

The resin was regenerated by washing with methanol to remove the strongly adsorbed picric acid.

Analytical Methods. Chromatographic column effluents were collected with a Beckman Model 132 fraction collector. The ultraviolet absorbance of the effluents was monitored at 247 mμ with a Gilford Model 2000 multiple sample absorbance recorder to locate hypoxanthine and its derivatives. Fractions were analyzed for total amino acids by the ninhydrin method of Cocking and Yemm (1954) and for carbohydrates by the anthrone method of Toennies and Kolb (1964).

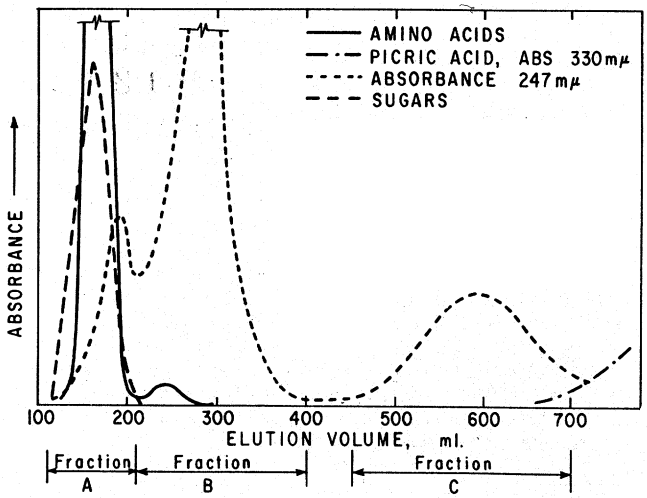

Figure 1. Chromatography on Amberlite XAD-2 of beef extract deproteinized with picric acid

Ultraviolet spectra were recorded with a Bausch and Lomb Spectronic 505 and absorbance in the visible region was measured with a Beckman Model B spectrophotometer. Amino acid analyses were performed according to Spackman *et al.* (1958) using a Phoenix automatic amino acid analyzer. Beef extract components were detected in the eluted fractions by thin-layer chromatography as described previously (Zaika *et al.*, 1968).

RESULTS AND DISCUSSION

Chromatography on polystyrene resin Amberlite XAD-2 of beef muscle extract containing picric acid (Figure 1) yielded separations similar to those previously obtained by chromatography of beef extract deproteinized by dialysis (Zaika *et al.*, 1968). Picric acid, strongly adsorbed on the polystyrene resin, began to emerge after inosine (ultraviolet absorbing peak No. 3) had been almost completely eluted. No components of beef extract had been found to elute after inosine (Zaika *et al.*, 1968). Thus, the removal of excess picric acid from the components of beef extract was essentially complete. The small amount of picric acid which emerged with inosine could be removed by rechromatography of the inosine fraction.

The eluted fractions A, B, and C (Figure 1) were subjected to thin-layer chromatography, ultraviolet spectrometry, and automatic amino acid analysis to establish their composition and to compare them with beef diffusate. The composition of these fractions is shown in Table I. Most components of beef extract were found in fraction A and may be recovered from a meat sample in a relatively short time.

A number of standard compounds known to be present in beef extract (diffusate) were also chromatographed on the polystyrene column for comparison. The elution volumes for these compounds are listed in Table II.

Since the purpose of this work was to isolate a meat flavor precursor fraction, the fractions eluted from Amberlite XAD-2 column were subjected to thermal decomposition (Zaika *et al.*, 1968) and the odors obtained were compared to the odor of beef diffusate produced in a similar manner. Fraction A yielded on pyrolysis an odor very much like that from beef diffusate. Fraction B also yielded an odor which was "grassy" in character, while fraction C yielded little, if any, odor. Fraction A, then, contains the important flavor precursors of the basic meaty aroma.

Table I. Composition of Fractions A, B, and C Eluted from Amberlite XAD-2 Column and of Beef Diffusate

Component	Diffusate[a]	Fraction A	Fraction B	Fraction C
Glucose	+	+	−	−
Fructose	+	+	−	−
Ribose	+	+	−	−
Glucose 6-$PO_4^{=}$	+	+	−	−
Unknown sugars	+	+	−	−
Lactic acid	+	+	−	−
Phosphates	+	+	−	−
Creatine	+	+	−	−
Creatinine	+	+	+	−
Urea	+	+	−	−
Taurine	+	+	−	−
Threonine	+	+	−	−
Serine	+	+	−	−
Asparagine-glutamine	+	+	−	−
Proline	+	+	−	−
Glutamic acid	+	+	−	−
Glycine	+	+	−	−
Alanine	+	+	−	−
Valine	+	+	−	−
Methionine	+	+	−	−
Leucine	+	−	+	−
Isoleucine	+	−	+	−
Tyrosine	+	−	+	−
Phenylalanine	+	−	+	−
Ornithine	+	+	−	−
Lysine	+	+	−	−
Histidine	+	+	−	−
Anserine	+	+	−	−
Carnosine	+	+	−	−
Arginine	+	+	−	−
Inosinic acid	+	+	−	−
Inosine	+	−	−	+
Hypoxanthine	+	−	+	−
Uric acid	+	−	+	−
Unknown, abs. max 265 mμ	+	−	+	−
Inorganic salts	+	+	−	−

[a] Zaika *et al.*, 1968.

Table II. Elution Volumes at Peak Concentrations

Compound[a]	Volume, Ml.	Recovery, %
Glycine	137	100
Glucose	140	99
Histidine	141	101
Inosinic acid	169	99
Tyrosine	247	100
Hypoxanthine	248	98
Inosine	579	98

[a] Compounds were applied to a column of Amberlite XAD-2, 2.9 × 34.5 cm. in aqueous or dilute HCl solutions in quantities of 2 to 10 mg. of each and eluted with water.

Precautions to be taken during protein precipitation have been mentioned by Hamilton and Van Slyke (1943). An excess of picric acid is to be avoided, otherwise loss of some basic components may result.

It seems unlikely that unknown flavor precursors have been removed either during treatment with picric acid or by chromatography on the polystyrene resin, since the column effluent corresponding to fractions A plus B gave on pyrolysis an odor indistinguishable from the odor of a meat flavor fraction deproteinized by dialysis.

Experiments with standard solutions of guanine and adenine showed, however, that these substances are eluted from columns of XAD-2 resin as very broad peaks with elution

volumes greater than that of inosine. Under the conditions of the present experiment these compounds would be only partially separated from picric acid. In the meat samples examined, however, the presence of guanine and adenine could not be demonstrated in the diffusates.

It is also unlikely that the flavor fraction eluted from the polystyrene resin column contained any picrates. These were not detected by thin-layer chromatography, nor did the spectra of eluted fractions show any absorption at wavelengths above 300 mμ.

The procedure described above offers a fast, convenient, inexpensive method for the isolation of the water-soluble low-molecular-weight components of meat as well as of other tissues. The fractions thus obtained are free from excess deproteinizing agent and are suitable for organoleptic evaluation and pyrolysis studies. Simultaneously, fractionation of meat extract components may be achieved, particularly of the purine derivatives. Also aromatic amino acids and the leucines may be separated from the other amino acids.

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