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SOME BIOCHEMICAL AND ELECTRON MICROSCOPIC
STUDIES OF THE PROTEIN PRESENT IN SEEDS
RECOVERED FROM TOMATO CANNERY WASTE.

The Ohio State University, Ph.D., 1974
Food Technology

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SOME BIOCHEMICAL AND ELECTRON MICROSCOPIC STUDIES OF
THE PROTEIN PRESENT IN SEEDS RECOVERED
FROM TOMATO CANNERY WASTE

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the
Graduate School of The Ohio State
University

By

Louise Karl Eggers, B.Sc., M.Sc.

* * * * *

The Ohio State University

1974

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DEDICATION

To my loving husband, Tom
ACKNOWLEDGMENTS

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The author wishes to thank her sister Ruth E. Karl for taking a part of the summer to help in the typing and preparation of this dissertation.

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INTRODUCTION

Pollution of the environment from industrial waste is increasing more rapidly than municipal waste and future emphasis will necessarily be placed on the abatement of this problem (Lacey and Keeler, 1970). These workers also stated that the United States fruit and vegetable processing industries alone generate and contribute millions of tons of waste annually to the environment. Recent USDA statistics indicate that the annual commercial crop of tomatoes for processing in the United States is approximately seven million tons, almost three million tons of which become processing waste (Rice and Stout, 1962; USDA, 1969). Ben-Gera and Kramer (1969) estimated that with the continued increased introduction of automation, the quantity of tomato cannery wastes will soon double.

The largest quantities of tomatoes are made into juice and juice products, i.e., catsup, paste, and puree. The residue or cannery waste, which consists of
peels, cores, culls, seeds, trimmings, and liquor, comprises 10 to 30% of the fruit depending on the cultivar and the end product produced. Disposal of this residue has consisted primarily of dumping, of use as land fill, and of flushing effluents into sewage plants, streams and rivers (Altschul, 1958).

Tomato product processing in Ohio is a seasonal operation. Many of these processing plants are small businesses and do not have the financial capabilities to purchase waste disposal equipment or are located in areas where solid waste disposal is a problem. This usually results in the processor trucking the waste to distant areas where large tracts of land are available for the spraying and spreading of waste. Disposal of tomato cannery waste in this manner has been a serious aesthetic problem to surrounding communities and is also an expense for the processor. Larger tomato processing operations, although they might have independent waste disposal facilities or access to municipal disposal, are also concerned with the problem and cost of tomato cannery waste disposal (Geisman, 1973).

Preliminary results obtained at The Ohio State University show that the tomato seeds alone comprise
approximately 51% of the total solid tomato product cannery waste. It has been known for some time that seeds synthesize and store large quantities of protein in the course of their development and that the proteins of seeds are of a complex mixture (Altschul, 1963 in Schultz and Anglemier, 1964). In 1969, Ben-Gera and Kramer claimed that the amino acid composition of tomato seed protein compared favorably with that of soybean protein. Hollingsworth and Greaves (1970) stated that the commonest of the nutritional deficiency diseases in the world today are the protein-calorie deficiency diseases and that new or unconventional sources of protein need to be investigated.

Ideally, a solution to the tomato cannery waste problem would:

1. decrease the amount of solid waste to be disposed, thereby decreasing cost for the tomato processor.
2. decrease pollution of the environment from industrial waste.
3. provide the tomato processor with a sellable and profitable by-product.
4. provide a product (perhaps a food or feed grade protein) that would be useful and economical from the consumer point of view.
This project was undertaken at the Department of Horticulture, The Ohio State University in an attempt to learn more about the proteins in tomato seeds. Specific objectives included:

1. separation of seeds from whole cannery waste.
2. determining the effects of pH and ionic strength on protein solubility.
3. determining the effect of various solvent media on the extraction of tomato seed protein.
4. demonstrating the molecular weight range of the various proteins present in aqueous extracts of tomato seed protein through the use of gel filtration.
5. demonstrating the heterogeneity or the numbers of protein species in the protein extracts through the use of polyacrylamide gel electrophoresis.
6. conducting preliminary amino acid analysis on whole ground tomato seed meal.
7. photographing the membrane bound protein bodies occurring in tomato seeds and observing the changes taking place in these protein bodies throughout the course of a germination series through the use of electron microscopy.
These specific objectives were completed and the results and implications are reported in this dissertation. It is hoped that these investigations will provide a basis for the utilization of tomato seeds as a source or food or feed grade protein.
REVIEW OF THE LITERATURE

According to Hülme (1971), the tomato, which is indigenous to the lower slopes of the Andes in South America, is a member of the potato family Solanaceae, belonging to the relatively small genus Lycopersicon. The economic importance of the tomato is considerable and world production of the fruit at 24 million metric tons was only surpassed by grapes at 51 million, citrus fruit at 31 million and pome fruit at 26 million (F.A.O., 1968).

Tomato Cannery Waste Utilization

The recovery of tomato pomace was studied by Edwards et al. in 1952. They passed tomato waste through a cyclone and dried and pressed it into a cake. Analyses of the dried cake were reported as follows: moisture, 8%; crude protein, 22.15%; fat, 14.2%; fiber, 29.6%; ash, 3.3%; and nitrogen free extract, 22.4%. Altschul (1958) reported the use of such dried tomato pomace in dog food and in feeds for fur-bearing animals. Stewart (1931) and Tomhave (1931) stated that tomato pomace used in cattle and dairy cow feeding provided good weight gain and milk production.
Three interesting reports published in the 1940's dealt with animal and human feeding studies. Researchers reported that consumption of dried tomato pomace produced an anti-diarrheal effect. The reasons for this effect were not stated (McCay and Smith, 1940; Smith, 1941; and Morris, 1946).

Auten in 1968 found that fresh tomato waste was also an acceptable ruminant feed. Its nutritional value was intermediate between alfalfa and corn silage. Chick feeding studies were conducted by Esselen and Fellers in 1939 and the tomato waste was reported to be an adequate feed supplement.

Economic studies into the utilization of tomato waste have also been conducted. Templeton (1947) and Edwards et al., (1952) suggested that the most profitable method of utilizing whole tomato waste was dehydration followed by direct feeding to animals. Dennison (1968), however, found that due to the large amount of water present in the waste, dehydration becomes too expensive and this was in agreement with the work of Autin (1968). Murthy et al., (1959) reported that the resins extracted from tomato peel could be economically used for varnishes. Casazza (1942) stated that the resins could specifically be used as varnishes for lining tin cans in the food industry.
Tomato seed oils extracted from cannery waste have been successfully used in the manufacturing of salad oils and margarine (Cavalcanti, 1941 and Dabrowska, 1970). Although the literature does not show that tomato cannery waste has ever been used as a substrate for single cell protein cultivation, workers have reported using other vegetable and fruit wastes for economical production of protein from fungi (Rogers, 1972).

Kwee (1970) noted in his work that along with protein, other potential by products could be obtained from tomato vines, leaves and fruit waste. For example, by spray or freeze drying, a red tomato powder that was rich in tomato flavor and high in protein could be produced from the whole cannery waste. Kwee proposed the use of this product in pasta products or as a breakfast drink. He also compared tomato cannery waste protein concentrate to the soy protein isolate, Ralston Purina Supro 610, and found the functional properties of the two proteins to be similar.

**Nutritional Properties**

The amino acids are the fundamental units of protein and there are eight essential amino acids (amino acids not synthesized in vivo or not synthesized in adequate amounts) for the adult human being: valine, leucine,
isoleucine, threonine, methionine, phenylalanine, tryptophan and lysine (Bigwood, 1972). These amino acids must be provided in the diet and failure to do so results in negative nitrogen balance representing a loss of nitrogen and mobilization of body protein reserves (Bigwood, 1972).

Rose et al., (1955), Leverton (1959) and Swendseid et al., (1956a, 1956b) conducted and reported studies concerning the quantitative and qualitative essential amino acid requirements for adult humans. The essential amino acids identified by these workers are: leucine, isoleucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine, tryptophan and valine. According to Lewis and Boorman (1970) and Irwin and Hegsted (1971) cystine is formed in the body from methionine and if dietary cystine is insufficient to meet body requirements, an increased demand or requirement for methionine occurs. Phenylalanine is converted into tyrosine in vivo and an insufficient amount of dietary tyrosine leads to an increased requirement in phenylalanine (Lewis and Boorman, 1970, Irwin and Hegsted, 1971). In other words, the dietary requirement for phenylalanine is a function of the total aromatic amino acid content of the diet.
Evidence indicating the importance of the non-essential amino acids also exists. Snyderman et al., (1968) found that administration of nonessential nitrogen in the form of glycine or urea could restore normal weight gain and nitrogen retention in infants after milk protein was reduced to the point where infant weight gain and nitrogen retention were adversely affected. Stucki and Harper (1962) reported that there was an optimal level of essential amino acid intake in rats beyond which no further weight gains are made and, if nonessential amino acid nitrogen is lacking, growth is inhibited.

According to Rosenfield (1971), the amino acid composition of protein foods determines the quality of the protein. Galizzi (1967) studied the amino acid content of tomato by-products and found glutamic acid to be the most abundant amino acid in the seed meal while glycine was the most abundant amino acid in the tomato skins. Ben-Gera and Kramer (1969) stated that the amino acid composition of tomato seed protein compared favorably with that of soybean protein. Vecchiotti and Piva (1964) reported that methionine was the limiting amino acid when tomato seed protein was used in pig feeding studies. Oren (1973) stated
that the sulphur containing amino acids were low in tomato seeds.

Nutritional studies utilizing tomato pulp, a dehydrated product of tomato culls, have been reported. Chapman et al., (1958) found that tomato pulp gave satisfactory results for fattening grazing steers. Ammerman et al., (1963, 1965) studied the nutritive value of tomato pulp for steers, lambs and chicks and reported favorable results as far as animal weight gain was concerned.

According to Kwee (1970), tomato cannery waste protein concentrates were slightly deficient in isoleucine, methionine and tryptophan as compared to the FAO provisional pattern and these deficiencies were more pronounced in the fractions extracted at low pH. Kwee (1970) conducted nutritional evaluations using *Tetrahymena pyriformis* W and his results showed that the protein quality of the extracts made at pH 4.0 and 4.8 were similar to the protein in meat, while fractions extracted at pH 3.5 were similar to the protein from maize and wheat. Oren (1973) conducted amino acid analysis on whole tomato cannery waste and reported low levels of lysine, cystine and methionine.

According to Hulme (1970), it was not possible to give a useful average amino acid content for whole
ripe tomatoes depending on the condition of growth and ripening. He did state, however, that glutamic acid was always predominant and that proline was virtually absent.

**Protein Bodies**

Osborne (1924) stated that as early as 1855, researchers were aware of the numerous intracellular protein granules contained in seed tissues. Hartig, in 1855, isolated these protein granules from oilseeds and named them "aleurone grains". Altschul et al., 1961 claimed that about 75% of the total soluble proteins of the peanut occurred as protein bodies in the seed. From the results published by Varner and Schidlosky in 1963, it has been estimated that about 50% of the total soluble proteins in peas are particle bound or occur in protein bodies. It has been more recently estimated that about 70% of the protein in soybeans resides in protein bodies (Tombs, 1967 and Saio and Watanabe, 1966).

In recent years, intact protein bodies have been prepared and studied from several ungerminated seeds: peanuts (Altschul et al., 1964 and Dieckert, 1962), cottonseed (Yatsu and Jacks, 1968), soybean (Saio and Watanabe, 1966 and Tombs, 1967 and Wolf,
1970), hempseed (St. Angelo et al., 1968), lima beans (Morris et al., 1970), corn (Christianson et al., 1969), wheat (Graham et al., 1963), rice (Mitsuda et al., 1969), barley (Tronier et al., 1971), and germinating peas (Matile, 1968 and Varner and Schidlovsky, 1963).

Bagley et al., (1963) studied the peanut (Arachis hypogaea) which contained about 25% of its protein in the cotyledons. They stated that the enormous variation in protein content of seeds was probably due to quantities of biologically active proteins in some seeds and to the presence in some seeds of large quantities of reserve proteins. They also showed that the parenchyma and cotyledon tissue of certain seeds had protein bodies that went through an organized system of change during germination; first, coalescence, followed by fragmentation, and finally, disappearance. Positive stains for protein confirmed that protein was present in these vacuoles.

Electron microscopic techniques have been employed to determine the purity and integrity of seed preparations and to examine them for substructures. In general, under the electron microscope, protein bodies of oilseeds are rather similar in appearance.
Dieckert (1962) and Lui and Altschul (1967) reported that the protein bodies of oilseeds varied in size from 1 to 20 microns in diameter, and that they contained seed storage proteins enclosed by a single membrane. These workers also stated that the protein bodies stain evenly with both osmic acid (OsO₄) and potassium permanganate (KMnO₄) and that some bodies contain globoids, the sites of phytic acid storage. Tombs (1967) however, found no evidence for localization of phytate in globoids within the protein bodies as noted with other seeds. He suggested that phytate was combined with proteins rather than being compartmentalized. Tombs' work with the electron microscope showed that the aleurone grains or protein bodies were large (2 to 10 microns) spherical particles while the oil was located in much smaller particles (0.2 to 0.5 microns in diameter) designated as spherosomes (Tombs, 1967).

The protein bodies were found to be surrounded by a membrane consisting of phospholipids and further, the membrane was stable to diethyl ether and to hexane (Tombs, 1967). Protein bodies also contain crystalloids (Ory et al., 1968 and St Angelo et al., 1968). Crystalloids were reported to be pure crystals of the
major seed storage protein and surrounded by spherosomes (storage organelles containing the oil of the seed) which fill the remainder of the cells. Hempseed was one seed having crystalloids within the protein bodies. The protein bodies were surrounded by spherosomes and contained globoids as did cottonseed. However, hempseed differed from other oilseeds studied in that the protein bodies contained distinct crystalloid-type inclusions. St. Angelo et al., (1968) isolated and characterized these crystalloids. They found them to be pure crystals of edestin, the major storage protein. The protein body itself was then found to be the major site of enzymatic activity.

Morphologically, the protein bodies of cereals are different from the protein bodies observed in oilseeds. In cereal seeds, the protein bodies contained neither globoids nor crystalloids of storage protein and they were generally circled by a single ring of spherosomes which also lined the inner wall of the cell (Graham et al., 1963; Khoo and Wolf, 1970; Mitsuda et al., 1969; Ory and Henningsen, 1969).

The enzymatic make-up and activity of seeds is another area of interest. Yatsu and Jacks (1968) isolated intact protein bodies by homogenizing and
fractionating dehulled cottonseed in water-free glycerol and through electron microscopic techniques and in vitro chemical tests confirmed the presence of acid hydrolytic activity in these organelles. Recently, Morris et al., (1970) examined protein bodies isolated from ungerminated lima beans and showed the presence of acid protease and acid phenylphosphatase activity. Phytase activity was absent in dormant seed preparations but was detected after four days of germination.

The preparation of seeds for study under the electron microscope has proven to be extremely difficult. Mollenhauer and Totten (1971) stated that inadequate methods and chemicals for the preservation of seeds for electron microscopy has hindered studies in this area. They reported that fixative and plastic penetration is inhibited by the density of cell walls and secreted slimes as well as by the dehydrated state of the seed tissues. The literature to date showed no evidence of the tomato seed being investigated under the electron microscope.

Methods Used To Study And Analyze Plant Proteins

According to Osborne (1924), plant proteins were
being studied and considered for use as or in foods as early as 1773 by two French experimenters, Rouelle and Parmentier. Throughout the 1800's basic work continued in the areas of animal, plant, and seed proteins (Dumas and Cahours, 1842; Hartig, 1855, 1856; Denis, 1859; Weyl, 1876, 1877). In 1924, Osborne published his classic work on protein classification. Osborne based his principal means for identifying and differentiating protein on solubility. His classification is still used today (Shultz and Anglemer, 1964). Classification of seed proteins according to Osborne's method included albumins (water soluble), globulins (insoluble in water but soluble in dilute neutral salt solutions), prolamines (ethanol soluble) and glutelins (insoluble in neutral salt solutions or in alcohol but soluble in dilute alkali or acid).

Shultz and Anglemer reported (1964) that the major proportion of seed proteins are the reserve or storage proteins, the seed enzymes being a minor proportion. They also stated that the proteins of seeds are a complex mixture. Some of the crystalline proteins or those purified by salt fractionation were grossly heterogeneous and evidence indicated overlapping of
protein species among the various solubility classes (Shultz and Anglemier, 1964).

Polyacrylamide gel electrophoresis provides a versatile way to analyze proteins and nucleic acids. Smithies (1955, 1959) stated that the purpose of a polyacrylamide gel was twofold. First, the gel matrix provides a homogenous support to eliminate gravity induced disturbances arising from density differences due to either local concentration changes or to ohmic heating from the passage of current. Second, gels of appropriate concentration have effective spacings in their matrices (pore sizes) on the same order of size as macromolecules and they act as molecular sieves in which smaller molecules move more freely than larger ones. In gel electrophoresis this effect on motility is superimposed on that of an electric field of charged molecules to obtain resolving power. Smithies (1959) also mentioned that acrylamide readily forms gels over a wide range of concentrations from approximately 2% (useful for macromolecules on the order of one or two million daltons) up to greater than 20% (useful for providing sieving effects for molecules as small as 1000 daltons).
He also reported that the acrylamide gels are formed by covalent bonding and therefore strong hydrogen bond breaking agents like urea could be included in the gels.

To date, no published results on the electrophoretic patterns of the proteins of tomato seeds have appeared in the literature. Recently, however, proteins of bean cotyledons have been fractionated into globulins, acid-soluble albumins and alkaline-soluble albumins. These fractions were subjected to sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis and the banding patterns obtained revealed information on the complexity of the proteins present, on molecular weight determinations and on the binding of protein units (McLeester, 1973). Protein electrophoresis has also been used to aid in cereal variety identification (Calif. Agr. St., 1973).

As reported by Lehninger (1970), isopycnic sucrose density gradient centrifugation is a versatile procedure used for determining sedimentation coefficients and for separating all types of macromolecules, organelles, and viruses. It has an advantage in that it is simple to perform and has good resolving power. Lehninger
(1970) described the method as follows. A sample (a mixture of macromolecules) is dissolved in a light solvent and layered on the top of a preformed sucrose gradient and then subjected to high speed centrifugation in a swinging bucket rotor. Each type of macromolecule sediments down the sucrose gradient at its own rate, depending on size, shape, weight, and density and forms into bands that can be recovered and then studied or fractionated by other methods. Sucrose was the gradient of choice because it was inexpensive, soluble, dialysable and it was harmless to most proteins (Anderson, 1966). The literature to date showed no published works dealing with tomato proteins being subjected to sucrose density gradient centrifugation.

Since the introduction of a cross-linked dextran (Sephadex) in 1959, molecular-sieve chromatography (also known as gel-filtration or exclusion chromatography) has been developed extensively and is now one of the principal techniques in the purification and characterization of proteins (Porath, 1962). As stated by Porath and Flodin (1959), separations by this technique depend on the fact that molecules larger
than the largest pores of the swollen Sephadex, i.e., above the exclusion limit, cannot penetrate the gel particles and therefore pass through the bed in the liquid phase outside the particles, and therefore are eluted first. Smaller molecules, however, penetrate the gel particles to a varying extent depending on their size and shape. Molecules are eluted from a Sephadex bed in the order of decreasing molecular size. Although tomato seed proteins have not been chromatographed on Sephadex columns, other seed proteins have. For example, the molecular sizes of the water extractable soybean proteins have been resolved by gel filtration (Hasegawa, et al., 1963).
METHODS AND MATERIALS

Tomato Seeds for Experimentation

Whole tomato waste was obtained from the Minster Canning Company, Minster, Ohio in the Fall of 1973. The seeds were separated from the whole waste by floatation and allowed to dry for 48 hours in a forced air oven at 28 C. Additional tomato seeds for experimentation were obtained from Dr. W. George, Department of Horticulture, The Ohio State University. Seeds for protein extraction studies were of mixed greenhouse and field cultivars while the tomato seeds used for electron microscopy studies were specifically Campbell 28(F) Lot #412-006, Petoseed-1972. Specific greenhouse cultivars 909-13, Mo-533 and M-R13 were used in an experiment to determine the amount of protein in different tomato seed cultivars. The seeds were then either stored at room temperature until further use or were ground in a Wiley mill fitted with a #40 mesh screen. When whole seed meal was to be used, it was freshly ground for each experiment. For most experiments, however, the ground seed meal was defatted with a 2:1 (v/v) ratio of chloroform and methanol.
Twenty grams of seed meal was mixed with 200 ml of the chloroform-methanol mixture and blended for 3 minutes in a Waring Blender. The slurry was then filtered through a Buchner funnel fitted with No. 1 Whatman filter paper. The seed meal was dried and the solvent allowed to evaporate in a forced air oven for 48 hours at 28°C.

To determine the weight of seeds contained in whole waste, similar portions of whole waste were forced air dried at 28°C for 48 hours and then weighed. The waste was then subjected to floatation and the seeds separated from the skins, cores, and peels. The two fractions were again dried, weighed and the percent weight of seeds contained in whole tomato cannery waste calculated.

Determination of Protein

The amount of protein present in the liquid samples obtained throughout these studies was determined by the method of Lowry (Lowry et al., 1951) and by the Biuret method (Stickland, 1951). In both methods, standard curves were constructed for each experiment. Examples of these sample curves appear in the appendix in Figures 5 and 6. The standard proteins used were bovine serum albumin (fraction V) and zein. Distilled water was used
in all control tubes. Absorbance was read at 555 nm in a Gilford Model 2400 Spectrophotometer that had been fitted with a rapid sampling device and a digital print-out. The micro-Kjeldahl method, as described by Ma and Zuazaga (1942), was used to determine the amount of crude protein in the dry ground seed samples. All protein determinations were carried out in triplicate for each experiment conducted.

**pH and Ionic Strength Studies**

The extractability of tomato seed protein as a function of pH was obtained by stirring defatted seed meal in distilled water (a meal water ratio of 1:10 (w/v) at room temperature for 30 minutes. Next, 1 N HCl or NaOH was added to vary pH. The extracts were centrifuged for 10 minutes at 12,000 g's and then analyzed for protein. The effect of solubility as a function of ionic strength was determined by stirring defatted seed meal (meal water ratio of 1:10 (w/v) in NaCl solutions of varying ionic strength, centrifuging and again analyzing the extracts for protein.

**Extractability Studies**

Protein was extracted from defatted tomato seed meal by mixing a 1:10 (w/v) ratio of seed meal with either distilled water; 0.1 M NaCl, 0.5 M NaCl and 0.25 M ascorbic
acid or 0.5 M sucrose. The slurry was slowly stirred on a magnetic stirrer for 30 minutes at room temperature. All extracts were then analyzed for protein.

Preparation of Extracts for Electrophoresis

Five grams of defatted seed meal were blended at room temperature with 50 ml of a solution containing 0.5 M NaCl and 0.25 M ascorbic acid. The filtrate was centrifuged at 20,000 g's for 30 minutes at 4 C. The supernatant was decanted and further clarified by two more centrifugations. To the final clear supernatant, an equal volume of distilled water was added. A white precipitate formed and was pelletted by centrifugation at 20,000 g's for 30 minutes. This fraction was then dissolved in 0.5 M NaCl and was designated fraction one. The remainder of the supernatant was dialyzed overnight at 4 C against a large volume of distilled water. A white precipitate formed and it was pelletted by centrifugation at 20,000 g's for 30 minutes. This pellet was designated fraction two and was dissolved in 0.5 M NaCl. The supernatant gave no further precipitate on prolonged dialysis and was designated the acid albumin fraction. Extracts for electrophoresis were also prepared by stirring a 1:10 (w/v) ratio of seed meal with distilled water or 0.1 N Tris. The extracts were centrifuged and then subjected to dialysis at 4 C.
overnight.

**Electrophoresis**

The discontinuous electrophoretic system used in these studies was according to the method of Davis (1964). The samples to be analyzed were mixed with 20% sucrose and layered on top of 3, 5, 7.5, and 10% (w/v monomer) acrylamide gels. Bovine serum albumin and normal human serum were used as controls. The amount of protein applied to the gel was varied to yield clear banding patterns. Tris-glycine buffer, pH 8.7, was used in both the upper and lower reservoirs. Bromophenol blue was used as the marker dye and was added to the upper reservoir buffer. The power supply was connected and 3 to 5 mA/tube was applied until the bromophenol blue marker dye had migrated the length of each running tube. After electrophoresis, the gels were removed from the glass running tubes by forcing cool tap water along the inside edges of the tube with a syringe and needle. All gels were stained with 0.5% Naphthalene Black in 7% acetic acid (v/v) for approximately two hours and then allowed to de-stain overnight in fresh 7% acetic acid in a recirculating destaining apparatus.

**Electron Microscopy**

Tomato seeds (Campbell 28, Lot #412-006, Potoseed-
1972) were germinated for various times on moist filter paper pads in plastic petri dishes at room temperature. After the appropriate germination time, the seeds were dissected and small pieces of cotyledon were excised from several seeds. The cotyledon pieces were then immediately placed in the appropriate fixatives.

Three different methods of fixation were used and compared. In the first method, tissues were fixed for three hours (room temperature) in 6% glutaraldehyde which had been prepared in 2% phosphate buffer (pH 7.1). Following fixation, the tissues were washed for eight hours with several changes of phosphate buffer. The tissues were then postfixed in 2% phosphate buffered OsO₄ for two hours at room temperature. Following the postfixation period, the samples were rinsed in several changes of phosphate buffer and then dehydrated for ten minutes each in 25, 50, 75 and 95% ethanol and in three 10-minute changes of 100% ethanol.

In the second method, the seed tissues were fixed in 2% unbuffered KMnO₄ for two hours at refrigerator temperature. Washing and dehydration was conducted as described above.

The third method involved fixing tissues in a 3% glutaraldehyde, 1.5% paraformaldehyde, 1.5% acrolein
mixture made up in phosphate buffer (pH 7.1) for two hours at room temperature. After fixation the samples were rinsed with five 10-minute changes of buffer. Postfixation was accomplished by soaking the tissues in either 1.5% OsO$_4$ for two hours at room temperature or by soaking them in unbuffered KMnO$_4$ for fifteen minutes in an ice bath. Washing and dehydration were done as described in the first two methods.

Infiltration of the samples for the three different methods was done in the same manner. The dehydrated tissues were soaked for one hour in a 1 to 3 (v/v) ratio of Spurr's resin and ethanol, then in a 1 to 1 (v/v) ratio of resin to ethanol for three hours and finally in a 3 to 1 (v/v) ratio of resin and ethanol for twelve hours. The tissues were then transferred to 100% resin in plastic beam capsules and placed in a 70 C vacuum oven for fifteen minutes. The capsules were then placed in a 70 C oven at atmospheric pressure and left until hardened (sixteen hours). Ultra-thin sections were cut with an ultra-microtome that had been fitted with a diamond knife. Post staining of the thin sections was accomplished by floating the grids (specimen side down) on a drop of 2% uranyl acetate in 50% ethanol for four minutes followed by rinsing in distilled water. The specimen grids were then floated
on a drop of lead citrate for two minutes, rinsed in
distilled water and allowed to air dry. All grids were
viewed with a Zeis EM-9A electron microscope.

**Preparation of Extracts for Sucrose Gradients and**
**Gel Filtration**

Water extractable tomato seed protein was obtained
by stirring defatted meal with water (water meal ratio
10:1 (v/w) at room temperature for thirty minutes
and centrifuging for ten minutes at 4 C at 12,000 g's.
The clarified extracts were dialyzed in the cold for
twenty-four hours against several changes of phosphate
buffer to remove ultraviolet absorbing substances.

**Sucrose Density Gradient Centrifugation**

A continuous sucrose gradient (10 to 40%) was
preformed by an Isco Model 570 gradient former in
nitrocellulose tubes (1" X 31/2"). A 1.5 ml aliquot
of sample was carefully layered on top of the gradients
and run in a preparative ultracentrifuge for two hours
at 200,000 rpm's. An SW-27 swinging bucket rotor (bucket
capacity 38.5 ml) was used. After completion of the
run, the tubes were fractionated and the contents of
the tubes channeled through a Gilford UV Spectrophotometer
and analyzed for protein at 280 nm.

**Gel Filtration**

Sephadex G-200 (Pharmacia Fine Chemicals, Inc.,
New Jersey) was swelled on a boiling water bath for five hours and allowed to cool and then carefully packed into a 2.5 cm Bio-rad column. Phosphate buffer, containing 0.005% ethyl mercuric thiosalicylate to prevent microbial growth, was used as the eluent at an operating pressure of 16-cm H$_2$O or 0.12 ml/minute. The eluent was continuously fed into the column from two reservoirs. Blue Dextran 2000 was used to calculate void volume and riboflavin and hemoglobin were used to monitor the column. Approximately 1.5 ml of sample was layered on top of the column. The column was channeled through a Gilford UV spectrophotometer and continuously monitored for protein at 280 nm.

**Amino Acid Analysis**

The Division of Agronomy, The Ohio Agricultural Research and Development Center, Wooster, Ohio conducted and reported the quantitative amino acid analyses on dry ground tomato seed meal samples. All samples were hydrolyzed in 6 N HCl at 110 C for twenty-four hours and 0.4 ml of the diluted aliquot injected into the instrument. The instrument used was a Beckman Model 120-B amino acid analyzer.
RESULTS AND DISCUSSION

Tomato cannery waste obtained from the Minster Canning Company, Minster, Ohio consisted of seeds, cores, skins and peels. It was found in our laboratory that the seeds were easily separated from the waste by floatation and that the seeds comprised, on the average, 51% of the waste by weight. This is in close agreement with the work done by Johns and Gersdorff in 1922. They found that their tomato waste consisted of 46.1% seeds by weight. The food processing industry in Ohio is mainly a tomato industry and Ohio produces annually about 550,000 tons of tomatoes for processing (U.S.D.A, 1972). Of this 550,000 tons, about 42,000 tons of solid waste is generated and disposal of this waste is a problem. The recovery of seeds from tomato waste by floatation could be used by processors with little expense and technical ability. Recovery could also help reduce the problem of solid waste disposal and could provide a salable product for the tomato processor.

The possibility of tomato processors utilizing the seeds as a protein source has not extensively been investigated. In our laboratory, experiments were first
conducted to determine the amount of protein present in the tomato seeds. The tomato waste used in these studies was from tomatoes of mixed field cultivars. Whole cannery waste was found to contain approximately 15% protein while the tomato seed meal from the cannery waste seeds was found to contain on the average, 25.8% crude protein according to the micro-Kjeldahl method ($N \times 5.85$). Defatted seed meal from the cannery waste seeds contained 28.8% protein. Campbell-28, a field cultivar used only for electron microscopy in these studies, was determined to contain 25.6% protein. Tomato seeds from greenhouse cultivars, however, were found to contain on the average, a lower percentage of protein. These results are shown in Table 1.

Johnson et al., (1972) stated that protein content is under genetic control and is influenced by cultural practices and production environment. This could explain the range of protein (20.1% to 25.8%) between the greenhouse and field tomato seeds. Johnson et al., (1972) stated that it is not unusual to find wide differences in the protein content of grains and oilseeds. For example, protein values as high as 18% and as low as 7% were found in wheat in the United States (Johnson et al., 1972). Future research could
### TABLE 1
PROTEIN CONTENT OF WHOLE TOMATO WASTE AND A FEW SELECTED TOMATO SEED CULTIVARS AS DETERMINED BY THE KJELDAHL METHOD

<table>
<thead>
<tr>
<th>MATERIAL TESTED</th>
<th>% PROTEIN EXTRACTED</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Tomato Cannery Waste</td>
<td>15.0</td>
<td>14.8-15.3</td>
</tr>
<tr>
<td>Cannery Waste Seeds</td>
<td>25.8</td>
<td>25.3-26.0</td>
</tr>
<tr>
<td>(mixed field cultivars)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohio M-R13</td>
<td>25.3</td>
<td>24.9-25.5</td>
</tr>
<tr>
<td>909-13</td>
<td>20.1</td>
<td>19.9-20.8</td>
</tr>
<tr>
<td>Mo-533</td>
<td>22.8</td>
<td>21.5-23.0</td>
</tr>
<tr>
<td>Campbell-28</td>
<td>25.6</td>
<td>25.1-25.9</td>
</tr>
<tr>
<td>Defatted Seed Meal (seeds from cannery waste)</td>
<td>28.9</td>
<td>28.3-29.5</td>
</tr>
</tbody>
</table>

### TABLE 2
AMOUNT OF PROTEIN EXTRACTED FROM DEFATTED TOMATO SEED MEAL AS A FUNCTION OF pH

<table>
<thead>
<tr>
<th>pH</th>
<th>% PROTEIN EXTRACTED</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>7.7</td>
<td>7.1-7.9</td>
</tr>
<tr>
<td>3.2</td>
<td>3.7</td>
<td>3.5-3.9</td>
</tr>
<tr>
<td>4.5</td>
<td>3.0</td>
<td>2.7-3.3</td>
</tr>
<tr>
<td>5.3</td>
<td>4.0</td>
<td>3.5-4.2</td>
</tr>
<tr>
<td>5.8</td>
<td>5.2</td>
<td>5.0-5.8</td>
</tr>
<tr>
<td>6.0</td>
<td>6.0</td>
<td>5.9-6.5</td>
</tr>
<tr>
<td>6.5</td>
<td>7.7</td>
<td>7.0-8.1</td>
</tr>
<tr>
<td>7.2</td>
<td>9.1</td>
<td>8.5-9.6</td>
</tr>
<tr>
<td>8.5</td>
<td>9.7</td>
<td>9.0-9.9</td>
</tr>
<tr>
<td>11.2</td>
<td>11.0</td>
<td>10.8-11.6</td>
</tr>
</tbody>
</table>

The average of 15 samples is reported, three runs of each.
include investigating the quality and quantity of protein contained in the seeds of tomatoes as influenced by production environment and plant breeding.

Tomato seeds recovered from tomato product cannery waste have a protein content comparable to grain and oilseeds. Defatted tomato seed meal contains about 29% protein while whole rice contains 7.5%; cottonseed meal, 35%; whole grain wheat, 12.2%; low fat soybean flour, 44.7% and corn meal, 9.2% protein (Rosenfield, 1971).

Experiments were also conducted to determine the effect of pH and ionic strength on the solubility and extraction of tomato seed proteins. The effect of pH on the solubility of tomato seed proteins is shown in both Table 2 and Figure 1. A suspension of defatted tomato seed meal in water (w/v ratio of 1:10) had a pH of 5.8 and 5.2% protein was solubilized. Addition of acid decreased solubility and the least amount of protein extracted (3.0% protein) occurred at around pH 4.5. This was similar to the isoelectric point of soybean protein and to casein which occurred around pH 4.2 to 4.6 (Wolf and Cowan, 1971). Further addition of acid caused the resolubilization of the
FIGURE 1  EXTRACTABILITY OF PROTEIN IN DEFATTED TOMATO SEED MEAL AS A FUNCTION OF pH.
proteins below the isoelectric point. At pH 1.8, 7.7% protein was dissolved. Addition of alkali caused a constant increase in the amount of protein extracted from the tomato seed meal. At pH 11.2, 11.0% protein was dissolved.

The effect of ionic strength on the solubility of tomato seed proteins is shown in Table 3 and Figure 2. Protein solubility increased from 5.2% to 7.3% as the ionic strength was increased from 0 to 0.50. Dechary et al., (1961), Wolf and Cowan (1971) and McLeester et al., (1973) reported that the majority of seed proteins were globulins. They also reported that globulins are sensitive to changes in their ionic and pH environment in aqueous systems; being soluble in salt solutions and insoluble in water in the region of their isoelectric points. Since the majority of proteins in oilseeds are contained in membrane bound protein bodies, often the extraction of proteins from seeds has been difficult and incomplete (Tombs, 1967).

Experiments need to be conducted in order to determine a method for more efficient and complete extraction of tomato seed protein. The experiments conducted for this dissertation indicated that at a neutral pH of 7.2, 9.1% (or approximately 1/3 of the
### TABLE 3
AMOUNT OF PROTEIN EXTRACTED FROM DEFATTED TOMATO SEED MEAL AS A FUNCTION OF IONIC STRENGTH

<table>
<thead>
<tr>
<th>IONIC STRENGTH</th>
<th>% PROTEIN EXTRACTED</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>7.3</td>
<td>6.8-7.5</td>
</tr>
<tr>
<td>0.10</td>
<td>6.6</td>
<td>6.1-6.9</td>
</tr>
<tr>
<td>0.05</td>
<td>6.0</td>
<td>5.4-6.6</td>
</tr>
<tr>
<td>0.02</td>
<td>5.5</td>
<td>5.0-5.7</td>
</tr>
<tr>
<td>0.01</td>
<td>5.2</td>
<td>5.0-5.8</td>
</tr>
<tr>
<td>distilled water</td>
<td>5.2</td>
<td>4.9-5.5</td>
</tr>
</tbody>
</table>

### TABLE 4
PROTEIN EXTRACTABILITY STUDIES

<table>
<thead>
<tr>
<th>EXTRACTION METHOD</th>
<th>% PROTEIN EXTRACTED</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M NaCl</td>
<td>5.6</td>
<td>5.0-5.9</td>
</tr>
<tr>
<td>0.5 M sucrose</td>
<td>7.0</td>
<td>6.7-7.3</td>
</tr>
<tr>
<td>0.5 M NaCl and 0.25 M ascorbic acid</td>
<td>5.7</td>
<td>4.9-5.5</td>
</tr>
<tr>
<td>distilled water</td>
<td>5.2</td>
<td>4.7-5.3</td>
</tr>
</tbody>
</table>

b The average of 15 samples is reported, three runs of each.
FIGURE 2  EXTRACTABILITY OF PROTEIN IN DEFATTED TOMATO SEED MEAL AS A FUNCTION OF IONIC STRENGTH
total protein available in the tomato seeds) can easily be made soluble. It was also found that a NaCl solution (0.50 ionic strength) was effective in extracting 7.3% crude protein but these samples required extensive and time consuming dialysis after extraction in order to remove the NaCl. Subjecting the ground tomato seed to heat or to solvents utilizing a combination of pH and ionic strengths could perhaps increase the amounts extracted and the ease of protein extraction. Various solvents used in these studies for protein extraction included 0.5 M sucrose, 0.1%M NaCl, distilled water and a solution of 0.5 M NaCl and 0.25 M ascorbic acid. The 0.5%M sucrose solution was effective in extracting 7.0% crude protein. These results are shown in Table 4.

Experiments were then conducted in order to demonstrate the numbers of proteins present in aqueous extracts of the tomato seed meal and to demonstrate the ranges in molecular weight. Results from gel filtration on Sephadex G-200 appear in Table 5 and Figure 3.

A mixture of Blue Dextran, bovine serum albumin and tomato seed protein extract was layered on top of the column. It was expected that the tomato seed protein would be fractionated into several components, depending on size and molecular weight. The tomato seed protein,
however, consistently eluted with the Blue Dextran at around 27 ml while the bovine serum albumin eluted at about 42 ml. Blue Dextran is prepared from dextran, a high molecular weight branched polysaccharide of bacterial origin, with an average molecular weight of 2,000,000 by the introduction of a polycyclic chromophore (Pharmacia Fine Chemicals AB, 1972). Because of its high molecular weight, Blue Dextran is completely excluded from the interstitial spaces of all Sephadex G-types. Any protein with a molecular weight as large or larger than the Blue Dextran would also be excluded from the Sephadex, passing straight through the column without being fractionated. These results indicated that the tomato seed protein in the extract was of a very high molecular weight.

It is unlikely that any seed globulin or albumin would have molecular weights in the range of 2,000,000 daltons therefore another reason for the proteins early elution with the dextran is probable. It has been known for some time that plant and seed proteins undergo complex association-dissociation reactions that make analysis difficult. In 1959, while working with protein extracts of cabbage leaves, Heitefuss and co-workers reported complex protein aggregation reactions occurring due to the formation of hydrogen bonding,
### TABLE 5
GEL FILTRATION

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>SAMPLE</th>
<th>NUMBER OF ML IN WHICH SAMPLE ELUTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mixture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.2, 42.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Mixture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.8, 42.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Blue Dextran</td>
<td>26.5</td>
</tr>
<tr>
<td>4</td>
<td>Blue Dextran</td>
<td>27.4</td>
</tr>
<tr>
<td>5</td>
<td>Blue Dextran</td>
<td>27.0</td>
</tr>
<tr>
<td>6</td>
<td>Blue Dextran</td>
<td>27.6</td>
</tr>
<tr>
<td>7</td>
<td>ATSPE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.6</td>
</tr>
<tr>
<td>8</td>
<td>ATSPE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.8</td>
</tr>
<tr>
<td>9</td>
<td>ATSPE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.3</td>
</tr>
<tr>
<td>10</td>
<td>ATSPE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sample consisted of a mixture of blue dextran, bovine serum albumin and an aqueous extract of tomato seed protein.

<sup>b</sup> aqueous tomato seed protein extract

<sup>c</sup> The first figure is the number of ml to elute the blue dextran and the ATSPE<sup>b</sup> while the second figure is the number of ml to elute bovine serum albumin.
FIGURE 3 GEL FILTRATION OF TOMATO SEED PROTEIN, BLUE DEXTRAN AND BOVINE SERUM ALBUMIN.
ionic bonding and Van der Waals forces. Wolf et al. (1962) reported that some soybean globulins in the molecular weight range of 350,000 daltons were capable of forming disulfide polymers and associating to higher molecular weight units or dissociating into subunits. They also reported changes in molecular size and weight depending on the solvent used in extracting the proteins. Tombs (1967) reported similar problems in fractionating and characterizing seed proteins. He suggested that the extracted proteins undergo dimerization but that the dimerization is reversible. Wolf and Cowan (1971) discussed the fact that plant and seed proteins undergo association-dissociation reactions and that the exact nature of these reactions were unknown.

In order to determine whether or not the tomato seed proteins were undergoing association or dimerization into very high molecular aggregates, the experiments involving sucrose density gradient centrifugation were conducted. With sucrose gradient centrifugation, more factors were involved in causing separation of proteins than with gel filtration. Sucrose density gradient centrifugation takes advantage of the molecular size, shape, weight and density in the separation of
molecules. It is known that the presence of polyhydroxy compounds such as sucrose, prevent or reverse aggregation of proteins (Heitefuss et al., 1959). Results in Figure 4 show that at least 3 peaks or protein species of medium molecular weight were present in the tomato seed protein extract. These peaks did not appear in the Sephadex gel filtration column, suggesting that there were protein aggregation and dimerization reactions occurring. A fourth peak, as indicated by a broken line of circles on Figure 4, appeared as the bottom contents of the sucrose gradient tubes were analyzed for protein. This would indicate still higher molecular weight proteins and protein aggregates. This fraction could be further studied and fractionated by subjecting it to a higher percent sucrose gradient (30-70%). Availability of equipment and expense prevented the completion of the 30-70% gradients.

Evans et al., (1962) stated that the total proteins of a seed or even of a crude fraction of the total proteins contains a number of protein species. Electrophoresis is one of the methods of choice in preliminary protein studies and in demonstrating the numbers of protein species and their molecular weights.
FIGURE 4 SUCROSE GRADIENT (10-40%) PROTEIN FRACTIONS
Maizel (1971) stated that the concentration of polyacrylamide provides a sieving effect that aids in the separation of protein molecules. He also stated that a standard gel (7.5% acrylamide) separates the majority of proteins ranging from 10,000 to 1,000,000 molecular weight, with maximum resolution from 30,000 to 300,000 daltons while small pore gels (15 to 30% acrylamide) are for materials with molecular weights below 10,000. The 3.75 to 7.0% gels are best for materials with molecular weights above 1,000,000.

In the electrophoretic experiments conducted with tomato seed protein extracts, gels with 3.5, 5.0, 7.5 and 10% acrylamide were used. This was done to achieve good resolution and to obtain an idea of the molecular weight range of the protein species. These experiments were conducted under mild conditions of pH and no reagents such as urea were present that might possibly create additional components. The best resolution was achieved by the discontinuous system of Davis (1964). Photographs of representative electrophoretic patterns on polyacrylamide gels are shown on Plates 1 through 4. Plate 1 shows two electrophoretic runs of a 0.1 M tris extract of tomato
seed protein. Four bands can be seen indicating four different protein species. Some trailing or smearing can be seen and this could indicate over-loading of sample or the presence of other very low molecular weight substances. Plate 2 shows a water extract of tomato seed protein on gels of 3.5, 5.0, 7.5 and 10% acrylamide concentration. The 10% gel provided the best resolution. An interesting difference appears on these gels. Five bands can be seen in the upper half of the gels and on the top of the gels, a larger more dense band can be seen. This more dense band can be seen moving into the gel bed of the 3.5 and 5.0% acrylamide tubes. This would indicate a high molecular weight protein or an aggregation or dimerization of proteins that occurs with protein extraction in water but not in 0.1 M tris. These results support the findings of the gel filtration and sucrose density gradient centrifugation experiments.

Plate 3 demonstrates the 0.5 M NaCl, 0.25 M ascorbic acid protein extract fractionated on 3.5, 5.0, 7.5 and 10% gels. McLeester et al., (1973) found that an ascorbic acid, NaCl medium facilitated the extraction and fractionation of the seed globulins and albumins from bean seeds. The best resolution
was obtained on the 7.5% acrylamide gels. Five distinct bands appear and there is no evidence of trailing and smearing or of a high molecular weight species sitting on the top of the gel.

In all cases, the proteins were best resolved on standard 7.5 and 10% gels indicating that the proteins were in the 30,000 to 300,000 molecular weight range. The protein band that appeared moving into the 3.5% gel would indicate a protein aggregation or a protein species with a molecular weight above 1,000,000.

Plate 4 demonstrates the effect of extensive dialysis on the separation of the tomato seed proteins. By decreasing the salt concentration through dialysis, the globulins were precipitated while the acid soluble albumins remained in solution. Gel 2 is globulin fraction number one while gel 3 is globulin fraction two and gel 4 is the acid soluble albumins and perhaps a globulin species that remains soluble even after extensive dialysis.

The information obtained from the electrophoresis experiment is valuable in that it demonstrates the number of proteins present in aqueous extracts. It also indicates that the method of extraction affects
the form in which the protein is extracted. That is, whether or not the protein is extracted as separate species or as large aggregates or dimers. The form of protein extracted determines its characteristics in a food system and this would be of interest to the food technologist.

As already mentioned, there are eight essential amino acids for man; tryptophan, phenylalanine, methionine, lysine, valine, threonine, isoleucine and leucine. Kretovich (1966) pointed out the importance of studying the amino acid composition of the whole protein complex occurring in a particular foodstuff. He stated that only with this approach can correct data on amino acid composition be obtained and consequently on the feeding value, because individual proteins may be incomplete in their essential amino acid composition. In this study, whole defatted ground tomato seed was analyzed for amino acid composition. Seed meals remaining after extraction with various solvents were also analyzed. The results from these experiments are found in Table 6, page 60.

Tomato seed protein, in comparison to soy flour, was about 13% higher in lysine, 42% higher in arginine and about 49% higher in threonine. Tomato seed protein however, contained only about one-ninth the amount of
Plate 1. A 0.1 M tris extract of tomato seed protein electrophoresed on 7.5% acrylamide gels.
Plate 2. A water extract of tomato seed protein electrophoresed on 3.5, 5.0, 7.5 and 10% acrylamide gels.
Plate 3. A 0.5 M NaCl, 0.25 M ascorbic acid extract of tomato seed protein electrophoresed on 3.5, 5.0, 7.5 and 10% acrylamide gels.
Plate 4. Electrophoresis of tomato seed protein fractions after extensive dialysis on 7.5% acrylamide gels.

gel 1 - BSA
gel 2 - globulin fraction 1
gel 3 - globulin fraction 2
gel 4 - acid soluble albumins
cystine and only about one-half the amount of methionine as did the soy flour. Similarly, in comparison to corn (opaque-2), the tomato seed protein was high in lysine, threonine and arginine but low in methionine, cystine and leucine. It is interesting to note that tomato seed protein contains about 60% more threonine per 100 grams of protein than does egg or milk (cow) protein. Lysine amounts in egg, milk and tomato seed protein are about the same. Amount of sulfur containing amino acids present in tomato seed protein are low in comparison to egg and milk protein. Tryptophan was not analyzed in these studies.

Proteins deficient or low in amino acids can be corrected in whole or in part by protein supplementation. A protein presenting a poor amino acid balance could be mixed with another protein which contained an amino acid limiting in the first protein. Therefore, each tends to make up for the deficiencies of the other. Bressani et al., (1971) have stated that the cereal grains are mainly deficient in lysine although other essential amino acids are also limiting. The findings of these experiments point to the possibility of using tomato seed protein
as a supplementing protein since the tomato seed appears to contain high amounts of lysine.

It can also be seen from the differences in amino acid composition on Table 6, that the choice of solvent does have an effect on the protein extracted. Sample one, which was extracted once with distilled water, was much lower in phenylalanine, tyrosine and threonine than the original unextracted tomato seed meal while there was little change in the amount of lysine. Samples two and three, which were extracted with 0.5 M NaCl and 0.5 M NaCl, 0.25 M ascorbic acid respectively, were lower in lysine as well as the other amino acids. These differences in amino acid composition indicated that different amounts and kinds of proteins can be extracted by utilizing different solvents.

Cotyledon tissue in resting and germinating seeds contains a large number of storage bodies in which much of the seed protein is concentrated. These protein stores or reserves of the resting seed have been found to make up anywhere from 25% to 70% of the protein of the seed and were present in protein bodies (Tombs, 1967 and Evans et al., 1962). The function of
### TABLE 6

**AMINO ACID COMPOSITION OF VARIOUS TOMATO SEED MEALS**

<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>SAMPLE 1</th>
<th>SAMPLE 2</th>
<th>SAMPLE 3</th>
<th>SAMPLE 4</th>
<th>SAMPLE 5</th>
<th>SAMPLE 6</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LYSINE</td>
<td>6.0</td>
<td>3.4</td>
<td>3.4</td>
<td>6.6</td>
<td>5.8</td>
<td>4.8</td>
</tr>
<tr>
<td>HISTIDINE</td>
<td>2.9</td>
<td>1.5</td>
<td>1.5</td>
<td>2.9</td>
<td>2.3</td>
<td>3.3</td>
</tr>
<tr>
<td>ASPARAGINE</td>
<td>7.6</td>
<td>5.5</td>
<td>5.8</td>
<td>10.3</td>
<td>5.8</td>
<td>8.5</td>
</tr>
<tr>
<td>ASPARTIC ACID</td>
<td>8.0</td>
<td>6.3</td>
<td>4.1</td>
<td>6.7</td>
<td>-</td>
<td>10.8</td>
</tr>
<tr>
<td>THREONINE</td>
<td>2.5</td>
<td>1.1</td>
<td>1.4</td>
<td>7.8</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>SERINE</td>
<td>1.8</td>
<td>1.3</td>
<td>0.9</td>
<td>2.1</td>
<td>-</td>
<td>4.8</td>
</tr>
<tr>
<td>GLUTAMIC ACID</td>
<td>7.9</td>
<td>3.3</td>
<td>0.7</td>
<td>11.9</td>
<td>-</td>
<td>17.5</td>
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<tr>
<td>PROLINE</td>
<td>5.5</td>
<td>5.7</td>
<td>4.3</td>
<td>6.0</td>
<td>-</td>
<td>7.6</td>
</tr>
<tr>
<td>GLYCINE</td>
<td>2.4</td>
<td>0.9</td>
<td>1.2</td>
<td>3.3</td>
<td>-</td>
<td>4.8</td>
</tr>
<tr>
<td>ALANINE</td>
<td>5.0</td>
<td>3.8</td>
<td>4.8</td>
<td>5.3</td>
<td>-</td>
<td>6.6</td>
</tr>
<tr>
<td>CYSTINE</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.9</td>
<td>1.7</td>
</tr>
<tr>
<td>VALINE</td>
<td>4.9</td>
<td>3.5</td>
<td>3.5</td>
<td>4.6</td>
<td>4.2</td>
<td>5.1</td>
</tr>
<tr>
<td>METHIONINE</td>
<td>0.5</td>
<td>0.4</td>
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<td>0.1</td>
<td>2.0</td>
<td>2.1</td>
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<td>ISOLEUCINE</td>
<td>4.3</td>
<td>3.1</td>
<td>3.1</td>
<td>4.4</td>
<td>4.7</td>
<td>3.4</td>
</tr>
<tr>
<td>LEUCINE</td>
<td>2.6</td>
<td>2.0</td>
<td>1.7</td>
<td>2.6</td>
<td>6.6</td>
<td>9.1</td>
</tr>
<tr>
<td>TYROSINE</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>3.4</td>
<td>4.1</td>
<td>4.0</td>
</tr>
<tr>
<td>PHENYLALANINE</td>
<td>0.8</td>
<td>0.7</td>
<td>0.7</td>
<td>3.9</td>
<td>5.7</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Sample 1 Whole defatted ground tomato seed after one extraction with distilled water.

Sample 2 Whole defatted ground tomato seed after one extraction with 0.5 NaCl.

Sample 3 Whole defatted ground tomato seed after one extraction with a 0.5 M NaCl, 0.25 M ascorbic acid medium.

Sample 4 Whole defatted ground tomato seed.

Sample 5 Soybean flour. Data from Meyer (1968).

Sample 6 High lysine corn (opaque-2). Data from Mertz (1972).
<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>TSP</th>
<th>EGG&lt;sup&gt;a&lt;/sup&gt;</th>
<th>COW'S-MILK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucine</td>
<td>4.4</td>
<td>6.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.6</td>
<td>8.8</td>
<td>9.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>6.6</td>
<td>6.4</td>
<td>7.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.9</td>
<td>5.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.4</td>
<td>4.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.2</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.1</td>
<td>3.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Threonine</td>
<td>7.8</td>
<td>5.1</td>
<td>4.6</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>-</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Valine</td>
<td>4.6</td>
<td>7.3</td>
<td>6.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from Bigwood (1972).
the storage proteins is to supply nitrogenous material to the growing seedlings on germination. It has been assumed that the protein body is digested enzymatically on germination, whereupon the amino acids and peptides are made available for protein synthesis. The protein body eventually disappears (Dechary et al., 1961).

As stated by Mollenhauer and Totten (1971), chemicals and methods for the fixation of seed tissue remain inadequate to this day. The seed tissue's cell walls and secreted slimes pose a barrier to fixative and plastic penetration. Three separate methods of processing tomato seed cotyledons at various stages of germination were examined in this study. The first method, as described in the section on Methods and Materials, involved fixation in 6% glutaraldehyde for three hours at room temperature followed by 2% OsO₄ for two more hours at room temperature. No electron-micrographs were obtained from this method. The tissues were not evenly fixed and infiltration was poor.

The second method, however, gave good results. In this method, the seed tissue was fixed in 2% un-
buffered KMnO₄ for two hours at 4 °C. Examples of the KMnO₄ fixation are seen on plates 5, 6, 8, 9, 10 and 11. With the KMnO₄, there is a deposition of electron-opaque manganese dioxide at the sites of membranes thereby fixing membranes well. It does not however, preserve ribosomes or chromosomal material well. Contrast was rather poor on these plates even after post-staining with uranyl acetate and lead citrate. This is due to the low contrast usually obtained when using Spurr's low viscosity embedding plastic. Fixation and infiltration was even throughout the tissue and sectioning was possible on the one through four day germination samples. The six through eight day samples were not infiltrated well and sectioning was not possible.

Method three involved a fixative mixture of glutaraldehyde to maintain cellular form, acrolein for deep penetration and paraformaldehyde for the preservation of protein bodies. This was followed by subjecting the tissues to either OsO₄ or KMnO₄. Examples of this technique can be seen on plates 7, 12, 13, 14 and 15. Plate 7 shows that there was preservation of subcellular components; mitochondrion, endoplasmic
reticulum and chloroplasts can be seen. With the other methods, this detail could not be seen. Plates 12 through 15 show cotyledon cells from tomato seeds germinated for five days. The specimens did not seem to be fixed as well as the twelve hour sample and sectioning of this older germinating tissue was difficult. As can be seen, the quality of the electron micrographs is not as good as those obtained from the younger germinating samples of method two or three. Longer fixation times and also a longer infiltration schedule could remedy this problem.

It is evident from these results that one method of fixation alone is inadequate for preparing tomato seed germination series for electron microscopy. Throughout the course of germination, the seeds become hydrated and complex biochemical reactions and tissue changes take place. These tissue and biochemical changes affect the rate of penetration of fixatives and embedding materials. Although method two was acceptable for the one through three day germination series, it was not adequate for the older germinating cotyledon tissue. Method three worked well for the younger germinating seeds but was less acceptable for the five through seven day tissue.
In summary, method two or three would be the methods of choice for the younger germinating tissues while a modification of method three would be chosen for the five through seven day samples. The choice of methods would depend on the type of seed and the particular seed tissue being studied.

It was of major interest in this study to observe cytological changes in sections of tomato seed cotyledons on germination. The sequence of changes in the protein bodies are shown on plates 5 through 15. A cotyledonary cell twelve hours after the start of germination typically looks like the cells seen on plates 5, 6, 7 and 8. The protein bodies, which stained evenly with KMnO₄ or OsO₄, are enclosed by a single membrane. Surrounding the protein bodies are numerous sac-like or spherical particles. These are somewhat smaller than the protein bodies and are the locations of lipid and enzyme deposits. These particles are designated as spherosomes and they can be seen to be membrane bound. In Plate 1, it can be seen that some protein bodies have within them small vacuoles. It is impossible to determine whether these are globoid or crystalloid inclusion vacuoles as are often found
in oilseed protein bodies or whether these are tears or artifacts due to the method of fixation. It is of interest to note the subcellular detail in Plate 4. Mitochondria, endoplasmic reticulum and young chloroplasts can be seen. At three days after the start of germination the protein bodies appear to swell or enlarge and canities around the outer edges of the protein bodies are obvious. A loose sponge like structure of the protein mass also begins to appear. At five days, the protein bodies are beginning to coalesce (Plate 9). As germination proceeds, these protein bodies become one large loose mass of small fragments as shown in Plate 13. This was characteristic of the tomato seed cotyledon cells after 6 days of germination. Plates 14 and 15 show characteristic cells after the disappearance of the protein body structures (8 days).

In conclusion, tomato seeds are shown to contain tissues and cells that have accumulated and stored quantities of protein in membrane bound protein bodies. During the course of germination, these proteins are metabolized and the changes in and the final disappearance of the protein bodies can be seen via electron microscopy.
Plate 5. Tomato seed germinated for 12 hours. The cotyledon was prefixed with 6% glutaraldehyde and post-fixed in 2% KMnO₄.

Magnification = 9,621 x
PB = protein body
S = spherosome
CW = cell wall
ML = middle lamella
ER = endoplasmic reticulum
Plate 6. Tomato seed germinated for 12 hours. The cotyledon was prefixed with 6% glutaraldehyde and post-fixed in 2% KMnO₄.

Magnification = 20,145 x
PB = protein body
S = spherosome
CW = cell wall
ML = middle lamella
ER = endoplasmic reticulum
Plate 7. Tomato seed germinated for 12 hours. The cotyledon was prefixed with 3% glutaraldehyde, 1.5% paraformaldehyde and 1.5% acrolein. It was then post-fixed with 1.5% OsO$_4$ buffered in phosphate buffer.

Magnification = 26,690 x
PB = protein body
M = mitochondria
ER = endoplasmic reticulum
PBM = protein body membrane
Plate 8. Tomato seed germinated for 24 hours. The cotyledon was prefixed with 6% glutaraldehyde and post-fixed in 2% KMnO$_4$.

Magnification = 8,443 x  
S = spherosome  
SM = membrane around spherosome
Plate 9. Tomato seed germinated for 24 hours. The cotyledon was prefixed with 6% glutaraldehyde and post-fixed in 2% KMnO₄.

Magnification = 27,000 x  
S = spherosome  
SM = membrane around spherosome
Plate 10. Tomato seed germinated for 3 days. The cotyledon was prefixed with 6% glutaraldehyde and post-fixed in 2% KMnO₄.

Magnification = 6,050 x
PBB = protein body breakdown
M = protein body membrane
S = spherosome
CW = cell wall
ML = middle lamella
Plate 11. Tomato seed germinated for 3 days. The cotyledon was prefixed with 6% glutaraldehyde and post-fixed in 2% KMnO₄.

Magnification = 6,290 x
S = spherosome
ER = endoplasmic reticulum
PBB = protein body breakdown
M = protein body membrane
Plate 12. Tomato seed germinated for 5 days.

Magnification = 9,738 x
PB = protein body
Plate 12
Plate 13. Tomato seed germinated for 5 days.

Magnification = 5,000 x
PM = protein mass
Plate 14. Tomato seed germinated for 8 days.

Magnification = 4,500 x
Plate 15. Tomato seed germinated for 8 days.

Magnification = 4,140 x
Plate 15
SUMMARY AND CONCLUSIONS

Tomato waste is a major disposal problem for Ohio tomato product processing plants. Discussion within the Department of Horticulture at The Ohio State University and with representatives from the tomato product processing industry suggested the importance of the recovery and utilization of tomato seeds, a major component of this cannery waste. To obtain data on the quantity and characterization of the protein present in tomato seeds, the following specific objectives were investigated:

1. separation of the seeds from tomato product cannery waste.
2. determination of the amount of protein present in tomato seeds.
3. determination of the solubility and extractability of the protein in tomato seeds.
4. determination of the amino acid composition of tomato seed protein.
5. fixation and infiltration of tomato seed cotyledonary tissue for electron microscopy.
6. observation of changes occurring in the tomato seed protein bodies during germination through the use of electron microscopy.

Tomato cannery waste was subjected to floatation and, on the basis of weight, about 51% of the waste was recovered as tomato seeds. These seeds were analyzed for protein and were found to contain approximately 29% crude protein. Extractability of tomato seed protein was found to be influenced by pH, ionic strength, and solvent media. A pH of 7.2 extracted 9.1% protein while a NaCl solution (0.5 ionic strength) extracted 7.3% protein. A 0.5 M solution of sucrose was effective in extracting about 7% protein. Based on protein fractionation using electrophoresis, sucrose density gradient centrifugation and Sephadex gel filtration, four or five different species of proteins were found. Also, there was evidence of association or dimerization reactions occurring among these proteins, depending on the solvent medium used. Amino acid analysis conducted on ground tomato seed showed that in comparison to high lysine corn and to soy flour, tomato seed protein was high in lysine, threonine and arginine but low in the sulfur containing amino acids.
Electron microscopy was used to view the protein containing vacuoles (protein bodies) and to observe the change occurring in the protein bodies during germination. The protein bodies initially enlarged and the protein within the protein bodies took on a sponge-like appearance, indicative of initial protein breakdown. The protein then began to coalesce and this was followed by protein body membrane breakdown. Three techniques for fixation of tomato cotyledonary tissue were compared: (1) 6% glutaraldehyde for three hours followed by \( \text{OsO}_4 \) for two hours, (2) 2% unbuffered \( \text{KMnO}_4 \) for two hours, and (3) a mixture of glutaraldehyde, acrolein and paraformaldehyde for one hour followed by \( \text{OsO}_4 \) for one hour or \( \text{KMnO}_4 \) for fifteen minutes. The first method proved unsatisfactory for fixation of tomato seed cotyledon tissue. Method two gave even fixation and was acceptable for the one through three day germinating samples but was not acceptable for the older germinating seeds tissue. The third method of fixation provided even fixation and worked well for the younger germinating seeds. It was, however, less acceptable for the five through seven day tissue.

The electron microscopic studies suggest that the difficulty experienced in extracting the total
amount of protein present in the seeds could be related to large amounts of protein being membrane bound within storage vacuoles. This suggestion is amenable to experimental testing.
FIGURE 5  STANDARD CURVES FOR LOWRY (•–•) AND BIURET (○–○) DETERMINATION
FIGURE 6  STANDARD CURVES FOR BIURET WITH BSA (● ●) AND ZEIN (○ ○).
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