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Chemical, Anatomical, and Histochemical Studies on the Navy Bean Seed[1]

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SYNOPSIS. The seed coats, cotyledons, and embryonic axes constituted 7.7%, 90.5%, and 1.8% of the dry bean matter, respectively. Significant differences were found in the chemical composition of seed tissues. The migration of water through hydrated seed coats was demonstrated. The starch content of cotyledons was 39.3%. Starch granules were imbedded in protein matrices of parenchyma cells. The parenchyma cells of cotyledons possessed thick secondary walls with numerous paired pits.

INFORMATION on chemical composition of specific tissues, structure of cells, and localization of chemical constituents in cells of the mature navy bean (*Phaseolus vulgaris* L.) seed is a prerequisite for an explanation of physical and chemical changes in bean tissue during mechanical, thermal, chemical, and enzymic treatments. Although proximate analyses of whole bean meal (6, 13) and of the seed coat (12, 16) have been reported, no information has been found on the chemical composition of the cotyledon and the embryonic axes of the bean. Some general information on the gross structure and anatomy of the unprocessed bean seed has been included in textbooks by Winton (19), Eames and MacDaniels (5) and Esau (7), but detailed data on cell, cell-wall, and starch granule dimensions in tissues have not been reported. Further, the localization of chemical constituents in bean tissue by histochemical techniques has not been recorded in the literature.

This investigation was undertaken to obtain additional information on the chemical and structural properties of the unprocessed navy bean seed to provide a basis for agronomic, processing, and nutritional studies.

MATERIALS AND METHODS

Seeds of the Michelite variety were selected for these studies because they are grown extensively in Michigan for commercial canning and home baking.

Choice hand-picked Michigan-grown Michelite bean seeds, stored at about 4° C. and possessing moisture content of about 15% by weight, were used in all cases. Soaked bean seeds with dimensions 11–12 mm. long, 7–8 mm. wide, and 8–9 mm. deep were selected for the anatomical study.

All chemicals were C.P. grade and distilled water was used in all experiments.

Chemical Composition of Specific Seed Tissues

To facilitate the separation of seed coats, cotyledons, and embryonic axes for the determinations of their relative dry weights and chemical composition, the beans were soaked for approximately 12 hours at about 24° C. Under these conditions, the beans imbibed an optimum amount of water. Before the water imbibed by the specific tissues was determined, the free surface water on the tissues was removed by centrifuging at 2500 rpm and by blotting on absorbent paper. The tissues were dried to constant weight in an oven at approximately 97° C. under a pressure of not more than 100 mm. of mercury. Tissues for chemical analyses were dried under the conditions mentioned above and ground in a Wiley mill to pass through a 60-mesh screen.

The tissues were analyzed for percent nitrogen by the micro-Kjeldahl method (1) and a factor of 6.25 was used to convert the nitrogen values to percentages of protein. To determine the ash content, the tissues were charred in fused silica crucibles and ashed at about 290° C. The ether-extractable substances were determined according to the A.O.A.C. method (1).

To determine the total starch of the cotyledon, proteins and water-soluble carbohydrates were extracted with 0.4 *M* NaCl before acid hydrolysis was initiated. Approximately 2.5 g. of dried ground cotyledons were mixed with 200 ml. of 0.4 *M* NaCl in a 250 ml. centrifuge bottle and the suspension was allowed to stand for 1 hour at about 24° C., with frequent agitation. The suspension was centrifuged at about 400 *g* for 10 minutes and the supernatant liquid was removed. This extraction procedure was repeated twice. Microscopic examination of the supernatant liquid indicated that no starch granules were present. The residue was transferred to a 500 ml. flask with the aid of 200 ml. of water and 20 ml. of concentrated HCl was added. After refluxing for 25 hours, the suspension was neutralized by NaOH solution. The volume of the solution was brought up to 500 ml. and filtered through Whatman #3 paper. The reducing sugars were determined by the method of Somogyi (15). The total starch was determined by multiplying the total amount of reducing sugar by 0.9.

All of the analyses were carried out at least in duplicate.

Anatomical and Histochemical Studies on the Bean Cotyledon

To examine the anatomical structure of the cotyledon, sections with thicknesses between 85 and 100 microns were cut from beans pre-soaked for 12 hours at about 24° C. The dimensions of the cells were measured with the aid of a calibrated eyepiece micrometer at 430x magnification. The dimensions of about 300 cells were obtained for each plane, location, and cell type.

Transverse sections, with thicknesses between 75–125 microns, were used for the histochemical localization of proteins, amino acids, starch granules, and pectic substances *in situ* in the cotyledon cells.

A saturated solution of picric acid is an excellent precipitating agent for proteins and is capable of staining them to an intense yellow color (8). Sections were placed in a saturated picric solution for 24 hours and subsequently washed in distilled water. The histochemical techniques reported by Serra (14) have been used for the detection of specific amino acids in cotyledon tissues. The reaction of the modified Millon's reagent with tyrosine or tyrosine-containing protein will produce a brick red or rose color in the tissue. Sulfhydryl groups in protoplasm can be detected by the formation of a red or pink color with the nitroprusside reagent.

The iodine test was found satisfactory for locating starch granules in parenchyma cells. Sections of bean cotyledons were placed in an iodine solution (0.3 g. iodine, 1.5 g. potassium iodide and 100 ml. distilled water) until the granules attained a light blue color and then they were washed in distilled water. An alcoholic solution of congo red was used to stain the cellulosic cell walls (8).

Dye-staining reactions of pectins lack specificity and consequently a test based on the reaction of ester groups in pectin with aqueous alkaline hydroxylamine to form red-colored water-insoluble complexes was employed (11).

For the determination of starch-granule dimensions, beans were ground in a Wiley mill to pass through a 60-mesh screen to release the granules from the cells. The resulting meal was suspended in distilled water and representative samples were placed on slides for microscopic examination.

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RESULTS AND DISCUSSION

Chemical Composition of Specific Tissues

The seed coats, cotyledons, and embryonic axes constituted 7.7%, 90.5% and 1.8%, respectively, of the dry weight of mature navy beans (table 1). By weight, the two cotyledons of the seed are the most important components.

Table 1 presents percentages of protein, ether-extract, and ash of the seed coats. The protein content was 4.8% on a dry weight basis. Snyder (16) reported that seed coats of "soft" and "hard" Michigan pea beans contained 5.6% and 6.8% protein, respectively, while Ott and Ball (12) found 5.07% protein in the seed coats of mixed navy bean varieties. No information has been presented on the digestibility or biological value of the seed coat proteins. Snyder (16) has suggested that the amount of protein in the seed coats of beans may be related to hardshell character. However, the relationship between denatured proteins and the hardshell character should not be overlooked. The ether-extract of the seed coat was very low, namely, 0.48%. Presumably, this material exists on the outer portions of the seed coat as a wax-like material. The fat contents of two samples of Michelite were reported as 0.4% and 0.31%, respectively, by Snyder (16). The ash content of the seed coats was 8.44%, as shown in table 1. Snyder (16) found that seed coats with an ash content of 8.09% contained 2.47% calcium.

The seed coats from soaked beans possessed an average moisture content of 76.6%. This high capacity of the seed coat for water suggested the possibility of water migration through the seed coat for the hydration of other bean tissues during the soaking period. Consequently, a water-migration experiment was made. Pins were attached to each of 25 undamaged navy beans in the hilum area with waterproof cement. After the application of silicone grease to the upper halves of the bean surfaces, each bean was lowered from a support so that its lower uncoated surface (excluding the hilum, micropyle and caruncle) was exposed to distilled water at about 25° C. After 5½ hours, the average weight increase of the beans was found to be about 28%. Moreover, hydrated cotyledon tissue resided within the water-exposed areas. This indicates that water was capable of migrating through the hydrated seed coats. Snyder (16) has shown that in some bean seeds the entrance of water at ordinary temperatures was largely through the micropyle and germinal area. She found that Great Northern beans which had their micropyles and scars covered with cement gained 3.8% in weight compared to the control beans which gained 52% during a 24-hour soaking period. However, it was pointed out that a large proportion of pea-type beans with cemented germinal areas swelled during the soaking period, suggesting a more general permeability of the seed-coat surface for this class of beans.

During the development of the seeds in the pod, the endosperm cells are digested and, at maturity, the beans possess cotyledonary tissue but no endosperm cells. Seeds with no endosperm in the mature state are called exalbuminous (7). The results in table 1 indicate that the cotyledons contained 27.5% protein and 1.65% ether-extractables. Moreover, the starch content of the cotyledons was determined to be 39.3% on a dry-weight basis. Although no data on the percentage of starch in the cotyledons of beans have been found in the literature, studies by Eichelberger (6), and Peterson and Churchill (13) indicated that the entire navy bean contained 35.22% and 35.2% starch, respectively. The ash content of cotyledons was found to be 3.50% (table 1), while Snyder (16) found an ash content of 4.18% and a calcium content of 0.0373% in beans without the seed coats. The cotyledons of soaked beans possessed an average moisture content of 53.8% (table 1). Presumably, most of the water, both bound and free, resides in the proteinaceous and cellulosic portions of the cells. Although the starch granules in the cells of the cotyledon probably do not contribute to the volume increase of the cotyledon during the soaking period, they undoubtedly imbibe water, since starch granules, in general, are capable of imbibing 25 to 30% water without appreciable swelling (9).

The hypocotyl and epicotyl constitute the axis of the bean embryo. The embryonic axes were found to contain 45.6% protein, 3.11% ether-extractables, and 3.58% ash (table 1). The high moisture content of 70.4% in the embryonic axes of the soaked beans may be explained by the large amount of hydrophilic protein in the cells.

Anatomical and Histochemical Studies on the Bean Cotyledon

With respect to weight and volume, the two cotyledons are the most important components of the bean seed. Moreover, textural characteristics and nutritive value of processed seeds presumably are influenced to a large extent by the size and shape of cells, dimensions of cell walls, and the localization of chemical constituents in the cotyledons.

The outermost layer of the cotyledon consists of epidermal cells. The inner and outer epidermal cells have been designated as those along the flat and curved surfaces of the cotyledon respectively (figures 1 and 2). Table 2 summarizes the dimensions of the epidermal cells in various locations and planes. In the transverse plane, the inner epidermal cells were, on the average, more than 3 times longer than those of the outer epidermis and varied widely in length as evidenced by the standard deviation. The data in table 2 indicate that, from a three-dimensional viewpoint, the inner epidermal cells were elongated cubes with the long axes in the transverse direction while the outer epidermal cells were somewhat cubical. The thickness of the outer surface cell-walls ranged from about 3.5 to 8 microns for the inner epidermal cells and from about 3.5 to 5 microns for the outer epidermal cells. Double-wall dimensions of adjacent cells in the epidermis were around 1.5 microns.

Microscopically, the cell contents of the epidermis appeared granular. Upon treating the sections with 0.3%

Table 1—Composition of bean tissues.

Component	Percentages of components in various tissues		
	Seed coat	Cotyledon	Embryonic axis
Amount of tissue in bean*	7.7	90.5	1.8
Protein in tissue*†	4.8	27.5	45.6
Ether-extract in tissue*	0.48	1.65	3.11
Ash in tissue*	8.44	3.50	3.58
Moisture in soaked beans	76.6	53.8	70.4

*Dry weight basis. † Protein calculated by 6.25 × % N.

Table 2—Dimensions of epidermal cells of bean cotyledons.

Location of cells	Plane of section	Length, microns		Width, microns	
		Mean SD	Range	Mean SD	Range
Inner epidermis	Transverse*	77 ± 22	152-35	23 ± 3	33-14
Inner epidermis	Longitudinal†	20 ± 4	37-11	17 ± 4	26-9
Outer epidermis	Transverse*	21 ± 4	30-13	17 ± 3	23-9
Outer epidermis	Longitudinal†	19 ± 3	32-11	15 ± 3	23-9

* Sections removed at the hilum. † Sections removed at the axis attachment.

iodine solution, the cell contents attained a uniform light yellow color which indicated that no starch granules were present and that presumably the granular structure was

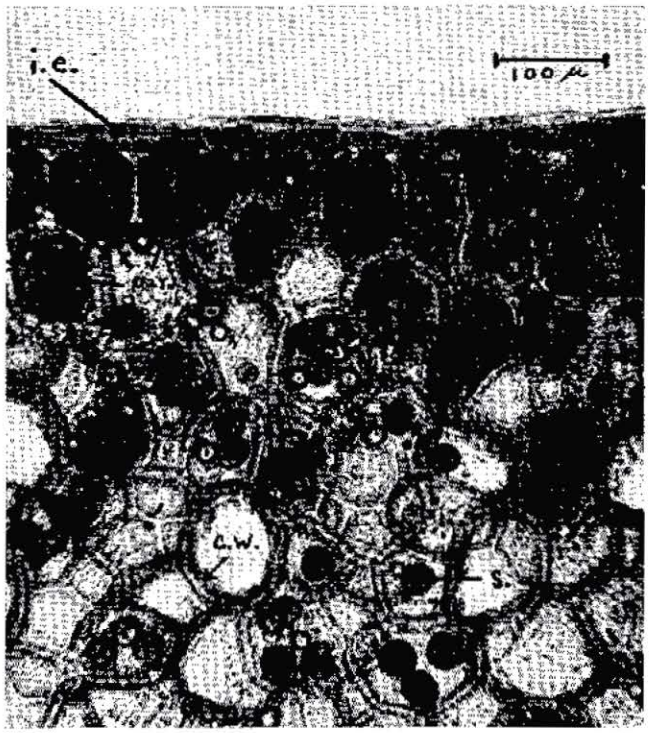

Figure 1—Transverse section of bean cotyledon tissue showing parenchyma and inner epidermal cells. i.e.—inner epidermis; par.—parenchyma cell with starch granules imbedded in protein matrix; c.w.—cell wall; s.—starch granule.

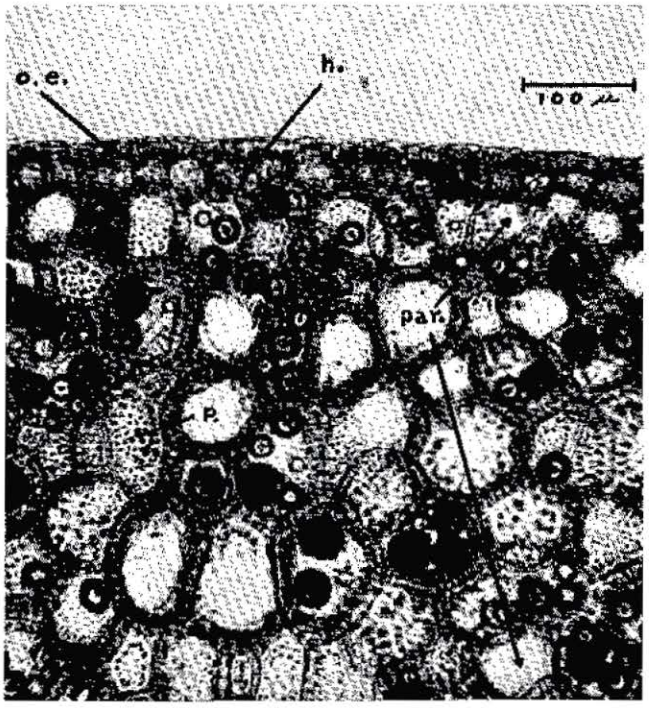

Figure 2—Transverse section of bean cotyledon tissue showing parenchyma, outer epidermal, and hypodermal cells. o.e.—outer epidermis h.—hypodermis; par.—parenchyma cell; p.—pit.

proteinaceous in nature. A positive reaction of picric acid with all tissues tested indicated that proteins were distributed throughout the granular matter of the inner and outer epidermal cells. Although the epidermal cells did not contain protoplasm with sufficient sulfhydryl groups to produce a positive reaction with nitroprusside reagent, the presence of tyrosine in these cells was indicated by the positive brownish coloration of the cell contents. Presumably free tyrosine was removed from the cells during the histochemical treatment and consequently the coloration of cell contents may be attributed primarily to tyrosine-containing proteins. This presumption is further strengthened by the fact that the intense coloration could not be attained by the relatively small amount of free tyrosine in the protoplasm. Color differences of the cell contents treated with Millon's reagents suggest that the outer epidermal cells contain more tyrosine than cells of the inner epidermis.

The layer of cells inward from the outer epidermis (figure 2) has been designated as the hypodermis. From the data in table 3, we may conclude that the hypodermal cells were larger than the outer epidermal cells and tended to be elliptical in shape. The standard deviations and ranges indicate a wide variation of cell size. The cell contents appeared granular but did not contain starch granules. Histochemical tests indicated that the granular matter was composed essentially of proteins. With Millon's test, the uniform purplish-rose color of the cell contents suggested that tyrosine-containing proteins were distributed throughout the granular mass. The double-wall thickness of adjacent cells was about 3 microns.

Parenchyma cells and vascular bundles constitute the remaining tissues of the cotyledon. A preliminary examination of parenchyma tissue indicated that cells near the outer hypodermis were somewhat smaller than the remainder of the parenchyma cells (figure 2). Table 4 indicates that the small parenchyma cells in this hypodermal region have a mean length and width of 71 and and 57 microns, respectively, and were not elongated in a particular direction. In general, smaller starch granules than those in the larger parenchyma cells were present in these cells. Although the parenchyma cells next to the inner epidermis were similar in size to the majority of parenchyma cells, many of them were elongated inwardly from the surface as shown in figure 2. The dimensions of the parenchyma cells in various planes and locations are presented in table 4. The cells in the transverse sections near the caruncle were apparently

Table 3—Dimensions of hypodermal cells of bean cotyledons.

Plane of section	Location of sections in bean	Length, microns		Width, microns	
		Mean SD	Range	Mean SD	Range
Transverse	At the hilum	38 ± 11	81-16	28 + 9	55-9
Longitudinal	At the axis attachment	36 ± 11	72-14	25 ± 8	58-11

Table 4—Dimensions of parenchyma cells of bean cotyledons.

Location of cells in section	Location of section in bean	Plane of section	Length, microns		Width, microns	
			Mean SD	Range	Mean SD	Range
Unicellular layer next to hypodermal layer	At the hilum	Transverse	71 ± 16	132-32	57 ± 14	95-28
Inward from inner epidermis to a boundary about 260 microns from outer epidermis	2 mm. from tip towards caruncle	Transverse	122 ± 17	173-84	92 ± 16	123-66
	2mm. from tip towards micropyle	Transverse	109 ± 15	153-70	82 ± 9	120-49
	At the hilum	Transverse	109 ± 17	155-65	83 ± 12	109-53
	At axis attachment	Longitudinal	110 ± 20	175-87	82 ± 15	149-39

somewhat larger than those in the hilum and micropyle regions. In the longitudinal section, the parenchyma cells, from middle lamella to middle lamella, were on the average 109 microns long and 82 microns wide. The average double-wall thickness was 8.8 microns with a standard deviation of 1. These thick walls are responsible to some extent for the rigidity of the soaked beans. Throughout the parenchyma tissue, small triangular intercellular spaces were observed.

The treatment of cotyledon sections with iodine solution showed that in each parenchyma cell, blue-colored starch granules were imbedded in a yellow-stained matrix composed chiefly of proteins. In fact, histochemical tests provided evidence that proteins were distributed throughout the matrix of each cell. Further, the reddish coloration of the matrix when treated with Millon's reagents indicated that tyrosine-containing proteins were distributed throughout the matrix. The presence of sulfhydryl groups (cysteine) in the matrix was indicated by the positive reaction of the nitroprusside test. Since the starch granules remained in the matrices of cells even after treatments with amino-acid detecting reagents, structural details of the matrices were obscured. Consequently, a method was developed to gelatinize and break down the starch granules. Sections, about 100 microns in thickness, were placed in a 30% sulfuric acid solution at 75° C. for 1 minute and subsequently washed in water. The sections were placed in a 0.1% iodine solution for one minute. The matrices acquired a deep yellow coloration and the presence of starch granules was not observed. With the breakdown of starch granules, the intact, rigid matrices possessed cavities which varied in size (figure 3). Each cavity was enveloped by dense proteinaceous matter; thus starch granules in the normal parenchyma cells presumably are not in intimate contact with each other. The rigidity of acid-treated matrix may be attributed to the strong intermolecular binding of protein molecules. Cox et al. (4), in their studies on corn, found that starch granules in the horny and floury endosperm were imbedded in proteinaceous matrices. They observed that the strands of protein in the matrix were birefringent and suggested that the majority of protein molecules were oriented.

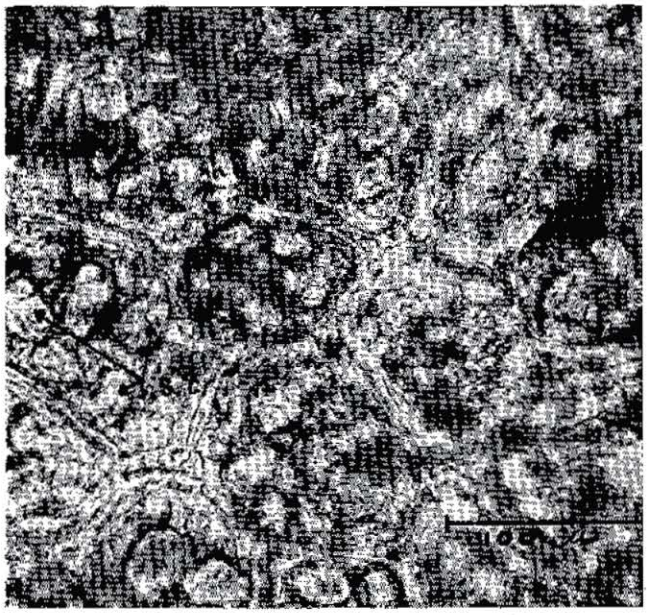

Figure 3—Parenchyma cells of the cotyledon. Starch granules were disintegrated by acid treatment. S.G.—starch granule cavity.

Knowledge of the physical characteristics of starch granules is valuable for the explanation of specific properties of the granules and changes in cells. From 541 measurements of liberated granules, the average length was calculated to be 28 microns with a standard deviation of 4 microns. Moreover, the length of the granules ranged from 9 to 48 microns. The shape of the liberated granules varied from round to elliptical and the smooth surface indicated a low compression upon the granules in the matrix during maturation and drying of the bean seeds. Faint striations were observed on the surfaces of the granules.

Large surface cavities with radiating fissures were apparent in both the *in situ* (figures 1 and 2) and liberated granules. Whistler and Thornburg (18) have reported that cavities of starch granules in undried corn kernels are rarely observed, but in the case of dried kernels, large cavities are present in the granules. Probably the cavities in the starch granules of navy bean seeds are formed during the drying period. In drying studies, Whistler et al. (17) found that as the moisture content of corn starch granules decreased, the number of cavitated granules increased; moreover, at specific moisture levels the percentage of cavitated starch granules dried at about 27° C. was much less than the percentages of cavities in granules which were dried at 45° C. and 60° C. Undoubtedly, the degree of cavitation is related to the structural and chemical changes of the granules during various treatments.

On a structural basis, plant cell walls consist of three fundamental parts; namely, the primary wall, middle lamella and secondary wall. In general, the primary wall consists of cellulose, hemicelluloses, noncellulosic polysaccharides and pectic substances (2). The middle lamella, consisting of calcium and magnesium pectates (2, 10), cements together adjacent primary walls. In many botanical studies, ruthenium red, as a stain for pectic substances, has been used for the detection of the middle lamella; however, the lack of specificity of ruthenium red for pectic substances has been stressed (13, 14). Differentiation of primary walls and middle lamella is sometimes difficult. Esau (7) has suggested the term "compound middle lamella" to include the two primary walls and the middle lamella. McCready and Reeve (11) using the specific test for pectic substances observed that the positive coloration was confined to the compound middle lamella in apple, lemon and potato tissues. In our studies, no color development in the compound middle lamella was observed after the bean tissues were treated according to the method of McCready and Reeve (11). Perhaps the negative results may be due to small amount and low methoxy content of the pectic substances in this region. Bettelheim and Sterling (3) reported that the site of pectic substances was not successfully located in potatoes by the method of McCready and Reeve due to the low methoxy content of pectic substances.

Further wall-thickening takes place after the cell has attained a specific size and shape. This wall has been designated as the secondary wall and consists essentially of cellulose and hemicelluloses (7). The presence of secondary walls in the epidermal and hypodermal cells could not be established. However, in the parenchyma cells, the secondary walls were observed to be very thick as compared to the primary wall and contained numerous small cavities called simple pits (figure 2). Each pit was opposite to an-

other pit in the adjacent secondary wall. Between each paired-pit, a thin pit membrane consisting of two primary walls and a middle lamella was observed. The presence of pits in the secondary wall undoubtedly facilitates the diffusion of water into the protoplasm during the soaking period.

SUMMARY

The seed coats, cotyledons and embryonic axes constituted 7.7%, 90.5% and 1.8%, respectively, of the dry matter of mature beans. Although a relatively small amount of protein was found in the seed coats, (4.8%) the cotyledons and embryonic axes were rich sources of protein with 27.5% and 45.6% respectively. The starch content of the cotyledons was determined to be 39.3%. Determinations of ash and ether-extractable matter in various tissues also have been included.

Beneath the epidermal and hypodermal cell layers, the cotyledons consist of parenchyma cells. Dimensions of the specific cells and cell walls, discussion of anatomical structure and histochemical observations have been presented. Within each parenchyma cell, starch granules were imbedded in a protein matrix. Dimensions and physical characteristics of starch granules have been reported.

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official Methods of Analysis. 8th Ed. 1955. Washington, D. C.
2. BONNER, J. Plant Biochemistry. 1950. Academic Press, Inc. New York, N. Y.
3. BETTELHEIM, F. A., and STERLING, C. Factors associated with potato texture. II. Pectic substances. Food Research, 20:118, 129. 1955.
4. COX, M. J., MACMASTERS, M. M., and HILBERT, G. E. Effect of the sulfurous acid steep in corn wet milling. Cereal Chem., 21:447–465. 1944.
5. EAMES, A. J., and MACDANIELS, L. H. An Introduction to Plant Anatomy. 1947. McGraw-Hill Book Company, Inc., New York, N. Y.
6. EICHELBERGER, M. The carbohydrate content of navy beans. J. Am. Chem. Soc., 44:1407–1408. 1922.
7. ESAU, K. Plant Anatomy. 1953. John Wiley and Sons, New York, N. Y.
8. JOHANSEN, D. A. *Plant Microtechnique*. 1940. McGraw-Hill Book Company, New York, N. Y.
9. KERR, R. W., Editor. Chemistry and Industry of Starch. 1950. Academic Press, Inc., New York, N. Y.
10. KERTESZ, Z. I. The Pectic Substances. 1951. Interscience Publishers, Inc., New York, N. Y.
11. MCCREADY, R. M., and REEVE, R. M. Test for pectin based on reaction of hydroxamic acids with ferriclon. J. Agr. Food Chem., 3:260–262. 1955.
12. OTT, A. C., and BALL, C. D. Some components of the seed coats of the common bean, *Phaseolus vulgaris*, and their relation to water retention. Arch. Biochem., 3:189–192. 1943.
13. PETERSON, W. H., and CHURCHILL, H. The carbohydrate content of the navy bean. J. Am. Chem. Soc., 43:1180–1185. 1921.
14. SERRA, J. A. Histochemical tests for proteins and amino acids. The characterization of basic proteins. Stain Technol., 21: 5–18. 1946.
15. SOMOGYI, M. Notes on sugar determination. J. Biol. Chem., 195:19–23. 1952.
16. SNYDER, E. B. Some factors affecting the cooking quality of the pea and great northern types of dry beans. Nebraska Agr. Exp. Sta. Res. Bul. 85. 1936.
17. WHISTLER, R. L., SPENCER, W. W., GOATLEY, J. L., and NIKUNI, Z. Effect of drying on the presence of cavities in corn starch granules. Cereal Chem., 35:331–336. 1958.
18. ——, and THORNBURG, W. L. Development of starch granules in corn endosperm. J. Agr. Food Chem., 5:203–207. 1957.
19. WINTON, A. L. Microscopy of Vegetable Foods. 1906. John Wiley and Sons, New York, N. Y.