the LSS-sorghum diet was higher than that of animals given the diets with sorghum grown on plots treated with NPK fertilizer whether expressed as concentration or total content of the mineral in the tissue. The manganese content of the LSS grain was higher than that of the NPK grain (Table I). Though the values for kidney manganese are statistically different, they are all within normal values reported in the literature (Underwood, 1971).

In addition to the elements listed in Table III, the grain and tissue samples are analyzed for Cd, Ni, Pb, and Cr. None of these were present in the animal tissues in detectable levels and only Cr was found in trace amounts in the grains. Thus, the application of sewage sludge to plots on which the crops were grown appeared to have no adverse effects on rats fed diets containing a high proportion of either corn, sorghum, or soybean grain.

SUMMARY

With the possible exception of Mn, Fe, and Cu, sewage sludge treatments applied to the crops had no effect on mineral composition of liver and kidney of rats fed diets containing about 80% of either corn, sorghum, or soybeans. Differences in mineral composition of the tissues, including Mn, Fe, and Cu, were within normal physiological ranges and no overt symptoms of toxicities or deficiencies were observed.

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LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis", 8th ed, 1955.
Boswell, F. C., *J. Environ. Qual.* **4**, 267 (1975).
Burns, R. E., *Agron. J.* **63**, 511 (1973).
Duncan, D. B., *Biometrics* **11**, 1 (1955).
Kirkham, M. B., *Environ. Sci. Technol.* **9**, 765 (1975).
Snedecor, G. W., "Statistical Methods", 4th ed, Iowa State College Press, Ames, Iowa, 1946.
Somers, E., *J. Food Sci.* **39**, 215 (1974).
Underwood, E. J., "Trace Elements in Human and Animal Nutrition", Academic Press, New York, N.Y., 1971, pp 177–207.
Wood, G. W., Glantz, P. J., Rothenbacher, H., Kradal, D. C., in "Faunal Response to Spray Irrigation of Chlorinated Sewage Effluent", Pennsylvania State University Research Publication No. 87, 1975.

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Isolation of Lipoxygenase from Split Pea Seeds, Snap Beans, and Peas

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Lipoxygenase was isolated from dried split pea seeds, frozen raw peas, and snap beans by ammonium sulfate fractionation, gel filtration, and ion-exchange chromatography. Split pea seed lipoxygenase was purified 19-fold; 22% of the original activity was recovered. Raw vegetable lipoxygenases were partially purified by a modified procedure using Ca^{2+} in the extraction, which appeared to stabilize the enzyme. Snap bean lipoxygenase was purified threefold, recovering 8% of the original activity. Pea lipoxygenase was purified ninefold, recovering 3% of the activity. The enzymes were characterized by determination of pH optima, behavior on polyacrylamide gels, and limited kinetic studies.

An active lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) system has been identified in many vegetables including legumes (Dillard et al., 1960) corn (Fritz and Beevers, 1955), potatoes (Galliard, 1970), flax seed (Zimmerman, 1966), alfalfa (Siddiqi and Tappel, 1956a), and peas (Lee and Wagenknecht, 1958). The bitterness of soybeans has been attributed to the action of lipoxygenase (Rackis et al., 1972). In frozen raw or unblanched vegetables, progressive deterioration during storage was associated with changes in the lipid fractions which were thought to be catalyzed by lipoxygenase (Wagenknecht and Lee, 1958).

Many of the purification procedures used for isolation of soybean lipoxygenase are based on the work of Theorell et al. (1947). Modifications of that method have been used for isolating green pea seed lipoxygenase by a number of workers (Mapson and Moustafa, 1955; Siddiqi and Tappel, 1956b; Wagenknecht and Lee, 1956; Dillard et al., 1961; Eriksson, 1967). More extensive purification of pea seed lipoxygenase was reported by Eriksson and Svensson (1970) and other investigators have used similar procedures (Haydar and Hadziyev, 1973; Anstis and Friend, 1974).

Differentiation of lipoxygenase isoenzymes is partially based on metal activation of the enzyme. According to Koch (1968) and Koch et al. (1971), calcium is required for activation of lipoxygenase in crude extracts from navy beans and soybeans. The presence of calcium activated lipoxygenase isoenzymes in soybeans has been reported by a number of workers (Holman et al., 1969; Christopher et al., 1972; Restrepo et al., 1973; Zimmerman and Snyder, 1974), but the way in which calcium ions function has not been clarified.

The present investigation was undertaken to develop a method for purifying lipoxygenase from seeds and raw vegetables for use in further studies of the effects of Ca^{2+} on lipoxygenase activity and of the effects of lipoxygenase systems on color, odor, and flavor changes in frozen raw or unblanched vegetables. The work described here deals with the partial purification and some properties of lipoxygenase isolated from dried split peas, frozen raw snap beans, and raw peas.

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EXPERIMENTAL SECTION

Materials and Reagents. Peas (variety Fordhook) and snap beans (variety Tendercrop) were grown at Urbana, Ill. during 1973. Vegetables were harvested before full maturity. Hulled peas and whole snap beans were sorted, washed, and frozen at −20 °C in 250 or 300 g lots in plastic freezer bags. Samples were withdrawn as needed and used within 1 year of storage. Dried split pea seeds (variety unknown) were purchased locally and kept at 25 °C until used.

Linoleic acid was purchased from The Hormel Institute (Austin, Minn.). Bovine serum albumin, Trizma Base, and Trizma HCl were obtained from Sigma Chemical Co. (St. Louis, Mo.) and Sephadex G-150 and DEAE-Sephadex A-50 were from Pharmacia Fine Chemicals (Piscataway, N.J.). All solutions were prepared using deionized distilled water.

Analyses. Protein determinations were performed by the biuret method (Clark, 1964) or by the Lowry method (Lowry et al., 1951) using crystalline bovine serum albumin as a standard. The absorbance of protein at 280 nm was used as an index of protein concentration in the column eluents.

Lipoxygenase activity was determined spectrophotometrically at 234 nm by the diene conjugation method ("Worthington Enzyme Manual", 1972). The assay was performed at 25 °C and at a pH of 7.2. Linoleic acid substrate was prepared by the method of Rackis et al. (1972). Linoleate concentration in the reaction mixture was 1.71 mM. One unit of lipoxygenase activity was defined as an increase in absorbance of 0.001 per min for a 4-min reaction period.

Disc gel electrophoresis was performed as described by Davis (1964). Proteins were stained with 1% Amido Black. The specific staining procedure for lipoxygenase described by Guss et al. (1967) was used.

Purification Procedure. *Sample Preparation.* Defatted and decolorized powders were prepared by extracting and washing ground split pea seeds or frozen raw vegetables with at least 4 to 6 volumes of cold (−20 °C) acetone. The dried acetone powder was pulverized and stored at −20 °C until used, usually less than 2 weeks.

Enzyme Extraction from Split Pea Seeds. Split pea seed acetone powder (60 g) was extracted with 300 ml of 100 mM Tris-HCl buffer (pH 7.2) for 1 to 2 h at 4 °C. The slurry was forced through two layers of cheesecloth and centrifuged at 2000*g* for 20 min. All centrifugations were done at 0 °C. The supernatant was brought to 30% saturation with solid $(NH_4)_2SO_4$ at 4 °C. The inactive precipitate was discarded and the supernatant brought to 50% saturation with respect to $(NH_4)_2SO_4$. Centrifugation was done at 12 000*g* for 30 min.

The precipitate was dissolved in about 25 ml of 50 mM Tris-HCl buffer (pH 7.2) and dialyzed at 4 °C overnight against two 500-ml portions of buffer. All succeeding steps were performed at 0 °C.

Gel Chromatography. The dialysate was applied to a Sephadex G-150 column (5 × 90 cm) equilibrated with 50 mM Tris-HCl buffer (pH 7.2). Fractions of 10 ml were eluted from the column with the same buffer, at a flow rate of 50 to 75 ml per h. Alternate fractions were assayed for lipoxygenase activity and protein. Fractions containing lipoxygenase activity were pooled and the enzyme was precipitated by addition of solid $(NH_4)_2SO_4$ to 50% saturation and centrifuged at 12 000*g* for 30 min. The protein was redissolved in approximately 20 ml of 50 mM NaCl in 50 mM Tris-HCl buffer (pH 7.2) and dialyzed overnight against two 500-ml aliquots of the same buffer.

Ion Exchange Chromatography. The dialysate was applied to a DEAE-Sephadex A-50 column (2.6 × 20 cm) and eluted with a linear salt gradient increasing from 50 to 500 mM NaCl in 50 mM Tris-HCl buffer, in a total volume of 500 ml, at a flow rate of 20 to 25 ml per h. Lipoxygenase active fractions were pooled and solid $(NH_4)_2SO_4$ was added to 50% saturation. This final preparation was stored at 0 °C.

Enzyme Extraction from Frozen Raw Vegetables. The procedure used for extracting the enzyme from split pea seeds was used initially for frozen raw vegetables. Modifications were later made to increase the recovery of enzyme activity and the degree of purification. The modified procedure was as follows. Acetone powder from 900 g of frozen raw beans or peas was extracted with 700 ml of 100 mM Tris-HCl buffer (pH 7.2) for 1 h 15 min at 4 °C. The slurry was forced through cheesecloth and centrifuged at 2000*g* for 30 min. One-sixteenth volume of 6.4% $CaCl_2$ was added to the supernatant and stirred for 1 h at 4 °C. This was centrifuged at 2000*g* for 30 min. The supernatant was brought to 30% saturation with solid $(NH_4)_2SO_4$. The inactive precipitate was discarded and the active enzyme was precipitated at 60% $(NH_4)_2SO_4$ saturation. The protein was dissolved in 100 mM Tris-HCl buffer containing 2 mM Ca^{2+}. Centrifugations were done at 15 000*g* for 30 min. Dialysis and gel and ion-exchange chromatography were performed as described above, except that all buffers contained 2 mM Ca^{2+}.

RESULTS AND DISCUSSION

Isolation of Split Pea Seed Lipoxygenase. The procedure used for purification of split pea seed lipoxygenase was similar to that used for pea seed lipoxygenase by Eriksson and Svensson (1970), Haydar and Hadziyev (1973), and Haydar et al. (1975). Some modifications were made in order to develop a reliable method, applicable to different seeds and fresh vegetables. Since one of the objectives of the investigation was to purify lipoxygenase in order to study Ca^{2+} effects, this ion was omitted from the extracting and eluting buffers. Restrepo et al. (1973) suggested that the use of Ca^{2+} during purification might nullify effects of Ca^{2+} as an activator. Preliminary work indicated that the degree of purification of split pea seed lipoxygenase in the absence of Ca^{2+} was approximately the same as that achieved using Ca^{2+}, so it appeared unnecessary to include Ca^{2+} in the procedure. Recently, Haydar et al. (1975) pointed out that inclusion of Ca^{2+} was detrimental in lipoxygenase extraction from pea seeds.

A second modification was the use of DEAE-Sephadex A-50 for ion exchange chromatography. This resulted in similar elution patterns and degrees of purification to those achieved with DEAE-cellulose. Only one ion-exchange step was employed, since the use of a second column by Eriksson and Svensson (1970) resulted in the loss of total activity without an increase in specific activity. This modification was also made by Haydar and Hadziyev (1973).

The course of the purification of split pea seed lipoxygenase is shown in Table I. During the isolation, an increase in total activity was noted after the first ammonium sulfate fractionation. Anstis and Friend (1974) reported a similar increase when working with pea seedlings. This was not obtained by other workers (Eriksson and Svensson, 1970; Haydar and Hadziyev, 1973). The anomalous increase in activity may be due to the removal of a naturally occurring inhibitor or an antioxidant present in the crude extract (Rhee and Watts, 1966; Pinsky et al., 1971). Another possibility is that an enzyme which cat-

Table I. Purification of Split Pea Lipoxygenase[a]

Fraction	Total act., units	Total protein, mg	Sp act., units/mg of protein	Deg of purification	% recovery
Crude extract	6.9×10^7	4724	15 200	1.0	100
30% $(NH_4)_2SO_4$ supernatant	17.8×10^7	4394	51 000	3.4	260
50% $(NH_4)_2SO_4$ precipitate	6.1×10^7	1451	47 400	3.1	89
Sephadex G-150 pooled fractions	3.1×10^7	220	162 400	10.7	45
DEAE-Sephadex pooled fractions	1.5×10^7	51	287 200	18.9	22

[a] Each value shown is the mean of four replications.

Table II. Purification of Snap Bean Lipoxygenase[a]

Fraction	Total act., units	Total protein, mg	Sp act., units/mg of protein	Deg of purification	% recovery
Crude extract	1.5×10^7	2697	5 400	1.0	100
Crude + $CaCl_2$	1.2×10^7	2395	4 900	0.9	81
30% $(NH_4)_2SO_4$ supernatant	2.2×10^7	1927	11 300	2.1	150
60% $(NH_4)_2SO_4$ precipitate	5.8×10^7	1193	49 000	9.1	400
Sephadex G-150 pooled fractions	1.0×10^7	322	30 500	5.7	68
DEAE-Sephadex pooled fractions	0.12×10^7	68	16 700	3.1	8

[a] Each value shown is the mean of two replications.

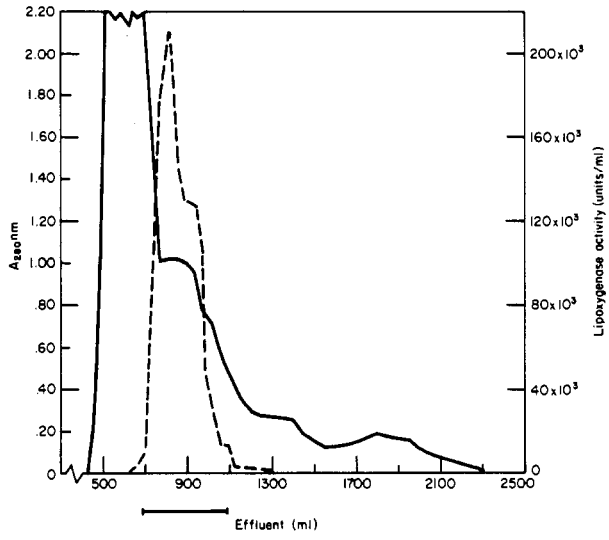

Figure 1. Separation of split pea lipoxygenase on Sephadex G-150: column dimensions, 5 × 90 cm; 1590 mg of protein applied; eluent used, 50 mM Tris-HCl (pH 7.2); (——) A_{280nm}; (- - -) lipoxygenase activity; (⊢⊣) pooled fractions.

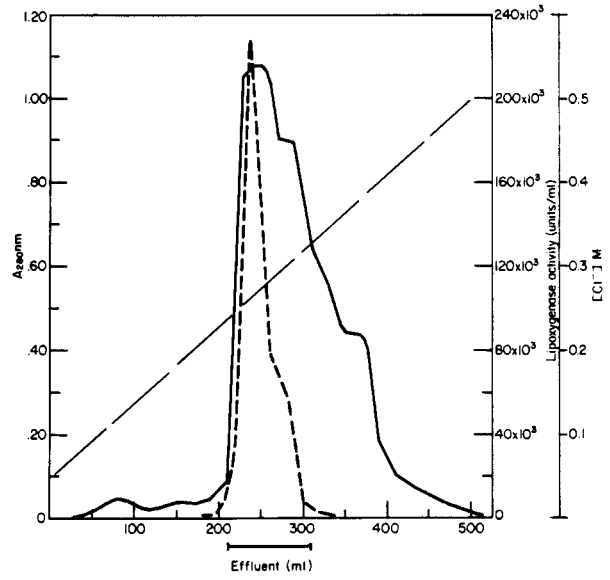

Figure 2. Purification of split pea lipoxygenase on DEAE-Sephadex: column dimensions, 2.6 × 20 cm; 409 mg applied; (- -) NaCl gradient, 50 to 500 mM NaCl in 50 mM Tris-HCl (pH 7.2); (——) A_{280nm}; (- - -) lipoxygenase activity; (⊢⊣) pooled fractions.

alyzes the destruction of conjugated dienes or hydroperoxides produced by the lipoxygenase reaction was removed in the fractionation.

After both ammonium sulfate fractionations, the specific activity of the crude split pea seed extract was increased about threefold. The separation of protein fractions on Sephadex G-150 is shown in Figure 1. Lipoxygenase activity was found in the second eluted peak. An 11-fold purification over the crude extract was achieved in this step. In ion-exchange chromatography on DEAE-Sephadex, lipoxygenase was eluted in the first major peak at a Cl^- concentration of 0.24–0.28 M (Figure 2). Twenty-two percent of the original lipoxygenase activity was recovered in the final preparation which had a specific activity approximately 19 times that of the crude extract.

Isolation of Frozen Raw Vegetable Lipoxygenases. The purification procedure used for split pea seed lipoxygenase did not give satisfactory results with frozen raw snap beans or peas. After both ammonium sulfate fractionations, a 12-fold purification of lipoxygenase was achieved with snap beans, and a twofold purification with peas. However, further purification of the 50% ammonium sulfate precipitate from the raw vegetable extracts by gel and ion-exchange chromatography resulted in almost total loss of activity.

The loss of activity of snap bean and pea lipoxygenase after the use of dextran columns suggested the removal of some cofactor required by the enzyme. Koch (1968) and Koch et al. (1971) reported that dialysis of crude extracts or the use of dextran columns resulted in the loss of lipoxygenase activity by navy bean and soybean extracts. The activity was restored to varying degrees by addition of Ca^{2+}.

In this study dialysis of the extracts did not affect lipoxygenase activity but inactivation of lipoxygenase was apparent after gel filtration. Addition of Ca^{2+} to the column effluents restored minor amounts of activity. Since Eriksson and Svensson (1970) and Haydar and Hadziyev (1973) used Ca^{2+} in the extraction procedure, modifications were made to include 2 mM $CaCl_2$ in the buffers. In addition, a $CaCl_2$ precipitation step was added to the procedure as described by Yamamoto et al. (1970). An-

Table III. Purification of Pea Lipoxygenase[a]

Fraction	Total act., units	Total protein, mg	Sp act., units/mg of protein	Deg of purification	% recovery
Crude extract	1.8×10^7	9248	1 900	1.0	100
Crude + $CaCl_2$	2.1×10^7	8597	2 400	1.3	117
30% $(NH_4)_2SO_4$ supernatant	2.0×10^7	3970	5 000	2.6	113
60% $(NH_4)_2SO_4$ precipitate	0.82×10^7	1606	5 100	2.7	47
Sephadex G-150 pooled fractions	0.27×10^7	649	4 200	2.2	15
DEAE-Sephadex pooled fractions	0.05×10^7	33	17 100	9.0	3

[a] Each value shown is the mean of two replications.

other modification was made to more completely recover enzyme activity by precipitating the protein at 60% ammonium sulfate saturation instead of 50%.

Using this modified procedure, lipoxygenase was isolated from frozen raw snap beans and peas. The summaries of the purifications are given in Tables II and III. After ammonium sulfate fractionations, a ninefold purification of snap bean lipoxygenase and a threefold purification of pea lipoxygenase were achieved. Use of gel chromatography resulted in a loss of specific activity of both raw vegetable lipoxygenases. Snap bean lipoxygenase decreased relatively more than did pea lipoxygenase.

The final preparation of snap bean lipoxygenase was about three times as active as the crude extract, while the pea lipoxygenase was about nine times as active. The specific activity of both enzymes was approximately the same: 16 700 units/mg of protein for snap bean lipoxygenase and 17 100 units/mg of protein for pea lipoxygenase. Both had much lower specific activities than the final split pea seed lipoxygenase which had 287 200 units/mg of protein. The elution patterns of all three lipoxygenases from the ion-exchange columns were similar. Raw pea and snap bean lipoxygenases were eluted from the DEAE-Sephadex column at an ionic strength of 0.15 to 0.22 M, slightly lower than the split pea seed lipoxygenase elution.

Use of $CaCl_2$ during the extraction and purification appeared to activate the raw snap bean and raw pea lipoxygenases, although purification and recovery were not as successful with frozen raw vegetables as with dry pea seeds. The raw vegetables used in this study were harvested at a stage appropriate for good eating quality and were not fully mature. Since isolation of pea seed lipoxygenase has been accomplished by several groups with only reasonable losses of activity, it appears that during development of the seed some stabilization of the enzyme may occur.

The role of calcium ions in lipoxygenase action remains unclear. Restrepo et al. (1973) and Zimmerman and Snyder (1974) suggested that calcium activation was due to an interaction with the linoleate substrate rather than with soybean lipoxygenase itself. This is in conflict with Koch et al. (1971) who found that Ca^{2+} appeared to interact with the enzyme rather than the substrate.

According to Restrepo et al. (1973), the use of Ca^{2+} during the preparation of soybean lipoxygenase minimized any activation effects during the enzyme assay. These researchers emphasized that Ca^{2+} effects should be studied in purified rather than crude preparations. When pea seeds were used as an enzyme source, Haydar et al. (1975) found that addition of Ca^{2+} to the assay mixture resulted in higher activity when no Ca^{2+} had been used in the isolation than when it had been. However, they reported that Ca^{2+} was inhibitory at concentrations ranging from 0 to 4 mM whether or not Ca^{2+} had been included during purification. The latter conclusion is in disagreement with earlier findings on soybean lipoxygenase.

The assay method used also affects the results of calcium dependency tests. When the diene conjugation method of determining lipoxygenase activity was used, Ca^{2+} activation was noted (Koch et al., 1971). However, when oxygen uptake methods, either manometric or polarographic, are used, contradictory findings occur.

In addition, Ben-Aziz et al. (1970) and Zimmerman and Snyder (1974) indicated that the presence of Tween 20 in the substrate preparation nullifies the activation effect of Ca^{2+}. Since Tween 20 is commonly used to help solubilize linoleate substrates, this supports the hypothesis that calcium interacts with linoleate in solution. It also casts some doubt on the identification of calcium activated lipoxygenases by a number of workers when Tween 20 was present in the substrate. Since Tween 20 was used in the present study for substrate preparation, the effect of Ca^{2+} may have been minimized.

Characterization of Lipoxygenases. Acrylamide gel electrophoregrams of the lipoxygenase preparations in this study indicated the presence of one major and one to two trace protein bands in raw pea and split pea seed lipoxygenase preparations and one major and one trace protein band in snap bean preparations, indicating that purification was not complete. Use of the specific staining technique of Guss et al. (1967) indicated that one band of intermediate mobility had lipoxygenase activity. Other workers have found several isoenzymes of pea or bean lipoxygenase (Hale et al., 1969; Haydar and Hadziyev, 1973; Arens et al., 1973; Anstis and Friend, 1974). However, Haydar et al. (1975) noted that after prolonged storage of pea seeds, lipoxygenase activity was apparent in only one band.

The pH profile of split pea lipoxygenase showed an activity range between pH 4.0 and 8.0, with maximum activity occurring at pH 6.0. Wei (1970) found that the pH optimum for crude split pea seed extracts was between 6.5 and 7.0. Siddiqi and Tappel (1956b) reported a pH optimum of 6.9 for pea seed lipoxygenase. The enzyme purified by Arens et al. (1973) had a pH optimum of 6.3. These are in general agreement, when one considers differences in assay procedures and buffering systems used. A slightly higher optimum pH of 7.2 was reported by Haydar and Hadziyev (1973) and Haydar et al. (1975).

Maximum activity for raw pea lipoxygenase also occurred at pH 6.0. The pH profile resembled that found for split pea seed lipoxygenase, having a relatively narrow range with little or no activity below pH 3.5 or above 7.5. Snap bean lipoxygenase had an extremely narrow pH range, with activity between pH 6 and 8, and maximum activity at pH 7.5.

Limited kinetic studies with split pea seed lipoxygenase indicated that, at pH 7.2, the reaction rate increased almost linearly with substrate concentration. At pH 6.0, the oxidation more closely obeyed the Michaelis–Menten equation, but a decrease in reaction rate occurred at high substrate concentrations. Smith and Lands (1970) suggested that this might be due to increased random binding

of the substrate to the enzyme, blocking the site for product formation. The K_M calculated from the best straight line in a Lineweaver–Burk plot was 3.08×10^{-4} M. This is lower than the K_M of 2.3×10^{-3} M reported for pea seed lipoxygenase (Haydar and Hadziyev, 1973). The discrepancy is probably due to differences in enzyme source and assay method.

The objectives of this investigation were only partially realized. The presence of an active lipoxygenase in frozen raw snap beans and peas was confirmed. A satisfactory degree of purification of the enzyme could not be achieved by using the same method for raw vegetable and seed lipoxygenases. However, the inclusion of Ca^{2+} in the extraction increased the effectiveness of the purification procedure for raw vegetable lipoxygenases. The enzyme appears to be less stable in raw immature vegetables than in seeds and therefore, a method of further stabilizing the enzyme must be developed to minimize loss of lipoxygenase activity during the purification. Work on isolation of lipoxygenase from seeds and vegetables of the same variety is needed to determine if there are changes in the enzyme during germination and maturation.

LITERATURE CITED

Anstis, P. J. P., Friend, J., *Phytochemistry* **13**, 567 (1974).
Arens, D., Seilmeier, W., Weber, F., Kloos, G., Grosch, W., *Biochim. Biophys. Acta* **327**, 195 (1973).
Ben-Aziz, A., Grossman, S., Ascarelli, I., Budowski, P., *Anal. Biochem.* **34**, 88 (1970).
Christopher, J., Pistorius, E., Axelrod, B., *Biochim. Biophys. Acta* **284**, 54 (1972).
Clark, J., "Experimental Biochemistry", W. H. Freeman and Co., San Francisco, Calif., 1964, p 94.
Davis, B. J., *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
Dillard, M. G., Henick, A. S., Koch, R., *Food Res.* **25**, 544 (1960).
Dillard, M. G., Henick, A. S., Koch, R., *J. Biol. Chem.* **236**, 37 (1961).
Eriksson, C. E., *J. Food Sci.* **32**, 438 (1967).
Eriksson, C. E., Svensson, S. G., *Biochim. Biophys. Acta* **198**, 449 (1970).
Fritz, G., Beevers, H., *Arch. Biochem. Biophys.* **55**, 436 (1955).
Galliard, T., *Phytochemistry* **9**, 1725 (1970).
Guss, P. L., Richardson, T., Stahmann, M. A., *Cereal Chem.* **44**, 607 (1967).
Hale, S. A., Richardson, T., Von Elbe, J. H., Hagedorn, D. J., *Lipids* **4**, 209 (1969).
Haydar, M., Hadziyev, D., *J. Sci. Food Agric.* **24**, 1039 (1973).
Haydar, M., Steele, L., Hadziyev, D., *J. Food Sci.* **40**, 808 (1975).
Holman, R. T., Egwim, P. O., Christie, W. W., *J. Biol. Chem.* **244**, 1149 (1969).
Koch, R. B., *Arch. Biochem. Biophys.* **125**, 303 (1968).
Koch, R. B., Brumfiel, B. L., Brumfiel, M. N., *J. Am. Oil. Chem. Soc.* **48**, 532 (1971).
Lee, F. A., Wagenknecht, A. C., *Food Res.* **23**, 584 (1958).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Mapson, L. W., Moustafa, E. M., *Biochem. J.* **60**, 71 (1955).
Pinsky, A., Grossman, S., Trop, M., *J. Food Sci.* **36**, 571 (1971).
Rackis, J. J., Honig, D. H., Sessa, D. J., Moser, H. A., *Cereal Chem.* **49**, 586 (1972).
Restrepo, F., Snyder, H. E., Zimmerman, G. I., *J. Food Sci.* **38**, 779 (1973).
Rhee, K. S., Watts, B. M., *J. Food Sci.* **31**, 669 (1966).
Siddiqi, A. M., Tappel, A. L., *Plant Phys.* **31**, 320 (1956a).
Siddiqi, A. M., Tappel, A. L., *Arch. Biochem. Biophys.* **60**, 91 (1956b).
Smith, W. L., Lands, W. E. M., *Biochem. Biophys. Res. Commun.* 41, 486 (1970).
Theorell, H., Holman, R. T., Åkeson, Å., *Acta Chem. Scand.* 1, 571 (1947).
Wagenknecht, A. C., Lee, F. A., *Food Res.* **21**, 605 (1956).
Wagenknecht, A. C., Lee, F. A., *Food Res.* **23**, 25 (1958).
Wei, C. K., M.S. Thesis, University of Illinois, 1970.
"Worthington Enzyme Manual", Worthington Biochemical Corporation, Freehold, N.J., 1972.
Yamamoto, A., Yasumoto, K., Mitsuda, H., *Agric. Biol. Chem.* **34**, 1169 (1970).
Zimmerman, D. C., *Biochem. Biophys. Res. Commun.* **23**, 398 (1966).
Zimmerman, G. I., Snyder, H. E., *J. Agric. Food Chem.* **22**, 807 (1974).

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