

1984

Preliminary Evaluation of Lectins as Fluorescent Probes of Seed Structure and Composition

S. Shea Miller

S. H. Yiu

R. G. Fulcher

I. Altosaar

Follow this and additional works at: <https://digitalcommons.usu.edu/foodmicrostructure>



Part of the [Food Science Commons](#)

Recommended Citation

Miller, S. Shea; Yiu, S. H.; Fulcher, R. G.; and Altosaar, I. (1984) "Preliminary Evaluation of Lectins as Fluorescent Probes of Seed Structure and Composition," *Food Structure*: Vol. 3 : No. 2 , Article 5.

Available at: <https://digitalcommons.usu.edu/foodmicrostructure/vol3/iss2/5>

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Food Structure by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



PRELIMINARY EVALUATION OF LECTINS AS FLUORESCENT PROBES OF SEED STRUCTURE AND COMPOSITION

S. Shea Miller¹, S.H. Yiu², R.G. Fulcher³, and I. Altsosaar¹

¹Department of Biochemistry, University of Ottawa,
Ontario, Canada K1N 6N5

²Food Research Institute, Agriculture Canada,
Ottawa, Ontario, Canada K1A 0C6

³Ottawa Research Station, Agriculture Canada,
Ottawa, Ontario, Canada K1A 0C6

Abstract

Several commercially available fluorescein isothiocyanate and rhodamine isothiocyanate-conjugated plant lectins have been applied to cereal and oilseed tissues to permit identification and localization of specific structures and carbohydrates by fluorescence microscopy. Ulex europaeus Agglutinin I (UEA I) and Ricinus communis Agglutinin I (RCA I) showed specificity for the amyloids in rapeseed cotyledonary cell walls. Wheat Germ Agglutinin (WGA) bound to rapeseed coat mucilage, as well as fungal hyphae in infected wheat. Lens culinaris Agglutinin (LCA) bound only to starch in cereal sections, and at higher magnifications of isolated starch granules, the annular structure was clearly visible.

Introduction

Lectins are sugar-binding proteins or glycoproteins of non-immune origin which agglutinate cells and/or precipitate glycoconjugates (Goldstein et al. 1980). Although most lectins have been isolated from plants, they have also been found in bacteria, fungi, lichens, invertebrates, and vertebrates. The carbohydrate specificity of lectins is usually directed to a single monosaccharide or structurally related monosaccharides, and lectins may bind to their complementary monosaccharides whether they occur as free sugars, or as terminal groups on various types of glycoconjugates (Clarke and Hoggart 1982). Interest is increasing in the use of lectins as microscopic probes to investigate the nature and distribution of carbohydrate-containing components of both plant and animal tissues and cells (for examples see Rougier et al. 1979, Vermeer and McCully 1981, Pena et al. 1981, Holthofer et al. 1981, Baldo et al. 1982 a,b,c, and Sato and Spicer 1982).

The chemical nature of polysaccharides is often difficult to determine *in situ*. Solvent extraction frequently yields mixtures which require complex purification procedures (for example, see Siddiqui and Wood 1977b), and often yields little information about the original location of the extracted components within the tissues. The availability of purified lectins provides a potential tool for identifying and determining where carbohydrates and/or carbohydrate-containing components are situated within tissues and cells. In addition, the specificity of lectins can extend beyond sugar residues, to include the glycosyl linkages in an oligosaccharide. For example, Lens culinaris agglutinin is specific for α -glucosyl groups, while Ricinus communis agglutinin is specific for β -galactosyl groups.

Commercially available labelled lectins include several, which are useful in bright field, fluorescence, and electron microscopy (EM). We have chosen fluorescent-labelled lectins in our study because of the sensitivity of fluorescence microscopy as compared to bright-field methods,

Initial paper received April 12 1984.
Final manuscript received October 1 1984.
Direct inquiries to R.G. Fulcher.
Telephone number: (613) 995-9428.

Key Words: Lectins, fluorescence microscopy, seed structure, cell walls, carbohydrates, amyloid, mucilage.

Contribution No. 613 of Food Research Institute,
Contribution No. 747 of Ottawa Research Station,
Agriculture Canada.

and the ease of preparation and examination of samples as compared to EM. The range of lectin specificities is quite broad (Clarke and Hoggart 1982) and their availability is increasing. As such, it may become possible to select lectins with specific affinities to detect and localize components of particular interest in raw materials, and follow the fate of these components as materials are processed and consumed. This approach could aid in evaluation of the availability of carbohydrates in processed materials from a nutritional standpoint. The use of lectins in routine laboratory examination of foods is not practical due to their cost, but their potential as probes in fundamental investigations of carbohydrates in food materials, both raw and processed, is considerable. This preliminary examination of various lectins demonstrates clear structural specificities, for which the underlying chemical specificities are proposed.

Materials and Methods

Samples

Mature seeds of *Brassica campestris* L. cultivars Echo, Candle, and Sarson R500 were used to examine lectins with binding specificities for rapeseed. Mature kernels of oats (*Avena sativa* L. cv. Hinoat) and wheat (*Triticum aestivum* L. cv. Fredrick) were used for investigation of cereal starch-binding lectins. Grains of *T. aestivum* L. cv. Concorde which were naturally contaminated with the head blight fungus *Fusarium graminearum* Schwabe were also used to assess the affinity of various lectins for fungal hyphae in cereals. Although the level of *Fusarium* infection varied from kernel to kernel in the sample tested, on average the level of contamination within the sample was very high, as indicated by the unusually high levels (7 ppm) of the fungal metabolite deoxynivalenol (vomitoxin). Infected material was similar to that used in a milling and baking study described earlier (Young et al 1984).

Sectioning

Frozen Sections. Whole, unfixed rapeseeds were soaked in cold water for at least 30 minutes, mounted in Tissue Tek II O.C.T. Compound (Lab Tek Products, Miles Laboratories Inc., Naperville, Illinois), and frozen on dry ice-cooled mounting blocks. Frozen sections 10 μ m thick were cut using steel knives in a cryostat set at -20°C and mounted on glass slides pre-coated with Albumin Fixative (Fisher Scientific Company, Fairlawn, New Jersey).

Glycol Methacrylate (GMA) Sections. For higher resolution, samples were fixed and embedded in glycol methacrylate plastic as described by Fulcher and Wong (1980). Briefly: samples were fixed in 6% glutaraldehyde in 0.025 M Na phosphate buffer pH 7.0 at 4°C for 72 hours. Samples were dehydrated sequentially in methyl cellosolve, ethanol, *n*-propanol, and *n*-butanol, infiltrated with GMA monomer (Feder and O'Brien 1968) for 72 hours, and polymerized at 60°C (overnight) or at room temperature under U.V. light (3 to 4 days). Sect-

ions 2-4 μ m thick were cut using glass knives and mounted on slides.

Lectins

Purified lectins labelled with fluorescein isothiocyanate or rhodamine isothiocyanate were obtained from Cedarlane Laboratories (Hornby, Ontario). The following lectins were tested: Wheat Germ Agglutinin (WGA), with a reported specificity for N-acetylglucosamine and its oligosaccharides, *Lens culinaris* Agglutinin (LCA), with reported specificities for α -D-glucosyl groups, *Ulex europaeus* Agglutinin I (UEA I) which is specific for L-fucosyl groups, and *Ricinus communis* Agglutinin I (RCA I), with a reported specificity for β -D-galactosyl groups (Clarke and Hoggart 1982).

For staining, sections were incubated with 1.0 mg/ml of lectin in 10 mM HEPES buffer containing 0.15 M NaCl and 0.04% sodium azide at pH 6.1-8.5. (depending on the lectin used, as recommended by the supplier) for 30 minutes in a moist chamber. Sections were then washed twice for 15 minutes each in a solution containing 0.001 M each of Ca^{2+} , Mg^{2+} , and Mn^{2+} (Baldo et al 1982a) at pH 7.0 and monitored microscopically to ensure that all excess lectin had been removed. Slides were air-dried and mounted in non-fluorescent immersion oil for microscopic examination. Some sections were counter-stained after lectin binding for 5 minutes in Fast Green (0.01% in 5% acetic acid, pH 2.5) or Evans Blue (0.01% in distilled water, pH 7.0).

Tests for inhibition of binding of UEA I and RCA I were performed by adding L-fucose, D-galactose, or D-glucose (to 0.2 M) to the lectin solution and pre-incubating for 30 minutes before applying to sections.

Microscopic Examination

Sections were examined using a Zeiss Universal Research Microscope (Carl Zeiss Canada Ltd.) equipped with a III RS epi-illuminating condenser for fluorescence analysis. The condenser contained fluorescence filter combinations (FC's) with dichromatic beam splitters, and exciter/barrier filters with maximum transmission at 450-490 nm/ 520 nm (FC I) for examination of fluorescein-labelled material, and 546 nm/ 590 nm (FC II) for examination of rhodamine-labelled material (Fulcher and Wong 1980). Photomicrographs were obtained using 35 mm Kodak Tri-X pan film, ASA 400.

Results and Discussion

Of the lectins investigated, several exhibited specificities for carbohydrate-containing components, making them potentially useful for identifying and locating particular components in foods. Preliminary studies on rapeseed and cereal grains are presented here as examples of the possible uses of lectins in determining the distribution of specific carbohydrates in raw materials.

Rapeseed cotyledonary cell walls have been described previously using iodine/potassium iodide

and the fluorochromes Calcofluor White M2R New and Congo Red (Yiu et al (1983)). While these reagents and dyes have proved to be useful in the examination of whole rapeseed and its products, their specificity is somewhat limited. Iodine/potassium iodide, often used to localize amyloid (Kooiman 1957, Johansen 1940) which is the carbohydrate found in rapeseed cotyledonary cell walls, also stains a number of other polysaccharides, including starch, glycogen, hydrolyzed cellulose, and chitin (Johansen 1940). Congo Red has also been shown to have an affinity for mixed-linkage β -glucans in cereal cell walls (Wood and Fulcher 1978, Fulcher and Wong 1980, Fulcher et al 1984), but in rapeseed the dye binds to the seed coat mucilage as well as to the cotyledonary cell walls. Calcofluor is similar to Congo Red in its staining affinities, and binds to a wide range of materials in addition to rapeseed cell walls, including cellulose, chitin, and a variety of other β -linked polymers (Maeda and Ishida 1967, Hughes and McCulley 1975, Fulcher and Wong 1980, Wood et al 1983).

Of the lectins tested, UEA I and RCA I, with reported specificities for L-fucose and D-galactose respectively (Clarke and Hoggart 1982), showed significant specific binding to rapeseed cotyledonary cell walls. (Figs. 1, 2, 3). In thin GMA sections (Fig. 1), binding of UEA I occurred only in the cotyledonary cell walls, and distribution of the fluorescent lectin was not uniform throughout the cell wall. Whether this was due to incomplete binding of the lectin (perhaps due to interference by the GMA embedding medium), or to variation within the cell wall itself, has not yet been determined. More uniform distribution of fluorescence was observed in the thicker, unembedded cryostat sections treated with either RCA I (Fig. 2) or UEA I (Fig. 3). No other cell walls within the seeds had any affinity for these lectins, suggesting that fucoamyloid and amyloid are both confined to cotyledonary cell walls.

L-fucose and D-galactose occur as terminal residues in fucoamyloid and amyloid isolated from rapeseed meal (Siddiqui and Wood 1971, 1977a, 1977b). The suggestion that UEA I was in fact binding primarily to the fucoamyloid is supported by the significant decrease in lectin binding to the cell wall after the lectin was pre-incubated with L-fucose for 30 minutes prior to staining (Fig. 4). No such decrease was observed when the lectin was preincubated with D-glucose or D-galactose. In comparison, Fig. 3 shows the very intense fluorescence observed when pre-incubation of the UEA I lectin with L-fucose is omitted. Similar inhibition studies on RCA I show loss of binding ability when the lectin was pre-incubated with D-galactose while cell wall fluorescence was still visible when pre-incubated with D-glucose or L-fucose.

Mucilage, a carbohydrate of some rapeseed and most mustard seed coats or hulls (Weber et al 1974, Vose 1974, Yiu et al 1982, Van Caeseele and Mills 1983) exhibits a different lectin affinity than the cotyledonary cell walls. When applied to rapeseed or mustard sections, GMA, which has a reported specificity for N-acetylglucosamine

(Clarke and Hoggart 1982), showed a pronounced affinity for the seed coat mucilage, with no other binding observed, as shown in Fig. 5. Mucilage is an acidic polysaccharide consisting primarily of cellulose and uronic acids, notably galacturonic acid (Weber et al 1974, Vose 1974), as well as glucose, arabinose and xylose. Galactose and an uncharacterized glycoprotein have also been detected (Aspinall 1976, Aspinall and Krishnamurthy 1976). Whether this protein is in fact present in the mucilage, and whether it contains N-acetylglucosamine, a common saccharide in plant glycoproteins (Sharon 1974), remains to be determined.

When applied to sections of cereal grains, LCA stained only the starch granules, as shown in the oat section in Figure 6. This marked specificity of LCA for starch suggests that it may provide a potentially useful probe into the nature and distribution of starches in processed (especially cooked) foods, where it can no longer be identified on the basis of morphology or birefringence.

The internal structure of starch has been examined frequently using both light and electron microscopy (Wivinis and Maywald 1967, Moss 1976, Gallant and Sterling 1976, Hood and Liboff 1983). Although the superior resolution of EM provides a very detailed structural picture of the starch granule, extensive pretreatments are usually used to visualize "growth rings", including enzyme or acid hydrolysis, oxidation with periodic acid, and/or treatment with thiosemicarbazide (Gallant and Sterling 1976, Evers 1979, Hood and Liboff 1983). In contrast, the binding of fluorescein-conjugated LCA to starch granules - a simple, fairly rapid procedure - clearly shows the annular organization, as shown in the large wheat starch A granules in Fig. 7. The smaller B granules also show intense staining of a central core, but do not exhibit the ring structure with this method. An envelope or halo is also visible around many of the granules. The chemical nature of this envelope is as yet unclear, and may in part be due to refraction artefacts at the granule surface. Because the two major components of starch, amylose and amylopectin, both contain α -D-glucosyl chains, one of the reported specificities of LCA (Clarke and Hoggart 1982), it is likely that both starch polymers are capable of binding the lectin to some extent. Possible differences in the LCA binding specificities of the two polysaccharides remain to be determined, but the pronounced affinity of LCA for starch emphasizes its potential utility in examining the distribution and fine structure of starch in both raw and processed materials.

Fungal infection is a common problem in food systems, especially where raw materials such as grains or oilseeds are stored in bulk for extended periods of time. Determination of fungal infection is usually accomplished using isolation and cultural techniques, or by assaying for fungal metabolites such as chitin (Tuite and Foster 1979) or ergosterol and deoxyvalenol (Young et al 1984). These techniques can, however be quite expensive and time consuming, and often yield little information about the actual extent of fungal penetration into the material.

Similarly, low levels of fungal penetration of a grain are also frequently difficult to detect microscopically although this problem has recently been addressed in rapeseed by Schans et al (1982) and Van Caeseele and Mills (1983) using the fluorochrome acridine orange to visualize fungal hyphae with the fluorescence microscope. Although not exclusively specific for fungal hyphae, the high sensitivity of the fluorochrome offers improved detectability. In comparison, WGA showed specificity only for fungal hyphae in *Fusarium*-infected wheat tissues (Figs. 8, 9, 10). In Fig. 8, the fluorescein-labelled hyphae are visible within the pericarp, and in a packed mass between the two cuticular layers of the testa in the early stages of infection. In this section, the infection appears to have been superficial, involving only the outer layer of the grain. In Fig. 9, infection has progressed into the sub-aleurone region of the grain, and individual hyphae are also readily detected using WGA as a fluorescent probe. In this case, the hyphae have penetrated into individual starch granules, presumably aided by the high amylolytic activity in this particular kernel which was selected from a sample of partially sprouted (germinated) grains. In Fig. 10 however, there was no initial indication of sprouting and the WGA-labelled hyphae are clearly visible in the central endosperm as they have invaded the storage protein matrix between the starch granules.

N-acetylglucosamine, one of the reported specificities of WGA (Clarke and Hoggart 1982), is a common cell wall polysaccharide of many fungi (Sturgeon 1974) and the affinity of WGA for fungal hyphae has been demonstrated by Mirelman et al (1975). The utility of WGA in detection of cereal-contaminating organisms is clearly demonstrated in Figs. 8-10. The binding is quite specific, very sensitive, and obviously capable of detecting individual hyphae. N-acetylglucosamine-binding lectins may also prove useful in examining fundamental processes of food contamination and perhaps in assessing related processes such as mold-ripening of cheeses.

Although the cost of lectins renders them impractical for routine (e.g., quality control) analyses, the results presented here are but a few selected examples of their many potential applications in fundamental investigations of the distribution of carbohydrates in food materials. Use of these probes should lead to a greater understanding of the relationships between composition, structure and function in cells and tissues, and they should also allow monitoring of changes in morphology and distribution of carbohydrates during processing of raw materials. As fluorescent probes, lectins are both highly sensitive and specific markers which should find many future applications in food science.

Figure Captions

All figures are fluorescence micrographs. With the exception of Figures 3 and 4, all were stained with fluorescein-labelled lectins, and were photographed using FC I. Figures 3 and 4 were stained with rhodamine-labelled lectin, and were photographed using FC II. Scale bars in μm .

Figure 1. UEA I treated GMA section of Sarson R500 rapeseed showing fluorescent staining of cotyledon cell walls (arrow). Autofluorescent protein bodies (p) are visible inside the cells.

Figure 2. RCA I treated cryostat section of Echo rapeseed showing fluorescence of cotyledon cell walls.

Figures 3 and 4 illustrate the inhibition of UEA I binding by L-fucose. When the lectin was pre-incubated for 30 minutes with 0.2 M L-fucose before applying to the section, significantly less lectin was bound (Fig. 4) than when lectin alone was applied to the section (Fig. 3). Both figures are cryostat sections of Echo rapeseed.

Figure 5. WGA-treated cryostat section of Candle rapeseed, counterstained with Fast Green, showing fluorescent lectin binding to the seed coat mucilage (arrow). No specific fluorescence is visible in the cotyledon (C).

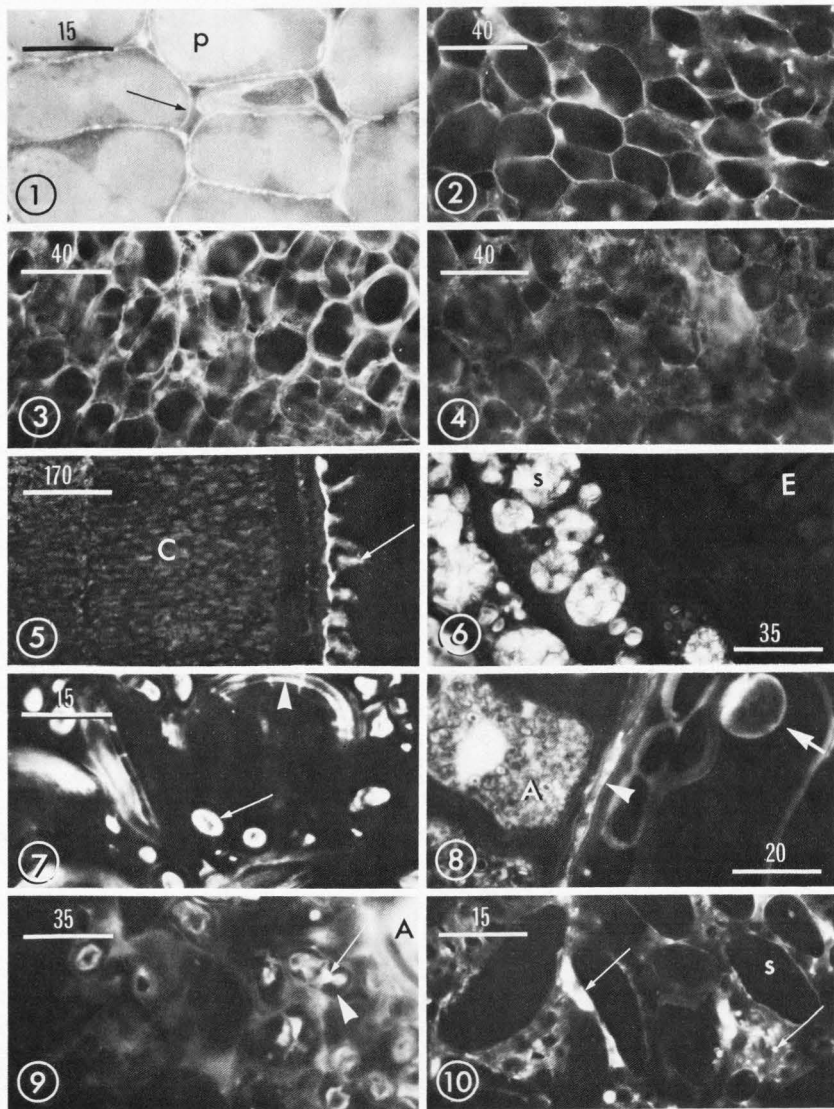
Figure 6. LCA-treated section of oat showing lectin binding to starch granules (S). No fluorescence is visible in the embryo (E) or in the endosperm matrix surrounding each compound starch granule.

Figure 7. LCA-treated GMA section of starch granules from wheat showing growth rings (arrow-head) in the large type A granules. Lectin binding also occurs in the smaller type B granules (arrow) but no growth rings are visible. "Halos" are discernible around both types of granules.

Figure 8. WGA binding to fungal hyphae penetrating the pericarp (arrow) and packed between the cuticular layers of the testa (arrowhead) of wheat. Fluorescence in the aleurone cells (A) is autofluorescence, and is not due to lectin binding.

Figure 9. GMA section of sprouted wheat showing the fungal hyphae (arrow), after WGA treatment, penetrating the non-fluorescent starch granule (arrowhead). Autofluorescence is visible in the aleurone layer (A).

Figure 10. WGA binding to fungal hyphae (arrows) between starch granules (S) in the starchy endosperm of wheat. Individual hyphae are readily distinguishable.



Acknowledgements

The patience and expert technical assistance of Mr. F. Wong are gratefully acknowledged. This research was supported in part by the Canadian Council of Canada CUAP grant 83-4.

References

- *Aspinall, G. O. (1977) Polysaccharides of rapeseed hulls. I. Isolation of polysaccharide fractions, in: Essays in Rapeseed Seed, Oil, Meal, and Mill Fractions, 4th Progress Report, Rapeseed Association of Canada Publication No. 46, p. 15.
- *Aspinall, G. O., Krishnamurthi, T. N. (1976) Polysaccharides of rapeseed hulls. 2. Study of inter-sugar linkages, in: Research in Rapeseed Seed, Oil, Meal, and Mill Fractions, 4th Progress Report, Rapeseed Association of Canada Publication No. 44000, pp. 146-147.
- Baldo, B. A., Boniface, P. A., Simmonds, D. H. (1982a) Lectins as cytochemical probes of the developing wheat grain. I. Fluorescein-labelled lectin covering a range of specificities with emphasis on those that bind D-galactose. Australian J. Plant Physiol. **9**: 647-661.
- Baldo, B. A., Boniface, P. A., Simmonds, D. H. (1982b) Lectins as cytochemical probes of the developing wheat grain. II. Reaction of wheat-germ lectin with the succinate epidermis. Australian J. Plant Physiol. **9**: 663-675.
- Baldo, B. A., Boniface, P. A., Simmonds, D. H. (1982c) Lectins as cytochemical probes of the developing wheat grain. III. Reaction of potato and wheat-germ lectins with wheat protein bodies. Australian J. Plant Physiol. **9**: 677-688.
- Clarke, A. E., Hoggart, R. A. (1982) The use of lectins in the study of glycoproteins, in: Antibody as a tool, the applications of Immunocytochemistry, J. J. Marchalonis, G. W. Warr (eds) John Wiley and Sons, N.Y. pp 347-401.
- Evers, A. D. (1979) Cereals starches and proteins, in: Food Microscopy, J. G. Vaughan (ed) Academic Press London, pp. 139-1191.
- Feder, N., O'Brien, T. P. (1981) Plant Microtechniques: Some principles and new methods. Amer. J. Bot. **55**: 123-142.
- Fulcher, R. G., Wong, S. I. (1980) Inside cereals - a fluorescence microchemical view, in: Cereals for Food and Beverages, G. E. Inglett and L. Munck (eds) Academic Press, London, pp. 1-26.
- Fulcher, R. G., Wood, P. J., Yiu, S. H. (1984) Insights into food carbohydrates through fluorescence microscopy. Food Technol. **38**(1): 101-106.
- Gallant, D. J., Sterling, L. (1976) Electron microscopy of starch and starch products, in: Examination and Analysis of Starch and Starch Products, J. A. Radley (ed) Applied Science Publishers Ltd., London pp. 33-559.
- Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T., Sharon, N. (1980) What should be called a lectin? Nature **285**: 66.
- Holthofer, H., Virtanen, I., Pettersson, E., Tornroth, T., Alfthan, O., Linder, E., Miettinen, A. (1981) Lectins as fluorescence microscopic markers for saccharides in the human kidney. Lab. Invest. **45**(5): 391-399.
- Hood, L. F., Liboff, M. (1983) Starch Ultrastructure, in: New Frontiers in Food Microstructure, D. B. Bechtel (ed) American Association for Cereal Chemists, Inc., St. Paul. pp 341-370.
- Hughes, J., McCully, M. E. (1975) The use of an optical brightener in the study of plant structure. Stain Technology **50**(5): 319-329.
- Johansen, D. A. (1940) Plant Microtechnique. McGraw-Hill, New York. Chapter XV.
- Koiman, P. (1957) Amyloids of plant seeds. Nature **179**: 107-109.
- Maeda, H., Tshida, N. (1967) Specificity of binding of hexopyranosyl polysaccharides with fluorescent brightener. J. Biochem. **62**(2): 276-278.
- Mirelman, D., Gulun, E., Sharon, N., Lotan, R. (1975) Inhibition of fungal growth by wheat germ agglutinin. Nature **256**: 414-416.
- Moss, G. E. (1976) The microscopy of starch, in: Examination and Analysis of Starch and Starch Products, J. A. Radley (ed) Applied Science Publishers Ltd., London. pp 1-32.
- Pena, S. D. J., Gordon, B. B., Karpati, G., Carpenter, S. (1981) Lectin histochemistry of human skeletal muscle. J. Histochem. Cytochem. **29**(4): 542-546.
- Rougier, M., Kieda, C., Monsigny, M. (1979) Use of lectin to detect the sugar components of maize root cap slime. J. Histochem. Cytochem. **27**(4): 878-881.
- Sato, A., Spicer, S. S. (1982) Ultrastructural visualization of galactose in the glycoprotein of gastric surface cells with a peanut lectin conjugate. Histochem. J. **14**: 125-138.
- Schans, J., Mills, J. T., Van Caeseele, L. (1982) Fluorescence microscopy of rapeseeds invaded by fungi. Phytopathology **72**: 1582-1586.
- Sharon, N. (1974) Glycoproteins of higher plants, in: Plant Carbohydrate Biochemistry, J. B. Pridham (ed), Academic Press, London, pp 235-252.
- Siddiqui, I. R., Wood, P. J. (1971) Structural investigation of water-soluble rapeseed (*B. campestris*) polysaccharides. Part I. Rapeseed amyloid. Carbohydr. Res. **17**: 97-108.
- Siddiqui, I. R., Wood, P. J. (1977a) Structural investigation of sodium hydroxide-soluble rapeseed (*B. campestris*) polysaccharides. Part V. Fucoamyloid. Carbohydr. Res. **53**: 85-94.
- Siddiqui, I. R., Wood, P. J. (1977b) Carbohydrates of Rapeseed: A review. J. Sci. Fd. Agr. **28**: 530-538.
- Sturgeon, R. J. (1974) Chemical and biochemical aspects of fungal cell walls, in: Plant Carbohydrate Biochemistry, J. B. Pridham (ed) Academic Press, London, pp 219-233.

Discussion with reviewers

- Tuite, J., Foster, G. H. (1979) Control of storage diseases of grain. *Ann. Rev. Phytopathol.* 17: 343-366.
- *Van Caeseele, L., Mills, J. T. (1983) Mucilage in canola seeds. Rapid detection and interaction with storage fungi, in: *Research on Canola Seed, Oil, Meal and Meal Products*, 7th Progress Report. Canola Council of Canada Publication No. 61. pp 167-173.
- Vermeer, J., McCully, M. E. (1981) Fucose in the surface deposits of axenic and field grown roots of *Zea mays* L. *Protoplasma* 109: 233-248.
- Vose, J. P. (1974) Chemical and physical studies of mustard and rapeseed coats. *Cereal Chem.* 51: 658-665.
- Weber, F. E., Taillie, S. A., Stauffer, K. R. (1974) Functional characteristics of mustard mucilage. *J. Food Sci.* 39: 461-466.
- Wivinis, G. P., Maywald, E. C. (1967) Photographs of starches, in: *Starch: Chemistry and Technology*, Vol. II. *Industrial Aspects*. R. L. Whistler and E. F. Paschall (eds) Academic Press, New York. pp 649-685.
- Wood, P. J., Fulcher, R. G. (1978) Interactions of some dyes with cereal β -glucans. *Cereal Chem.* 55(6): 952-966.
- Wood, P. J., Fulcher, R. G., Stone, B. A. (1983) Studies on the specificity of interaction of cereal cell wall components with Congo Red and Calcofluor. Specific detection and histochemistry of (1,3),(1,4)-D-glucan. *J. Cereal Sci.* 1: 95-110.
- Yiu, S. H., Poon, H., Fulcher, R. G., Altosaar, I. (1982) The microscopic structure and chemistry of rapeseed and its products. *Food Microstructure* 1(2): 135-143.
- Yiu, S. H., Altosaar, I., Fulcher, R. G. (1983) The effects of commercial processing on the structure and microchemical organization of rapeseed. *Food Microstructure* 2(2): 165-173.
- Young, J. C., Fulcher, R. G., Hayhoe, J. H., Scott, P. M., Dexter, J. E. (1984) Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. *J. Agric. Fd. Chem.* 32: 659-664.

F.W. Sosulski: Does C. reich with small starch granules as compared with the large starch granules? Would the differences in LCA binding reflect variations in molecular packing within the granule?

Authors: We cannot comment on differences in intensity, as the methods to measure them are not available. We can only note differences in staining patterns.

F.W. Sosulski: The authors emphasize the potential of using LA affinity as a tool in the investigation of the structure of starch in processed foods. Could the authors comment on the nature of LCA binding with gelatinized or retrograded starch?

Authors: We have not yet examined processed starches except to note that gelatinized starches appear to retain an affinity for the appropriate lectin.

J.M. Faubion: Do you think it possible to label with two lectins simultaneously, fluorescein-labelled WGA and rhodamine-labelled LCA for example?

Authors: Simultaneous labeling should be possible, providing the specificities of the two lectins are different.

J.M. Faubion: Have you examined any waxy or high amylose cereals for the interaction of their starches with LCA?

Authors: Yes... LCA binds to these starches as well.

*Available from Canola Council of Canada,
301-433 Main Street, Winnipeg,
Manitoba, Canada R3B 1B3

Acknowledgements

The patience and expert technical assistance of Mr. F. Wong are gratefully acknowledged. This research was supported in part by Canola Council of Canada CUAP grant no. 83-42.

References

- *Aspinall, G. O. (1976) Polysaccharides of rapeseed hulls. 1. Isolation of polysaccharide fractions, in: Research on Rapeseed, Seed, Oil, Meal, and Meal Fractions, 4th Progress Report. Rapeseed Association of Canada Publication No. 40. p. 145.
- *Aspinall, G. O., Krishnamurthy, T. N. (1976) Polysaccharides of rapeseed hulls 2. Study of inter-sugar linkages, in: Research on Rapeseed, Seed, Oil, Meal, and Meal Fractions, 4th Progress Report. Rapeseed Association of Canada Publication No. 40. pp. 146-147.
- Baldo, B. A., Boniface, P. A., Simmonds, D. H. (1982a) Lectins as cytochemical probes of the developing wheat grain. I. Fluorescein-labelled lectins covering a range of specificities with emphasis on those that bind D-galactose. *Australian J. Plant Physiol.* **9**: 647-661.
- Baldo, B. A., Boniface, P. A., Simmonds, D. H. (1982b) Lectins as cytochemical probes of the developing wheat grain. II. Reaction of wheat-germ lectin with the nucellar epidermis. *Australian J. Plant Physiol.* **9**: 663-675.
- Baldo, B. A., Boniface, P. A., Simmonds, D. H. (1982c) Lectins as cytochemical probes of the developing wheat grain. III. Reaction of potato and wheat-germ lectins with wheat protein bodies. *Australian J. Plant Physiol.* **9**: 677-688.
- Clarke, A. E., Hoggart, R. M. (1982) The use of lectins in the study of glyco-proteins, in: Antibody as a tool, The Applications of Immunochemistry. J. J. Marchalonis, G. W. Warr (eds) John Wiley and Sons, N. Y. pp 347-401.
- Evers, A. D. (1979) Cereal starches and proteins, in: Food Microscopy, J. G. Vaughan (ed) Academic Press, London. pp. 139-191.
- Feder, N., O'Brien, T. P. (1968) Plant Microtechniques: Some principles and new methods. *Amer. J. Bot.* **55**: 123-142.
- Fulcher, R. G., Wong, S. I. (1980) Inside cereals - a fluorescence microchemical view, in: Cereals for Food and Beverages, G. E. Inglett and L. Munck (eds) Academic Press, London. pp. 1-26.
- Fulcher, R. G., Wood, P. J., Yiu, S. H. (1984) Insights into food carbohydrates through fluorescence microscopy. *Food Technol.* **38**(1): 101-106.
- Gallant, D. J., Sterling, C. (1976) Electron microscopy of starch and starch products, in: Examination and Analysis of Starch and Starch Products, J. A. Radley (ed) Applied Science Publishers Ltd., London pp. 33-59.
- Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T., Sharon, N. (1980) What should be called a lectin? *Nature* **285**: 66.
- Holthofer, H., Virtanen, I., Pettersson, E., Tornroth, I., Alifhan, O., Linder, E., Miettinen, A. (1981) Lectins as fluorescence microscopic markers for saccharides in the human kidney. *Lab. Invest.* **45**(5): 391-399.
- Hood, L. F., Liboff, M. (1983) Starch Ultrastructure, in: New Frontiers in Food Microstructure, D. B. Bechtel (ed) American Association for Cereal Chemists, Inc., St. Paul. pp 341-370.
- Hughes, J., McCully, M. E. (1975) The use of an optical brightener in the study of plant structure. *Stain Technology* **50**(5): 319-329.
- Johansen, D. A. (1940) Plant Microtechnique. McGraw-Hill, New York. Chapter XV.
- Koolman, P. (1957) Amyloids of plant seeds. *Nature* **179**: 107-109.
- Maeda, H., Ishida, N. (1967) Specificity of binding of hexopyranosyl polysaccharides with fluorescent brightener. *J. Biochem.* **62**(2): 276-278.
- Mirelman, D., Gulun, E., Sharon, N., Lotan, R. (1975) Inhibition of fungal growth by wheat germ agglutinin. *Nature* **256**: 414-416.
- Moss, G. E. (1976) The microscopy of starch, in: Examination and Analysis of Starch and Starch Products, J. A. Radley (ed) Applied Science Publishers Ltd., London. pp 1-32.
- Penas, S. D. J., Gordon, B. B., Karpati, G., Carpenter, S. (1981) Lectin histochemistry of human skeletal muscle. *J. Histochem. Cytochem.* **29**(4): 542-546.
- Rougier, M., Kieda, C., Monsigny, M. (1979) Use of lectin to detect the sugar components of maize root cap slime. *J. Histochem. Cytochem.* **27**(4): 878-881.
- Sato, A., Spicer, S. S. (1982) Ultrastructural visualization of galactose in the glycoprotein of gastric surface cells with a peanut lectin conjugate. *Histochem. J.* **14**: 125-138.
- Schans, J., Mills, J. I., Van Caeseele, L. (1982) Fluorescence microscopy of rapeseeds invaded by fungi. *Phytopathology* **72**: 1582-1586.
- Sharon, N. (1974) Glycoproteins of higher plants, in: Plant Carbohydrate Biochemistry, J. B. Pridham (ed), Academic Press, London. pp 235-252.
- Siddiqui, I. R., Wood, P. J. (1971) Structural investigation of water-soluble rapeseed (*B. campestris*) polysaccharides. Part I. Rapeseed amyloid. *Carbohydr. Res.* **17**: 97-108.
- Siddiqui, I. R., Wood, P. J. (1977a) Structural investigation of sodium hydroxide-soluble rapeseed (*B. campestris*) polysaccharides. Part V. Fucoamyloid. *Carbohydr. Res.* **53**: 85-94.
- Siddiqui, I. R., Wood, P. J. (1977b) Carbohydrates of Rapeseed: A review. *J. Sci. Fd. Agri.* **28**: 530-538.
- Sturgeon, R. J. (1974) Chemical and biochemical aspects of fungal cell walls, in: Plant Carbohydrate Biochemistry, J. B. Pridham (ed) Academic Press, London. pp 219-233.

Discussion With Reviewers

- Tuite, J., Foster, G. H. (1979) Control of storage diseases of grain. *Ann. Rev. Phytopathol.* 17: 343-366.
- *Van Caeesle, L., Mills, J. T. (1983) Mucilage in canola seeds: Rapid detection and interaction with storage fungi, in: Research on Canola Seed, Oil, Meal and Meal Products, 7th Progress Report. Canola Council of Canada Publication No. 61. pp 167-173.
- Vermeer, J., McCully, M. E. (1981) Fucose in the surface deposits of axenic and field grown roots of *Zea mays* L. *Protoplasma* 109: 233-248.
- Vose, J. P. (1974) Chemical and physical studies of mustard and rapeseed coats. *Cereal Chem.* 51: 658-665.
- Weber, F. E., Taillie, S. A., Stauffer, K. R. (1974) Functional characteristics of mustard mucilage. *J. Food Sci.* 39: 461-466.
- Wivinis, G. P., Maywald, E. C. (1967) Photographs of starches, in: Starch: Chemistry and Technology, Vol. II. Industrial Aspects. R. L. Whistler and E. F. Paschall (eds) Academic Press, New York. pp 649-685.
- Wood, P. J., Fulcher, R. G. (1978) Interactions of some dyes with cereal β -glucans. *Cereal Chem.* 55(6): 952-966.
- Wood, P. J., Fulcher, R. G., Stone, B. A. (1983) Studies on the specificity of interaction of cereal cell wall components with Congo Red and Calcofluor. Specific detection and histochemistry of (1 3),(1 4)-D-glucan. *J. Cereal Sci.* 1: 95-110.
- Yiu, S. H., Poon, H., Fulcher, R. G., Altosaar, I. (1982) The microscopic structure and chemistry of rapeseed and its products. *Food Microstructure* 1(2): 135-143.
- Yiu, S. H., Altosaar, I., Fulcher, R. G. (1983) The effects of commercial processing on the structure and microchemical organization of rapeseed. *Food Microstructure* 2(2): 165-173.
- Young, J. C., Fulcher, R. G., Hayhoe, J. H., Scott, P. M., Dexter, J. E. (1984) Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. *J. Agric. Fd. Chem.* 32: 659-664.

F.W. Sosulski: Does LCA react with small starch granules as intensely as with the large starch granules? Would these differences in LCA binding reflect variations in molecular packing within the granule?

Authors: We cannot comment on differences in intensity, as methods to measure them are not available. We can only note differences in staining patterns.

F.W. Sosulski: The authors emphasize the potential for using LCA affinity as a tool in the investigation of the structure of starch in processed foods. Could the authors comment on the nature of LCA binding with gelatinized or retrograded starch?

Authors: We have not yet examined processed starches except in noting that gelatinized starches appear to retain an affinity for the appropriate lectin.

J.M. Faubion: Do you think it feasible to label with two lectins simultaneously, fluorescein-labelled WGA and rhodamine-labelled LCA for example?

Authors: Simultaneous labelling should be possible, providing the affinities of the two lectins are different.

J.M. Faubion: Have you examined any waxy or high amylose cereals for the interaction of their starches with LCA?

Authors: Yes. LCA binds to these starches as well.

*Available from Canola Council of Canada, 301-433 Main Street, Winnipeg, Manitoba, Canada R3B 1B3