

Protein pattern changes in the germinating bean seed* —

T. E. G. LEE**, O. J. CROCOMO***

COMPENDIO

Se investigaron los cambios de proteínas solubles totales, glicoproteínas y sus respectivas subunidades en los cotiledones de granos de Phaseolus vulgaris L. en germinación. Se usaron técnicas de electroforesis de gel poliacrilamida para detectar estas proteínas y subunidades. La desaparición de estas bandas de proteína se encontró estar en secuencia con la proteína tipo legúmina como la primera que es hidrolizada, seguida por la vicilina-I, y finalmente por la vicilina-II. La síntesis de proteínas nuevas en los cotiledones de las semillas de frijol en germinación fue también detectada.

Introduction

It has been known for many years that seed germination involves the breakdown of storage protein and their conversion into smaller, more soluble nitrogenous compounds, such as amides and amino acids. These compounds are then translocated to the growing embryo where they are utilized (9). In the *Phaseolus vulgaris* beans, the protein component generally make up about 20 to 25 per cent of the dry weight in which 55-60 per cent are storage proteins (11). They are vicilin-like protein (which include vicilin-I and vicilin-II) and legumin-like protein (11). The proportion of vicilin-like protein to legumin-like protein was estimated as 7 : 1 and vicilin-I to vicilin-II as 1 : 8 (11). The remaining proteins are mainly albumins (20-25%) and glutamins (10-15%)† Various proteases and peptidases have been demonstrated in different germinating seeds (4). Although the physiological role of these enzymes is still not apparent, the observations that proteolytic activity increased as germination proceeds and as protein decreases in the cotyledons suggests that they may preferentially hydrolyze the storage protein such as vicilin and legumin (1, 9). The study described herein was performed to evaluate the changes in protein patterns that occur in the total soluble protein and glycoprotein during germination of bean seeds.

Materials and Methods

Plant material: Seeds of *Phaseolus vulgaris* L. cv. 'Goiano precoce' were soaked in a 20 per cent Q-Boa solution which is a germicide containing 5.2 per cent of active Cl for one hour and rinsed 3 to 4 times in distilled water. The seeds were then sown in moist silicon and maintained in a growth chamber. The germination conditions were controlled with a day temperature as 28 C. and night temperature as 22 C. A combination of 2500-3000 ft-c of fluorescent and incandescent light was provided for 13 hr each day with the remaining hr in dark. The germination was carried out for periods of 3, 6 and 10 days. The germinating seeds were sprayed daily with distilled water and abnormal seedlings were discarded. At the end of each germinating period, samples of seedlings were collected and after removal of the seed coat and seedling axis, the cotyledons were ground directly in a mortar with addition of liquid nitrogen. Ungerminated seeds with the seed coat and axis attached, about the same size as the seeds used for germination, were ground in a ball-mill (Spex Mixer-Mill N° 8000).

Protein samples: A crude total soluble protein preparation was obtained by extraction of the cotyledon mills (1 g) with 20 ml of 0.2 N NaCl in 0.05 M tris (hydroxymethyl) aminomethane buffer (pH 8.1) in the cold for 30 min. The homogenates were centrifuged at 23000 g for 30 min at 0 C. and the resulting supernatants were then dialyzed overnight against excess tris-NaCl buffer in a cold room. Protein content was determined by the Lowry's method (12) and the volume of each sample necessary to give approximately 300 μ g of protein for electrophoresis was calculated.

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** Visiting professor, Universidade Federal da Paraíba, Centro de Ciência e Tecnologia, Campus de Areia, 58397, Areia, PB, Brazil

*** Head, Seção de Bioquímica de Plantas, Centro de Energia Nuclear na Agricultura, 13400, Piracicaba, SP, Brazil

† Lee and Crococo, unpublished data.

Disc electrophoresis: Polyacrylamide gel columns (7%) were prepared as described by Davis (6). Electrophoresis using about 0.3 mg protein sample was usually carried out for 50 min in a tris-glycine buffer with a current of 5 mA per gel column. Detection of the separated protein components was achieved by staining the gels for 2 hr with 0.5 per cent aniline blue black in 7 per cent acetic acid and destained by diffusion with several changes of 7 per cent acetic acid.

SDS gel electrophoresis and molecular weight determination: SDS gel electrophoresis was performed by the method of Laemmli (10) using 10 per cent gel. Electrophoresis was carried out with a current of 1 mA per gel until the bromophenol blue marker reached almost to the bottom of the gel (about 4 hr). The gels were then cut at the tracking dye band and stained overnight with 0.05 per cent coomassie brilliant blue R-250 in methanol: acetic acid: water (25:7:68 by volume) and diffusion-destained by repeated washing in the same solvent.

Molecular weight of the subunits were estimated from the positions of the polypeptides on the gels in relation to the marker protein as described before (11)

Detection of glycoproteins. The periodic acid-Schiff (PAS) technique was applied for the detection of glycoproteins following electrophoresis on acrylamide gels. The procedures used were mainly according to the method of Zacharius *et al.* with slightly modifications (16). Gels derived from electrophoresis were first immersed in a 12.5 per cent Trichloroacetic acid (TCA) solution for 30 min (50 ml/gel), rinsed lightly with distilled water and then immersed again in 1 per cent periodic acid (made in 7 per cent acetic acid) in dark for 60 min at 31 C. The gels were then washed overnight with distilled water with constant shaking and a few changes (100 ml/gel) to remove the periodic acid remained in the gels. After exhausting washing, the gels were stained with fuchsinulfite reagent (4) in dark at 31 C for 50 min. The gels were then washed with freshly prepared 0.5 per cent metabisulfite 3 times for 10 min each (50 ml/gel) and washed again with distilled water with frequent changes and motion until excess stain is removed. After this step, the gels were stored in 7 per cent acetic acid.

Sample weight: Fresh weight was taken immediately after the sample was collected. Dry weight was measured after the sample was dried in a 70 C oven for 3 days. Ten samples were randomly collected each time for the weight determination.

Results

The seeds start to emerge after 3 days. The increase in fresh weight of the cotyledons occurs at this time too (Fig. 1-B). The fresh weight then start to decline mainly caused by a loss of water in the tissue. Dry weight is constantly decreasing during the whole germination period (Fig. 1-B).

Changes in protein content: Figure 1-A shows the protein content changes during the germination of bean seeds. The content of this tris-NaCl extractable protein in the cotyledons of seedling decreased with increasing of the germination age. Steady decline of protein was found during the first 3 days of germination followed by a more rapid loss.

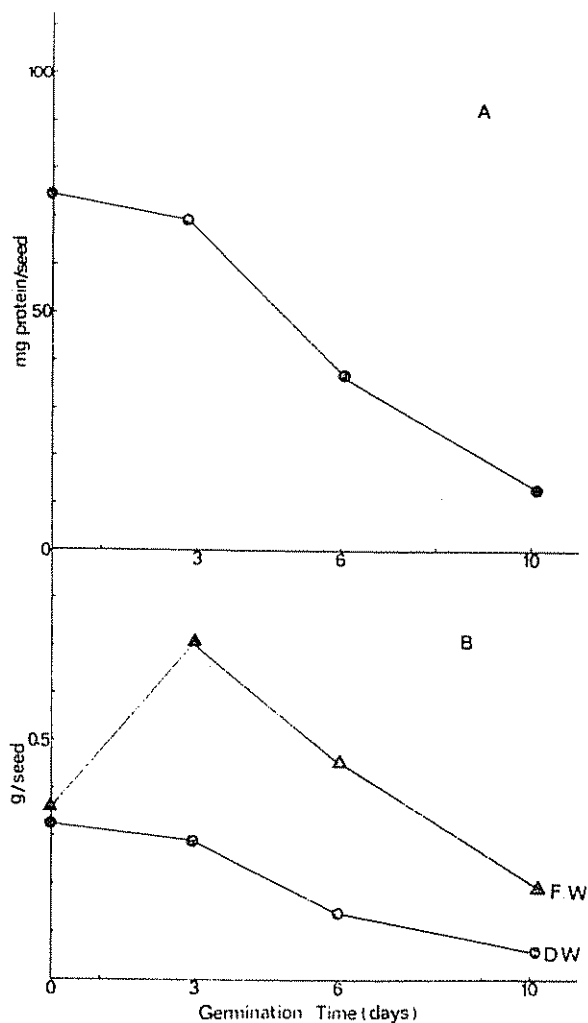


Fig. 1 - A — Protein changes occurring in germinating cotyledons of *Phaseolus vulgaris* beans

B — Weight changes occurring in germinating cotyledons of *Phaseolus vulgaris* beans (F.W.: Fresh weight; D.W.: Dry weight).

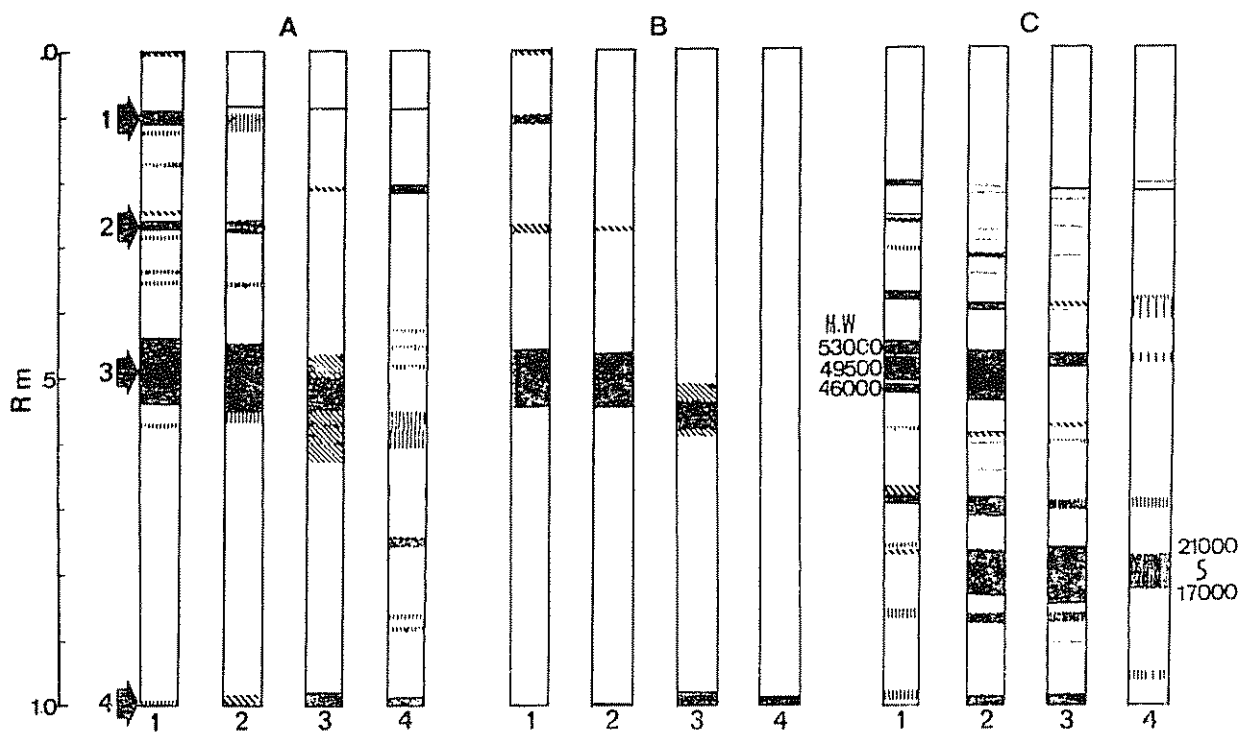


Fig. 2—A—Disc gel electrophoretic patterns of total soluble protein components in *Phaseolus vulgaris* beans following germination
 B—Disc gel electrophoretic patterns of glycoproteins in *Phaseolus vulgaris* beans following germination

C—Subunit patterns of total soluble proteins in *Phaseolus vulgaris* beans following germination 1) 0 days germination; 2) 3 days germination; 3) 6 days germination; 4) 10 days germination M.W.: Molecular weight

Changes of the total soluble protein patterns: Nineteen distinguishable tris-NaCl buffer extractable protein bands were observed in ungerminated seeds by disc gel electrophoresis (Fig. 2, gel A1). This was used as a reference to represent the total soluble protein pattern. It was observed that most of the bands were relatively slow-moving proteins situated in the upper part of the separation gels. Four major bands were named by the number (N^o 1 to 4). The darkest bands were found to be band 1, 2 and 3. These bands were the major storage proteins existed in the Brazilian beans (11). Band 1 (Rm=Relative mobility to the front band; 0.10) is legumin-like protein. Band 2 (Rm=0.27) is vicilin I and band 3 (Rm=0.50) which contains the greatest amount is vicilin II (11).

With increasing age of the seedlings, the number of protein bands and their concentration in the cotyledons decreased (Fig. 2, gel A2, A3 and A4). The disappearance of individual proteins generally appeared to exhibit a sequence with the band 1 (legumin-like protein) as the first one to be hydrolyzed followed by band 2 (vicilin I) and then band 3 (vicilin II). When one protein is being hydrolyzed the first sign it shows was change of dark sharp band to a faint diffused band. After 3 days of germination, legumin-like protein was becoming very diffused and after 6 days of germination, it had been disappeared completely. Vicilin I started to show the sign of being hydrolyzed

but stayed as a dark band after day 3 and disappeared at about day 6 too. Vicilin II at day 6 has become a very diffused band but only disappeared after about 10 days of germination (trace amount may still be observed at this time). Band 4 which migrates with the bromo-phenol blue marker increased in relative intensity until day 6 and then decreased a little. The number and concentration of those small faint bands (mainly albumins) decreased sharply by day 3 and by day 6 most of them were undetectable. The appearance of several new protein bands was also observed by day 10 (Fig. 2, gel A4). 3 of them with Rm 0.09, 0.21 and 0.76 were found to be most dominant.

Changes of the glycoprotein patterns: Figure 2, gel B shows the glycoprotein patterns of bean cotyledons during germination. Band 1, 2 and 3 (see Fig. 2, gel A1) were found to be stained by the PAS reagent in the ungerminated seeds. This indicated that they are glycoproteins. After germination, the intensity of the color stained on band 1 and 2 decreased especially band 1 which was hard to detect even at day 3. At day 6, only a weak diffused band 3 was stained and by day 10, this band also disappeared. The sharp front band of Rm 1.0 (band 4), which migrates with the bromo-phenol blue marker, was very concentrated at both day 6 and day 10.

Changes of the protein subunit patterns: The subunit patterns of proteins from the germinating seeds were shown in Figure 2-C. The 3 subunits of vicilin II (M.W. 53000, 49500 and 46000) had disappeared after about 10 days of germination. The most significant changes are the increase in low molecular weight material which form relatively diffused bands. The most important one is a broad band of apparent molecular weight about 21000-17000 which sharply increased at day 3 and shows a decrease only at day 10.

Discussion

The results of the present investigation indicated that the major components in the cotyledon of bean seed are the 3 storage proteins; legumin-like protein, vicilin I and vicilin II (11). Previous studies by Lee and Crocorno (11) indicated that these proteins can be extracted and reasonably purified by zonal-isoelectric precipitation method. Legumin-like protein is a homogeneous protein while vicilin-like protein was found to be heterogeneous and could be further separated to vicilin I and vicilin II by gel electrophoresis. The subunits of vicilin-like protein and legumin-like protein had been determined by Lee and Crocorno (11). Analysis of amino acid composition of these proteins indicated that legumin-like protein is nutritionally more important than vicilin-like protein since it contains much higher sulfur-aminoacids than vicilin-like protein (11).

The decrease of these proteins during germination is in sequence with the legumin-like protein as the first one to be hydrolyzed followed by vicilin I and then vicilin II. Apparently certain protease begins to hydrolyze the legumin-like protein immediately after germination started. The most important sign that shows the protein being breakdown in gel is the change of its sharp dark band to faint diffused band. Changing to a greater electrophoretic mobility was also observed especially for the vicilin II protein. Jou and Stotzky (8) had indicated that globulins and basic proteins in beans decreased rapidly during 11 days of germination while a marked decrease in albumin occurred only in the first 2 days. Apparently the sharp decrease of proteins in the cotyledons after day 3 (Fig. 1-A) was caused by a decrease of globulin storage protein as can be seen in our gels. The overall results in our study was in general agreement with that of Beevers (1), Catsimpooulas *et al* (3) and Juo and Stotzky (8). Although Racusen and Foote (13) in a study of the major glycoprotein reported that there was little change in the major glycoprotein or in the total soluble protein in the germinating bean seeds, it must be pointed out that their studies were conducted for only 114 hr which is less than 5 days. The glycoprotein extracted by Racusen and Foote (1) was glycoprotein II which is the similar protein as vicilin II observed in our study (11). Our results indicated that this protein is the largest protein reserve and is not significantly hydrolyzed until day 6. Subunit pattern analysis also indicate that the 53000, 49500 and 46000 components of vicilin II do not disappear until day 6. By viewing this, our results are in agreement with

Racusen's and apparently, the vicilin II which composes the major part of the soluble proteins is used only sluggishly until some later period of plant development.

The protein breakdown process during germination have been reported (1, 9). Daussant (5) suggests that a progressive deamidation of storage protein take place as a first step in degradation, and that this is followed by cleavage of disulphide bonds when these are present. Further decrease in the size of the proteins is brought about by protease systems. The polypeptides released by these successive steps are then available for progressive hydrolysis by the endo- and exo-peptidases of the seed to their constituent amino acid (14). Beevers and coworkers (2) had reported that both total and specific activity of protease in cotyledon extracts of peas increased during germination. The appearance of several new protein bands in the gel of 10 days germination cotyledons, none of them could be stained by PAS reagent, also suggest a synthesis of new proteins in this stage. Since enzyme induction during germination has been reported (7), the appearance of new bands may reflect increase in enzymes. Beside this, band 4 which is the mobile material (indicator) that constituted the front, this band could also contain small molecular peptide components derived from hydrolyzation of larger protein molecules and the results showed that this band become much darker and concentrated after 6 days of germination which may also indicate intensive protease and peptidase activities. Thus, enzymes that specifically hydrolyze these storage proteins seem to be actively involved in the process of germination.

Summary

The changes of total soluble proteins, glycoproteins and their subunit patterns in the cotyledons of germinating *Phaseolus vulgaris* L beans have been investigated. Polyacrylamide gel electrophoresis techniques were used to detect these proteins and subunits. The disappearance of these protein bands was found to be in sequence with the legumin-like protein as the first one to be hydrolyzed followed by the vicilin-I, and finally the vicilin-II. The synthesis of new proteins in the cotyledons of germinating bean seed was also detected.

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Notas y Comentarios

Vacas en engorde hacen leche rica en calcio

El contenido de calcio soluble de la leche de la vaca baja en la primavera, cuando se la suelta al pastoreo después de comer alimentos conservados durante todo el invierno. El Agricultural Research Council (ARC) de Gran Bretaña informa que un grupo de científicos ha encontrado que la cantidad de calcio en la leche depende de la presencia de una sustancia ligadora del calcio, involucrada en la síntesis de la grasa. Espera que sea posible usar esta información para aumentar la estabilidad de la leche durante el procesamiento y el almacenamiento.

La variación estacional en el calcio soluble salió a la luz por primera vez en 1977, cuando Carl Holt y Donald Muir, del Instituto de Investigación Hannah in Ayr, Escocia, llevaron a cabo un reconocimiento de las lecherías del sudoeste de Escocia (*Journal of Dairy Research*, vol. 46, p. 433). Para verificar esta variación, los investigadores permitieron a ciertas vacas comer sólo alimento conservado, y entonces las soltaron al pastoreo para ver lo que sucedería al contenido de calcio de su leche. Conforme lo esperado, ese contenido decreció.

La explicación, creen Holt y sus colegas, está ligada a la síntesis de la grasa que realiza la vaca, la que a su vez está ligada a la forma en que la vaca almacena el calcio. Las células productoras de leche utilizan un sistema de bombeo enzimático para transportar el calcio a través de la membrana celular. Otras células especializadas tales como el retículo sarcoplásmico y los mitocondrios funcionan en una manera similar, pero ellos eventualmente alcanzan un estado constante en el que el calcio que se difunde pasivamente fuera de la célula iguala al que está bombeándose hacia adentro. Las células productoras de leche, en contraste, retienen el calcio ligándolo a tres sustancias fuertemente fijadoras de calcio: la caseína, el fósforo y el citrato.

El citrato está también involucrado en la producción de grasa en la célula secretora mamaria. Durante los períodos cuando la vaca está haciendo y almacenando grasa, aumentan los niveles de citrato en las células productoras de leche y aumenta el contenido de calcio de la leche concomitantemente. Pero durante la primera parte de la estación de pastoreo

hay una caída en los niveles de citrato en las células productoras de leche, y el contenido de calcio soluble baja también. Los científicos del ARC encontraron que podían reducir el contenido de calcio de la leche haciendo comer a las vacas alimentos conocidos como depresores de la síntesis de la grasa interna.

La leche es más susceptible de alterarse al calentarse si tiene altos niveles de calcio soluble. Los investigadores del ARC están ahora estudiando si las vacas que comen tales dietas producen leche que permanece más estable cuando se calienta al procesarse. Si ellos pudiesen conseguir esto, las máquinas que procesan la leche podrían permanecer más limpias y la industria lechera sería capaz de reducir el costo de operación de sus plantas.

Estos alimentos reductores de la grasa podrían también tener el efecto de prolongar la vida en los anaqueles de ciertos productos lecheros en climas calurosos, no un factor tan crucial en el clima británico, pero muy importante en países del Tercer Mundo que reciben leche evaporada de Europa.

Recuperación de la plata de material fotográfico

La recuperación de la plata parecería no tener importancia para la agricultura, pero hay que recordar que el yoduro de plata se usa cada vez más para "sembrar" las nubes en zonas áridas para aumentar las lluvias.

Un nuevo proceso químico ha sido patentado en Tel Aviv, Israel, para una recuperación sencilla de la plata de materiales fotográficos de desecho. Las películas para cámaras y otros materiales similares sensibles a la luz, constituyen uno de los principales usos industriales de la plata. Con la constante alza en el precio de este metal, 600 por ciento en poco más de un año (relativamente más que el oro), su recuperación en gran escala de material de desecho se torna en un asunto cada vez más atractivo (*Innovation* N° 51, Febr. 1980).

Michael S. Howard, de Tel Aviv, ha perfeccionado un nuevo proceso, que consiste en lavar el material y llevar la plata presente en el agua de lavado a una forma soluble en forma de haloide o sendohaloide. El compuesto de plata se recupera y las sales de plata se purifican.

Notas y Comentarios

Intento de erradicar al picudo del algodón

El picudo del algodón (*Anthonomus grandis*), la notoria plaga del algodón, podría ser eliminada completamente en los Estados Unidos, si tienen éxito los entomólogos del Departamento de Agricultura y unos 1600 millones de dólares de parte de los agricultores y contribuyentes de ese país. Una operación piloto, que comenzó en 1978 en 8000 hectáreas de algodón alrededor de Fayetteville, Carolina del Norte parece que ha tenido éxito (*The Economist* October 13th, 1979 p. 87). Los científicos consideran que si pueden eliminar al insecto allí, una de las zonas más severamente afectadas, ellos podrían hacer lo mismo en cualquier parte.

La ofensiva de Fayetteville comenzó con la aplicación de productos tóxicos, para reducir el número de picudos que irán a invernar durante el invierno. Se reanudó este año cuando los campos fueron asperjados varias veces con diflubenzurón (nombre registrado, Dimilin, un producto desarrollado por la North American Phillips Corporation) Dimilin mata la descendencia de los gorgojos en el estado de huevo o durante las mudas del desarrollo larval.

Entre las aplicaciones de Dimilin, por lo menos 100 machos estériles por cada picudo que se calcula existen en el campo se dejaron caer desde el aire, con el objeto de hacer improductiva la crianza. Observadores entrenados han tenido últimamente dificultades para encontrar gorgojos, y esta evidencia de éxito ha sido confirmada por el escaso recojo de 30 000 trampas que se pusieron en los campos. Cada una fue cebada con una feromona, pero las trampas sólo atraparon siete picudos.

Se están ahora trazando planes, con precisión militar, para llevar la acción a través de los 14 estados algodoneros de los Estados Unidos. Las operaciones de búsqueda y destrucción comenzarían en las Carolinas y se moverían hacia el sur a la frontera mexicana. Se establecería, además, al sur del Río Grande, una ancha zona libre de picudos, para negarle santuario al insecto.

La erradicación del picudo (que puede hacer daño de US\$ 300 millones en un verano) reforzaría los otros factores que hacen ahora al algodón más competitivo contra los textiles artificiales: los altos precios petroquímicos y la invención de una camisa de algodón que no necesita más cuidado que una "lave y use" de poliéster.

Aún así, los agricultores pueden resistirse a pagar su cuota de US\$ 800 millones para pagar el costo de la campaña. Los resultados de intentos pasados de erradicar completamente una población insectil han sido notoriamente de corta duración. Aún en Mississippi, donde el picudo tiene una gran intensidad, los agricultores están mostrando un agudo interés en alternativas a la erradicación total: programas dirigidos simplemente a mantener los números de insectos en niveles tolerables.

Publicaciones

Carta Informativa Agrícola Con fecha setiembre 1978 ha aparecido el primer número de este informativo del Instituto de Investigación Agropecuaria de Panamá (IDIAP), destinado a dar a conocer las actividades, resultados de ensayos sobre la investigación agrícola. Hace pareja con la *Carta Informativa Pecuaria*, aparecida en 1977, (Cf. *Turrialba* 29: 58) dividiéndose así las dos disciplinas del IDIAP.

El primer número tiene una descripción de la institución, y un artículo sobre avances de la soya en Panamá, además de noticias breves. El segundo número tiene fecha agosto 1979 y tiene artículos sobre plagas del arroz en Panamá, nuevas variedades de pimiento, nematología general, y siembra del plátano. La editora actual es Elizabeth de Ruiloba y la dirección es: Apartado 58, Santiago de Veraguas, Panamá.

Aspartame, un nuevo edulcorante

Un nuevo nombre, aspartame (APM), se ha unido a la sacarina y al todavía prohibido ciclamato como edulcorante artificial. Los franceses fueron los primeros en poder comprar el producto, que fue desarrollado por G. D. Searle de los Estados Unidos. El APM fue aprobado en Francia en agosto de 1979 y en febrero de 1980 se puso a la venta con el nombre de Canderelle, pero por ahora sólo en forma de tabletas para su uso en la mesa. La aprobación en Canadá y en los Estados Unidos se espera que sigan poco después (*The Economist* 16 de febrero de 1980, p. 96).

La aprobación, sin embargo, no será ilimitada. El APM, un péptido sintetizado a partir de dos aminoácidos, ácido aspártico y el éter metílico de fenilamina, no es tan estable como la sacarina, por ejemplo. Tiende a descomponerse en soluciones ácidas. La mayoría de las bebidas gaseosas y de alimentos enlatados, ambos de gran interés para los fabricantes de edulcorantes, acidifican. El APM es también insuficientemente estable a altas temperaturas como para ser usado en alimentos cocinados. Por eso, al principio, será vendido sólo en tabletas (esto es, para usarse con el café o té); como endulzador para bebidas en polvo, que se mezclan con agua en el momento de usarse; y para cereales de desayuno endulzados, que también se venden en estado seco.

El problema con los ciclamatos, y posiblemente con la sacarina (Cf. *Turrialba* 29:202); es que, después de haber cumplido su misión de endulzar el paladar, son absorbidos por el aparato circulatorio y después excretados por la orina, potencialmente causando cáncer. Una forma de evitar la posibilidad de cáncer consiste en agregar el ion estimulante de sabor dulce a una molécula lo suficientemente grande para que no pueda ser absorbida dentro del cuerpo. Sería entonces excretada sin ser afectada por el proceso digestivo, con las heces. Si fuera además estable en presencia de ácidos diluidos y de las temperaturas de cocción (evitando los inconvenientes del APM), tanto mejor.

Una compañía que trabaja en ese material es Dynapol, una del creciente número de empresas microbiológicas de California, que también trabajan en ingeniería genética. El trabajo con un edulcorante es sólo una parte de programa general para fabricar aditivos para alimentos que no pueden ser absorbidos por el cuerpo.

No sólo se intenta unir la parte activa del aditivo a una molécula demasiado masiva para ser absorbida por el cuerpo, sino también que tenga un enlace químico que el cuerpo no pueda quebrar. Dynapol ha completado pruebas sobre un nuevo preservativo, un antioxidante, al que se le ha unido un polímero. Está trabajando con un colorante amarillo y a fines de 1980 proyecta tener listo un nuevo color rojo. El costo es todavía alto, pero la idea de que los aditivos que dan sabor, color y mayor conservación a los alimentos, pasen por el tracto digestivo sin ser absorbidos, es lógica, ahora que los aditivos (y algunas sustancias naturales) son cada vez más sospechosos de ser dañinos a la salud.

Publicaciones

Boletín de Nuevas Adquisiciones El Centro Regional para la Educación Superior en la América y el Caribe (CRESALC), organismo de la Unesco localizado en Caracas, ha iniciado una publicación trimestral, *Boletín de Nuevas Adquisiciones*, dedicado a difundir la documentación sobre educación superior que se genera en la región. Está a cargo de Servicio de Información y Documentación, cuya directora es Natacha Márquez. El primer número, del volumen primero tiene fecha octubre de 1979 y contiene 43 páginas de referencias, de unas 10 citas por página. Ofrece servicio de fotocopia y anuncia que en diciembre publicaría un *Boletín de Resúmenes*, con síntesis de los documentos más relevantes. La dirección es: Apartado 62090, Caracas 106.