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# Interaction of Gossypol with Gossypin (11S Protein) and Congossypin (7S Protein) of Cottonseed and Glycinin (11S Protein) of Soybean. 1. Reaction Kinetics, Binding Stoichiometry, and Reversibility Studies

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Interaction of gossypol with gossypin, congossypin, and glycinin at pH 9.0, 8.0, and 7.6, respectively, followed by a difference spectral method, indicated that the gossypol–protein complexes were characterized by difference absorption maxima at 430–432 nm (gossypin), 436–437 nm (congossypin), and 430 nm (glycinin). The reaction was time dependent and was complete by 2 h with gossypin, 3 h with congossypin, and 10 h with glycinin. The number of binding sites in protein ($n$) was 4.0 for both gossypin and congossypin and 5.0 for glycinin. Thus, gossypin and congossypin bind a maximum of 4 gossypol molecules/mol of protein, while glycinin binds 5. Interaction was completely reversible, suggesting that only nonconvalent interactions were involved. The low association constants ($K$) suggested that the binding was of weak type and involved nonconvalent interactions.

Cottonseed proteins are widely recognized as a potential source of nutrients for human consumption. But their utilization for food uses is limited because of the presence of a toxic polyphenolic pigment, gossypol [1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-2,2′-binaphthalene-8,8′-dicarboxaldehyde], which is known to interact with proteins during heat processing of the cottonseed kernels (Clark, 1928). Martinez and Frampton (1958), Conkerton and Frampton (1959), Markman and Rzhekhin (1965), and Damaty and Hudson (1975b) have produced evidence to indicate that a major form of binding is the formation of Schiff bases by condensation of the formyl groups of gossypol with $\epsilon$-amino groups of lysine. However, Bressani et al. (1964) suggested that other factors must be involved in addition to Schiff base formation. Damaty and Hudson (1979) have established that application of heat on flours and isolates containing free gossypol resulted in the formation of insoluble, unhydrolyzable products due to irreversible copolymerization between gossypol and cottonseed proteins. It is evident from the literature that the exact nature of the interaction has not yet been clearly established, and no systematic study of the interaction of gossypol with cottonseed proteins, under controlled conditions of temperature, pH, etc., has been made. Further, these studies have been made with total proteins, and such studies with total proteins do not enable one to draw conclusions on the nature of binding sites on protein molecule, etc. Therefore, a study of the interaction of gossypol with isolated 11S (gossypin) and 7S (congossypin) proteins of cottonseed (Mohan Reddy and Narasinga Rao, 1988), which constitute about 65% of the total proteins, was initiated. A difference spectral method was used to follow the interaction. A study of the spectral properties of the gossypol–protein complex, reaction kinetics, binding stoichiometry, and reversibility of the interaction is presented. The nature of interaction was predicted from the thermodynamic constants calculated from the difference spectral data. Though the 11S and 7S proteins of cottonseed used in this investigation were free from gossypol impurities (Mohan Reddy and Narasinga Rao, 1988), interaction with another oilseed protein, soybean glycinin, which does not contain any gossypol, was followed for the purpose of comparison. Glycinin resembles 11S or 7S protein of cottonseed in its

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amino acid composition, molecular weight, and conformation (Catsimpoolas et al., 1971; Mohan Reddy, 1985).

## EXPERIMENTAL SECTION

**Materials.** Cottonseeds (*Gossypium herbaceum* var. Jaydhar) were obtained from Karnataka State Seeds Corp., Mysore, India. Soybean seeds (*Glycine max* var. Bragg) cultivated in a farm near Mysore, India, were purchased. Gossypolacetic acid was obtained from Sigma Chemical Co., St. Louis, MO (Lot No. 51F-4013). 2-Mercaptoethanol (2-Me) was purchased from Fluka. All other chemicals used in the study were of reagent grade. Ethyl alcohol was distilled twice before use. Deionized glass-distilled water was used in the experiments.

**Isolation of Gossypin and Congossypin.** Hexane-defatted (Mohan Reddy et al., 1982) and acetone-degossypolized (Damaty and Hudson, 1975a) cottonseed flour were used for the isolation of gossypin and congossypin. Proteins were isolated from low-gossypol cottonseed flour by the method described previously (Mohan Reddy and Narasinga Rao, 1988). The proteins were found to be homogeneous by sedimentation velocity, gel electrophoresis, gel filtration, and ion-exchange chromatography.

**Isolation of Soybean Glycinin.** Glycinin was isolated from defatted flour by the method of Appu Rao and Narasinga Rao (1977). The homogeneity of the lyophilized protein was tested by sedimentation velocity and gel electrophoresis and found to be better than 90% pure, with only traces of 7S and 16S proteins as contaminants. Since the formation of 7S and 16S fractions from glycinin is known to occur during the process of lyophilization (Wolf et al., 1962; Sureshchandra, 1984), the presence of these two fractions in glycinin may not represent true heterogeneity. Therefore, the above protein preparation was used without further purification.

**Protein Solutions.** Absorptivity values of 7.6, 6.0, and 7.9 at 280 nm for a 1% solution were used for determining the concentration of gossypin, congossypin, and glycinin, respectively (Appu Rao and Narasinga Rao, 1977; Mohan Reddy and Narasinga Rao, 1988). A stock solution of gossypin was prepared by dissolving freshly purified protein in 0.05 M pyrophosphate–0.05 M $NaHCo_3$ buffer, pH 9.0. Lyophilized protein was used to prepare a stock solution of congossypin in 0.1 M phosphate buffer, pH 8.0, and that of glycinin in 0.1 M phosphate buffer, pH 7.6, containing 0.1% 2-ME. Protein solutions at a concentration of $2 \times 10^{-5}$ M were used in the experiments, since this concentration was found to be optimum from preliminary experiments. Molecular weights of 240 000 and 140 000, respectively, for gossypin and congossypin (Mohan Reddy, 1985) and 320 000 for glycinin (Badley et al., 1975) were used.

**Gossypol Solutions.** Gossypol concentration in ethanol was estimated from a molar extinction coefficient ($\epsilon$) of 31 318 at 289 nm. This was determined as follows. The gossypolacetic acid (20 mg, dried under vacuum at 30 °C) was accurately weighed and dissolved in 50 mL of ethanol. Different dilutions of suitable concentrations were made from this stock solution, and absorbance at 289 nm was measured. A plot of absorbance at 289 nm vs gossypol concentration (mg/mL) was constructed, and $\epsilon$ was computed from this plot with a value of 518.5 for the molecular weight of gossypol.

A stock solution of gossypol in ethanol ($\sim 1 \times 10^{-2}$ M) was freshly prepared by dissolving gossypolacetic acid ($\sim 5$ mg; $M_r$ 578.5) in distilled ethanol (0.8 mL). A working solution of $1 \times 10^{-3}$ M gossypol was prepared by diluting the stock solution with the desired buffer, and this had about 8% ethanol. Gossypol concentrations between $2 \times 10^{-5}$ and $18 \times 10^{-5}$ M (20 and 180 $\mu$M) were used. The concentration of alcohol in the final solutions varied from 0.16 to 1.44%. Gossypol solutions degrade with time. Hence, $Na_2SO_3$ at a concentration of 0.05 M was added to prevent degradation. Stock solutions of gossypol in ethanol were always used within 1 h after their preparation. Addition of gossypol to protein did not alter the pH of the assay mixture.

**Measurement of Binding.** The interactions of gossypol with gossypin, congossypin, and glycinin were followed by using a difference spectral method (Gorman and Dornall, 1981). Difference spectra were recorded on a Beckman DU-8B spectrophotometer with temperature control and kinetics attachment, using a pair of matched tandem cells of 1-cm path length. The sample cell contained an equilibrium mixture of gossypol and protein in one compartment and buffer in the other. The reference cell contained an identical concentration of gossypol in one compartment and protein in the other. Difference spectra were recorded in the range 500–360 nm. The difference absorption peaks occurred in the 375-nm region, where gossypol (reference cell) absorbs strongly, and near the 430–437-nm region where the gossypol–protein complex (sample cell) absorbs strongly. In a typical experiment, 0.40-mL aliquots of $1 \times 10^{-4}$ M protein solution followed by 0.20 mL of 0.5 M $Na_2SO_3$ were added to a set of 2-mL volumetric flasks. Different aliquots, ranging from 0.04 to 0.36 mL of $1 \times 10^{-3}$ M gossypol working solution, were added to flasks, made to volume with buffer, mixed gently by inverting the flasks three times, wrapped in a aluminum foil, and incubated in the dark at room temperature ($\sim 26$ °C). Similar concentrations of protein, gossypol, and buffer, all containing 0.05 M $Na_2SO_3$, in three different sets of 2-mL volumetric flasks were also included as above. The difference spectra were recorded at the end of each incubation period. Each set of experiments was repeated at least twice.

**Reaction Kinetics.** A reaction mixture containing $2 \times 10^{-5}$ M protein and $20 \times 10^{-5}$ M gossypol (mole to mole ratio of 1:10) was prepared, and reaction kinetics were followed by measuring difference absorbance at 430 nm for gossypin and glycinin and at 437 nm for congossypin, in a Beckman DU-8B spectrophotometer. The spectrophotometer was programmed to maintain a temperature of 30 ± 0.1 °C and to record the change in absorbance at 1-min intervals during the first hour of reaction and at 5- or 10-min intervals, subsequently. The optimum time of reaction was computed from a plot of absorbance vs reaction time.

**Binding Stoichiometry.** This was determined by Job's method of continuous variation (Huang, 1982). The sum of the concentrations of protein and gossypol was held constant at $20 \times 10^{-5}$ M, their relative concentrations being varied. Difference absorbance at 430 or 435–437 nm, a measure of complex formation, was plotted against the mole fraction of protein or gossypol. A special point (maximum $\Delta A$) was determined from the plot and the binding stoichiometry or the number of binding sites ($n$) was calculated from the ratio of mole fractions of gossypol and protein at that special point.

**Reversibility Studies.** Reversibility of protein–gossypol interaction was determined as follows. A reaction mixture containing $2 \times 10^{-5}$ M protein and $20 \times 10^{-5}$ M gossypol incubated for the desired time at room temperature was diluted with a protein solution of the same concentration, so as to get a series of solutions containing $2 \times 10^{-5}$ M protein and 3, 6, 9, 12, 15, and $18 \times 10^{-5}$ M gossypol, and the difference spectra of these were recorded.

The reaction is said to be reversible if both direct and reverse difference spectra are identical.

**Treatment of Binding Data.** The binding data were analyzed by eq 1 (Lee et al., 1975), where $K$ is the intrinsic binding constant, $\beta = \Delta A/\Delta A_{max}$, $C_f = C - n\beta P$, $\Delta A$ is the

$$K = \frac{\beta}{1-\beta}\frac{1}{C_f} \quad (1)$$

observed difference absorbance of the gossypol–protein complex, $\Delta A_{max}$ is the maximal difference absorbance of the complex, $C_f$ is the molar concentration of unbound or free gossypol, $C$ is the total molar concentration of gossypol, $P$ is the molar concentration of protein, and $n$ is the binding stoichiometry. The value of $K$ is given by the slope of a plot of $\beta/(1-\beta)$ against $C_f$. $\Delta A_{max}$ was determined by extrapolation of a plot of $1/\Delta A$ against $1/C$ to $1/C = 0$ (Lehrer and Fasman, 1966). Since the plots of $1/\Delta A$ vs $1/C$ and $\beta/(1-\beta)$ vs $C_f$ were linear, the data were analyzed by the method of least squares, using a TI programmable 58 calculator.

## RESULTS AND DISCUSSION

To determine ligand binding to proteins, generally techniques such as equilibrium dialysis, gel filtration, etc., are used (Klotz, 1953; Hummel and Dreyer, 1962; Steinhardt and Reynolds, 1969). With the equilibrium dialysis technique it is possible to determine the number of moles of ligand bound/mole of protein as a function of free ligand concentration. Analysis of binding data by the Scatchard plot (Scatchard, 1949) yields the maximum number of binding sites on the protein molecule and the binding constant. Thus, the data do not need to be supplemented by the use of other techniques.

Spectroscopic methods have also been used to follow ligand–protein interactions (Bensi and Hildebrand, 1949; Person, 1965; Deranleau, 1969; Bergeron and Roberts, 1975; Gorman and Darnall, 1981). These techniques generally yield only the binding constant. Determination of the binding constants at different temperatures allows the energetics of the interactions to be evaluated. It is also possible to determine the effect of pH, salts, and other additives on the interaction.

Equilibrium dialysis technique, taking 48–72 h to attain equilibrium, was not found suitable to follow the interaction of gossypol, with gossypin, congossypin, and glycinin, since gossypol undergoes changes with time and is not stable. Maliwal et al. (1985) used CD spectroscopy and difference spectroscopy to follow gossypol binding by bovine serum albumin. They found that the binding constants determined by the two techniques were comparable. Since difference spectral measurements are less time consuming, this technique has been used in this investigation. It was observed that the results were reproducible and the standard deviations in the calculated association constants were small.

**Absorption Spectrum of Gossypol.** The absorption spectra of gossypol at pH 8.0 and 9.0 (Figure 1) show absorption maxima at 385–387, 295, and 238–239 nm and minima at 310 and 210 nm. The spectral region between 340 and 500 nm is useful for monitoring interactions with proteins, since the contributions of proteins to the absorption spectrum in this region are negligible. In the region below 340 nm it is significant. Upon addition of protein, spectral properties of gossypol changed. In difference spectroscopy, in the region 340–500 nm, the gossypol–protein system was characterized by a difference absorption ($\Delta A$) maximum at 430–435 nm and minima at 365–370 nm (Figure 2). The $\Delta A$ values at 430–435 nm

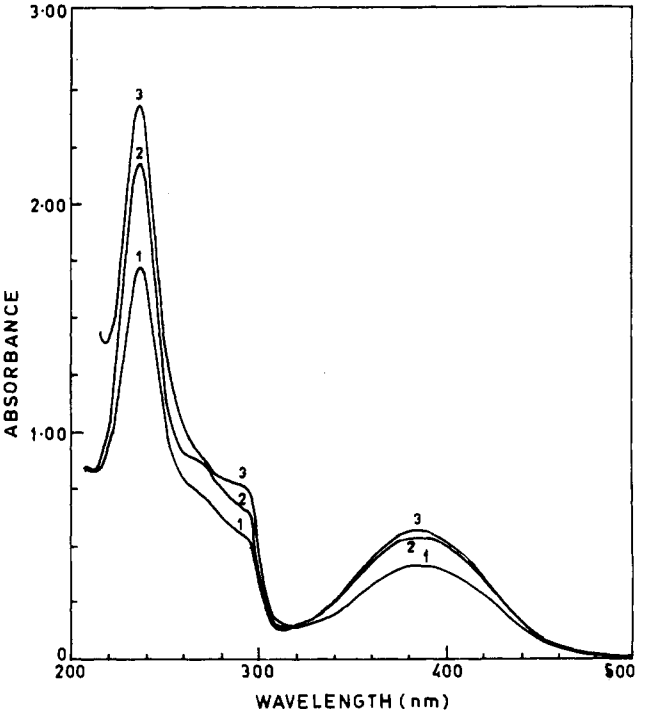


**Figure 1.** Absorption spectra of gossypol: (1) 0.1 M phosphate buffer, pH 7.6; (2) 0.1 M phosphate buffer, pH 8.0; (3) 0.05 M pyrophosphate–0.05 M $NaHCO_3$ buffer, pH 9.0.

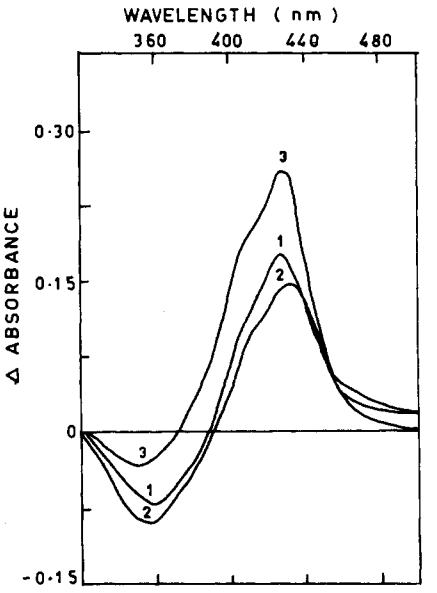


**Figure 2.** Difference spectra: (1) gossypin–gossypol mixture in 0.05 M pyrophosphate–0.05 M $NaHCO_3$ buffer, pH 9.0; (2) congossypin–gossypol mixture in 0.1 M phosphate buffer, pH 8.0; (3) glycinin–gossypol mixture in 0.1 M phosphate buffer, pH 7.6, containing 0.1% 2-ME. All contain 0.05 M $Na_2SO_3$.

varied with gossypol and protein concentration, and this peak was probably due to the formation of protein–gossypol complexes. Therefore, $\Delta A$ at 430–435 nm has been used to follow the interaction of gossypol with gossypin, congossypin, and glycinin.

**Stability of Gossypol.** Preliminary studies indicated that gossypol in aqueous solutions underwent oxidation with time. Oxidation was more pronounced under alkaline conditions. Therefore, the interaction studies were conducted at the lowest possible pH values: 7.6 for glycinin, 8.0 for congossypin, 9.0 for gossypin. The pH for each protein was selected depending upon its solubility in aqueous solutions. $Na_2SO_3$ was added to prevent oxidation

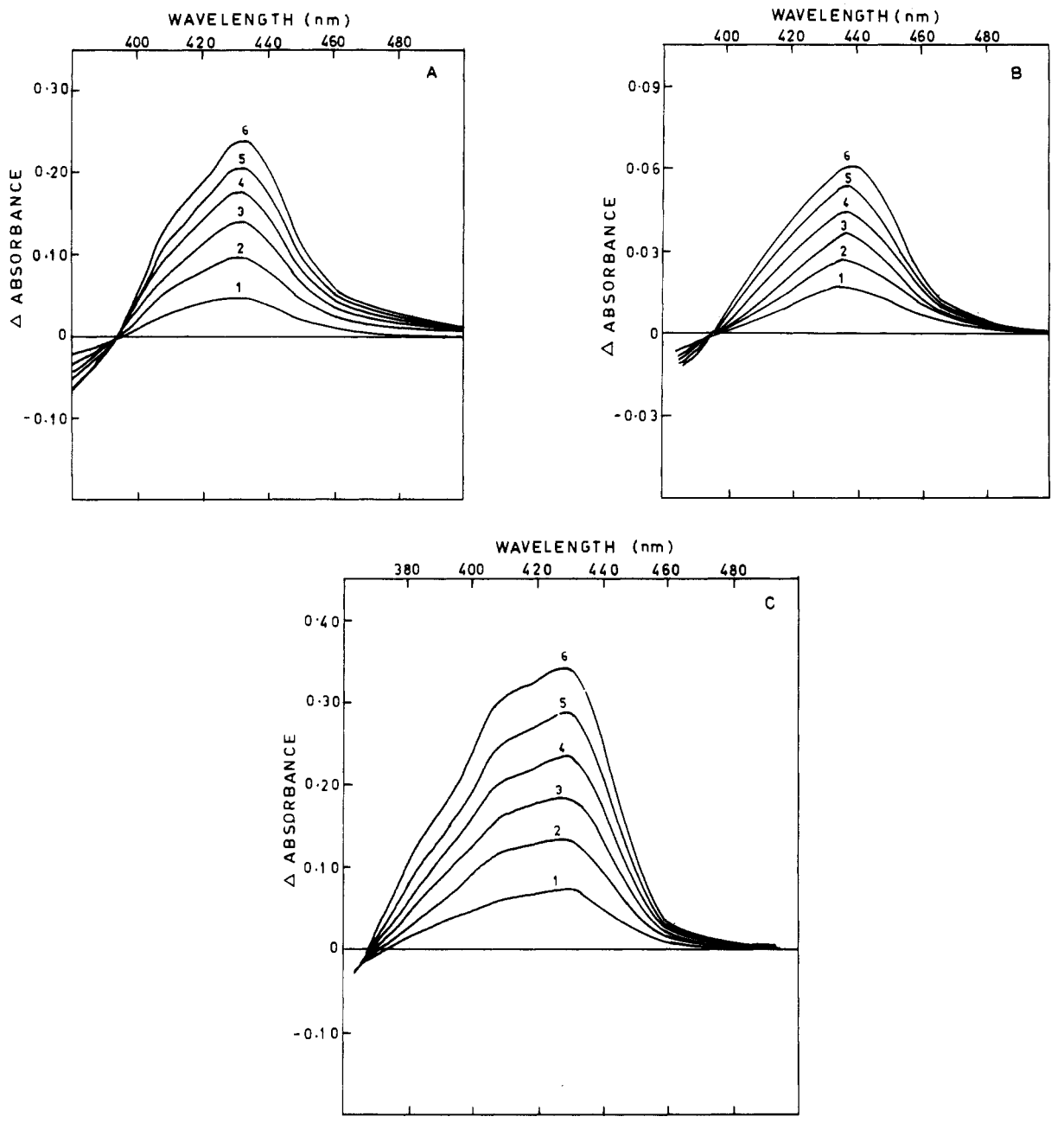


**Figure 3.** Effect of gossypol concentration on the difference spectra of (A) gossypin at 30 °C, (B) congossypin at 26 °C, and (C) glycinin at 26 °C. Gossypol concentrations: (1) $3 \times 10^{-5}$ M; (2) $6 \times 10^{-5}$ M; (3) $9 \times 10^{-5}$ M; (4) $12 \times 10^{-5}$ M; (5) $15 \times 10^{-5}$ M; (6) $18 \times 10^{-5}$ M.

of gossypol under these conditions. Stability of gossypol was tested at different concentrations of $Na_2SO_3$ by measuring its absorbance at 385 nm, and it was observed that gossypol was reasonably stable for nearly 11 h at 0.05 M $Na_2SO_3$. Thus, all the solutions contained 0.05 M $Na_2SO_3$ and measurements were completed within 11 h after the preparation of solutions.

**Interaction of Gossypol with Gossypin, Congossypin, and Glycinin.** Difference spectra of gossypol–gossypin, gossypol–congossypin, and gossypol–glycinin complexes, as a function of gossypol concentration, are shown in Figure 3. The spectra were characterized by difference absorption maxima at 430–432 nm for gossypin, 436–437 nm for congossypin, and 430 nm for glycinin. With an increase in the concentration of gossypol, $\Delta A$ values increased and the spectra exhibited an isobestic point at 390–393 nm for gossypin, 388–392 nm for congossypin, and 370–372 nm for glycinin. Therefore, $\Delta A$ at the appropriate wavelength maximum was used as a measure of complex formation to follow the reaction kinetics and binding stoichiometry.

**Reaction Kinetics.** Reaction kinetics of interaction of gossypol with gossypin, congossypin, and glycinin were followed at pH 9.0, 8.0, and 7.6, respectively (Figure 4). The $\Delta A$ values increased with time. The reaction proceeded at a faster rate initially, with a steep increase in $\Delta A$ up to 40 min for gossypin, 90 min for congossypin, and 180 min for glycinin, and it reached a constant value around 120 min for gossypin, 180 min for congossypin, and 600 min for glycinin. Thus, the reaction appeared to be complete in about 2 h with gossypin, 3 h with congossypin, and 10 h with glycinin. Therefore, in each case the mixture of gossypol and protein was incubated for the required interval of time, before the spectra were recorded.

The data of Figure 4 were analyzed to determine whether they fit a first-order reaction kinetic plot. The logarithmic plots are given in Figure 4C. The values of $\Delta A$ at $t = 0$ ($\Delta A_0$) and $\Delta A$ at infinity ($\Delta A_\infty$) were obtained

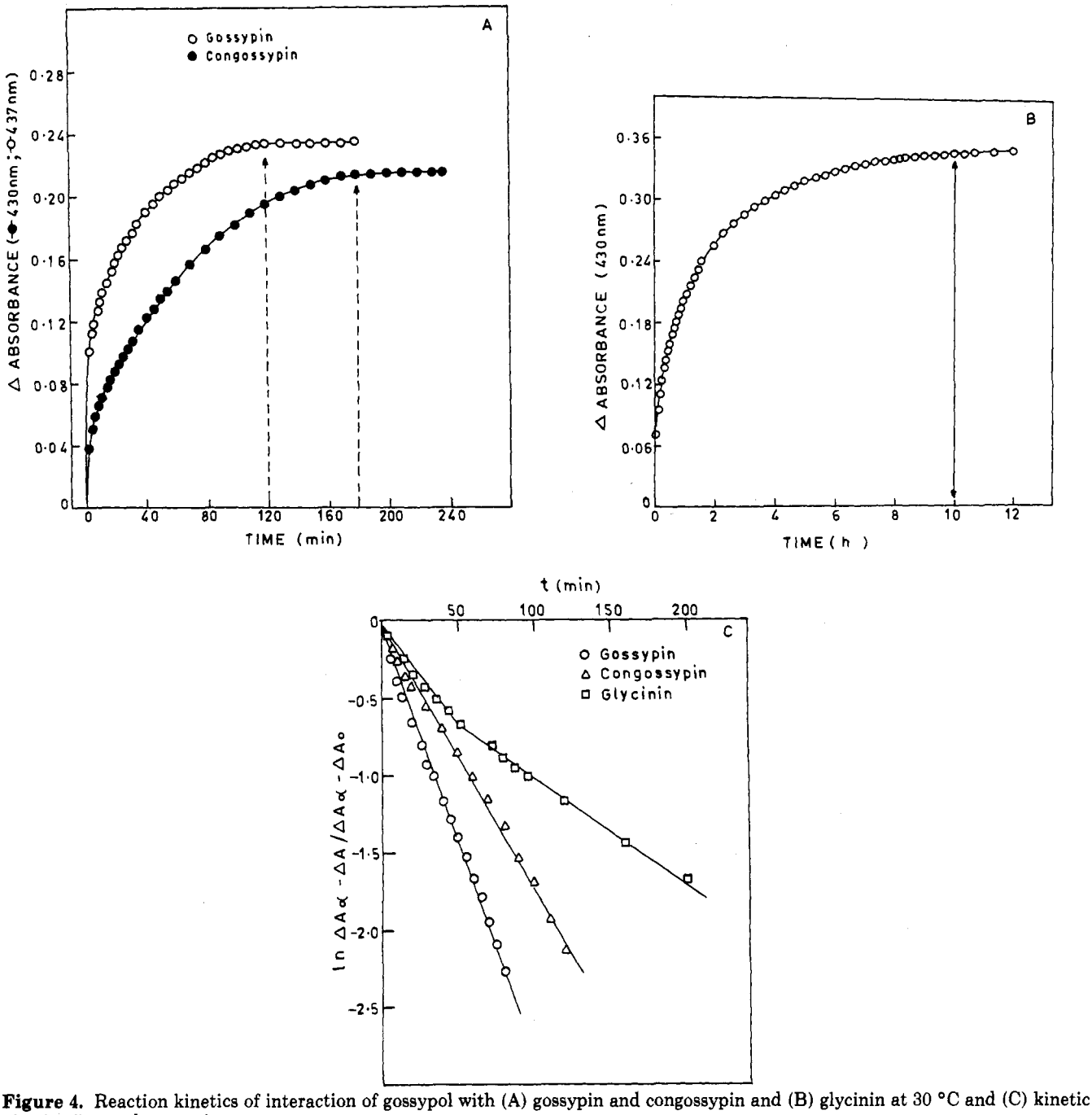


**Figure 4.** Reaction kinetics of interaction of gossypol with (A) gossypin and congossypin and (B) glycinin at 30 °C and (C) kinetic plot for first-order reaction.

by extrapolating the data of Figure 4A,B to $t = 0$ and from the plateau region, respectively. In the case of gossypin and congossypin the reaction followed first-order kinetics, yielding a single straight line in each case. However, with glycinin two straight lines were obtained indicating the reaction kinetics were complex. The reaction rates, calculated from the slopes, were $3.85 \times 10^{-2}$ min$^{-1}$ for gossypin and $1.67 \times 10^{-2}$ min$^{-1}$ for congossypin. For glycinin it was $1.4 \times 10^{-2}$ min$^{-1}$ initially and $6.6 \times 10^{-3}$ min$^{-1}$ at a later stage. Possibily glycinin undergoes some conformational change with time in the presence of gossypol.

**Binding Stoichiometry.** Binding stoichiometry of the interactions of gossypol with gossypin, congossypin, and glycinin is shown in Figure 5. The curves obtained were not symmetrical, and hence the maximum $\Delta A$ point was determined from the experimental curves directly, instead of from the intersection point of "extended tangents" or "limiting slopes" of the experimental curves. The latter method gives correct values only in systems involving the formation of a 1:1 complex (Gilbert, 1950). The binding stoichiometry or the number of binding sites ($n$) calculated from the ratio of mole fractions of gossypol and protein at the maximum $\Delta A$ point was 4.0 for both gossypin and congossypin and 5.0 for glycinin. Thus, gossypin and congossypin bind a maximum of 4 gossypol molecules/mol of protein, while glycinin binds 5. Therefore, for the purpose of calculating $C_f$ (eq 1), values of $n = 4$ for gossypin and congossypin and $n = 5$ for glycinin were used.

Binding stoichiometry of the proteins seems to be related to their subunit composition. Gossypin and congossypin are suggested to contain a large number of repetitive acidic and basic subunits bound together by disulfide bonds (Cherry and Leffler, 1984). Gossypin and congossypin contain 6–8 and 8 nonidentical subunits, respectively (Cherry and Leffler, 1984; Mohan Reddy, 1985). Glycinin consists of 12 subunits, 6 acidic and 6 basic subunits (Peng et al., 1984). It is suggested that these subunits alternate in the same layer and are held together

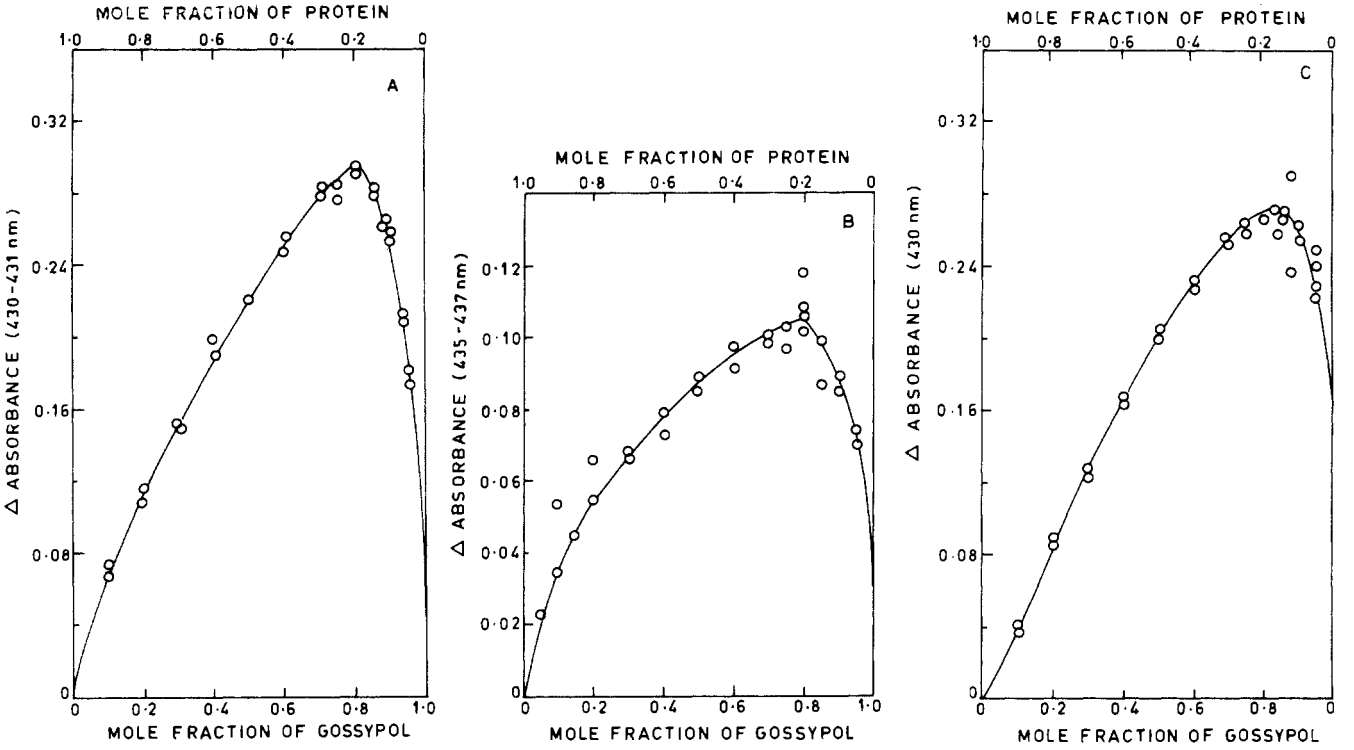


**Figure 5.** Binding stoichiometry of the interaction of gossypol with gossypin, congossypin, and glycinin. The Job plot: (A) gossypin in 0.05 M pyrophosphate–0.05 M $NaHCO_3$ buffer, pH 9.0, 30 °C; (B) congossypin in 0.1 M phosphate buffer, pH 8.0, 26 °C; (C) glycinin in 0.1 M phosphate buffer, pH 7.6 containing 0.1% 2-ME, 26 °C. All contain 0.05 M $Na_2SO_3$.

by hydrophobic and disulfide bonds. Therefore, binding stoichiometry or the number of binding sites of the proteins ($n = 4$ for gossypin and congossypin and $n = 5$ for glycinin) may suggest that gossypol binds preferentially to either acidic or basic subunits.

**Reversibility of Binding.** In order to determine the covalent or noncovalent nature of the binding and for proper thermodynamic analysis of the binding data, it was essential to determine whether the binding of gossypol to the proteins was reversible. These experiments were done at pH 9.0, 8.0, and 7.6 for gossypin, congossypin, and glycinin respectively.

Direct and reversed binding isotherms of gossypol to gossypin, congossypin, and glycinin, as plots of difference absorbance, $\Delta A$, as a function of gossypol concentration, $C$, are shown in Figure 6. In each case, the direct and reverse points fit the same curve within the limits of experimental error, indicating the reversible nature of binding.

The binding isotherms of gossypol to gossypin, congossypin, and glycinin as plots of $\Delta A$ against $C$ are shown in Figure 7A. The same data are presented in the form of double-reciprocal plots of $1/\Delta A$ against $1/C$ (Figure 7B) and mass–action plots of $\beta/(1-\beta)$ against $C_f$ (Figure 7C).

The $\Delta A$ values increased with an increase in gossypol concentration and were the highest for glycinin, followed by gossypin and congossypin. The plots of $1/\Delta A$ vs $1/C$ and $\beta/(1-\beta)$ vs $C_f$ were linear in all cases. Therefore, the intercept and slopes could be obtained by the method of least squares.

The maximal difference absorbance ($\Delta A_{max}$), intrinsic binding constant ($K$), and corresponding free energy change ($\Delta G$) for the binding of gossypol to gossypin, congossypin, and glycinin are given in Table I. The $K$ values with glycinin was considerably higher than that with gossypin or congossypin. Also the standard deviation in each case was of low magnitude, indicating the accuracy in the determination of $K$ values. $\Delta A_{max}$ was nearly the

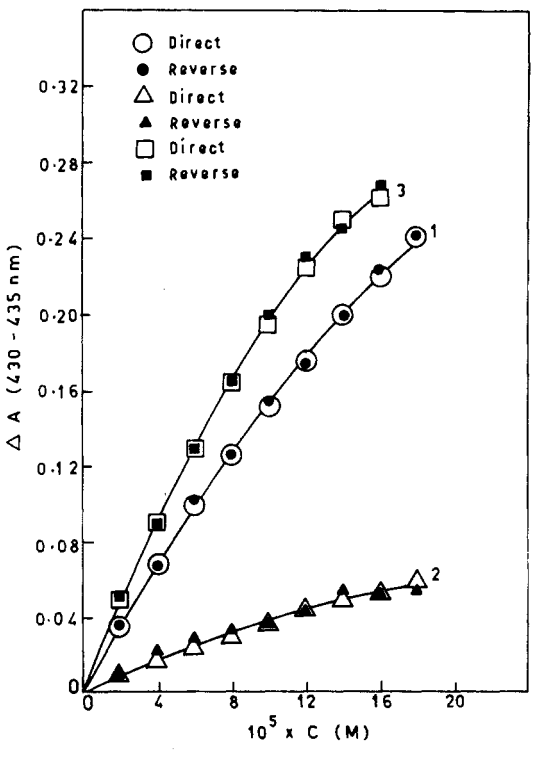


**Figure 6.** Reversibility of the interaction of gossypol with (1) gossypin, (2) congossypin, and (3) glycinin as plots of $\Delta A$ against $C$.

**Table I. Thermodynamic Constants and $\Delta A_{max}$ Values for the Binding of Gossypol to Gossypin, Congossypin, and Glycinin**

| protein (pH/temp, °C) | $\Delta A_{max}$ | $K$, $M^{-1}$ | $\Delta G$, kcal·$M^{-1}$ |
|---|---|---|---|
| gossypin (9.0/30) | 0.85 | $(2.53 \pm 0.04) \times 10^3$ | −4.7 |
| congossypin (8.0/26) | 0.19 | $(2.90 \pm 0.10) \times 10^3$ | −4.7 |
| glycinin (7.6/26) | 0.82 | $(4.17 \pm 0.08) \times 10^3$ | −4.9 |

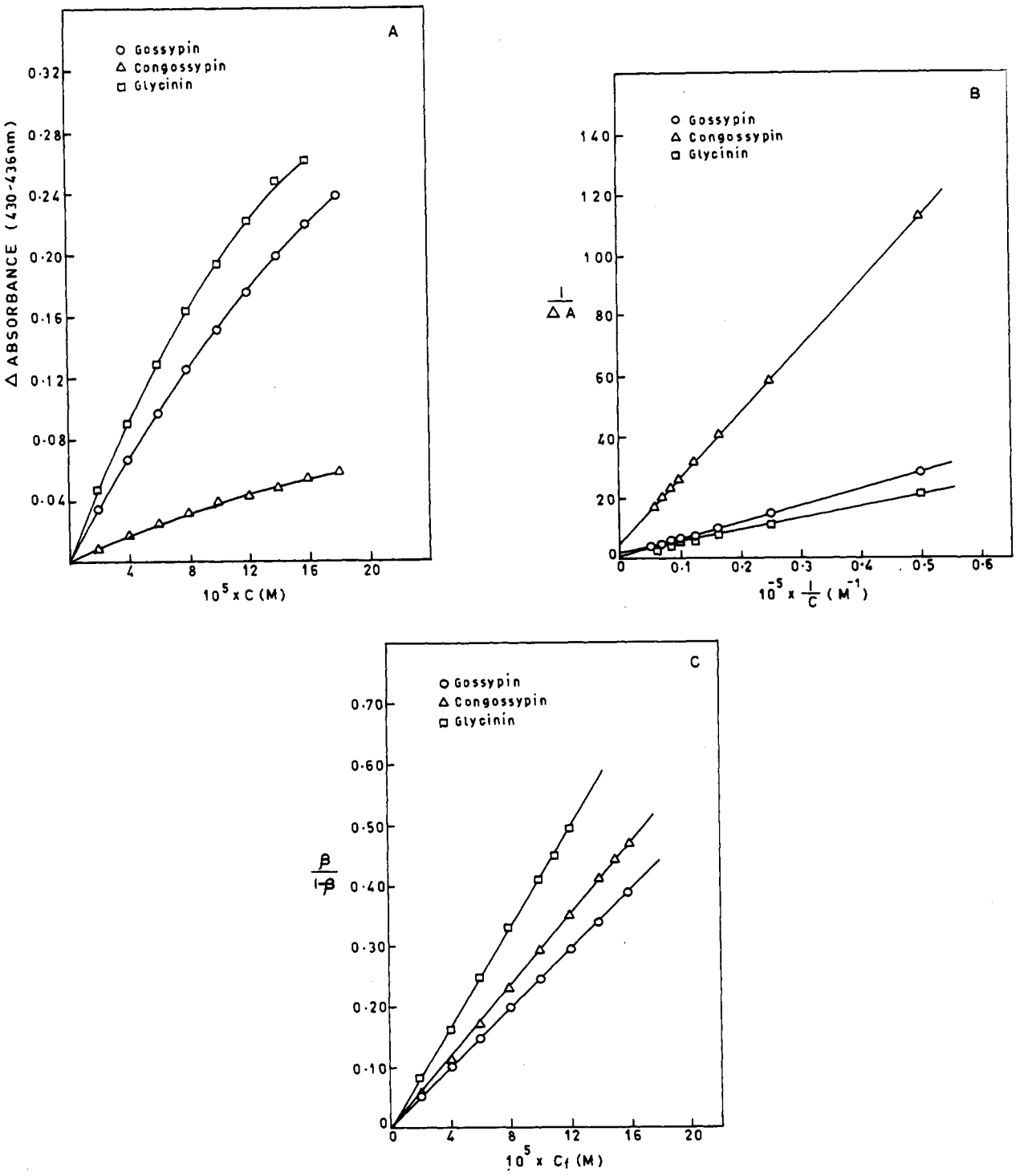


**Figure 7.** Interaction of gossypol with gossypin, congossypin, and glycinin: (A) plot of $\Delta A$ against $C$; (B) plot of $1/\Delta A$ against $1/C$; (C) mass-action plot of $\beta/(1-\beta)$ against $C_f$. Conditions: gossypin, pH 9.0 and 30 °C; congossypin, pH 8.0 and 26 °C; glycinin, pH 7.6 and 26 °C.

same for gossypin and glycinin. This may mean that gossypol is bound at the same type of sites on these two proteins and with the same geometry (Appu Rao and Cann, 1981). However, the value was much lower with congossypin. Since the number of binding sites on gossypin and congossypin is the same ($n = 4$), this difference in $\Delta A_{max}$ may be due to a difference in the geometry of binding.

The above results suggest that the binding affinity of gossypin, congossypin, and glycinin for gossypol varied as reflected in reaction kinetics (Figure 4), binding stoichiometry (Figure 5), and binding parameters (Table I) although these proteins resemble one other in amino acid composition, conformation, and other physicochemical characteristics (Catsimpoolas et al., 1971; Mohan Reddy, 1985). Reaction of gossypol with gossypin and congossypin was complete much faster (2–3 h) than with glycinin, which took 10 h. Gossypin and congossypin have equal numbers of binding sites ($n = 4$), whereas with glycinin the number was 5. It is not clear whether this is due to a change in the nature of binding sites or whether it reflects differences in the geometry of binding sites. Variation in $\Delta A_{max}$ values (Table I) and slopes of double-reciprocal plots (Figure 7) superficially would suggest differences in geometry of binding sites and binding affinities of these proteins to gossypol.

The reversible nature of binding of gossypol to these proteins strongly suggests that only noncovalent interactions are involved. The low binding constants (Table I) also suggest that the binding is of a weak type and involves noncovalent interactions. However, covalent interaction between gossypol and cottonseed proteins was observed by earlier workers (Clark, 1928; Markman and Rzhekhin, 1965; Damaty and Hudson, 1979), where more drastic conditions such as high temperatures (and pressures) were used. Also estimation of "available" lysine was used to follow the interaction. We are unable to commet on the sensitivity of this method. Possibly the drastic conditions facilitate covalent interaction.

The higher binding constant ($4.17 \times 10^3$ $M^{-1}$) in the case of glycinin indicates that the affinity of the protein for gossypol is greater than that of gossypin and congossypin, whose binding constants are almost the same. This may not be due to any gossypol bound to cottonseed proteins in situ.

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**Registry No.** Gossypol, 303-45-7.

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# Characterization of Sweet Potato Stillage and Recovery of Stillage Solubles by Ultrafiltration and Reverse Osmosis

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Sweet potatoes were fermented to ethanol. After ethanol was distilled, residual stillage was separated into filter cake, centrifuged solids, and stillage solubles. The protein in filter cake was much less soluble than that in sweet potato. Of the nitrogen in stillage solubles, 91% passed through a 10 000 molecular weight cutoff membrane. Permeate from stillage solubles processed by combined ultrafiltration and reverse osmosis had much lower nitrogen, solids, and ash contents than that of stillage solubles. Thus, ultrafiltration combined with reverse osmosis can be used to recover sweet potato stillage solubles for potential food or feed uses while providing a permeate that can be reused for water or safely discarded.

Sweet potato (*Ipomoea batatas*) is one of the most promising crops for energy production; Jones et al. (1983) estimated yields of 570–760 and 712–1140 gal of ethanol/acre for Jewel and HiDry sweet potatoes, respectively. Matsuoka et al. (1982) reported alcohol fermentation of raw sweet potato in a one-step process. Chua et al. (1984) used no heating or low-temperature heating to convert sweet potato starch for ethanol fermentation. Wu and Bagby (1987) reported effects of commercial pectinases on viscosities of sweet potato slurries before fermentation and on maximum ethanol concentrations and presented proximate and amino acid compositions of fermentation products from sweet potatoes with normal (18–24%), relatively high (27–30%), and very high (35% and up) dry-matter contents.

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