Aqueous Solvents for Extracting Glanded Cottonseed Protein without Gland Rupture[1]

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ABSTRACT

The presence of pigment glands has thwarted attempts to extract edible cottonseed protein aqueously from glanded seeds or gland-rich meals, probably because of the widely held belief that glands rupture on contact with aqueous media. We found several aqueous salt solutions in which glands did not rupture. Glands remained intact in saturated (2 *m*) sodium sulfate, but not in saturated 2 *m* or 4 *m* solutions of sulfates, chlorides, and nitrates of other Group IA elements as well as sodium chloride and sodium nitrate. Glands also remained intact in saturated solutions of sulfates of aluminum, ammonium, cadmium, copper, magnesium, nickel, and zinc, and chlorides of calcium, iron, and magnesium. Some of these solutions were diluted to $< 50\%$ saturation before glands started rupturing. Cottonseed protein in the liquid cyclone underflow fraction (gland-rich fraction) was soluble in sodium sulfate and magnesium sulfate, but not in calcium chloride or sodium phosphate. Its solubility in sodium sulfate was investigated further with the following results: Alkalinity of sodium sulfate solution had no effect on solubility; ratio of solid to solvent had no effect in the range of 1:3.5-1:60 (wt:vol); 80% saturated sodium sulfate was optimal for solubility without gland rupture; the period of contact of meal and solvent had no effect on protein solubility in the range of a few minutes to 2 hr. These results indicate that the extraction of cottonseed protein with aqueous solvents in the presence of pigment glands appears technically feasible.

INTRODUCTION

Cottonseed pigment glands are spherical structures about 100-400 μ in diameter that contain gossypol and gossypol-related pigments. Each gland is circumscribed by a layer of tangentially flattened cells, and within the matrix of each gland are spherules about 0.1-1.5 μ diameter that contain the pigments (1). When finely comminuted glanded cottonseed meats are processed with the liquid cyclone (LCP) (2-4), the underflow fraction contains virtually all of the glands from the meats and nearly half of the protein. The presence of pigment glands in this underflow fraction and in other gland-rich meals renders the meals unsuitable as sources of edible cottonseed protein. Currently, edible protein is prepared by aqueous processing of gland-free meals only (5-8). Similar attempts with gland-rich meals have probably been thwarted by the widely held belief that glands are labile and readily rupture in aqueous media (9). In this communication, we describe several aqueous solutions in which pigment glands do not rupture. Furthermore, we found that cottonseed protein is readily soluble in at least some of these nondisrupting solutions, indicating that cottonseed protein may be isolated from gland-rich meals with aqueous solutions.

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EXPERIMENTAL PROCEDURES

The pigment gland-rich fraction (underflow) from LCP fractionations of Texas High Plains cottonseed was obtained from H.K. Gardner, Jr., of this laboratory. Glands were isolated from the underflow fraction by flotation (9). Solvents used for density adjustments were mixtures of hexane and carbon tetrachloride, or hexane and Freon 113. Isolated glands were rinsed in hexane, dried, and stored over silica gel in vacuo.

Rupture of pigment glands was determined by suspending glands with gentle stirring in solutions contained in depression-well slides, placing a cover slip over the well, and observing the glands continually with a low-power binocular light microscope for at least 2 hr. Gland rupture was sometimes difficult to judge when it occurred slowly. Often the glands burst immediately with rapid extravasation of contents. Sometimes, however, glands slowly ruptured over several hours, with slow streaming of contents. Thus we decided that if readily noticeable rupture occurred after 2 hr of contact, the glands would be considered ruptured. If very little rupture, or if only small amounts of streaming occurred, "rupture" would still be our decision, but a modifier such as "extremely slow" or "very slow" would be added. In these cases, many of the glands would still be intact. If no rupture occurred after 2 hr, the solution was considered nondisruptive.

The solubility of cottonseed protein in aqueous solutions that did not rupture pigment glands was examined, with the LCP underflow fraction as the source of protein. Generally, 1 g of underflow was dispersed into 20 ml or 40 ml of solvent and the mixture was incubated for 2 hr with continuous shaking. In other experiments where noted, the ratio of underflow fraction to solvent and the period of incubation differed from the above. The mixture was centrifuged at 27,000 x *g* for 10 min, and the resultant supernatant was filtered with a fritted glass Buchner funnel of medium porosity to ensure removal of suspended matter. However, filtration was found unnecessary, because the nitrogen content of the supernatant with or without filtration was essentially identical. Nitrogen content was measured by Kjeldahl analysis.

RESULTS

To ascertain whether pigment glands rupture in aqueous salt solutions, glands were suspended in saturated solutions and observed with the light microscope. Table I shows results with sulfates, chlorides, and nitrates of Group IA elements (excluding francium). Gland rupture was extremely slow in saturated lithium sulfate, beginning gradually after contact for over 1 hr; however, the only solution in which glands remained intact was saturated sodium sulfate. Comparisons of molalities of the saturated solutions indicated that ionic strength was not a factor for intactness of glands. Since saturated sodium sulfate is about 2 *m* at room temperature (ca. 25 C), 2 *m* concentrations of other salt solutions were also tested where possible. Concentrations of 4 *m* were included to account for the fact that, since sulfates are divalent whereas chlorides and nitrates are monovalent, equimolal solutions of sulfates would provide twice as many cations as would those from chlorides and

TABLE I

Effects of Saturated Solutions of Acids and Salts of Group IA Elements on Pigment Glands

Compound	Molality at saturation[a]	Gland rupture	Compound	Molality at saturation[a]	Gland rupture	Compound	Molality at saturation[a]	Gland rupture
H_2SO_4	18.0[b]	yes	HCl	11.7[b]	yes	HNO_3	15.6[b]	yes
Li_2SO_4	3.0	yes[c]	LiCl	19.9	yes	$LiNO_3$	13.0	yes
Na_2SO_4	2.9	no	NaCl	6.2	yes	$NaNO_3$	11.3	yes
K_2SO_4	0.7	yes	KCl	5.0	yes	KNO_3	4.5	yes
Rb_2SO_4	2.0	yes	RbCl	8.1	yes	$RbNO_3$	5.5	yes
Cs_2SO_4	5.1	yes	CsCl	11.7	yes	$CsNO_3$	1.7	yes

[a]At 30 C.
[b]Molarity of concentrated reagent.
[c]Extremely slow rupture.

TABLE II

Effects of Saturated Solutions on Pigment Glands

Solution	Molality at saturation[a]	Glands		Solution	Molality at saturation[a]	Glands	
		Rupture	Intact			Rupture	Intact
$Al_2(SO_4)_3$	1.2		x	$AlCl_3$	5.2[b]	x[c]	
$CaSO_4$	0.1	x		$CaCl_2$	9.2		x
$CdSO_4$	3.7[d]		x	$CdCl_2$	7.2	x	
$CuSO_4$	1.6		x	$CuCl_2$	6.0	x	
$Fe_2(SO_4)_3$	-	x		$FeCl_3$	5.6[d]		x
$MgSO_4$	3.8		x	$MgCl_2$	5.7[d]		x
$MnSO_4$	4.5	x[c]		$MnCl_2$	6.4	x	
$(NH_4)_2SO_4$	5.9		x	NH_4Cl	7.7	x	
$NiSO_4$	2.7		x	$NiCl_2$	5.3	x[e]	
$ZnSO_4$	3.4[d]		x	$ZnCl_2$	31.7[f]	x[c]	
$Al(NO_3)_3$	1.7[f]	x		NaH_2PO_4	8.9		x
$Ca(H_2PO_4)_2$	0.1	x		Na_2HPO_4	1.5	x	
$Ca(NO_3)_2$	9.3	x		NaI	12.7	x	
$Mg(C1O_4)_2$	4.5[f]	x		$NaNO_2$	10.8	x	
Na_3citrate	2.6	x[c]		$SbCl_3$	46.8	x	
Na_4EDTA[g]	2.7	x		SDS[h]	0.4	x[e]	
Na_2CO_3	4.8	x[c]		Sucrose	6.4	x	
$NaHCO_3$	1.3	x		Urea	19.9[f]	x	

[a]At 30 C, unless stated otherwise.
[b]Molality of saturated solution at 15 C.
[c]Extremely slow rupture.
[d]Molality of saturated solution at 20 C.
[e]Slow rupture.
[f]Molality of saturated solution at 25 C.
[g]Sodium ethylenediaminetetraacetic acid.
[h]Sodium dodecyl sulfate.

nitrates. In certain cases, e.g., cesium nitrate, 2 *m* or 4 *m* concentrations could not be obtained (see molalities at saturation in Table I). We found that glandular rupture also occurred in 2 *m* and 4 *m* concentrations.

Table II shows results of testing saturated inorganic salt solutions of other than Group IA elements for their ability to maintain glandular structure. Glands often did not rupture in solutions of metallic sulfates; other than that observation, no factor appeared to be common to nondisrupting salt solutions. For instance, the wide ranges of ionic strengths and molalities that exist among the nondisruptive salts include values for these parameters of the disruptive salts. Also, surface tensions of the salts in which glands remained intact are generally higher than those of salts in which glands ruptured, but too many exceptions occur for surface tension to be a factor. Values for activity coefficients of the salt solutions also appear to be unrelated to response of glands to the solutions.

Table II also shows responses of glands to some saturated solutions of organic compounds. Of particular interest is the rupture of glands in saturated sucrose solution as well as in isotonic sucrose, which indicates that osmotic properties of the solutions were not involved.

The level to which the nondisruptive salt solutions could be diluted yet still maintain glandular integrity was examined. Gland ruptures that occurred in diluted solutions of saturated salts in which they had not ruptured when the concentration was higher were of three types: I. slow rupture, occurring when solution was diluted to ca. 40% saturation, and rapid rupture, occurring at ca. 10% saturation; II. slow rupture at 80% saturation and rapid rupture at ca. 30% saturation; III. slow rupture at ca. 80% saturation, rapid at 55%, back to slow at ca. 10%, very slow at 5%, then rapid at a few percent saturation. Salts yielding the three responses were: I. aluminum sulfate, magnesium

TABLE III

Dissolution of Cottonseed Protein in Nondisruptive Solutions

Salt solution	Protein dissolved (%)
90% Saturated Na_2SO_4	51
80% Saturated PO_4^{3a}	4
90% Saturated $CaCl_2$, pH 6.9	9
90% Saturated $CaCl_2$, pH 2.2	6
100% Saturated $MgSO_4$	51
60% Saturated $MgSO_4$	67

[a]Sodium phosphate solutions when neutral, strongly alkaline (containing 1.9 N NaOH), or strongly acidic (containing 1.2 N HCl) yielded the same values.

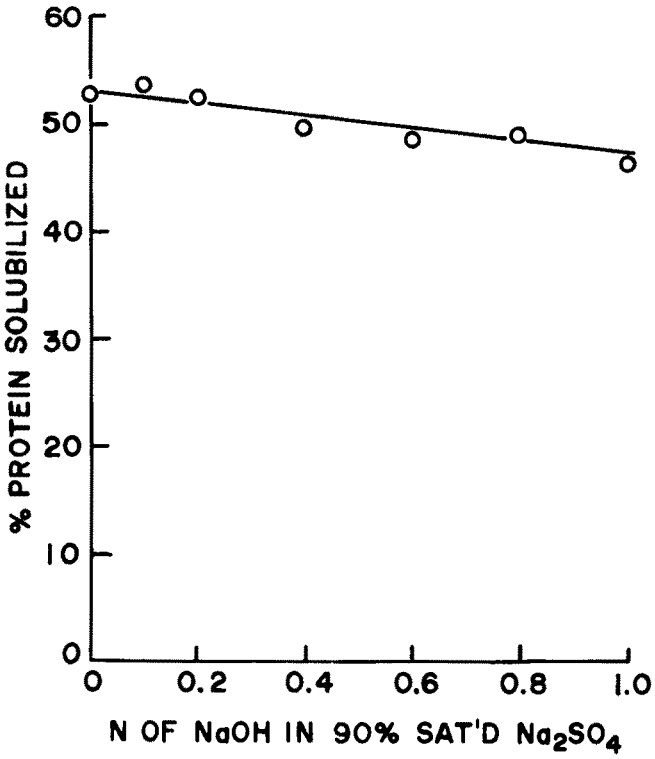

FIG. 1. Effect of alkalinity on the solubility of cottonseed protein in 90% saturated sodium sulfate.

sulfate, sodium dihydrogen phosphate, ammonium sulfate, zinc sulfate; II. cadmium sulfate, copper sulfate, sodium sulfate, nickel sulfate; III. calcium chloride.

Since these results established that pigment glands do not rupture in certain salt solutions, the solubility of cottonseed protein in the nonrupturing solutions was determined. The only nonrupturing solutions tested were those in Tables I and II that were both innocuous and relatively inexpensive. Table III shows solubilities of cottonseed protein in four salt solutions at differing saturation levels and pH values. Solubilities in calcium chloride and sodium phosphate were slight; but in sodium sulfate and magnesium sulfate they were sufficient to warrant further investigations.

The solubility of cottonseed protein as a function of pH and of cottonseed and other seed globulins in alkaline solutions is well known (10-12). Thus, the effect of alkalinity on the solubility of cottonseed protein in 90% saturated sodium sulfate (containing from 0.01 to 0.10 N NaOH) was of interest. Results in Figure 1 indicate that solubility decreased slightly as alkalinity increased. Thus sodium sulfate, which is ca. pH 4, need not be alkaline for maximal solubility of cottonseed protein; this is fortunate, because

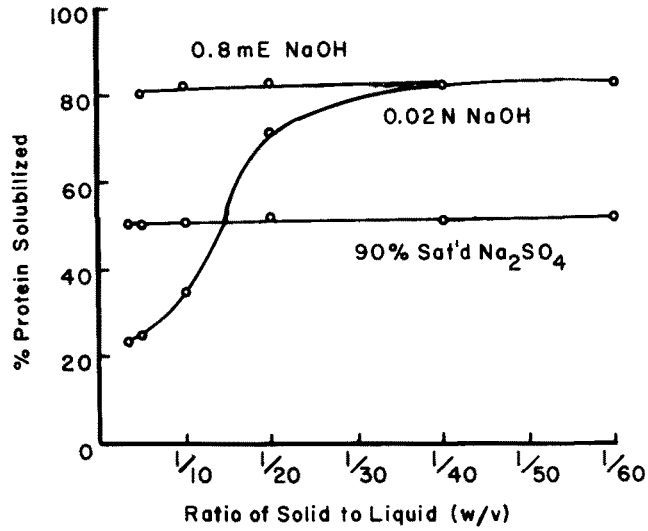

FIG. 2. Effect of solid-to-liquid ratio on the solubility of cottonseed protein in 0.8 mEq of NaOH, 0.02 N NaOH, and 90% saturated sodium sulfate.

pigment glands rupture in alkaline solutions.

The ratios of the amount of solid (cottonseed meal) to the volume of solvent were examined to determine the amount of protein solubilized. Solvents tested were 90% saturated sodium sulfate, 0.02 N NaOH (standard protein solvent), and a solution that contained 0.8 mEq of NaOH irrespective of its volume. Results in Figure 2 show that ratios from 1:5 to 1:60 (wt:vol) affected the amount of protein solubilized slightly (from 81% to 85%) when the NaOH mEq value was held constant. In contrast, ratios in the range of 1:3.5-1:40 had a profound effect on the amount solubilized (21-83%, respectively) when 0.02 N NaOH was the solvent. Unfortunately, this standard protein solvent readily disrupts pigment glands. The ratios were not a factor for solubility in 90% saturated sodium sulfate within the limits shown in Figure 2 (1:3.5-1:60). However, handling a high ratio of solid to solvent is impractical.

The solubility of cottonseed protein in nondisrupting sodium sulfate as a function of salt concentration was investigated. Results in Figure 3 indicate that solubility of protein without rupture of pigment glands was maximal with ca. 80% saturated sodium sulfate. At lower saturation, pigment glands ruptured; above it, protein might have started to salt out.

Solubility of cottonseed protein in sodium sulfate as related to contact time of meal with solvent was also determined. When 80% saturated sodium sulfate was mixed with meal for a few, 30, 60, 90, or 120 min, the percent of protein dissolved was 52, 54, 55, 55, and 55, respectively (Fig. 4). Thus, a brief moment of contact resulted in extraction of protein; extended periods of incubation did not increase dissolution.

DISCUSSION

The difficulty of grading the response of glands to a given salt solution as rupturing or remaining intact was noted in the Experimental Procedure section. The decision to grade the response as "rupture," with modifiers such as "extremely slow" or "very slow" when only a relatively few number of glands ruptured or when rupture and streaming occurred slowly during the course of 2 hr, was based on practical implications. If gland-rich meals or flours were to be used as sources for protein extraction with nonrupturing salt solutions, then maintenance of glandular integrity (with maximum solubility of protein) would be a

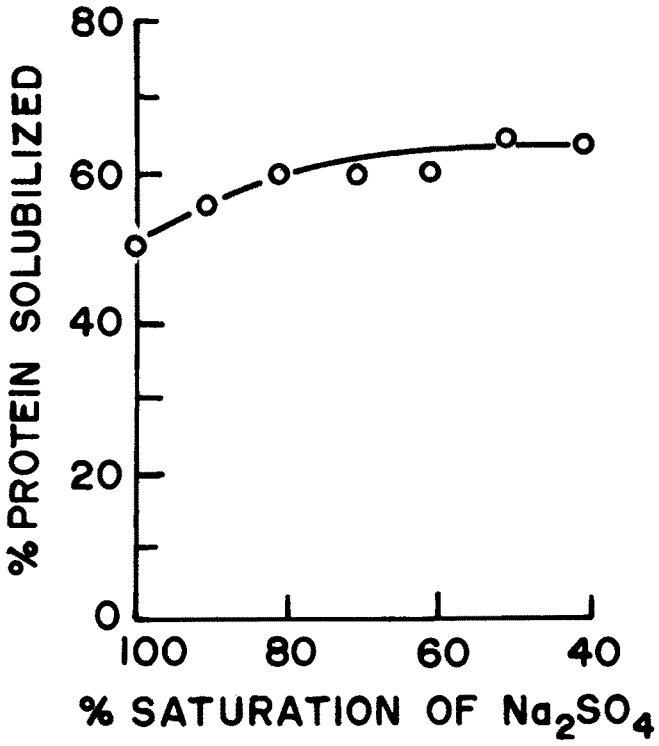

FIG. 3. Effect of concentration of sodium sulfate on solubility of cottonseed protein.

foremost consideration. Several nondisruptive solutions have been identified (Tables I and II). If a small amount of gland rupture is deemed not serious, then slow-rupturing solutions identified in the tables could be used. In this regard it is of interest that we have observed ruptured pigment glands in the relatively gossypol-free overflow fraction of the LCP (unpublished results).

The reason that glands ruptured in certain solutions but remained intact in others is unknown. Several attempts to explain this phenomenon, based on properties of the solutions, were unworkable. No correlation was found between response of glands and molalities, ionic strengths, osmotic strengths, pH values, surface tensions, or activity coefficients of the solutions. A seemingly unrelated observation, however, might have a bearing on these results. Many seeds have mucilaginous coats that rapidly expand on contact with water (13). We found that the coat of such a seed, *Capsella bursa-pastoris*, did not expand upon contact with the nondisruptive solutions that we tested (unpublished results). This phenomenon might be related to gland response, but further experimentation is needed to establish the relationship.

Cottonseed protein was soluble in certain nondisruptive solutions but not in others (Table III). The insolubility in sodium phosphate and calcium chloride might be useful if extraction of nonprotein matter by these solutions without gland rupture is desirable before extraction of protein with other, nondisruptive solutions.

The solubility of cottonseed protein, based on nitrogen analyses, in nondisruptive sodium sulfate was examined in detail. Sodium sulfate did not extract more than 60% of the nitrogen in the meal, whereas the standard solvent, 0.02 N NaOH, dissolved 80% of the nitrogen (Figs. 1-4). Identification of the nitrogenous matter, perhaps a species of protein, insoluble in sodium sulfate remains for future investigation. It is of interest that certain types of nucleic acids are insoluble in salt solutions of high ionic strength.

The ineffectiveness of alkalinity to enhance the solubility of cottonseed protein in sodium sulfate (Fig. 1) appears to contradict earlier studies that showed cottonseed protein to be more soluble in alkaline solutions of low ionic strength than in corresponding neutral solutions (10-12). Perhaps the protein species that was rendered soluble by alkali was already in solution in sodium sulfate, so that there was no enhancement by base.

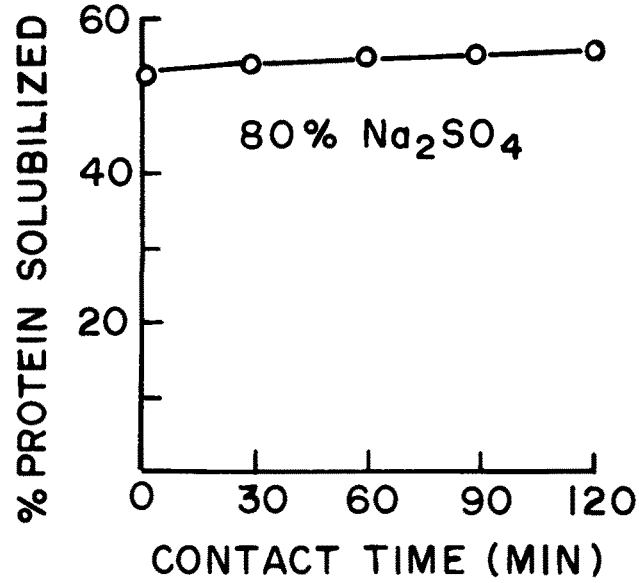

FIG. 4. Effect of contact time on solubility of cottonseed protein in 80% saturated sodium sulfate.

The amount of solvent needed for complete extraction of protein from a given amount of meal is limited only by processing practicability. For instance, 3.5 ml of 90% saturated sodium sulfate extracted as much protein from 1 g of meal as did 60 ml of the solvent (Fig. 2). However, the mixture of 1 g per 3.5 ml is viscous, and pastes result when lesser amounts of solvent are used.

The optimal concentration of sodium sulfate for maximum extraction of cottonseed protein with minimum gland rupture was near 80% saturation (Fig. 3); below that concentration, pigment glands ruptured. The decrease in extractability, however, does not appear to preclude use of higher concentrations, particularly because glands began to rupture near 80% saturation.

The period of contact of solvent and meal required for thorough extraction of protein is also limited only by processing practicability. The amount of protein extracted in a few minutes' contact was as much as was extracted in 2 hr (Fig. 4). This observation, particularly in view of the solid-to-liquid ratio requirement (Fig. 2), indicates that a rapid continuous-flow method such as percolation should suffice for thorough extraction of protein.

In conclusion, we discovered that pigment glands did not rupture or ruptured very slowly in several aqueous solutions, in two of which cottonseed protein was readily extractable.

Separation of protein, glands, and oil from full-fat cottonseed, separation of protein and glands from solvent-extracted cottonseed, and extraction of protein from the gland-rich underflow of the LCP might be technically feasible with these solutions.

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