RECENT DEVELOPMENTS IN THE PRODUCTION OF SUNFLOWER PROTEIN ISOLATES

By

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There is an increasing recognition of the potentiality of oilseeds to bridge the protein gap in the diets of much of the world's population. This has stimulated the attention and interest of many food scientists and food industries around the world. Although a major proportion of the food product development has centered on products derived from soybeans, reason dictates that the potential for food use of all the major oilseeds be carefully investigated.

Presently, sunflower seed is the fourth largest source of oilseed protein in the world (Table 1) (1). It is estimated that in 1969 about 1,750 million kg of protein was produced as sunflower seed. Russia produced about 65% of the crop and Europe and Argentina accounted for most of the rest (2). However, since the development of the high oil varieties there is a rising interest in sunflower seed protein as a potential crop in many other areas of the world, including the United States. There is also interest in sunflower seed protein as a potential source of food.

Biological studies reviewed by Smith (3) and by Clandinin (4) show that sunflower protein is highly digestible (90%) and possesses a high biological value (60%). These studies show that high quality sunflower protein is superior to most vegetable proteins (equal to soybean protein) in terms of digestibility and is quite comparable in biological value. Sunflower flour, because of its high protein content and high digestibility, is considered to be a suitable food for use in infant nutrition in addition to its suitability as feed for livestock and poultry (4). A search of the literature, however, reveals that a systematic and comprehensive study of conditions affecting the extraction of sunflower proteins is lacking. One of the advantages of sunflower seed protein in addition to its good quality, is that, to date at least, no antibiological factors such as trypsin inhibitors or compounds similar to gossypol have been reported. However, it is not without problems. A major problem in making a protein isolate from sunflower seed meal by the conventional method of extracting at alkaline pH followed by precipitation at the isoelectric pH is the development of a dark green color. This is believed to result from the oxidation of chlorogenic acid, a tannin-like compound which is ubiquitous in the plant kingdom (5) and is present in appreciable amounts in the sunflower seed (6). Even the isolates obtained by extracting the protein with neutral salt solution are brownish in color. These colors would certainly limit the use of sunflower protein isolates in the food industry.

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Attempts by Smith et al. (7) to remove chlorogenic acid from sunflower meal by solvent extraction severely denatured the protein. One method (8) that has been reported to remove chlorogenic acid without appreciable denaturation of the protein is rather involved and does not appear to be commercially feasible. The present work was undertaken to develop a method for preparing a sunflower seed protein isolate having an acceptable color by a process having commercial potential and to investigate the effect of several variables on the extraction of protein from undenatured, defatted sunflower seed meal.

Experimental Procedures

Hexane extracted meal was prepared under mild conditions from decorticated and flaked seeds of the Armavirec variety. The meal was extracted with 0.25% aqueous sodium sulfite at a pH of 10.5. The pH was adjusted during the extraction with 0.5 N NaOH. The extraction was carried out at room temperature for one hour with constant stirring using a meal to water ratio of 1:10 (w/v). The extract was filtered by squeezing through cheese cloth and the filtrate was centrifuged for 20 minutes at 2000 xg. The clear extract was adjusted to pH 5 with 0.5 N HCl. About 80% of the extracted protein precipitated and was separated by centrifugation. One portion of the precipitate was washed twice with distilled water and then freeze-dried. The other portion was extracted twice for a brief period of time with 50% aqueous isopropylalchol (IPA), once with distilled water, and then freeze-dried.

A pH-nitrogen solubility profile on the isolates was obtained as follows: One gram samples of the isolates were extracted with 50 ml of water each over a pH range from 2 to 10.5. The pH was adjusted during the extractions with 0.5 N NaOH or 0.5 N HCl. Clear extracts were obtained by centrifugation for 20 minutes at 4600 xg. A 25 ml aliquot from each extract was taken to determine nitrogen by the Kjeldahl method. The results are reported as the percentage of total nitrogen extracted.

The amino acid composition of the meal and the two isolates was determined by the method of Spackman et al. (9) using a Beckman Model 120C analyzer.

In the studies on the effect of variables on the extractability of sunflower seed protein, all protein solubility tests were carried out at room temperature, unless otherwise specified, using a meal-to-solvent ratio of 1:20 (w/v). In the solubility experiments, the desired pH was obtained by the careful addition of either 0.5 N HCl or 0.5 N NaOH. Any increases in volume as a result of pH-adjustment were accounted for in final calculations. The extracts were centrifuged for 30 minutes at 4600 xg and nitrogen was determined on the resulting supernatant solution by the Kjeldahl method.

Results and Discussion

The isolate obtained using traditional extraction procedures was very green. That prepared by extraction with alkaline sodium sulfite and water washed was much lighter in color, being a light gray. That which was washed with 50% IPA was nearly white. Aqueous ethanol (50%) appeared to be more effective in color removal than is aqueous IPA. Higher concentrations of IPA and ethanol induce denaturation of the

protein. Further investigations are in progress with other reducing agents and other organic extractants to evolve the most economical process.

It is believedthat the use of alkaline sodium sulfite for extraction probably prevents the accumulation of quinones, possibly by reducing them as soon as formed, rather than preventing the oxidation of chlorogenic acid *per se*. Smith *et al*. (7) have reported that other reducing agents such as dithionite and ascorbate also prevent the accumulation of quinones. It has been demonstrated that colorless isolates can be produced by alkaline extraction using 0.25% sodium sulfite, potassium meta bisulfite, or ascorbic acid followed by two washings of the isoelectric precipitate with 50% IPA. Evidently a reducing agent is necessary in the extraction because chlorogenic acid is readily oxidized to quinone, both non-enzymatically by oxygen at alkaline pH and enzymatically in the vicinity of neutral pH by the enzyme polyphenol oxidase (5). Quinone, once formed, readily enter into covalent linkages with various groups on protein molecules, resulting in chromophoric products.

The color reducing properties of the aqueous ethanol and IPA probably are due to their ability to break hydrogen bonds. Polyphenols are reported (10) to combine with proteins at acidic pH's through unusually strong hydrogen bonds. Thus, stronger H-bond breaking compounds in which chlorogenic acid is soluble should be more effective in its removal. Indeed, this has been found to be the case with dioxane. However, such compounds may, as does dioxane, denature the protein. The challenge is to find the compound that most effectively eliminates the residual chromophores without damage to the protein. The isolate obtained by the method described, although nearly white, still contains compound(s) which impart a tan to brown color to the protein solution at progressively more alkaline pH's. However, the color does not develop at pH's below 7.5.

A comparison of the pH-nitrogen solubility profile (Figure 1) of the isolate obtained by IPA extraction with that of sunflower meal shows that, insofar as solubility may be considered a criterion, very little or no denaturation of the protein had occurred. The lower amount of extractable nitrogen in the isolate over the pH range of 3-7 is due to the loss of water soluble proteins and other nitrogenous compounds in the preparation of the isolate. The profile of the isolate prepared without IPA extraction shows greater solubility at various pH's than the other isolate or the meal. This may be due to sulfitolysis of disulfide bonds resulting in more soluble protein-thiosulfate. The solubility profiles of isolates prepared with 0.1% reducing agents followed by 50% IPA washings show little change in the solubility properties of the protein when compared to the profiles obtained with 0.25% reducing agents. If anything, they tend to be slightly more soluble.

Amino acid analyses (Table II) show that there is a decrease in the cysteic acid value in the isolates, while lysine, tyrosine and phenylalanine are increased. In the IPA extracted isolate, proline is somewhat lower. These changes may be due to the loss of water soluble proteins not precipitated by acid, and perhaps to some loss of the prolamine fraction.

The results of the study of the conditions affecting extraction of sunflower proteins produced the following information: Table III presents a proximate analysis of defatted sunflower meal. The meal contained 54% protein and the other components were well within the expected range for

an oilseed meal.

Table IV shows the Osborne solubility classification for the various nitrogen fractions. As can be seen, some 56% of the nitrogen is soluble in neutral salt solution. This value is in agreement with earlier reports which show globulin to be the major protein fraction of sunflower seed. In addition to the large globulin fraction, there are appreciable quantities of albumin, the water soluble component, and also the alkaline soluble glutelins. The other protein components and non-protein nitrogen make rather minor contributions to the overall nitrogen content. Perhaps it should be pointed out that while the Osborne classification is a useful schematic of the distribution of various nitrogen fractions, these values can be rather arbitrary unless the conditions of extraction are rigorously maintained.

Since the principal protein fraction, based on the Osborne classification is globulin, a series of exploratory investigations were begun with the idea of finding the optimum conditions for solubilizing sunflower protein. These studies were conducted at room temperature using a 1.0 M aqueous solution of sodium chloride at pH 7.0 as the extracting solvent.

The first approach was to determine the meal-to-solvent ratio at which protein extraction is complete under the defined conditions. As can be seen in Table V, there was no increase in % nitrogen extracted when the meal-to-solvent ratio was increased from 1:10 up to 1:25. To obtain these data, the total volume of 1.0 M NaCl used in each extraction was also used in making final calculations. The data shown as % nitrogen recovered were calculated on the basis of solvent recovered after centrifugation. As would be expected, at lower meal-to-solvent ratios, there is a greater retention of the liquid of solvation per unit weight of meal, and this is reflected by the fact that considerably more nitrogen can be recovered using a broader meal-to-solvent ratio. For all succeeding extractions, a ratio of 1:20 was used.

Using time as the variable under the same conditions, results shown in Table VI give evidence that maximum extraction had occurred at 15 minutes. However, for subsequent work 60 minute extraction periods were arbitrarily used.

When temperature was used as the variable, it was found (Figure 2) that there was a surprising broad range at which near optimum extraction could be accomplished with only slight increase from 5 to 60°C. While a slight depression in solubility occurred at 75°, one would expect a marked reduction in this temperature, due to heat denaturation of the proteins. However, as can be seen, even at 90°C, extraction was still near maximum.

The next investigations were directed toward a determination of the effects of varying salt concentrations over a wide pH range. As shown in Figure 3, when aqueous calcium chloride is used as solvent, some 30-40% of the sunflower protein is soluble in the pH range 3 to 4. However, at pH 5.0 there is a marked increase in protein solubility, particularly at calcium chloride concentrations greater than 0.25 molar. Only slight increases were noted at higher pH values, but from these data it would appear that maximum extraction occurred with 0.75 molar calcium chloride at pH 7.0.

When sodium chloride was used as the extracting salt (Figure 4), less protein was soluble at low pH values, than with calcium chloride. Again, there was a marked increase in extractable protein at pH 5.0 and from these data it can be seen that maximum extraction occurred with 1.0 molar sodium chloride at pH 8.0. At pH values greater than 7.5, there was little difference in the extent to which the two salt solutions extract protein. In summary, it appears that maximum extraction occurs at 0.75 molar calcium chloride at pH 7.0 and with 1.0 molar sodium chloride at pH 8.0

The data presented in Figure 5 are rather interesting, and should be examined in some detail. Data indicated by the squares represents the solubility of sunflower proteins in 1.0 molar sodium chloride on the previous slide. Points represented by the circles show the solubility profile for sunflower protein in water. There are indeed, some interesting relationships between pH and protein solubility in the two solvents. With sodium chloride at pH 6.0, some 75% of the protein is soluble. This would presumably represent the albumin and globulin fractions. In contrast, there is a very broad pH range of minimum solubility with water with only 23% being soluble at pH 6.0. This low solubility in the pH range 3-8 has been reported to be due to the presence of chlorogenic acid which is found in the sunflower at rather high concentrations. This polyphenol is thought to form strong hydrogen bonds with the carbonyl oxygen of the peptide bonds with the carbonyl oxygen of the peptide bonds and thus reduce solubility. It is interesting to note with dilute salt solutions this effect disappears. Maximum solubility with water was obtained at pH 10.0. There is much contention that protein becomes rather severely denatured at pH 11.0 and higher. The data represented by the triangles suggest this is not the case with sunflower proteins. To obtain this information, aliquots of the material soluble in water at pH 11.0 were adjusted to increasing lower pH's with 0.5 N HCl. These data show that once the protein is in solution, it tends to remain in solution to a slightly greater extent even at the pH of minimum solubility. Considerably more of this protein was in solution at pH 3 and lower.

Reducing agents have been reported to increase protein extraction supposedly by preventing the formation of disulfide bonds. Results in Table VII with several reducing agents in a buffered 1.0 M sodium chloride solution show this is not the case with sunflower proteins. As a matter of fact, a decrease in protein solubility with some of the reducing agents was noted.

However, reducing agents have been found to be rather effective in preventing the formation of complexes between chlorogenic acid and sunflower proteins.

Summary and Conclusions

The results reported here are considered to point a direction rather than to signal an accomplishment. Much more obviously needs to be done just to define an optimum potential process for the preparation of a useful sunflower protein isolate. However, an ancillary benefit from this research is that it suggests approaches to the solution of color problems in other protein isolates. Preliminary investigations indicate that the presently disclosed procedure does in fact greatly improve the color of glandless cottonseed protein isolate. There are undoubtedly many other potential useful applications of the general principles involved.

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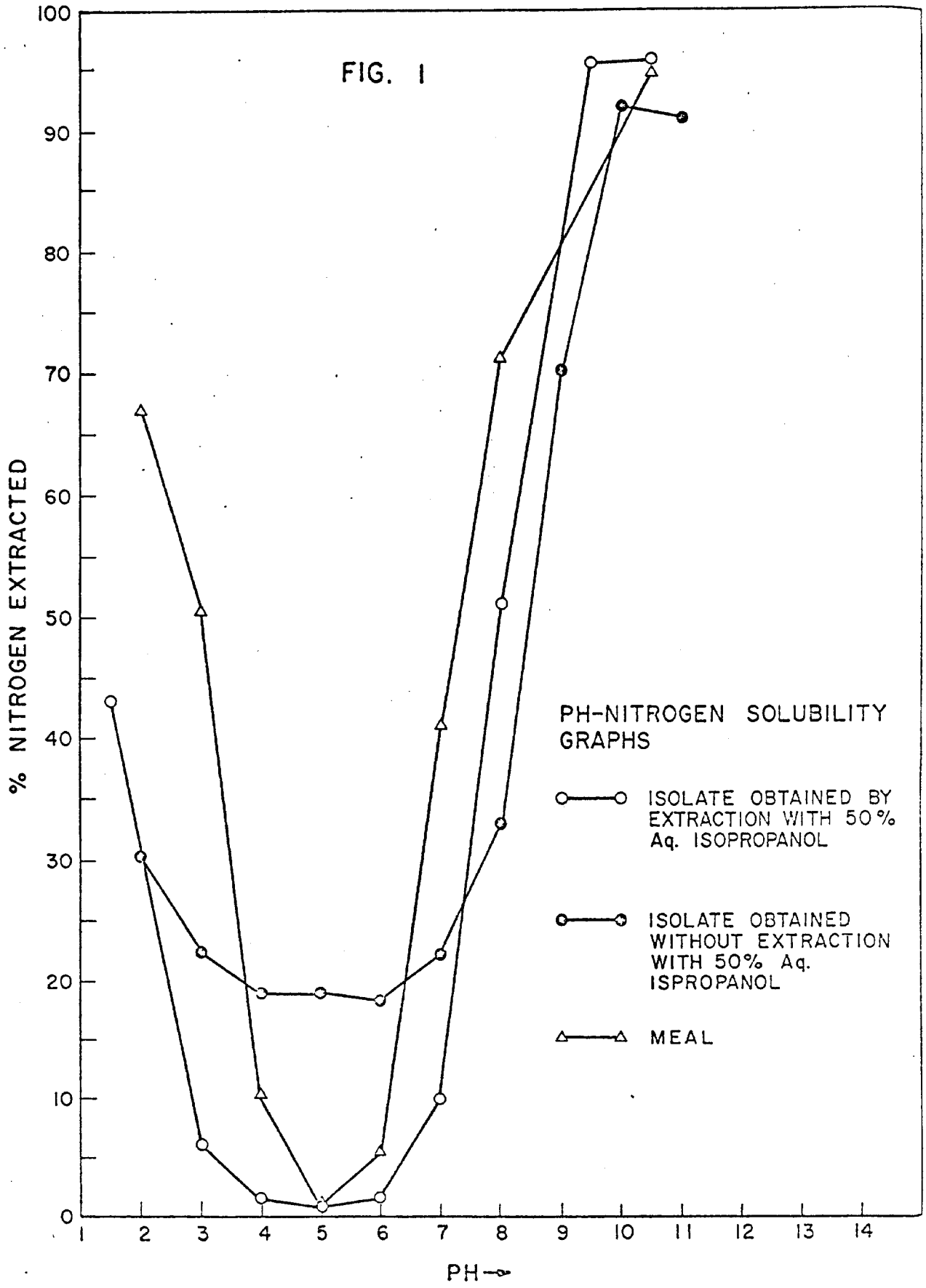


FIG. 1
% NITROGEN EXTRACTED
PH
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
PH-NITROGEN SOLUBILITY GRAPHS
ISOLATE OBTAINED BY EXTRACTION WITH 50% Aq. ISOPROPANOL
ISOLATE OBTAINED WITHOUT EXTRACTION WITH 50% Aq. ISPROPANOL
MEAL

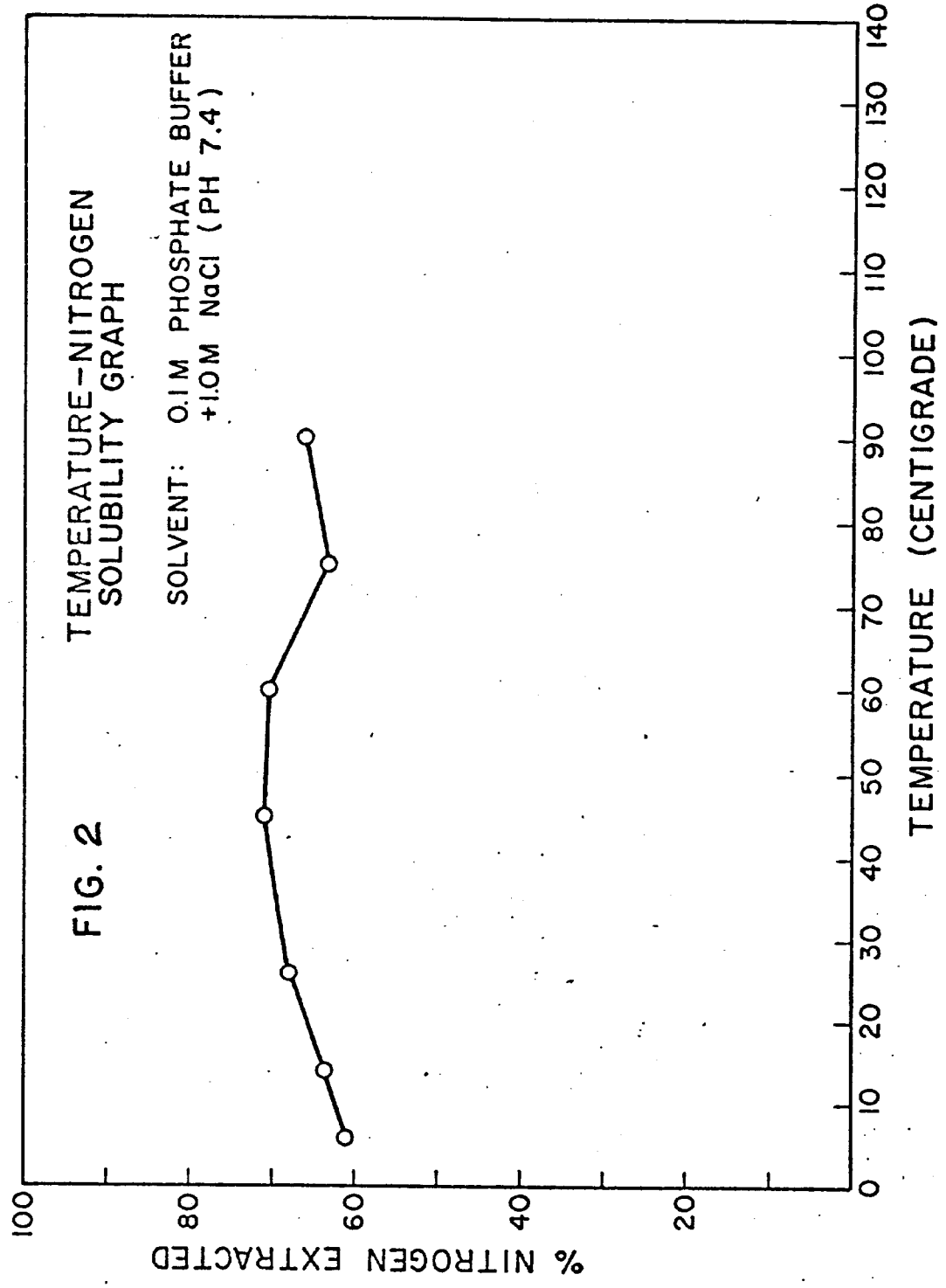


FIG. 2
TEMPERATURE-NITROGEN SOLUBILITY GRAPH
SOLVENT: 0.1M PHOSPHATE BUFFER +1.0M NaCl (PH 7.4)
% NITROGEN EXTRACTED
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
TEMPERATURE (CENTIGRADE)

FIG. 3

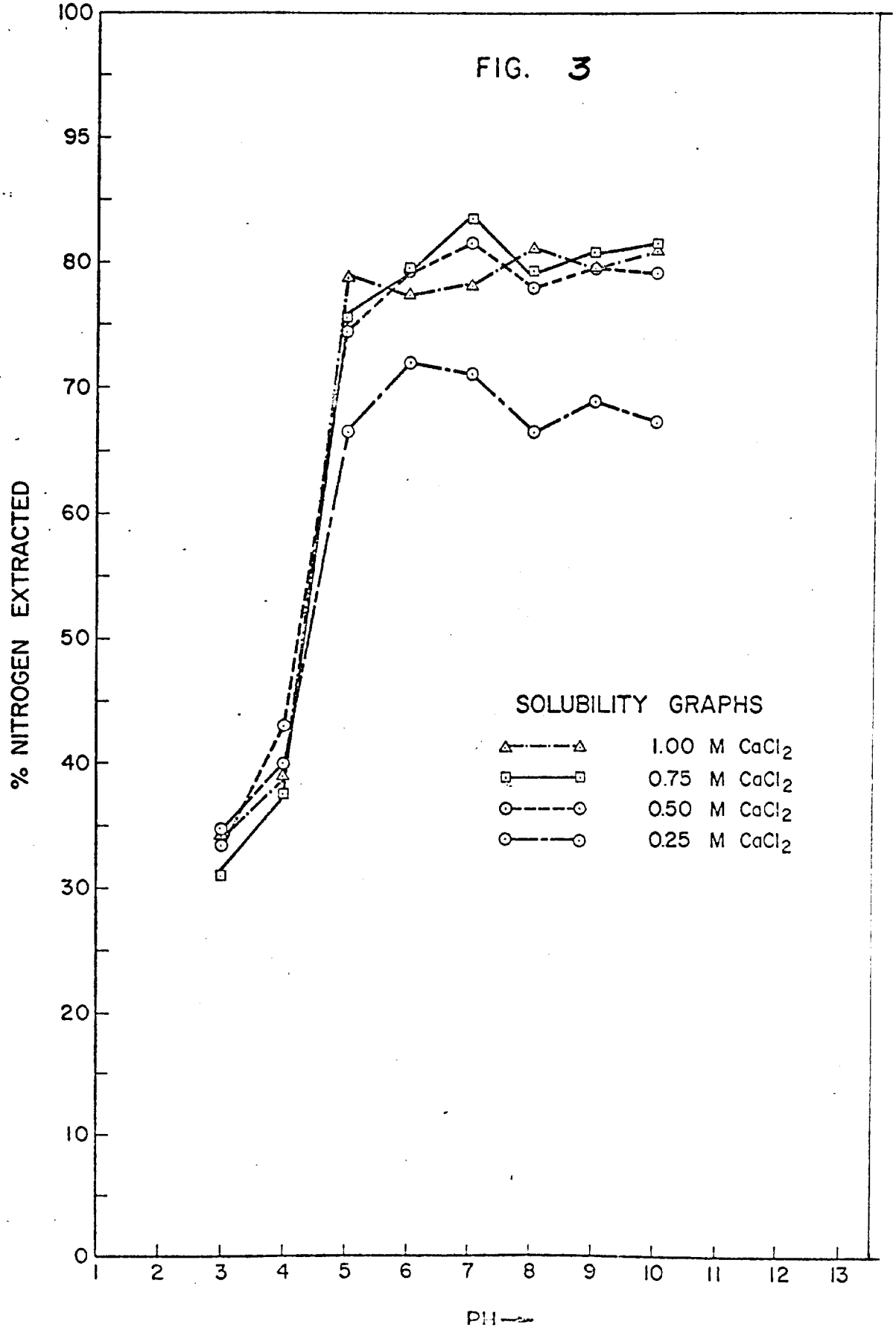
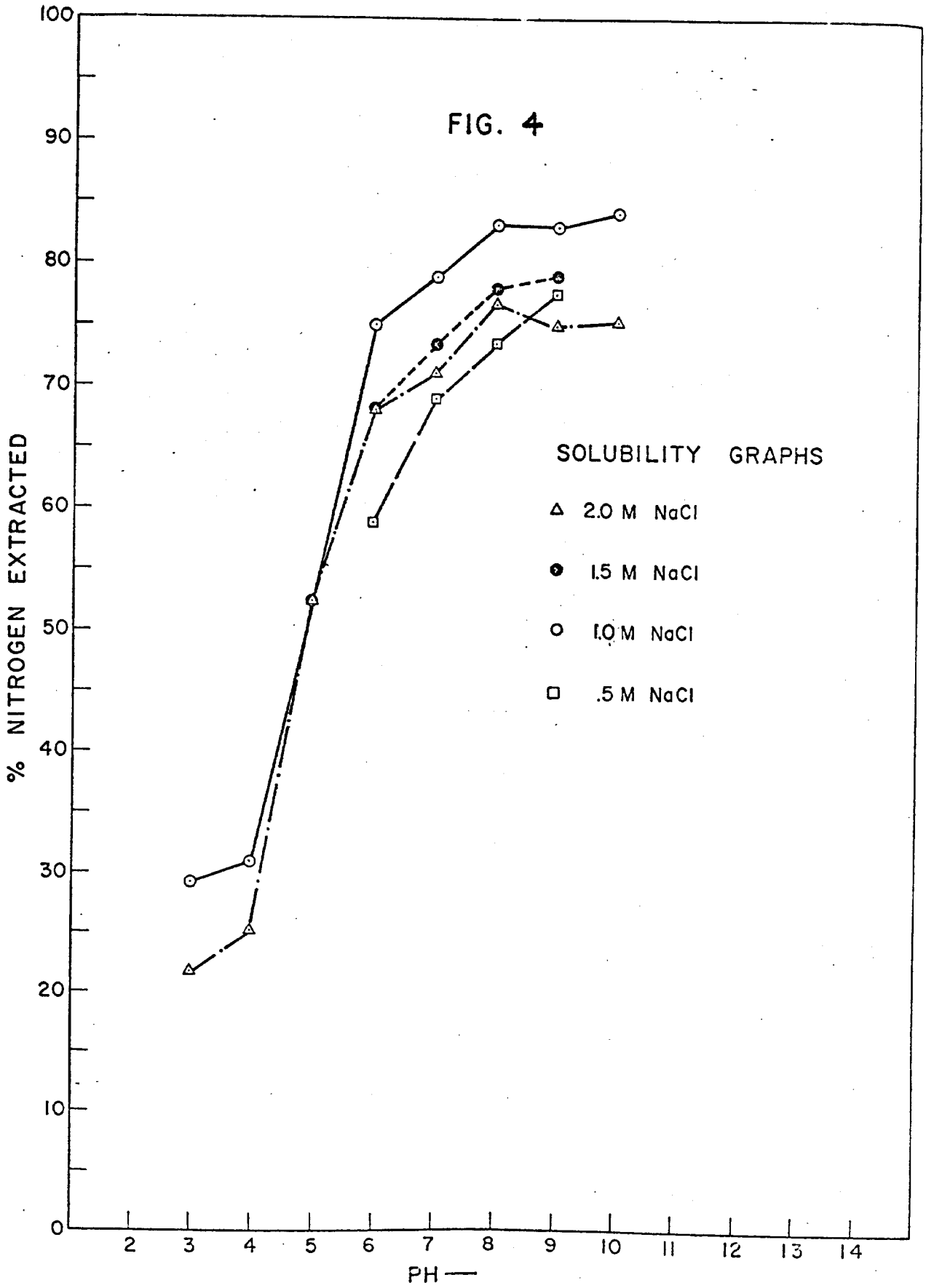

100
95
80
70
60
50
40
30
20
10
0
% NITROGEN EXTRACTED
1
2
3
4
5
6
7
8
9
10
11
12
13
PH
SOLUBILITY GRAPHS
1.00 M CaCl2
0.75 M CaCl2
0.50 M CaCl2
0.25 M CaCl2

FIG. 4


% NITROGEN EXTRACTED
100
90
80
70
60
50
40
30
20
10
0
2
3
4
5
6
7
8
9
10
11
12
13
14
PH
SOLUBILITY GRAPHS
2.0 M NaCl
1.5 M NaCl
1.0 M NaCl
.5 M NaCl

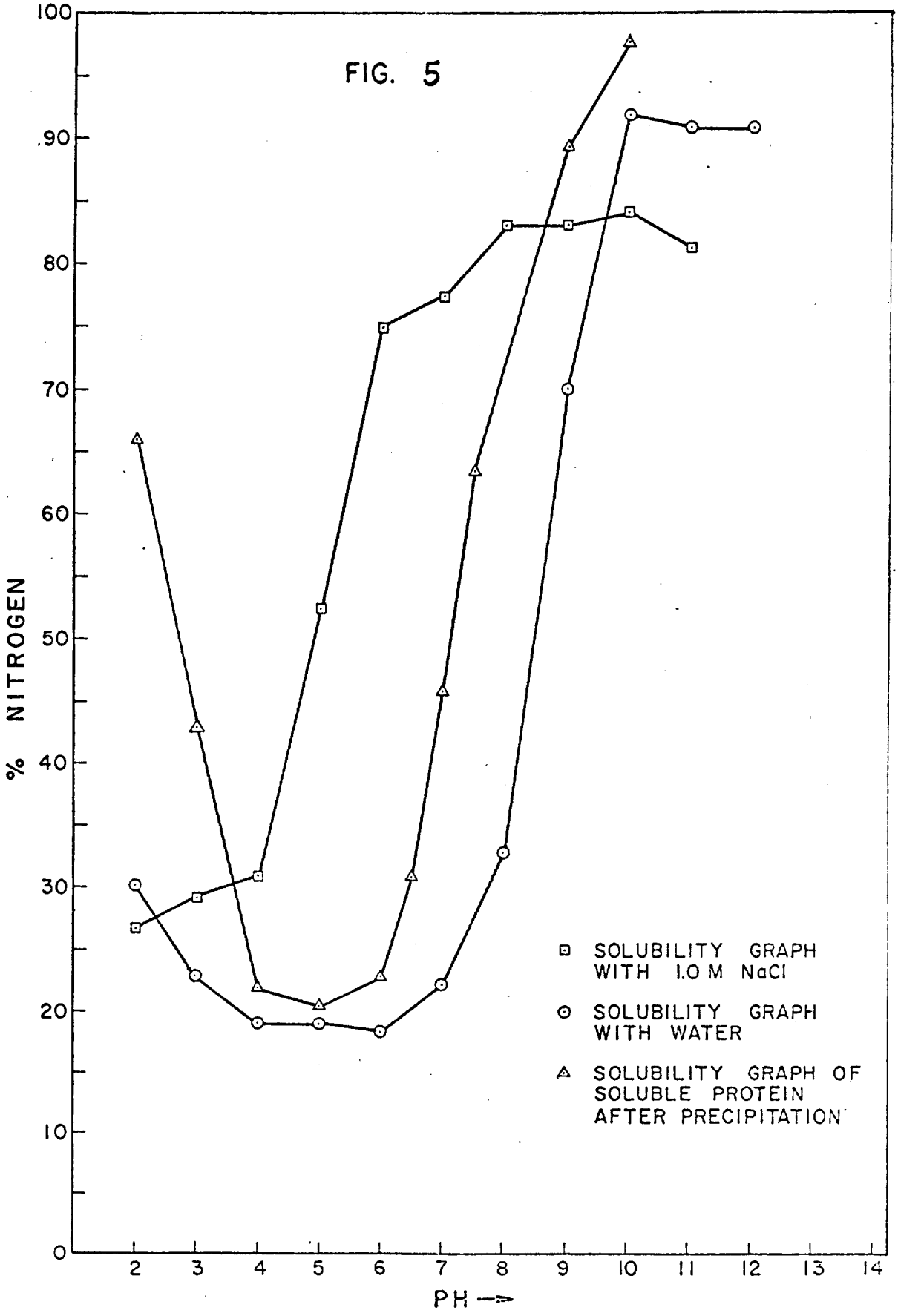


FIG. 5
% NITROGEN
100
90
80
70
60
50
40
30
20
10
0
2
3
4
5
6
7
8
9
10
11
12
13
14
PH →
SOLUBILITY GRAPH WITH 1.0 M NaCl
SOLUBILITY GRAPH WITH WATER
SOLUBILITY GRAPH OF SOLUBLE PROTEIN AFTER PRECIPITATION

TABLE I

World Production of Major Oilseed Proteins

	World Production of Oilseeds[1] (million metric tons)	Estimated Protein Content (%)	World Production of Oilseed Proteins (million kg)
Soybeans	40.6	40	16,240
Cottonseed	21.9	21	4,600
Peanuts	16.9	19	3,210
Sunflower Seed	9.2	19	1,750
Rapeseed	5.2	20	1,040
Flaxseed	3.6	25	900

[1]World Agriculture Production and Trade, February 1970.

TABLE II

AMINO ACID COMPOSITION OF THE ISOLATE AND SUNFLOWER MEAL[1]

	Amino Acid Content on the Basis of Percent of Protein[2]		
Amino Acids	Meal	50% aqueous isopropanol extracted isolate	Isolate prepared without 50% aqueous isopropanol
Lysine	3.15	3.04	3.00
Histidine	2.20	2.58	2.59
Arginine	9.16	9.54	9.64
Cysteic Acid	1.57	0.64	0.96
Methionine Sulfone	2.47	1.82	1.92
Aspartic Acid	9.32	10.80	10.79
Threonine	3.59	3.89	3.96
Serine	4.08	4.44	4.46
Glutamic Acid	23.53	23.98	24.62
Proline	4.47	3.95	4.69
Glycine	5.64	5.12	5.09
Alanine	4.29	4.90	4.83
Valine	5.15	5.93	5.98
Methionine	2.05	2.13	2.04
Isoleucine	4.45	4.90	4.93
Leucine	6.45	7.49	7.58
Tyrosine	2.84	3.29	3.54
Phenylalanine	4.67	6.10	6.16

[1]Data reported here are the result of single determination.
[2]Percent protein of the meal = 54.6
Percent protein of isopropanol extracted isolate = 95.94
Percent protein of the isolate prepared without isopropanol = 90.00

TABLE III

PROXIMATE ANALYSIS OF SUNFLOWER SEED MEAL

% Moisture and Volatile Materials	% Oil	% Nitrogen	% Protein	% Crude Fiber	% Ash
10.9	0.8	8.7	54.0	4.3	5.9

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TABLE IV

PROTEIN CLASSIFICATION
(Based on Solubility)

Fractions	Conditions of Extraction	Percent of Total Nitrogen
Water soluble-Albumin	CO_2-free water pH (6.6)	22.0
1 M NaCl soluble-Globulin	pH (7.0) adjusted by NaOH	56.2
Alcohol soluble Prolamine	70% aqueous ethanol	1.0
Glutelin	NaOH solution pH (11.0)	17.0
Insoluble residue		3.7
Non-protein Nitrogen	12% tungstate solution as protein precipitant	1.3

TABLE V

EFFECT OF MEAL-SOLVENT RATION ON EXTRACTION OF NITROGEN

Solvent used: 1.0 M sodium chloride
pH: 7.0 (adjusted with NaOH)

Meal:Solvent	% Nitrogen Extracted
1:10	75.6
1:15	75.4
1:20	75.5
1:25	75.2

TABLE VI

EFFECT OF TIME ON EXTRACTION OF NITROGEN

Solvent used: 1.0 M sodium chloride
pH: 7.0 (adjusted with 0.5 N NaOH)

Time of Extraction in Minutes	% Nitrogen Extracted
15	74.5
30	75.6
45	75.3
60	75.0
75	75.5
90	74.5

TABLE VII

EFFECT OF REDUCING AGENTS ON THE EXTRACTION OF NITROGEN

Solvent: 0.1 M Phosphate buffer + 1.0 M NaCl + Reducing Agents
pH: 7.0

	% Nitrogen Extracted		
Reducing Agents	0.25%	0.50%	1.0%
Ascorbic Acid	68.2	69.2	68.5
Sodium Sulfite	71.4	71.1	69.2
Sodium Bisulfite	70.5	68.7	67.4
Sodium Hydrosulfite	67.1	66.5	64.0
B - Mercaptoethanol	63.5	65.9	42.5

Control gave a percentage extraction value of 68.7.