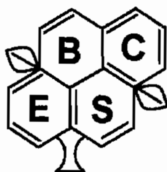

CHEMICAL STUDIES ON PHYTASE ACTIVITY AND PHYTATE IN THE SEEDS AND PROTEIN ISOLATES OF TWO SOYBEAN CULTIVARS

Journal

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ABSTRACT

The seeds of two new soybean cultivars (Giza 22, Giza 35) were studied; phytate concentration in cotyledons, hull and protein isolates, effect of pH on the solubility of phytate and protein, effect of germination on phytase activity and phytate, preparation of partially purified phytase from seeds germinated for 7 days, removal of phytate from protein isolates by partially purified phytase, and the functional properties of soybean defatted meals and their proteins isolates.

Preparation of low phytate - protein isolates (1.5-1.8% phytate) using the differences in solubilities of phytate and protein at acidic pH (5-5.5) was studied. The yield was lower compared with high phytate - protein isolates (2.7-3.1% phytate) precipitated at pH 4.5 with maximum yield.

No phytase activity could be detected in the dry seeds but appeared during germination to reach its maximum levels after 7 days of germination with concomitant decrease in phytate content. Partially purified phytases showed pH optimum at 5 and 5.5 for enzymes of Giza 22 and Giza 35, respectively. Incubation of partially purified phytase with high phytate-protein isolates at optimum pH and temperature 50^0C resulted in complete removal of phytate from protein isolates of Giza 22 and Giza 35 after 60 and 80 min, respectively. The functional properties determinations revealed that removed phytate soyprotein isolate (RP-SPI) exhibited functional

properties similar to or superior to those of other protein isolates or defatted meals.

INTRODUCTION

Human consumption of soybean and its products are increasing, due to the health benefits. Soybean is regarding as a highly nutritious food source for excellent oil (61% polyunsaturated fat and 24% monounsaturated fat) and protein (eight essential amino acids) content (Technical Bulletin of the American Soybean Association on Soybean Nutrition, 1999). The U.S. Food and drug administration has approved a label health claim that foods containing at least 6.25 g of soy protein preserving reduce the risk of cardiovascular disease (Food and Drug Administration, 1999).

The main concern about the presence of phytate in the diet is its negative effect on mineral uptake (Zhou and Eerdman, 1995). The formation of insoluble cation-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral bioavailability because these complexes are essentially non absorbable from the gastrointestinal tract. Especially zinc and iron deficiencies were observed as a consequence of high phytate attack.

Soybean isolates contain 90% protein, the major components being glycinin (11S protein) and β-conglycinin (7S protein) (Hermansson, 1978). These fractions constitute 61% of the isolate proteins (34 and 27 %, respectively); the rest consists of whey proteins, such as T-conglycinin, 7S basic globulin, lipoxygenase, β-amylase, agglutinins and trypsin inhibitors (Iwabuchi and Yamauchi, 1987 a and b). Isolates are the most defined form of soybean proteins, and they must have suitable functional properties to make the formulation successful, particularly with regard to consumer acceptability (Kinsella, 1979).

A major portion of phytate from soybean is coextracted and recovered with the protein fraction during the preparation of soy protein isolate. The phytate exists in the isolate as a complex with the proteins and polyvalent cations including Ca^{++}, Mg^{++}, and other ions such as Fe^{+++} and Zn^{++}, which has the effect of inhibiting their bioavailability (Maga, 1982 & Honig *et.al.*, 1984). Removal of dietary phytate significantly improves the bioavailability of these minerals.

The effects of germination on phytic acid in the seeds of four Egyptian legumes namely, field bean (*Vicia faba*), kidney bean

(*Phaseolus vulgaris*), pea (*Pisum Sativum*) and mungbean (*Vigna radiata*) were studied by Mahmoud and Fawzy (2003). In legume samples, the level of phytate decreased slightly during the first 2 days of germination with rapid decline between 3rd and 7th day. Phytate was disappeared completely from mungbean by the end of germination period. The overall reduction in phytate at the end of germination period (9 days) accounted for more than 90% for field bean, pea and kidney bean seeds. Lestienne *et.al.,* (2005) found that dehulling of soybean seeds led to a marked increase in phytate content, whereas milling favoured reactions between phytases and phytates.

Khalil (2006) found that probiotic fermentation treatments were effective in decreasing the phytate content for mung bean (MB) samples. Though, the combined germination and fermentation treatment reduced the phytate contents higher than those ungerminated only.

However, Azeke et.al. (2005) found that antinutrients, except phytic acid, were significantly reduced in African yam bean lactic acid fermentation with L. *Plantarum.* Thus, the results of Khalil (2006), it could that probiotic fermentation increased nutrient availability of the Egyptian MB.

In recent years, dietary phytate has received increased attention due to its role in cancer prevention and/or therapy and its hypocholesterolemic effect (Kuroda and Shamsuddin, 1995, Abd El-Rahman, 2005 & Abd El-Rahman *et.al.*, 2007). In turn, by binding an excess of free iron in the small intestine, phytates may prevent the formation of free radicals by the Fenton reaction in the colon and consequently decrease iron absorption for all that needs less iron.

Phytases are phosphatases that can hydrolyze phytic acid to inositol and free orthophosphate. These enzymes have been separated from germinating seeds (Griener *et.al.*, 2001 and 2002; Griener, 2002; Mahmoud and Fawzy, 2003 & Abdel Galil, 2003) in addition vegetables and fruits (Phillippy, 1998 & Phillippy and Wyatt, 2001), and their properties have been reported. Analysis of reports revealed species and cultivar differences for phytase characteristics.

Functionality has been defined as any property of a food ingredient (Kinsella, 1979), Functional properties are, however, often used to denote any property of proteins that affects their use, either as a processing aid or a direct contributor of product attributes. Most functional properties affect sensory characteristics of food and can

play a major role in the physical behavior of foods or food ingredients during their preparation, processing, and storage. Various forms of protein additives are added to foods to achieve functional, nutritional, or economic goals (Rakosky, 1989). Potential functional benefits include emulsification, foam formation, gelation, increased viscosity, and improved appearance, flavor, texture, and binding of fat or water. The selection of a specific protein depends on the function to be achieved in a finished product.

In the present study we determined: (a) levels of phytic acid in cotyledons and hull of two soybean cultivars as well as their protein isolates; (b) effect of pH on solubility of phytic acid and protein; (c) effect of germination on phytate and phytase activity ; (d) effect of partially purified phytase, separated from the germinated soybean seeds on protein isolates. Our ultimate goal of this work was to evolve the simplest treatment to obtain products with rich functional properties and better nutritional quality for human consumption.

MATERIALS AND METHODS

The seeds of Soybean (*Glycin max*) variety Giza 35 and Giza 22 were obtained from Agricultural Research Centre, Giza, Egypt. Soybean seeds were dehulled, flaked, ground in a coffee grinder and defatted by extraction with hexane. The soybean defatted meals (SDM) were kept in closed container at 4^0C till used.

Germination.

The samples to be germinated were surface sterilized with 1% sodium hypochlorite solution (Jood *et.al.*, 1985), washed and soaked for 2hr. The seeds were germinated in sterilized petri dishes lined with wet filter paper in an incubator at 25^0C. During germination, distilled water was sprinkled on seeds twice a day. Germinated samples were collected, at one day intervals during the germination period of ten days, dehulled, ground and lyophilized.

Extraction of phytic acid.

Phytic acid was extracted from defatted meals and protein isolates (Chang *et. al.*, 1977) with some modifications. The sample was blended with 3% trichloroacetic acid (TCA) in a blender for 2 min at ratio of 1:15 (meal: TCA). The legume slurry was placed in water bath at 60°C for 30 min with continuous stirring, followed by centrifugation at 8000 r.p.m. for 30 min. The clear supernatant was used for phytic acid determination.

Phytate phosphorous was determined by treating 5 ml of TCA soybean product extract with 1ml of N HCl solution containing 0.2%Fe^{+++} (as $FeCl_3$). The precipitate of ferric phytate was converted to ferric hydroxide (Wheeler and Ferrel, 1971). The $Fe(OH)_3$ was dissolved in hot 3.2 N HNO_3 and the iron was determined colorimetrically (Tabekhia and Luh, 1980). The phytic acid was calculated assuming a Fe: P molar ratio equal to 4:6.

The phytase enzyme activity was measured and extracted (Lolas and Markakis, 1977). Partial purified phytase was performed as described by Lolas and Markakis (1977). Solid $(NH_4)_2SO_4$ was added to the crude enzyme solution with continuous mechanical stirring to make it 35% ammonium sulphate saturation.the mixture was kept for 1 hr at 4°C followed by centrifugation at 10000 r.p.m.for 30 min. at 4°C. The residue was discarded and the supernatant was made 80% $(NH_4)_2SO_4$ saturation, followed by centrifugation as mentioned above. The protein precipitating between 35% and 80% saturation contained most of the phytase activity which was dissolved in 20 mM sodium acetate buffer, pH 5, and dialyzed against the same buffer for 24 hr at 4°C. Any Insoluble material was removed by centrifugation and the clear supernatant (partially purified phytase) was used as the enzyme source in all experimentsm

The iron phytate precipitated from the legume extract was converted to sodium phytate (Anderson, 1963). The hoogeneity of phytate was examined by descending paper chromatography with the solvent of Desjobert and Petek (1956) (n-propanol / ammonia / water; 5/4/1, v/v), and the phosphate reagent of Wade and Morgan (1955).

Determination of protein.

Protein was measured (Lowry *et.al.*, 1951) using bovine serum albumin as standard. Effect of pH on the solubility of phytic acid and protein was determined (Rham and Jost, 1979) with minor modification. The defatted flour was dispersed in water at ratio 1:10 (meal: water) and the pH was adjusted to desired value with 0.1 N NaOH or 0.1 N HCl. The suspension stirred for 45 min at room temperature, and after centrifugation at 7000 r.p.m. for 30 min aliquots of the supernatant were analyzed for protein and phytic acid.

Native isolate was prepared from defatted Soybean flour Giza 22 and Giza 35) as described by Sorgentini *et.al.*, (1995). The precipitate was washed with water, resolubilized in water by neutralization to pH

8 with 2 N NaOH at room temperature, and lyophilized. Five grams of each phytate containing protein isolate was dissolved in 100 ml of 0.1 M acetate buffer, pH 5 and the partially purified phytase (50 mg) was added and the mixtures were incubated in water bath at 50°C while being shaken. These conditions are the optimum pH (pH 5) and optimum temperature (50°C) At interval 2ml samples were withdrawn, placed in boiling water bath for 2 min to inactivate the enzyme, followed by placing in ice bath and the phytate content was determined as indicated before.

Functional property determinations.

The procedure described by Prinyawiwatkul *et.al.*, (1997) was used for determing water absorption capacity (WAC) and oil absorption capacity (OAC) with minor modification. Four grams of soy defatted meal (SDM) or soy protein isolate (SPI) were thoroughly mixed, without pH adjustment, with 20 ml of distilled water or in 50 ml centrifuge tubes. Suspension was stirred intermittently over a30 min period at 28°C. and then centrifuge at 1000 r.p.m. for 30 min at 28°C. The volume of decanted supernatant was measured, and water and oil absorption capacities were calculated. Emulsifying capacity (EC) and emulsifying stability (ES) were determined according to the method described by Yasumatsu *et.al.*, (1972). The sample, 1.5 g, was dispersed in 20 ml of distilled water with a magnetic stirrer. The dispersion was adjusted to pH 7 with 0.2 N NaOH and the volume made up to 25 ml with distilled water. Corn oil, 25 ml, was added and the mixture homogenized for 3 min at the highest speed. The emulsion so obtained was divided between 50 ml centrifuge tubes and centrifuged at 4000 r.p.m. for 5 min. The height of the emulsion layer, expressed as percent of the total height of fluid in the tude, was reported as EC.ES was determined in a similar manner, but heating (at 80°C in a water bath for 30 min.) and cooling of the emulsion (to room temperature) preceeded centrifugation Foaming capacity (FC) and foaming stability (FS) were measured according to the method described by Mitchell (1986). Distilled water (100 ml) was added to SDM or SPI (3 g) and the mixture was homogenized (Mechanika-preyzysna, Homogenizer type MBW-302, Poland) at the highest speed for 3 min at 28°C and transferred to a measuring cylinder. The volume of foam was calculated and increased volume is expressed as percent of foam capacity. The foam stability was determined by measuring the

decrease in volume of foam as a function of time up to a period of 30 min.

The functional properties tests were carried out at neutral pH and the results given are the mean of four determinations.

Statistical analysis.

Means and standard deviation of means were calculated for all data (triplicate determination) according to the procedures of statistical analysis system (SAS, 1989).

RESULTS AND DISCUSSION

Effect of some extracts on removal phytic acid from dehulled seeds.

Phytic acid is usually extracted with 0.5 N HCl or in dilute trichloroacetic acid (TCA). The effectiveness of these two extracts as well as water in removing phytic acid from soybean seeds were examined (Table 1). TCA at 60^0C was more effective than the other two extracts. Phytic acid extracted with water accounts for the level of water soluble phytate. Hydrochloric acids allows the extraction of soluble proteins which may subsequently in part form precipitates with phytic acid and thereby reduce the level of this compound in the extract.

Table (1): Effect of H_2O, 0.5 N HCl and 3 % TCA as extractant of phytic from soybean samples.

Extractant	Phytic acid	
	Giza 22	Giza 35
H_2O	1.2 ± 0.10	1.3 ± 0.12
0.5 HCl	1.5 ± 0.12	1.7 ± 0.14
3 %TCA	1.8 ± 0.18	2.1 ± 0.17

To produce accurate results, mild heating (near 60^0C) was an important step in yielding a clear extract. Although room temperature has been shown (Wheeler and Ferrel, 1971) to be satisfactory for extraction of phytate from wheat, it is not adequate for its extraction from legume seeds (Chang *et.al.*, 1977 & Mahmoud and Fawzy, 2003).

Effect of pH on solubility of phytic acid and protein in soybean extract.

For the different protein sources and different pH levels, the solubility profile in soybean samples was studied (Fig. 1). At acidic pH, protein and phytic acid curves overlap, while at pH 4 a minimum solubility for phytic acid occures in both soybean varieties. At higher pH values, solubility of phytic acid was greater than that of protein especially at pH levels between 4.5 and 6.5. At very alkaline pH, protein solubility remained high, but phytate solubility decreased sharply to less than 10 % above pH 11.5 (data not shown). Similar observation was found in faba bean (Carnovale *et.al.*, 1988) and soybeans (Rham and Jost, 1979 & Cheryan, 1980).

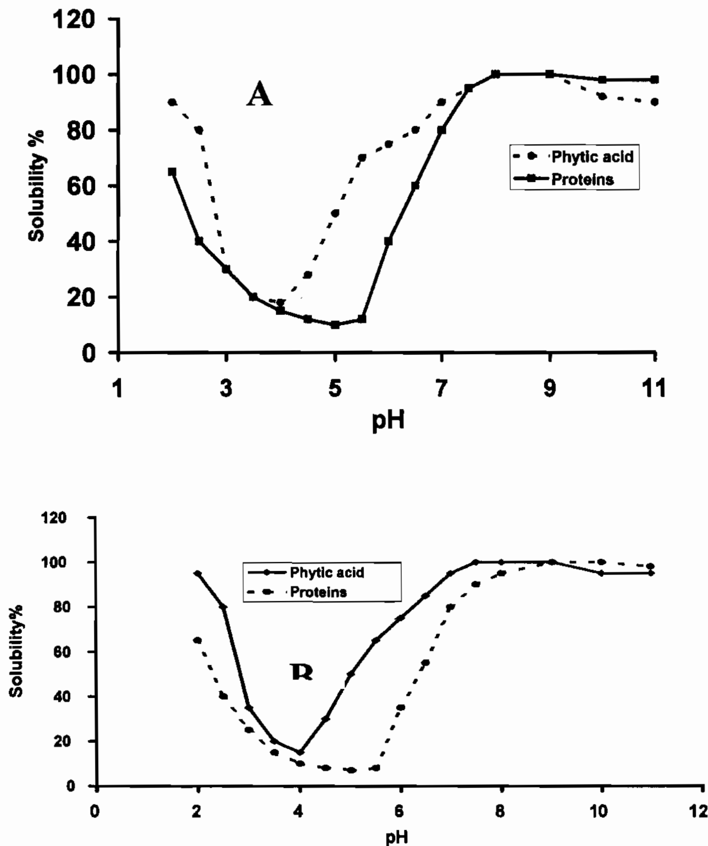

Fig (1): pH solubility profiles of phytic acid and protein in defatted soybean meal of Giza 22 (A) and Giza 35 (B).

The pH solubility profiles of soybean protein of both varieties used in this work revealed broad minima at 3.5 to 5.5; less than 15 % of the proteins were soluble (Fig. 1).

The difference in solubility at acidic pH used for preparing protein isolates with low-phytate content by precipitating soy protein isolate from a neutral soy extract at pH 5 to 5.5 (Fig. 1) instead of usual pH 4.5. The phytate levels were 2.7 % and 3.1 % for protein precipitating at pH 4.5, 1.8 % and 2.1 % for pH 5, 1.5 % and 1.8 % for pH 5.5. Yield decreased from 45 g (pH 4.5) to 37 g (pH 5) to 28 g isolate (pH 5.5) for 100 g of defatted meal. Thus maximum yields of protein isolates occur when precipitation is carried out near pH 4.5, the isoelectric region for the major proteins.

The low-phytate protein isolates were also characterized by reduced levels of trypsin inhibitors and rafinose family sugars compared to isolate precipitated at 4.5 as indicated by Rham and Jost (1979). When extraction was carried out at pH 11.5, centrifugation removed the insolubles containing most of the phytate, and acidification of the supernatant to pH 5.5 was conducted. Phytate levels in the isolates obtained from defatted meal of Giza 22 and Giza 35 were recorded to be 0.35 % and 0.6 %, respectively with protein recovery of about 42 g protein isolate from 100 g of defatted meal.

Phytic acid content in soybean seeds and protein isolates.

The levels of phytic in the two soybean samples (Table 2) revealed that phytic acid was predominantly located in the cotyledon and accounted for 1.8 % and 2.1 in Giza 22 and Giza 35, respectively. Phytic acid in the protein isolates was increased about 1.5-fold with respect to the starting dehulled seed flour (Table 2) and similar increases were observed for protein isolates of other legumes (Carnovale *et.al.*, 1988 & Mahmoud and Fawzy, 2003).

Paper chromatographic examination of phytate extracted from soybean samples used in this work revealed that phytate was primarily hexaphosphate with a barely detectable trace of other phosphates.

Table (2): Phytic acid content in soybean seeds and protein isolates.

Sample	% phytic acid			
	Whole seed	Hull	Dehulled	Protein isolate
Giza 22	1.6 ± 0.2	0.3 ± 0.01	1.8 ± 0.3	2.7 ± 0.4
Giza 35	1.9 ± 0.2	0.35 ± 0.01	2.1 ± 0.4	3.1 ± 0.4

Effect of germination on phytic acid and phytase.

No phytase activity was detected in the dry seeds of the two soybean varieties and the same is true for those germinated for one day. Neither significant increase in phytase activity nor decrease in phytate content (Fig. 2) was observed during the first two days of germination. This was followed by rapid in phytase activity as germination proceeded to reach its maximum level by the eighth day of germination in both varieties. Results (Fig. 2A and B) indicate that rise in phytase activity for both soybean varieties with a concomitant decline in phytate. The level of phytase was somewhat higher in Giza 35, which also reflected higher phytate content of 1.9 % compared to 1.6 % in Giza 22. By the end of germination the overall reduction in phytate accounted for 90 and 100 % for Giza 35 and Giza 22, respectively.

In accordance with many cereals and legumes, germination is a suitable method to increase phytase activity in legume seeds. Although large increase in phytase activities have been extensively reported in germinating seeds as well as in germinating pollen. The biochemical mechanism leading to this rise in phytase activity is not well understood. It was suggested that phytate degrading enzymes induced during germination may be synthesized from long lived, pre existing mRNA or by *de nova* synthesis (Griener *et.al*, 2001).

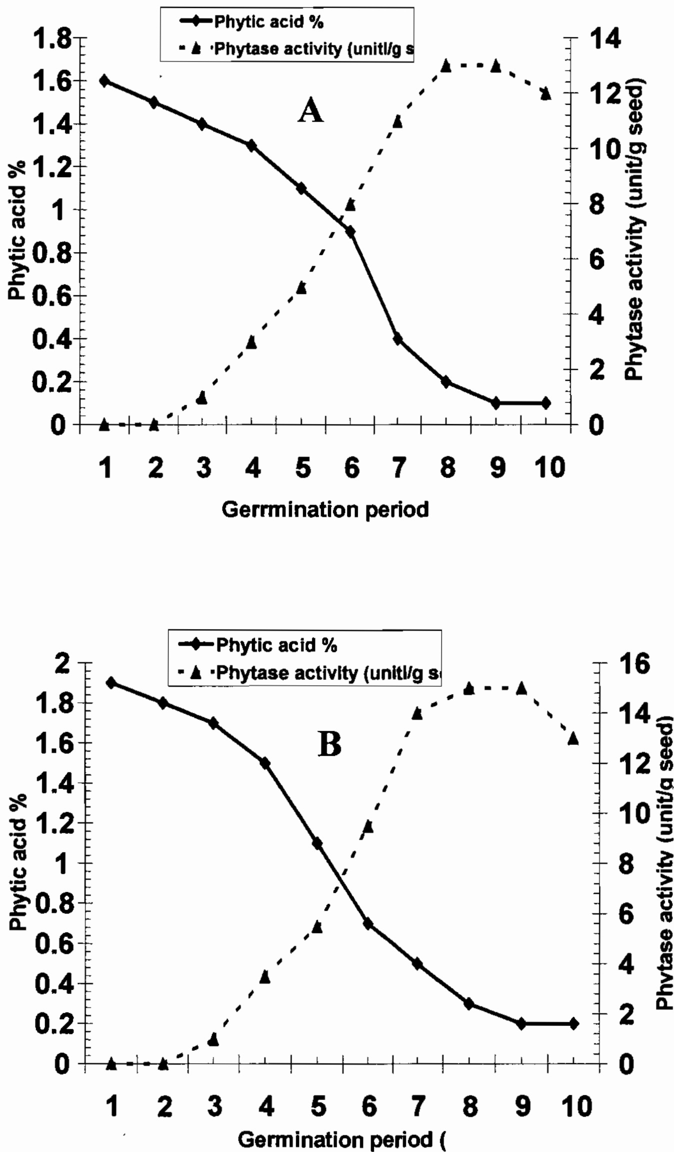

Fig (2): Effect of germination on phytase activity and phytic acid in soybean seed of Giza 22 (A) and Giza 35 (B).

It is evident from this study that simple germination of soybeans substantially decreases the phytate content due to the utilization of phosphorus during the increase in metabolic activity. This reduction of phytate should result in an improvement in nutritional quality of the soybean.

Soybean proteins are reported to have low calcium bioavailabilty (Lee *et.al.*, 1980 & Sato *et.al.*, 1986), probability due to phytate that binds too strongly with calcium and insolubilizing it. Therefore, it is better to remove phytate in order to increase calcium's.

Extraction and separation of partially purified phytase from germinated soybean seeds.

As indicated above, no phytase activity was detected in dry seed of both soybean varieties but the seed germinated for (eight) days contained the highest levels of enzyme activities, which were extracted and partially purified. Phytase activities of the partially purified enzymes preparation were determined after incubation in buffer with pH values ranging from 2.5 to 8. The separated enzyme was virtually inactive below pH 2.5, and increased as pH raised to reach highest levels at pH 5 and 5.5 for Giza 22 and Giza 35 phytase, respectively. Phytase activity was decreased gradually as pH increased to reach 35 % and 30 % at pH 7, 18 % and 10 % at pH 8 for Giza 35 and Giza 22, respectively (Fig. 3).

Two main types of phytate-degrading enzyme have been identified in plants: acidic phytate-degrading enzymes with optimum activity around pH 5.5 and alkaline phytate degrading enzymes with a pH optimum around pH 8. The phytate-degrading enzyme of *vicia faba* (Griener *et.al,* 2001), *phaseolus vulgaris* (Lolas and Markakis, 1977) are belong to the acidic ones.

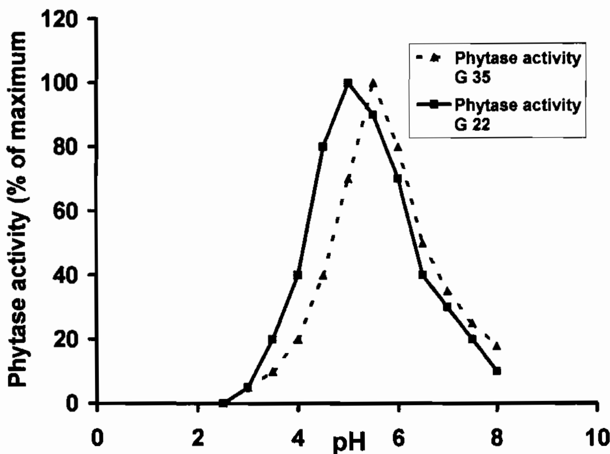

Fig (3): Effect of pH on the activities of partially purified phytases of Giza 22 and Giza 35.

The pH profile for phytase activities of phytase of fababean (*Vicia faba*) (Griener *et.al.*, 2001), pea (*pisum sativum*) (Beal and Mehta, 1985), navy beans (*phaseolus vulgaris*) (Lolas and Markakis, 1977) are belonging to acidic phytases. On the other hand, the phytase of germinating mung beans studied by Mandal *et.al.*, (1972) showed pH optimum of 7.5.

Removal of phytate from protein isolates by phytase.

Dephosphorylation of the protein isolates, separated from both soybean varieties, by the action of partially purified phytases extracted from the seeds germinated for (eight) days was studied. These protein isolates were characterized as high phytate- soybean protein isolates (HP-SPI) (3.1-2.7 %) since they extracted at pH 8 and precipitated by lowering the pH of the extract to 4.5.

Incubation of phytase separated from Giza 35 with protein isolates at pH 5.5 and 50^0C resulted in rapid decrease in phytate content of protein isolates of both soybean varieties to 36 % and 22 % of its level after 40 min of incubation for isolate of Giza 35 and Giza 22, respectively, (Fig. 4). Extending the incubation period with phytase revealed the complete removal of phytate from Giza 22 and Giza 35 protein isolates after 60 and 80 min, respectively (Fig. 4). Similar trend was also observed for Giza 22 phytase (data not shown). Thus, like other phytate degrading enzymes, the soybean phytase was effective in removal of phytate from soybean protein isolates. Similar observations were reported for phytate-degrading enzyme from other legumes (Griener *et. al.,* 2001 & Mahmoud and Fawzy, 2003). It has been suggested that phytate was hydrolyzed by the action of phytate degrading enzyme of field beans in a stepwise manner during degradation the hydrolysis rate decreased markedly.

The results indicate that germinated soybean seeds contain phytase activity comparing to that in germinated seeds of cereal such as wheat, spelt, and barley (Griener *et.al.*, 2000). The response to temperature and pH of phytate-degrading enzyme of soybean is similar to that of cereals (Griener *et.al.*, 1998, 2000, Konietzny *et.al.*, 1995 & Laboure *et.al.*, 1993) and soybean seeds (Gibson and Ullah, 1988).

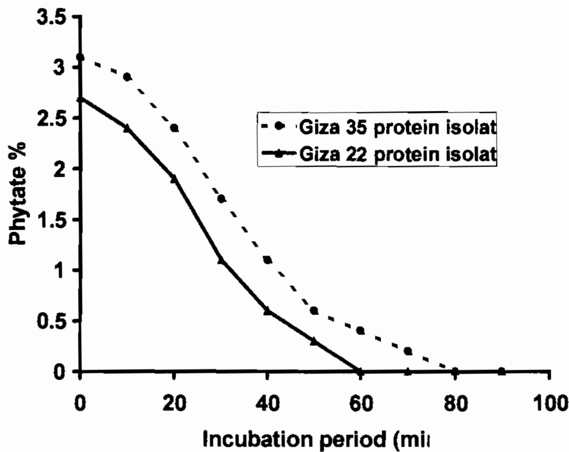

Fig (4): Removal of phytate from soybean protein isolates by partially purified phytase from germinated seeds of Giza 35.

The results indicate the preparation of low-phytate soybean protein isolates by precipitation from a neutral soy extract at pH 5-5.5 instead of the usual pH 5.5 resulted in decreased yield. Thus the total removal of phytate from protein isolates by using the differences in solubility at acidic pH, is difficult and therefore, phytic acid is present at low levels in protein isolate. Even through the reduction in mineral availability is the main nutritional effect of phytic acid, the nutritional and technological consequences of its effect on protein digestibility must also be taken into consideration.

Phosphorus in soybean is principally in the phytate from (Brooks and Morr, 1984). Phytate binds to soybean protein and interfere with the dietary mineral absorption in the small intestine by forming a complex and insolubilizing it (Erdman, 1979), Therefore, many researchers have made effects to remove phytate from soybean proteins using acidic and alkaline reagents, cations, EDTA, ultrafiltration, and anion-exchange resin (Kumagai et.al., 1998; Okubo et.al., 1975; Omosaiye and cheryan, 1979; Rham and Jost, 1979 and Brooks and morr, 1982).

Functional properties.

The functional properties were determined for defatted soybean meal (SDM), protein isolate precipitated at pH 4.5 (high phytate-soyprotein isolate) (HP-SPI) and removed phytate - soyprotein isolate (RP-SPI) (Table 3).

Table (3) Functional properties of SDM, HP-SPI and RP-SPI of the two soybean cultivars (Giza 22 and Giza 35).

Functional Properties*	Giza22			Giza 35		
	SDM	HP-SPI	RP-SPI	SDM	HP-SPI	RP-SPI
WAC (g/100g)	152 ± 4.00	156 ± 3.00	166 ± 4.00	157 ± 3.00	170 ± 4.00	171 ± 4.00
OAC (g/100g)	140 ± 3.00	138 ± 3.00	139 ± 4.00	143 ± 4.00	140 ± 3.00	141 ± 3.00
EC (%)	76 ± 2.00	81 ± 2.00	80 ± 2.00	80.00 ± 3.00	83 ± 3.00	81 ± 3.00
ES (%)	75 ± 2.00	76 ± 2.00	77 ± 3.00	77 ± 3.00	80 ± 2.00	80 ± 3.00
FC (%) No $CaCl_2$ + $CaCl_2$	182 ± 5.00 230 ± 6.00	195 ± 6.00 250 ± 7.00	191 ± 6.00 245 ± 6.00	185 ± 5.00 242 ± 7.00	199 ± 6.00 260 ± 5.00	193 ± 4.00 258 ± 5.00

***WAC:** water absorption capacity **OAC:** oil water absorption capacity
EC: Emulsifying capacity **ES:** Emulsifying stability **FC:** Foaming capacity.

The water binding protein is determining its degree of interaction with water. These are usually reported in literature as water absorption (WA) or water absorption capacity (WAC). The WAC for the protein isolates (156-171%) were higher than those for the defatted meals (152-157%) and the values recorded for Giza 35 products were higher than the corresponding Giza 22 products (Table 3). The higher level of WAC for SPI compared with SDM could be attributed to an increase of total protein in protein isolate, which has been the reason for increasing water retention (Hutton and Campbell, 1981). Contrarily, Prinyawiwatkul *et.al*, (1993) concluded that, the increase in water retention may not be associate to the total protein content.

The OAC is the binding of oil by non-polar side chains of protein. Oil absorption capacities for SDM and SPI (Table 3) accounted for 140% and 143% for SDM from Giza 22 and Giza 35, respectively. The OAC for protein isolates were slightly lower than those found for soybean defatted meals (Table 3). The ability of SDM and its protein isolate, like other proteins, to bind fat is likely due to non-polar side chains of protein molecules which considered to be

primary sites of lipid-protein interaction, thereby contributing to increased oil absorption (Zayas, 1997).

The emulsifying properties of SDM, HP-SPI and RP-SPI were studied by determining both the emulsifying activity and emulsion stability and the result is presented in Table (3). The emulsifying activity accounted for 76% and 80% for SDM from Giza 22 and Giza 35, respectively. These values were lower than those observed for SPI (Table 3)

The ability of SDM and SPI to emulsify oil and protein suspension into a mixture of fine fat globule dispersions is mainly attributed to soluble proteins. These soluble proteins are mainly inherently surface active due to their amphipathic nature and tendency to absorb at oil-water interfaces. However, Prinyawiwatkul *et.al.*, (1993) showed that the emulsifying capacity is more influenced by quality than quantity of soluble proteins. The emulsifying stability of SDM (75-77%) and SPI (76-80%) was similar to that of emulsifying capacity (Table 3).

Although the soluble protein of SPI was much greater than that of SDM, the emulsifying activities of both samples were close. This may be attributed to the fact that emulsifying activity is influenced more by quality than quantity of soluble protein (Prinyawiwatkul *et.al.,* 1993). Any emulsion is potentially unstable, and stable emulsion will remain unchanged without creaming, flocculation, coalescence, and/or phase inversion upon heating and/or shaking (Zayas, 1997).

The foaming capacity of soyprotein isolate was higher than that recorded for soy defatted meal (Table 3). In the presence of 0.5 % (W/V) $CaCl_2$, foaming capacity increased significantly from 182% to 242% and from 191% to 260% for SDM and SPI, respectively (Table 3).

After 5 min, FC decreased to values of 118% and 120% from initial values of 182% and 125% for SPM of Giza 22 and Giza 35, respectively and to values of 125%, 124%, 135%, and 130% for the initial values of 195%, 191%, 199% and 193% for HP-SPI and RP-SPI from Giza 22 and Giza 35, respectively. The foam collapsed at 60 to 70 min. similar changes were observed for FC of various soybean products in the presence of 0.5% $CaCl_2$.

The ability of protein foam and stabilize foams depends on several parameters such as types of proteins, degree of denaturation,

the presence or absence of calcium ions, pH, temperature, and whipping methods (Townsend and Nakai, 1983 & Fennema, 1996). The ability of $CaCl_2$ to enhance both foaming capacity and stability (Table 3) is due to stabilizing the effect of ionic binding between protein molecules (Fennema, 1996). Foam contributes smoothness, lightness, flavor dispersion, and palatability of foods. Foaming of protein solutions can be desired in many food applications.

Literature reports the influence of salt on protein foams are conflicting. Increased foaming capacity of proteins often observed at lower concentrations of NaCl has been attributed to increased protein solubility (Sosulski, 1977). Normally, salts destabilize protein foams primarily because they reduce the electrostatic forces between polypeptide chains by increasing the ionic strength of the medium (Kinsella, 1979).

In conclusion, using differences in solubility of protein and phytic for obtaining protein isolate with Low-phytate content resulted in lower yield of protein isolates. Precipitation of soybean extract at normal pH (4.5) near the isoelectric point of the major proteins resulted in maximum yield with high phytate content. Using the partially purified enzymes for removal of phytate from high phytate-protein isolate proved to be effective tool for obtaining protein isolates with functional properties similar to or superior to those commonly used soybean products. Because of its high protein content and good functional, RP-SPI has potential applications in new products formulation and fortification.

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دراسات كيميائية على نشاط الـ Phytase والفيتات والمعزول البروتيني في بذور صنفين من فول الصويا

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استخدم في هذه الدراسة بذور صنفين حديثين من فول الصويا همـا جيـزة 22 و جيـزة 35 وذلـك لدراسة:

- تركيز الفيتات في كل من الفلقات والقصرة ومعزول البروتين. 2-تأثير درجـة الحموضـة (pH) على ذوبان كل من حمض الفيتيك والبروتين.3-تأثير الإنبات على نشاط الفيتات ونشاط إنزيمPhytase 4- استخلاص إنزيم Phytase من البذور بعد إنباتها لمدة 7 أيام وتنقية هذا الإنزيم جزئيـا.5-اسـتخدم الـ Phytase المنقى جزئيا في إزالة الفيتات من معزول البروتين. ويمكن تلخيص نتائج الدراسة فيما يلي:

- تركيز حمض الفيتيك في بذور جيزة 22 (1.6 %) أقل منها في بذور جيزة 35 (1.9 %)ز ويزداد هذا التركيز بمقدار مرة ونصف في معزول البروتين من كلا من الصنفين.

- باستخدام خاصية اختلاف ذوبان كل من حمض الفيتيك والبروتين في وسـط حمـضي (pH 5 - 5.5) في ترسيب معزول البروتين الذي يحتوي على تركيز منخفض من حمض الفيتيـك. ولكـن الكميـة المتحصل عليها من هذا المعزول انخفضت بدرجة كبيرة مقارنة بالمعزول الذي رسب عند درجة pH 4.5 وبأعلى كمية ممكنة

- مستخلص البذور الجافة من كلا الصنفين لم يظهر أي نشاط لإنزيم Phytase الذي يؤدي الإنبـات لزيادة تركيزه ويصل إلى أعلى تركيز بعد 7 أيام من الإنبات

- استخدمت البذور التي تم إنباتها لمدة 7 أيام في الحصول على مستخلص الـ Phytase الخام الـذي تم تنقيته جزيئا بواسطة التفريد باستخدام التشبع بكبريتات الأمونيوم.

- بتحضين الإنزيم المنقى جزيئا عند درجة الحموضة المثلـى (5 - 5.5) والحـرارة المثلـى (50 درجة مئوية) أمكن التخلص كليتا من حمض الفيتيك بعد 60 دقيقة و80 دقيقة من التحضين وذلك لمعـزول بروتين جيزة 22 وجيزة 35 على التوالي.

- أوضحت الدراسة أن الصفات الوظيفية لمعزول البروتين المزال محتواه من حمض الفيتيك بواسطة إنزيم Phytase كانت مماثلة أو أفضل من تلك الصفات لمعزولات البروتين الأخرى أو وجبة فول الصويا الخالية من الدهن مما يشير إلى إمكانية استخدام هذا المعزول في تحضير بعض الوجبات الغذائية ذات القيمة الحيوية العالية.