Biological Forum – An International Journal **15(9): 707-711(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

In silico Study of Interaction Between Phytate and Seed Storage Proteins (SSPs) of Indian Mustard

Pant P.[1], Garg S.[2]*, Nain P.[2] and Punetha H.[4]

[1]M.Sc. Student, Department of Biochemistry, GBPUA&T, Pantnagar (Uttarakhand), India.
[2]Ph.D. Scholar, Department of Biochemistry, GBPUA&T, Pantnagar (Uttarakhand), India.
[3]Ph.D. Scholar, Department of Soil Science, College of Agriculture, GBPUA&T, Pantnagar (Uttarakhand), India.
[4]Senior Research Officer, Department of Biochemistry, GBPUA&T, Pantnagar (Uttarakhand), India.

(Corresponding author: Garg S.)*
(Received: 03 July 2023; Revised: 04 August 2023; Accepted: 04 September 2023; Published: 15 September 2023)
(Published by Research Trend)

ABSTRACT: The current study investigates the interactions between phytate and seed storage proteins found in Indian mustard seeds using an *in silico* method. Phytic acid is generally regarded as an undesirable constituent in diets and is found in substantial concentrations in mustard seeds. Although it is well known to contribute in mineral/protein deficiencies, it may also have potential physiological advantages. Interactions between biopolymers like proteins, phytic acid, and metal ions determine the negative and positive effects of this molecule. An objective comprehension of these interactions and how they impact the foods themselves is crucial in light of the growing market for plant-based diets in order to effectively manage and utilize phytates obtained from plants as well as to increase the accessibility of proteins from plant sources. In this study, the visualization of the interaction between napin and phytate produced lowest binding energy *i.e.*, 14.35 Kcal/mol and involved 3 H-bond interactions, while between cruciferin and phytate produced lowest binding energy *i.e.*, 13.99 Kcal/mol and involved 5 H-bond interactions. The findings of this study offer important insight into these IP6-related interactions, which shall aid in formulating strategies for using these plant-derived bioactive molecules for animal and human welfare. The incorporation of synthetic genes is ideal to improve bioavailability of protein fractions in diet, for which the *in silico* interactions are needed to be studied in greater detail, as like here. The candidate gene expression and thus silencing are yet to be achieved in this aspect of study related to Indian mustard.

Keywords: Cruciferin, Indian mustard, Molecular docking, Napin, Phytate, Seed Storage Proteins.

INTRODUCTION

According to estimates, up to a billion people worldwide, in both developing and developed nations, do not get enough protein in their diet which hinders their capacity to grow and increases their risk of getting diseases. In recent years, finding sustainable sources of protein for human and animal consumption is being emphasized in order to improve the protein supply chain in agriculture (Gacek *et al.,* 2018). The residual product obtained after oil extraction from mustard seeds proves to be a promising alternative for this situation as it is a rich source of protein having a well-balanced essential and sulfur-containing amino acid ratio and has the potential to be developed into a low-cost by-product (Joehnke *et al.,* 2018). Typically, mustard defatted meals contain 35–40% crude protein by weight weight (Verma *et al.,* 2019). In addition to being highly valuable for use in industrial processes and animal feed, these proteins may also be incorporated in human food items, helping to address the demand for nutritious proteins in vegetarian, flexitarian, or vegan diets (Fetzer *et al.,* 2020).

Rapeseed proteins have been studied for their nutritional benefits throughout the past few decades. The principal seed storage proteins *viz.,* cruciferin and napin were found and physiochemically described for the first time around 20 years ago (Von Der Haar *et al.,* 2014). The 12S globulin-type cruciferins, 2S albumin-type napins, oil-body proteins, oleosins and the recently discovered 7S vicilins make up the majority of protein in the *Brassica* seed, making up about 80–90% of the seed's total protein (Rahman *et al.,* 2021). Nutritional and anti-nutritional factors affect the utilization potential of *Brassica juncea*, *B. nigra* and *Sinapis alba* (Garg *et al.,* 2023).

Cruciferins are larger seed proteins comprising up to 50–70% of the total protein in seeds. They are salt-soluble neutral glycoproteins, also categorized as 12S globulins based on their sedimentation coefficient with molecular weights in range of 20-40 kDa (Kasprzak *et al.,* 2017). Cruciferins are composed of 2 polypeptide chains; α-chain (30 kDa) and β-chain (20kDa), joined by disulfide linkage**.** The other prominent SSPs present in seed meal of mustard are napins. These are water-soluble basic proteins, classified as 2S or 1.7S albumins based on their sedimentation coefficient (Ren *et al.,* 1999). Napins have low molecular weight ranging from 12-17 kDa and represents 20-40% of the total seed protein. Napins also are composed of 2 polypeptide chains; larger subunit of 10 kDa and smaller subunit of 4.5 kDa (Stone *et al.,* 2014). One of the major anti-

nutrients found in rapeseed mustard, phytic acid, which is mainly present as calcium, magnesium or potassium phytate is known to combine with proteins to create complexes, which reduces the functioning of the protein (Jithender *et al.,* 2019).

According to research, the interaction between phytate and protein is believed to begin during the development of seeds which forms a binary protein-phytate complex. Studies using gel filtration, ultrafiltration, and dialysis have demonstrated that phytate tend to bind to extracted or purified proteins such as bovine serum albumin (BSA), soy proteins, and α-globulin (Bye *et al.,* 2013). Evidences demonstrated by human and animal nutritionists suggested that once phytate precipitates with meal proteins and digestive enzymes, it shows indications of impaired utilization of amino acids (Selle *et al.,* 2012). Researchers have suggested that seeds might also possess ternary protein-metal-phytate complexes that are formed when phytate and proteins interact in the presence of divalent or multivalent cations. In this instance, a cationic bridge links a protein to a phytate molecule. However, further verification of the formation of this ternary protein-metal-phytate complex is necessary, most likely using ITC, FTIR, and other techniques (Wang and Guo 2021).

Since phytates are both free and protein-bound, it is likely that they are associated to or are surrounding proteins because they can contribute either positively or negatively to certain protein functionalities, such as solubility. Some studies have demonstrated that phytate causes proteins to agglomerate, producing turbidity or visible precipitates. However, several studies have also reported that proteins with an increasing phytate level showed more protein solubility when compared to proteins without phytate (Darby *et al.,* 2017). In light of the expanding market for plant-based foods, an objective understanding of these interactions and how they affect the foods themselves is essential for the smart control and utilization of phytates derived from plants and also for increasing the availability of proteins from plant sources.

MATERIAL AND METHODS

This study was performed to determine the possible interaction of cruciferin and napin proteins with a known anti-nutrient present in *Brassica* species called phytic acid which chelates metal ions and renders them unavailable for monogastric animals including humans.

A. Protein Molecule Modification by Bioinformatics Tools Utilization

Protein preparation is the process of modifying a macromolecular structure so that it can be employed in a computerized experiment. 3D structures of procruciferin (PDB ID: 3KGL), the closest-available 12S globulin protein and 2S albumin napin (PDB ID: 1PNB) from rapeseed (*B. napus*) were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB)**.**

The proteins required for this investigation were retrieved from Protein Data Bank in PBD format and protein file was prepared by AutoDock tools. Protein preparation was initiated by removing all the water molecules from the structure. Further, polar hydrogen atoms were added to the structure and Kollman charges were applied. After the completion of these steps, protein structure was saved as a PDBQT file.

B. Establishment of Grid maps

For 3KGL, grid spacing of 0.950 Å was used and the grid points in X, Y and Z axis were set at 122×116×112. The grid center coordinates were placed at X: – 44.078, Y: – 13.889 and Z: – 9.040. For 1PNB, grid spacing of 0.750 Å was used and the grid points in X, Y and Z axis were set at 54×56×54. The grid center coordinates were placed at X: –1.098, Y: – 0.115 and Z: – 5.129.

C. Ligand Molecule Preparation

2D structure of the phytate was obtained from the United States National Library of Medicine, National Center for Biotechnology Information server PubChem (NLM 2023). The spatial data file (SDF) format of the 2D structure of phytate was retrieved from the PubChem database and converted to the PDB format using Open Babel (Open Babel: 2023). This conversion was carried out because AutoDock requires the molecule to be in PDB format in order to prepare the ligand. After using AutoDock Tool to examine the ligand file, a PDBQT (Protein Data Bank, Partial Charge and Atom Type) file was exported. The ligand was now ready for docking.

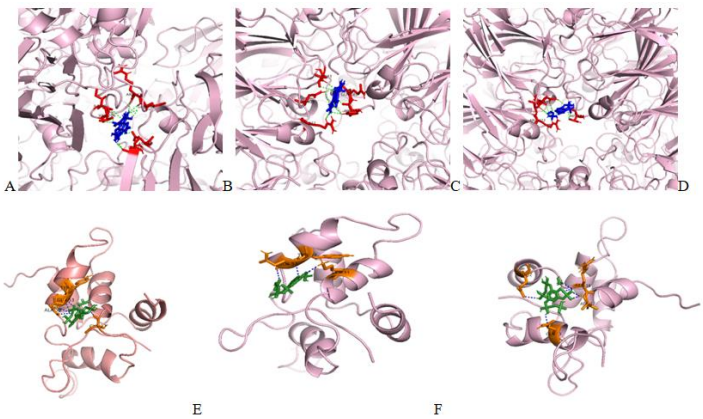


Fig. 1. A, B, C Different poses of docking of phytate with 3KGL (cruciferin); D, E, F Different poses of docking of phytate.

D. Protein-Ligand Docking and Generation of 2D interaction plots

Prepared protein and ligand files were uploaded in AutoDock Vina (Trott and Olson 2010) in PDBQT format and the process of blind docking was then executed. To determine if ligands and proteins are thermodynamically compatible, LigPlot was used to investigate hydrogen bonding and hydrophobic interactions between ligands and proteins (Wallace *et al.*, 1995).

RESULTS AND DISCUSSION

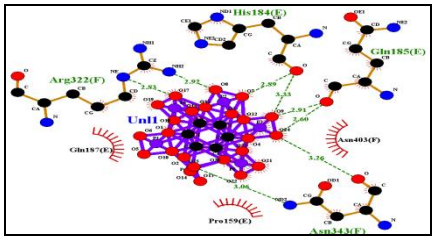
To check the interaction and determine the possible binding sites in seed storage proteins, crucifer in (3KGL) and napin (1PNB), were tested against phytate using AutoDock vina. Out of multiple structures produced for each protein-ligand docking, the structures with the lowest binding energies were selected and visualized using PyMol (Fig. 1). Interaction dynamics of all the docked structures were studied using LigPlot (Fig. 2).

Table 1: Docking energy, H-bonds, H-bond length (Å) and amino acid interaction interaction of 3KGL (cruciferin) with phytate.

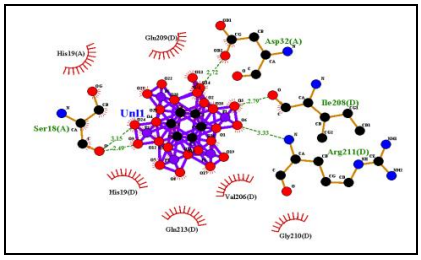
Protein	Ligand	Docking Energies (Kcal/mol)	H-Bond	Amino acids involved in H-bond interaction and other interactions (Amino acid-residue-Bondlength Å)
3KGL (cruciferin)	P phytate	-13.99.99	5	SER18(A) 3.15 Å, SER18(A) 2.49 Å, ASP32(A) 2.72 Å, ILE208(D) 2.79 Å, ARG211(D) 3.33 Å HIS19(A), HIS19(D), VAL206(D), GLU209(A), GLY210(D), GLN213(D)
		-13.63	8	HIS184(E) 2.89 Å, HIS184(E) 3.33 Å, GLN185(E) 2.91 Å, GLN185(E) 2.60 Å, ARG322(F) 2.83 Å, ARG322(F) 2.83 Å, ASN343(F) 3.26 Å, ASN343(F) 3.06 Å GLN187(E), ASN403(F), PRO159(E)
		-12.99	6	HIS184(C) 3.01 Å, THR402(A) 3.27 Å, THR402(A) 2.89 Å, ASN 403(A) 2.80 Å, ALA404(A) 3.29 Å, ARG322(A) 3.17 Å GLN185(C), GLN187(C), ARG 319(A)

Table 2: Docking energy, H-bonds, H-bond length (Å) and amino acid interaction interaction of 1PNB (napin) with.

Protein	Ligand	Docking Energies (Kcal/mol)	H-Bond	Amino acids involved in H-bond interaction and other interactions (AminoAcid-Residue-Bondlength Å)
1PNB (napin)	phytate	-14.35	3	GLN53(B) 2.91 Å, ALA55(B) 2.65 Å, ALA55(B) 2.74 Å LYS56(B), LEU30(B), TYR52(B), LYS31(B), CYS27(B)
		-13.62	8	CYS27(B) 3.33 Å, TYR52(B) 3.04 Å, TYR52(B) 2.91 Å, GLN53(B) 2.43 Å, GLN53(B) 2.94 Å, ALA55(B) 3.31 Å, ALA55(B) 2.88 Å, ALA55(B) 2.27 Å PRO28(B), LEU30(B), LYS31(B), LYS56(B)
		-13.17	6	GLU12(A) 3.29 Å, GLN13(A) 2.97 Å, GLN13(A) 3.04 Å, GLN13(B) 3.15 Å, ARG16(A) 2.93 Å, ARG16(A) 3.03 Å CYS14(B), LEU15(A), ALA17(A), GLU17(B)



G



H

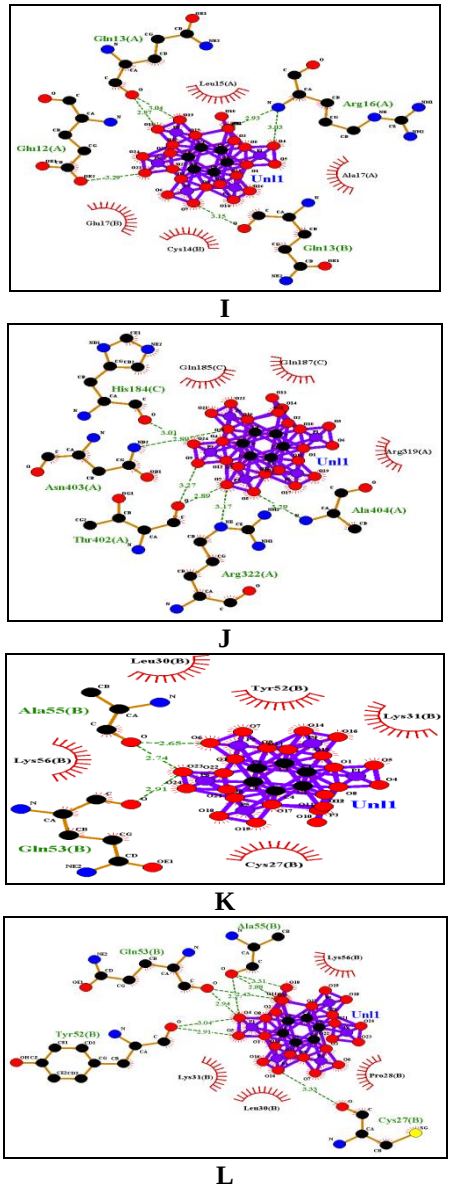


Fig. 2. G, H, I shows interaction dynamics of phytate with 3KGL (cruciferin); J, K, L shows Interaction dynamics of phytate with 1PNB (napin).

These illustrations depicted the interactions between the ligand and amino acids of target proteins as well as hydrogen bonds and hydrophobic bonds mentioned in Table 1 and 2.

CONCLUSIONS

As is already known, phytic acid has detrimental effects in the body by causing deficiency of minerals. However, it provides potential benefits by inhibiting oxidation and other unfavorable reactions that require metal ions. The interactions between IP6, metal ions, and biopolymers are what ultimately determine both the advantageous and detrimental consequences of phytic acid in the body. From the results obtained, it is clear that phytate is a highly reactive ligand with a propensity for interacting with a variety of cations, small molecules, and polymers like proteins. Understanding the functions phytate can play in human/animal digestion, food processing, polymer functionality, and many other domains requires a thorough understanding of its chemical and structural properties as well as how and when these interactions occur. Apart from the importance of studying these phytate-protein interactions in relation to seed proteins, they would also help in gaining insights into the underutilized beneficial effects of phytate on the functionality of food polymers.

FUTURE SCOPE

The *in silico* study might prove useful in understanding the genetic makeup of seed storage protein (SSPs), their synthesis and accumulation in the crop species and their interaction with other biochemical compounds in order to be able to control the quality and amount of seed proteins in mustard. In addition to this, it would be helpful in altering the SSP content of mustard in order to increase the quality, quantity, and digestibility of proteins in humans and animals and is one of the approaches that would be effective in establishing the use of these plant-derived bioactive molecules for animal and human welfare.

Acknowledgement. The authors are thankful to Dean, CBSH-GBPUAT & Department of Biochemistry, GBPUA&T, Pantnagar.
Conflict of Interest. None.

REFERENCES

Bye, J. W., Cowieson, N. P., Cowieson, A. J., Selle, P. H., & Falconer, R. J. (2013). Dual effects of sodium phytate on the structural stability and solubility of proteins. *Journal of Agricultural and Food Chemistry*, *61*(2), 290-295.

Darby, S. J., Platts, L., Daniel, M. S., Cowieson, A.J., Falconer, R. J. (2017). An isothermal titration calorimetry study of phytate binding to lysozyme: A multisite electrostatic binding reaction. *Journal of Thermal Analysis and Calorimetry*, *127*(2), 1201–1208.

Fetzer, A., Müller, K., Schmid, M., and Eisner, P. (2020). Rapeseed proteins for technical applications: Processing, isolation, modification and functional properties–A review. *Industrial Crops and Products*, *15*(8), 1129-1186.

Gacek, K., Bartkowiak-Broda, I., and Batley, J. (2018). Genetic and molecular regulation of seed storage proteins (SSPs) to improve protein nutritional value of oilseed rape (*Brassica napus* L.) seeds. *Frontiers in plant science*, *9*(1), 890-910.

Garg, S., Pant, U., Nain, P. and Puentha, H. (2023). Nutritional & Anti-Nutritional and Anti-Oxidative Profiling of Globally Utilized Diverse Seed Coat Color Mustards. *Biological Forum – An International Journal*, *15*(5), 1218-1221.

Jithender, B., Upendar, N. K. and Rathod, P. J. (2019). Nutritional and anti-nutritional factors present in oil seeds: an overview. *International journal of Conservation Science*, *7*(6), 1159-1165.

Joehnke, M. S., Sorensen, S., Bjergegaard, C., Markedal, K. E. and Sorensen, J. C. (2018). Effect of dietary fibre fractions on in vitro digestibility of rapeseed napin proteins. *Polish Journal of Food and Nutrition Sciences*, *68*(4), 335-345.

Kasprzak, M. M., Houdijk, J. G. M., Liddell, S., Davis, K., Olukosi, O. A., Kightley, S., G. A. White. and Wiseman, J. (2017). Rapeseed napin and cruciferin are readily digested by poultry. *Journal of animal physiology and animal nutrition*, *101*(4), 658-666.

NLM (National Library of Medicine), National Center for Biotechnology Information (NCBI). https://pubchem.ncbi.nlm.nih.gov.in/

Open Babel: The Open Source Chemistry Toolbox, http://openbabel.org.in/

Rahman, M., Guo, Q., Baten, A., Mauleon, R., Khatun, A., Liu, L. and Barkla, B. J. (2021). Shotgun proteomics of *Brassica rapa* seed proteins identifies vicilin as a major seed storage protein in the mature seed. *PloS one*, *16*(7), 253-384.

Ren, C. and Bewley, J. D. (1999). Developmental and germinative events can occur concurrently in precociously germinating Chinese cabbage (*Brassica rapa* ssp. Pekinensis) seeds. *Journal of Experimental Botany*, *50*(341), 751-1761.

Selle, P. H., Cowieson, A. J., Cowieson, N. P. and Ravindran, V. (2012). Protein–phytate interactions in pig and poultry nutrition: a reappraisal. *Nutrition research reviews*, *25*(1), 1-17.

Stone, A. K., Teymurova, A., Dang, Q., Abeysekara, S., Karalash, A., & Nickerson, M. T. (2014). Formation and functional attributes of electrostatic complexes involving napin protein isolate and anionic polysaccharides. *European Food Research and Technology*, *238*(5), 773-780.

Trott, O. and Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multi-threading. *Journal of Computational Chemistry*, *31*(2), 455-461.

Verma, A., Sharma, A., Rai, P. K. and Kumar, D. (2019). Effect of microwave pre-treatment on quality parameters in Indian mustard. *Journal of food science and technology*, *56*(11), 4956-4965.

Von Der Haar, D., Müller, K., Bader-Mittermaier, S., and Eisner, P. (2014). Rapeseed proteins–Production methods and possible application ranges. *Oilseeds and fats, Crops and Lipids*, *21*(1), 104.

Wallace, A. C., Laskowski, R. A. and Thornton, J. M. (1995). Ligplot: a program to generate schematic diagrams of protein-ligand interactions. *Protein Engineering, Design and Selection*, *8*(2), 127-134.

Wang, R and Guo, S. (2021). Phytic acid and its interactions: Contributions to protein functionality, food processing, and safety. *Comprehensive Reviews in Food Science and Food Safety*, *20*(2), 2081-2105.

How to cite this article: Pant P., Garg S., Nain P. and Punetha H. (2023). *In silico* Study of Interaction Between Phytate and Seed Storage Proteins (SSPs) of Indian Mustard. *Biological Forum – An International Journal*, *15*(9): 706-711.