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Relative Separation of Lunasin Peptide from Soya Protein and Checking Its Characteristics with Computational Tools (Based on Bioinformatics Findings)

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ABSTRACT

Bioactive peptides are small peptides that can exhibit various properties including anticancer properties. Lunasin peptide is a 43-amino acid peptide isolated from soy protein that has anticancer properties. In this study, soy protein was enzymatically hydrolyzed and has some properties similar to lunasin in in- silico . item It was checked The present study used commercial soybeans and soybean meal. In this research, the trypsin enzyme has acted on the substrate and the water quality has been measured. Protein extracted water was analyzed using methods such as electrophoresis and Bradford test to measure the quantity of protein.

In the second phase of the research, the properties of Lunasin were investigated using computational tools

The quality of water quality for commercial soybeans and soybean meal was 4.13 and 3.83, respectively. It was also determined by electrophoresis analysis that the peptides similar to lunasin were derived from soybean by trypsin enzyme. Bioinformatics results showed that acid Amines That At sustainability Role have, able change are. Results sign gave That this Peptide has Capabilities Anti cancerous Is That Maximum Score 87 % receive done Is .

Introduction of bioactive peptides

Dietary compounds have been isolated and identified to help maintain health and prevent chronic diseases such as cancer . More focus on active peptides Biologicals exist as components derived from food (which occur naturally or are produced through enzymes) that have a physiological effect in the body in addition to their nutritional value

Bioactive peptides are fragments of proteins that have a specific amino acid sequence and have favorable and positive effects on health and body function , which are usually regulated based on diabetic , anti - hypertensive . They are classified as anti[1] - their properties as anti - microbial , anti cancer and anti - oxidant . These peptides and their derived products from the most complex - sources of peptides . Bioactive peptides have also been isolated from various food materials such as soy , milk , meat , rice , and fish among many other substances have became

bioactive peptid		
Selective peptide	Source	Inhibitory activity
HVLSRAPR	HVLSRAPR	HT-29
WPP	Shell	PC-3/DU-145/HELLA/ H-1299
QPK	Sipa ink	DU-145/PC-3/LNCAP
RQSHFANAQP	Pea	Increased expression of P 53 protein in breast cancer cells
Glu-Glu-Arg-Pro-Arg	Rice bran	Colon-breast and liver cancer cells
Casein phosphopeptides	milk	ht-29/AZ-97
VECYGPNRPGF	Chlorella vulgaris	Stomach and colon cancer cells
CPCA	C.PRPENOIDOSE	G2PHe-2
Villaxation	D.Salina/Chorella ellipsoda	HCT-116
palmatoprotein	P.palmeta	HT-29
Y2	A.plantenis	mcf-7/Hepg-2
VPL	C.SOROKIANA	
VECYGPNRPGF	ضایعات ولگاریس	
Peptides obtained by hydrolysis of trypsin, alkalase and papain		Gastric cancer cells and colon cancer
Gly- pro/gly-leu	Caulerpa	Antioxidants

Bioactive peptides isolated from different sources

Bioactive peptides usually consist of 2 to 20 amino acids and have received much attention due to their biological activities and health benefits . In general , there are different methods to release bioactive peptides, such as dissolving in solvent, enzymatic water extraction and microbial fermentation. However, due to the lack of chemicals remaining in the final end products of peptides enzymatic denatured water is preferred, especially in the pharmaceutical and food industries . The , most commonly used peptide fractionation methods include repurification with different pore sizes sequential chromatography (for example , gel filtration chromatography and ,(and 30 kDa 10 ,5 ,3) ion exchange chromatography) and chromatography. The phase is reversed. Peptides can be identified
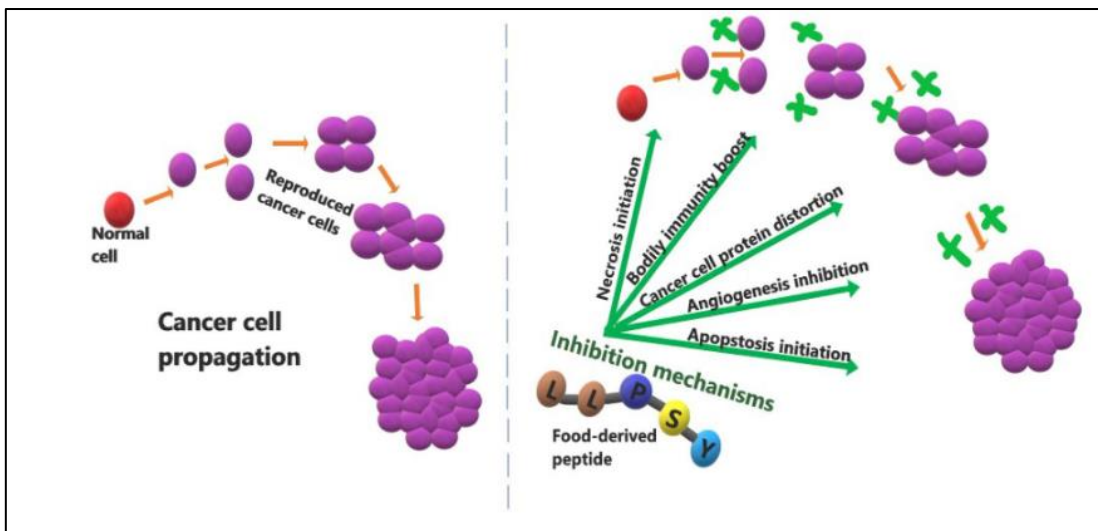
Identification of bioactive peptides

Over the years , bioactive peptides in foods through a classical approach or a bio approach Informatics have been discovered. The classical approach involves hydrolyzing proteins with , proteolytic enzymes to release multiple peptide fragments in the hydrogels. On the other hand proteins can be fermented by bacteria . Proteolytic enzymes of bacteria hydrolyze proteins and release peptides in the hydrolyzed water . Then the water of Kaftahs is checked in terms of ,biological activity in laboratory conditions . If the Kaftah waters show good biological activity

They are confirmed through in vivo testing . Then the biologically active caftan juice can be turned into a functional food and the bioactive peptides in the caftan juice can also be separated and purified into nutrients for non-drug treatment.

On the other hand, the bio method Informatics for the information contained in the database for Determining the frequency of breakdown of bioactive proteins in the target protein is used. Specific enzymes that can separate the identified parts from the main protein are selected to hydrolyze the peptides . This strategy increases the identification of known peptides from unknown proteins . A major challenge in creating bioactive peptides for therapeutic purposes has been the difficulty in establishing a cause and effect relationship between the consumption of bioactive peptides and their intended health effects in humans. However, as studies continue to confirm the therapeutic effects of bioactive peptides, a comprehensive review of recent advances in bioactive peptide research is necessary.

Most synthetic anticancer agents have been associated with nephrotoxic, neurotoxic, cardiotoxic and gonadotoxic side effects . For this reason, the search for bioactive anticancer peptides from food sources has increased.

**Mechanism of action of anticancer bioactive peptide**

Foods with high protein content are potential raw sources of choice for the synthesis of bioactive peptides . A diagram of the process with the limit values for the isolation of bioactive peptides based on food is given in Figure According to the reported research , the common methods are useful for the production of bioactive peptides from food materials Other rich sources of protein include protein breakdown using enzymes , fermentation with The best probiotics are directly selected using the chemical synthesis method In rare cases , bioactive peptides are isolated by unconventional methods.

Determining the sequence of the amino acid patterns in the obtained peptides and their characteristics is the last step to obtain the identity of the peptides and identify them One of the benefits of their nature is for health.
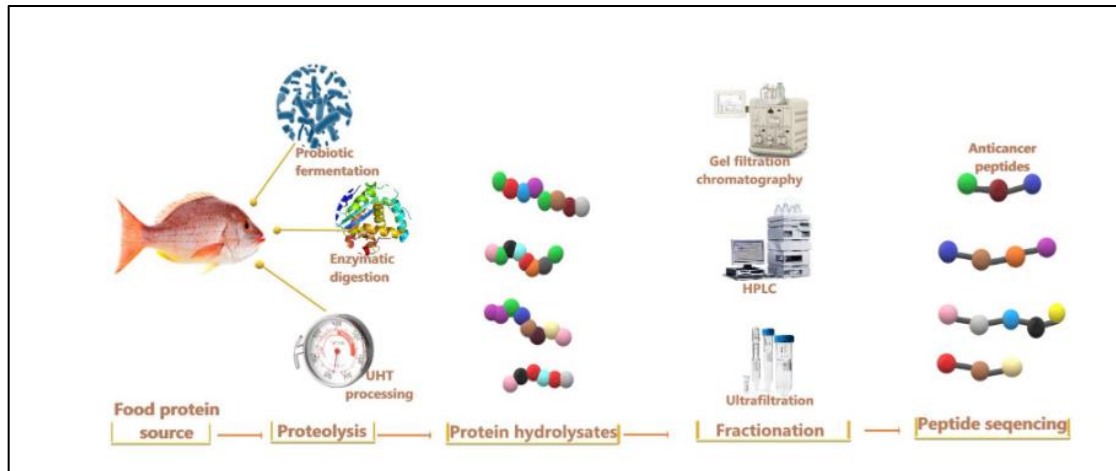
The process for identification and Determining the properties of anticancer bioactive peptides

Membrane properties cause penetration or inhibition They are medicated . Medicinal compounds or the location inside the membrane successively affects the therapeutic goals . Healthy cell membranes have zwitterion , phosphatidylcholine and sphingomyelin in the outer layer and in The outer layer has phosphatidylserine and phosphatidylethanolamine anionic molecules in the inner layer with asymmetric distribution . Internal layer with asymmetric distribution in First , by flippases (phosphatidylserine and phosphatidylethanolamine from the outer membrane to the inner membrane), flippases (phosphatidylcholine and cholesterol from the inner membrane to the outer membrane) and scramblas (facilitating the flip-flop of lipids). Stored . On the contrary, the cancer cell membrane loses this asymmetric distribution and changes in membrane fluidity , as a result of which the negative charge of phosphatidylserine is placed on the surface of the membrane. and also . the location of phosphatidyl -ethanolamine in the outer layer changes
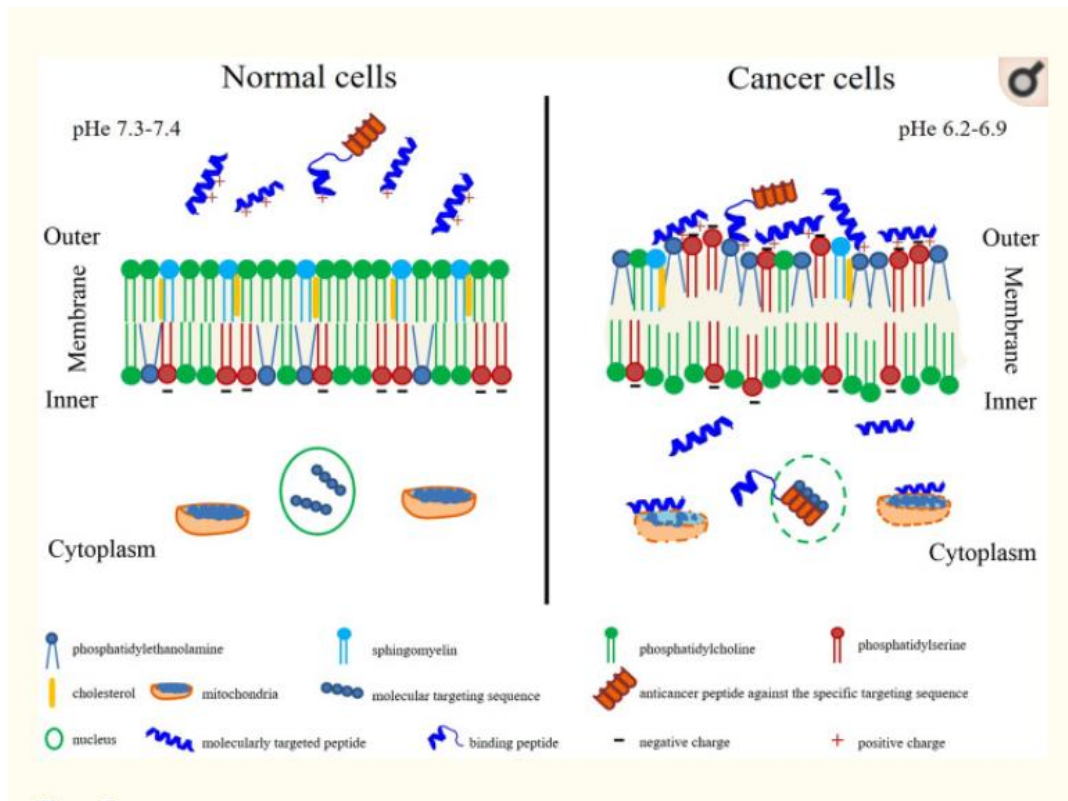
Comparison of three membrane properties and the action of anticancer peptides on healthy cells (left) and cancer cells (right

-Layer The outside of the healthy cell membrane creates a net charge, which leads to the non interaction of anticancer peptides on the surface of the healthy cell (left) , in Meanwhile , the net negative charge on the outer membrane of the cancer cell can interact with cationic anticancer peptides. In cancer cells , anticancer peptides , especially in alpha helical form , act as molecular targeting peptides that can target specific cancer cells . Or _ Penetrate and attach to organelle , membranes that lead to cancer cell apoptosis . Meanwhile , peptides that bind to anticancer drugs which have no anticancer properties , can identify and penetrate the cancer cell membrane.

The role of biocomputing in anticancer peptides

Since the active T*ACPs* , largely depend on the type , number and structure of their amino acids structural classification is the most popular classification method at present . _ _ _ . According to this classification , ACPs*can* be divided into four categories : α-*helical* ,*β-pleated* sheets *,* random coil and ring . Investigation of anticancer properties of peptides is done using different databases such as*CancerPPD* and*TumorHoP* e .
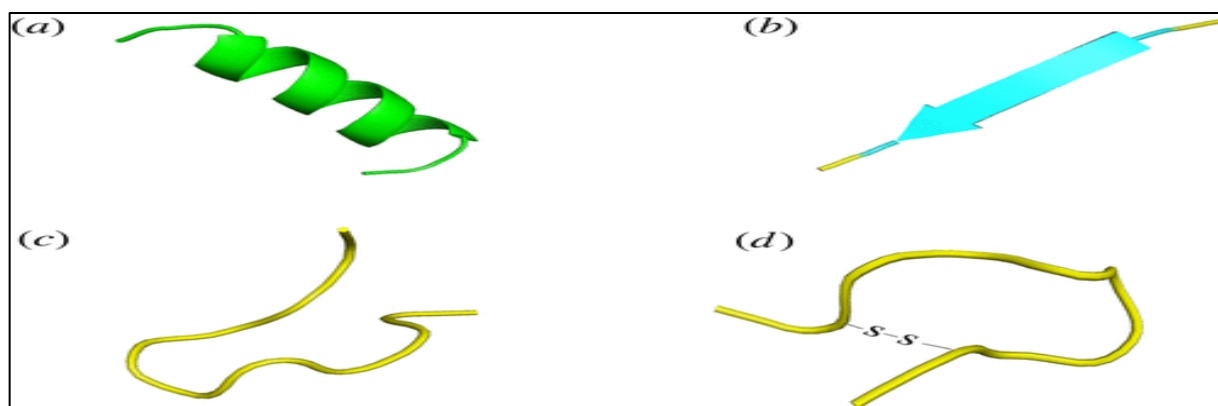
Structures of bioactive peptides

Introduction to the database *CancerPPD*

CancerPPD is a repository of dozens of peptides AnticancerACP and anticancer proteins have been tested . Data was collected manually from published research articles , patents and other databases The release of the verb .CancerPPD includes 3491 anticancer peptides and 121 anticancer proteins Each entry contains comprehensive information about a peptide such as its source , nature of the . peptide , anticancer activity , changesN terminal and-C terminal , it provides crack and so on -.

In addition CancerPPD information about 249 types of cancer cells and 16 different methods for testing.ACP is used . In addition to natural peptides , CancerPPD contains peptides withunnatural chemically modified and acidic residues The changes have been made . In addition to this primary , information , theCancerPPD database predicts tertiary structures and peptide sequences inSMILES format . Tertiary structures of peptides using the PEPstr method _ were predicted and secondary structures were assigned using DSSP_ In order to help users, a number of web tools have been created . These include keyword search , data review , sequence search and structural similarity CancerPPD researchers believe that it will be very useful in the design of anticancer drugs based on peptide D.
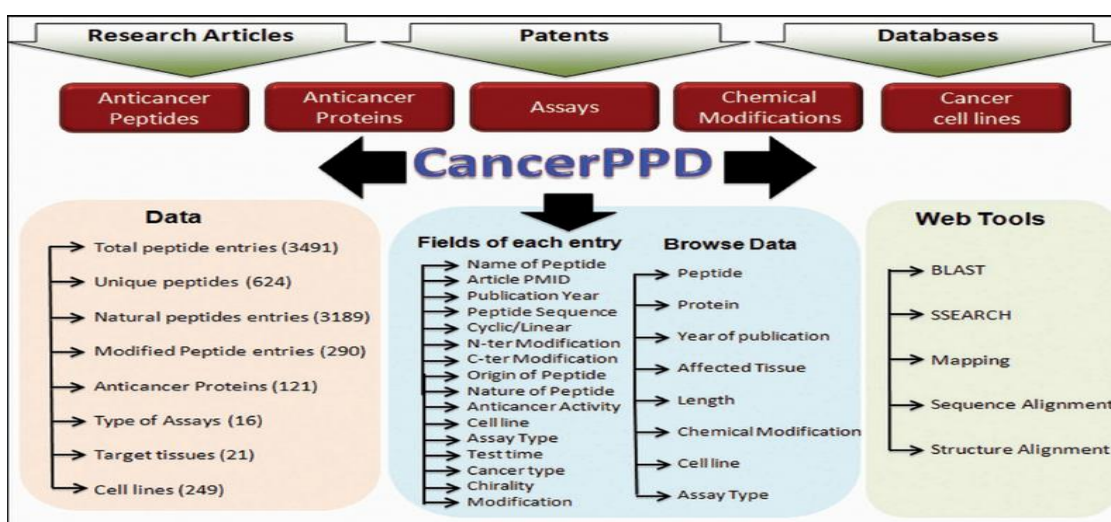
Database *CancerPPD*

Database Database *CancerPPD*

TumorHoPe database

TumorHoPe , database A manual database of experimentally validated tumor homing peptides specifically tumor cells and tumor - associated microenvironment ., that is , it diagnoses angiogenesis These peptides have been collected and compiled from published articles , patents and databases . The release of the verb*TumorHoPe* contains 744 peptides . _ _ Each entry contains , comprehensive information about a peptide , including its sequence , target tumor , target cell .identification techniques , peptide receptor , etc.

In addition , we obtain different types of information from these sequences We have obtained the peptide It includes the secondary / tertiary structure , amino acid composition , and the physical and chemical properties of peptides . It has been determined that the peptides in this database are of different types . Tumors include breast , lung , prostate , melanoma , colon , etc. These peptides have various motifs including There are motifs such as*RGD*) motif*Arg - Gly -Asp* and (*NGR motif*)*Asn-Gly-Arg* . Motifs that specifically identify tumor angiogenic markers ; (*TumorHoPe* comes with many web - based tools such as simple search / search , database browsing , and peptide mapping It 's integrated . These tools allow the user to search for tumor - inducing peptides based _ on amino acid composition , charge , polarity , hydrophobicity . slow down as a result*TumorHoPe* K is a unique database that provides comprehensive information on tumorigenic peptides and their target cells that have been experimentally confirmed . and presents . This database will be very

useful in the design of peptide - based drugs and drug delivery systems at *http://crdd.osdd.net/raghava/tumorhope/* It is available as a free version .

Predicting the anticancer properties of peptides

As peptides play a promising role in cancer treatment , an anticancer peptide design and prediction tool in silicon modeling is your daily routine . Has become . Although some measures have been reported for therapeutic pattern prediction based on the persistence of anticancer peptides , only one tool , AntiCP, has been used so far to design*ACP* is reported. This predictive tool is based on the SVM backing machine_ with the peptide shown as amino acid residues of the peptide and specifying the binary profile

However , the same study reported that a significant difference in the release of basic amino acids was observed between*ACP* and others*ACP* .exists

Therefore , in order to improve the prediction accuracy , in this work , an attempt has been made to , indicate the peptide through a vector and a protein - related tag that It includes leaving the bath the center and the remaining distribution of amino acids . In general , one of the places of poisons *ACP* . against cancer cells includes induction of apoptosis process

Table 1 Dataset distribution of peptides used in this study

Dataset	Anti-cancer peptides	Non-anti-cancer peptides
SA_TRAIN[a]	217	3,979
SA_IND[a]	40	40
SA_RAND[a]	—	2,000
ZOH[b]	138	206
TY1[c]	50	50
TY2[c]	50	50

[a] Dataset constructed in this study and available as supplementary D1
[b] Dataset from Hajisharifi et al. (2014)
[c] Dataset from Tyagi et al. (2013)

& Apoptosis performance prediction (Vijayakumar*Ptv*, 2015

was used to plot the hotspots of lunasin peptide HYPERLINK "https://loschmidt.chemi.muni.cz/hotspotwizard/" No regions for peptide functional region were .found in this database. In fact, the functional areas of lunasin peptide cannot be engineered Among the amino acids that can be effective in sustainability, there are points for engineering
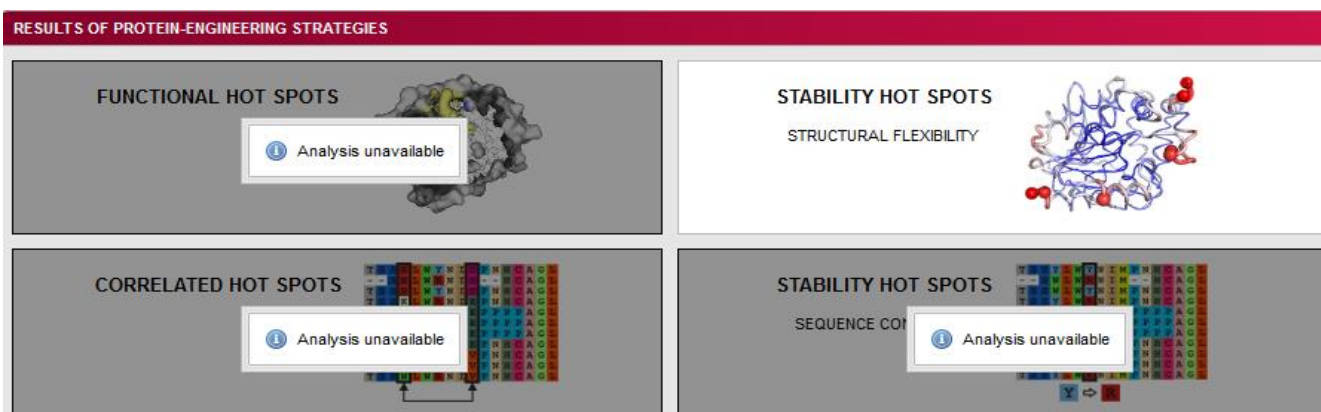
The possibility of changes in lunasin peptide .

In the second phase of the research, the properties of Lunasin were investigated using computational tools
,The quality of water quality for commercial soybeans and soybean meal was 4.13 and 3.83 respectively. It was also determined by electrophoresis analysis that the peptides similar to lunasin were derived from soybean by trypsin enzyme. Bioinformatics results showed that acid Amines That At sustainability Role have, able change are. Results sign gave That this Peptide has Capabilities Anti cancerous Is That*Khimg*. Maximum Score 87 % receive done Is

Protein extraction from soy

Soybean meal and commercial soybeans were procured from reputable centers. Protein extraction from commercial soybeans and soybean meal was done by three methods. The first method was protein precipitation using its isoelectric point. The second method was based on the chemicals used. In the third method, in addition to deposition at the isoelectric point, an ultrasonic bath was also used. A small amount of protein was measured by Bradford test in all three methods.

Soybean protein extraction by precipitation method at isoelectric point (first method)

10 grams of commercial soybeans and 10 grams of soybean meal were poured into separate jars and the pH was brought to 10 with the help of 1 M NaOH solution. This mixture was put on a stirrer for 1 hour and then it was centrifuged using a centrifuge at 3050 rpm at a temperature of 4 degrees Celsius. The supernatant solution was separated and the pH was adjusted to 4.3 using 1 M hydrochloric acid. This suspension was also placed on a stirrer and centrifuged again using a centrifuge at 3050 rpm at a temperature of 4 degrees Celsius. The resulting precipitate was

weighed with a scale with an accuracy of 0.01 and dissolved in water and the pH was adjusted to 7.

protein extraction by chemical method (second method)

0.3 grams of commercial soybeans and 0.3 grams of soybean meal were poured into separate jars. In each flask, 0.98 g of Tris-HCL, 0.5 g of SDS, 0.05 g of Dithiothreitol (DTT) were added and the volume reached 40 ml using distilled water. The pH of two suspensions was adjusted to 5.7. Using a centrifuge, it was centrifuged twice for 10 minutes at 4000 rpm at a temperature of 4 degrees Celsius. 25 ml of cold acetone (which was previously placed at -20 for 1 hour) was added to the

supernatant solution. It was centrifuged again using a centrifuge. Acetone and the supernatant were removed. The precipitate was dried at ambient temperature. The resulting precipitate was weighed on an ultra-sensitive scale and the Bradford test was performed to measure the amount of protein. Soybean protein extraction using precipitation at the isoelectric point and using an ultrasonic bath (third method)

10 grams of commercial soybeans and 10 grams of soybean meal were poured into separate jars and the pH was brought to 12 with the help of 1 M NaOH solution. This mixture was put on a stirrer for 1 hour and then it was centrifuged for 15 minutes using a 9000 RPM rotary centrifuge at a temperature of 4 degrees Celsius. The supernatant solution was separated and placed in the refrigerator for one hour and then placed in an ultrasonic bath for one hour at a temperature of 4 degrees Celsius. The pH was adjusted to 4 using 1M hydrochloric acid.

Bradford test to measure the amount of protein

This method is a quick and accurate method for estimating protein concentration, which is used in various fields of biology and biochemistry. The method originally described by Bradford has become the preferred method for protein quantification in many laboratories. This technique is simpler, faster and more sensitive than Lowry's method. In addition, compared to Lowry's method, it is subject to less interference by common reagents and non-protein components of biological samples. (Kruger, 2009)

Enzymatic Soybean Juice

Enzymatic soybean juice was made by trypsin enzyme. In one experiment, the effect of enzyme on soybean meal and commercial soybean was investigated, and in another experiment, the effect of trypsin enzyme on extracted soybean protein was investigated.

Checking the quality of the well water

The OPA method is one of the methods that can be used to measure the quality of ground water

Introduction of OPA method

In the OPA method, a reaction occurs between the orthophthalaldehyde reagent and the first amino group, which shows absorption at a wavelength of 340 nm in the presence of a wide range of thiol reagents such as beta-mercaptoethanol or dititritol. (Rutherfurd, 2010)
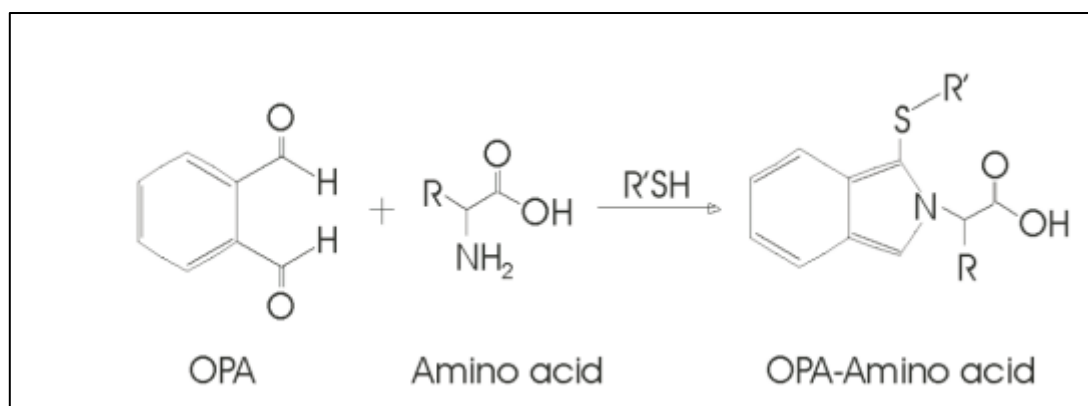
Reaction between amino acid group and orthophthaldialdehyde reagent (Rutherfurd, 2010)
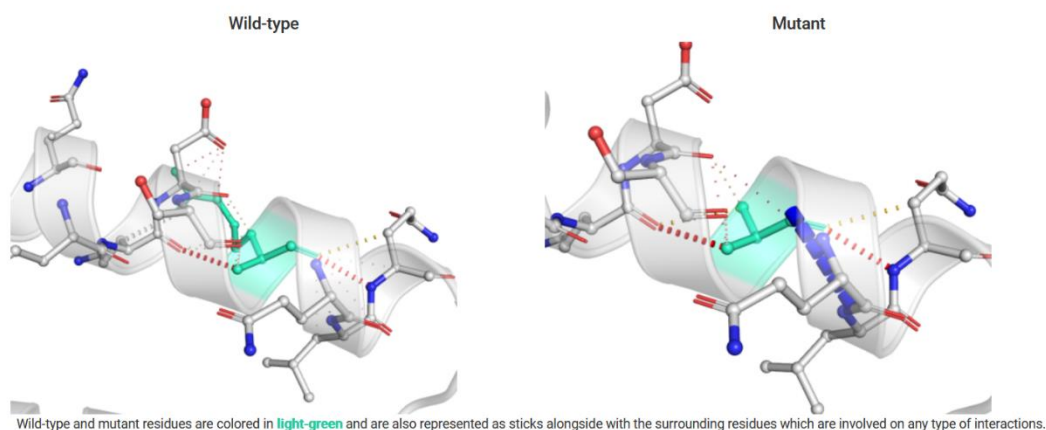
Dialysis and gel electrophoresis

During enzymatic digestion, soy proteins are converted into small peptides. In order to make the conversion of the protein into a visible peptide, and the lunasin peptide was visible at about 5 kilodaltons, the lunasin peptide was used by the SDS-PAGE method.
Studies have shown that lunasin peptide is associated with another 9 kilodalton protein, which is difficult to separate from each other. Therefore, for the relative purification of lunasin peptide, the 14 kilodalton band can also be used. (Vuyyuri, Shidal, & Davis, 2018)

MTT test

MTT assay is a color change method based on the reduction and breaking of colored crystals (tetrazolium) by the succinate dehydrogenase enzyme and the formation of purple insoluble crystals. Cell oxidoreductase enzymes after treatment for 0, 24, 48 or 72 hours, MTT (to a final concentration of 0.5 mg/ml) was added to each well and incubated for 3 hours at 37°C. Metabolically active cells reduced MTT to blue formazan crystals, which were dissolved in DMSO. Absorbances were measured at a wavelength of 560 nm. (Abel & Baird, 2018)

Examining peptidolonasin using computational tools

Lunasin peptide, which is isolated from soy albumin protein, has antimicrobial, anti-inflammatory and anti-proliferative properties. Lunasin is a small peptide that is difficult to purify 100% in laboratory conditions. Due to the small size of this peptide, it is difficult and costly to investigate its properties in laboratory conditions, therefore, in this research, the properties of lunasin were evaluated using computational tools. (Lafarga, Álvarez, Bobo, & Aguiló-Aguayo, 2018).

Wild-type and mutant residues are colored in **light-green** and are also represented as sticks alongside with the surrounding residues which are involved on any type of interactions.

Investigation of mutation in the type of molecular junctions. Wild-type and mutant residues are colored light green and are also shown as sticks next to surrounding residues involved in any interactions.

Δ Vibrational Entropy Energy | Visual representation

Free energy and entropy analysis due to mutation of arginine and alanine

Molecular docking

According to studies, lunasin peptide interacts with histone protein. The interaction between protein H3 and protein H4 and lunasin peptide has been investigated.

Mutations in lunasin mutable points
Changes in lunasin peptide were analyzed using dynamut server. In this server, for example, the amino acid arginine 11 was changed to alanine and the free energy change of peptidylonasin was studied and it was found that the change of arginine 11 to alanine causes protein instability.
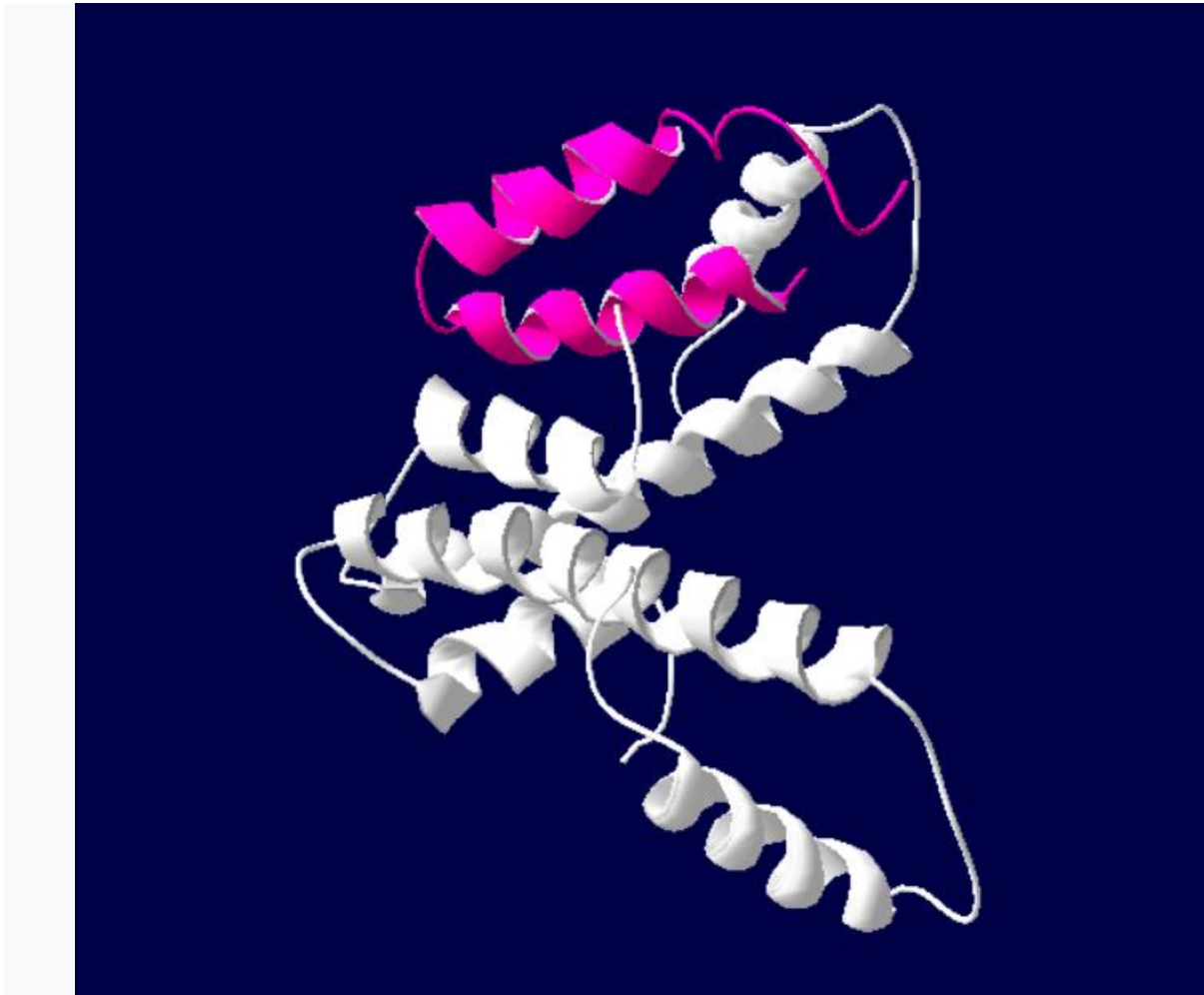
Checking the interaction between lunasin peptide and histone 3 and histone 4 proteins with the help of H-DOCK server, lunasin peptide is pink and histone protein is white.
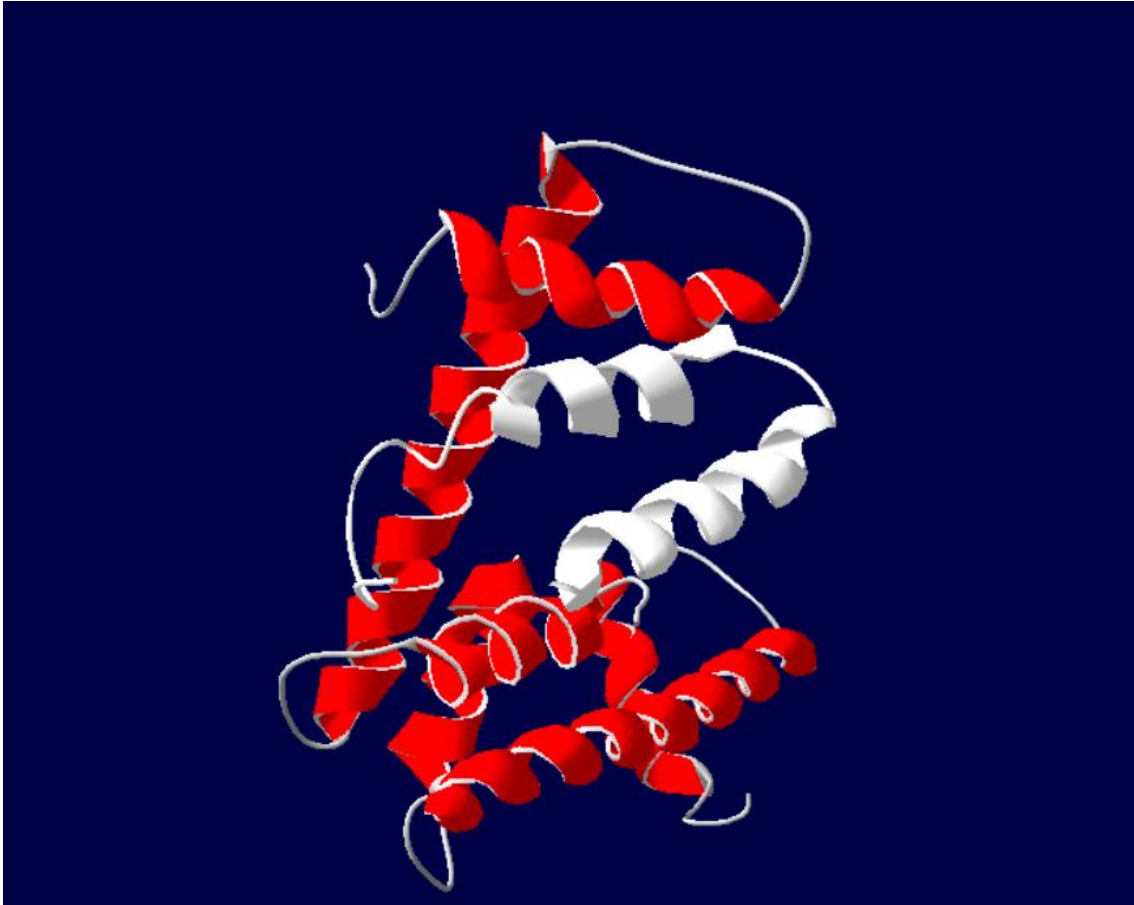
Interaction between lunasin peptide and histone protein with the help of Z-DOCK server, lunasin peptide has white color and histone alpha helices in red color and its coils in gray color.

Based on the obtained information, the histone protein interacts from the positively charged parts and the lunasin peptide interacts from the end and middle regions.

Discussion

According to studies*Chiangjang* Anticancer peptides mainly include glycine , lysine and Et al leucine amino acids . _ _ Are. And the terminal aspartic acid can increase the cationic property and .as a result the anticancer property *Chiangjong , Chutipongtanate , & Hongeng , 2020 (* Based on Lunacin peptide has 20% amino acids of lysine, leucine and glycine and 23.3 ,the obtained results amino acids of aspartic acid. The results are summarized in the figure

Based on the first structure of lunasin peptide, it can show anti-cancer properties. Also, based on the present observation, little similarity was observed between Lunasin sequence and other anticancer peptides. The studies of Zarandi Miandoab and Hosseinqoli showed that the lowest abundance of amino acids among plant anticancer peptides belongs to the amino acids methionine .and histidine
Chiangjang be composed of alpha study et al., the second structure of anticancer peptides can helices, beta sheets and random coils
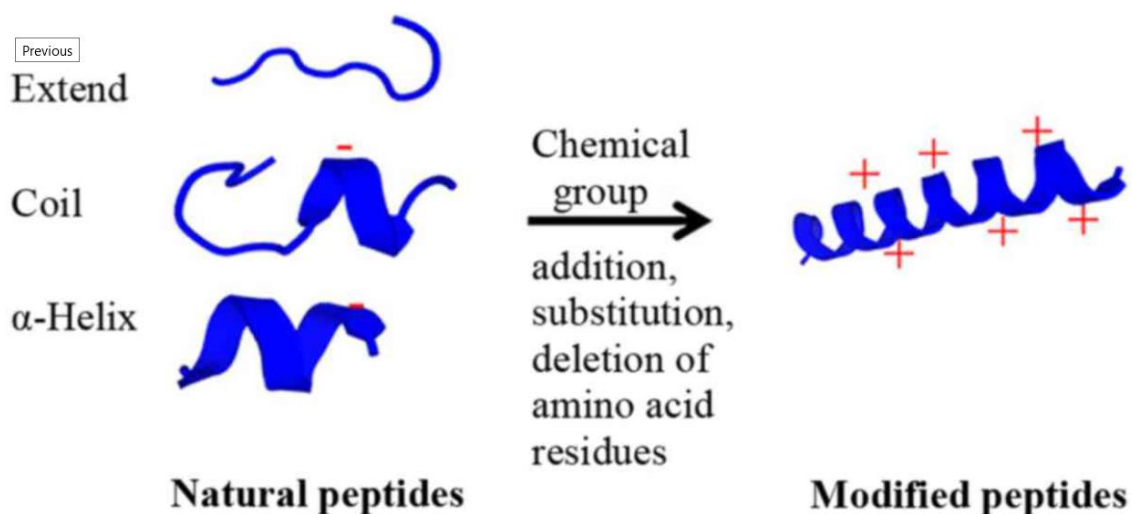
Types of secondary structures of anticancer peptides

The third structure of lunasin peptide was also modeled and the evaluation and optimization of the model was also done. The results obtained from*Coach* indicated that the functional areas of lunasin peptide are in the initial regions of the sequence. Also information obtained from the server *Hot Spot Wizard* showed that the functional parts of lunasin peptide cannot be changed and the *Dynamute server* also showed that substituting neutral amino acid instead of polar amino acid can reduce the stability of lunasin peptide.

Alves de Souza It also showed that the peptide of aspartic amino acids, terminal acid can play study a positive role in binding to chromatins, and lunasin peptide can inhibit the proliferation of cancer cells by acting on caspase 3 and activating the cellular cascade. In the study of*Alves de Souza* The properties of Lunasin have been studied and it has been determined that there are three areas of the spiralα _ _ includes survivalHis 5- Cys ,10Cys 22-Ile and 30Asp 35-Asp are present in 41 lunasin. This study showed that lunasin peptide is stable up to 90°C.

By combining*Coach information* and information obtained from docking by *H-DOCK* andZ - *DOCK servers* It can be concluded that lunasin peptide interacts with histone protein from the end regions of the sequence.

References

1. Abel, S. D. A., & Baird, S. K. (2018). Honey is cytotoxic towards prostate cancer cells but interacts with the MTT reagent: Considerations for the choice of cell viability assay. Food Chemistry, 241, 70–78.
2. Aleksis, R., Jaudzems, K., Muceniece, R., & Liepinsh, E. (2016). Lunasin is a redox sensitive intrinsically disordered peptide with two transiently populated α-helical regions. Peptides, 85, 56–62.
3. Andersen, C. A. F., Palmer, A. G., Brunak, S., & Rost, B. (2002). Continuum secondary structure captures protein flexibility. Structure, 10(2), 175–184.
4. Ashaolu, T. J., Yantiam, N., & Yupanqui, C. T. (2017). Immunomodulatory effects of pepsin-educed soy protein hydrolysate in rats and murine cells. Functional Foods in Health and Disease, 7(11), 889-900.
5. Chiangjong, W., Chutipongtanate, S., & Hongeng, S. (2020). Anticancer peptide: Physicochemical property, functional aspect and trend in clinical application. International Journal of Oncology, 57(3), 678–696.

6. Dai, Y., Cai, X., Shi, W., Bi, X., Su, X., Pan, M., … Qian, H. (2017). Pro-apoptotic cationic host defense peptides rich in lysine or arginine to reverse drug resistance by disrupting tumor cell membrane. Amino Acids, 49(9), 1601–1610.

7. Daliri, E. B.-M., Oh, D. H., & Lee, B. H. (2017). Bioactive peptides. Foods, 6(5), 32.

8. de Souza, S. M. A., Fernandes, T. V. A., Kalume, D. E., Lima, L. M. T., Pascutti, P. G., & de Souza, T. L. F. (2020). Physicochemical and structural properties of lunasin revealed by spectroscopic, chromatographic and molecular dynamics approaches. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1868(8), 140440.

9. Esteve, C., Marina, M. L., & García, M. C. (2015). Novel strategy for the revalorization of olive (Olea europaea) residues based on the extraction of bioactive peptides. Food Chemistry, 167, 272-280.

10. Fernández-Tomé, S., Sanchón, J., Recio, I., & Hernández-Ledesma, B. (2018). Transepithelial transport of lunasin and derived peptides: Inhibitory effects on the gastrointestinal cancer cells viability. Journal of Food Composition and Analysis, 68, 101–110.

11. Garnier, J., Osguthorpe, D. J., & Robson, B. (1978). Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. Journal of Molecular Biology, 120(1), 97–120.

12. Gonzalez de Mejia, E., Castañeda-Reyes, E. D., Mojica, L., Dia, V., Wang, H., Wang, T., & Johnson, L. A. (2021). Potential health benefits associated with lunasin concentration in dietary supplements and lunasin-enriched soy extract. Nutrients, 13(5), 1618.

13. Hernandez-Ledesma, B., Hsieh, C.-C., & Ben, O. (2009). Lunasin, a novel seed peptide for cancer prevention. Peptides, 30(2), 426–430.

14. Hoskin, D. W., & Ramamoorthy, A. (2008). Studies on anticancer activities of antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1778(2), 357–375.

15. Huang, Y., Wang, X., Wang, H., Liu, Y., & Chen, Y. (2011). Studies on mechanism of action of anticancer peptides by modulation of hydrophobicity within a defined structural framework. Molecular Cancer Therapeutics, 10(3), 416–426.

16. Jahangirian, H., Kalantari, K., Izadiyan, Z., Rafiee-Moghaddam, R., Shameli, K., & Webster, T. J. (2019). A review of small molecules and drug delivery applications using gold and iron nanoparticles. International Journal of Nanomedicine, 14, 1633.

17. Jeong, H. J., Jeong, J. B., Hsieh, C. C., Hernández-Ledesma, B., & de Lumen, B. O. (2010). Lunasin is prevalent in barley and is bioavailable and bioactive in in vivo and in vitro studies. Nutrition and Cancer, 62(8), 1113–1119.

18. Kawaguchi, K., Han, Q., Li, S., Tan, Y., Igarashi, K., Kiyuna, T., … Nelson, S. D. (2018). Targeting methionine with oral recombinant methioninase (o-rMETase) arrests a patient-derived orthotopic xenograft (PDOX) model of BRAF-V600E mutant melanoma: implications for chronic clinical cancer therapy and prevention. Cell Cycle, 17(3), 356–361.

19. Kruger, N. J. (2009). The Bradford method for protein quantitation. The Protein Protocols Handbook, 17–24.

20. Kong, X., Guo, M., Hua, Y., Cao, D., & Zhang, C. (2008). Enzymatic preparation of immunomodulating hydrolysates from soy proteins. Bioresource technology, 99(18), 8873-8879.

21. Kyte, J. (2006). Structure in protein chemistry. Garland Science.

22. Lafarga, T., Álvarez, C., Bobo, G., & Aguiló-Aguayo, I. (2018). Characterization of functional properties of proteins from Ganxet beans (Phaseolus vulgaris L. var. Ganxet) isolated using an ultrasound-assisted methodology. Lwt, 98, 106–112.

23. Maddocks, O. D. K., Athineos, D., Cheung, E. C., Lee, P., Zhang, T., van den Broek, N. J. F., … Kruiswijk, F. (2017). Modulating the therapeutic response of tumours to dietary serine and glycine starvation. Nature, 544(7650), 372–376.

24. Pimentel, F. B., Alves, R. C., Harnedy, P. A., FitzGerald, R. J., & Oliveira, M. B. P. P. (2019). Macroalgal-derived protein hydrolysates and bioactive peptides: Enzymatic release and potential health enhancing properties. Trends in Food Science & Technology, 93, 106–124.

25. Rodrigues, E. G., Dobroff, A. S., Taborda, C. P., & Travassos, L. R. (2009). Antifungal and antitumor models of bioactive protective peptides. Anais Da Academia Brasileira de Ciências, 81, 503–520.

26. Roy, S., & Teron, R. (2019). BioDADPep: a bioinformatics database for anti diabetic peptides. Bioinformation, 15(11), 780.

27. Ruth, T. K., Rumble, J. N., Lamm, A. J., & Ellis, J. D. (2020). How Consumers Process Complex Information Related to Food Biotechnology: The Case of Citrus Greening. Journal of Food Products Marketing, 26(2), 103–122.

28. Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of proteins in hydrolysates: a review. Journal of AOAC International, 93(5), 1515–1522.Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. Food Quality and Safety, 1(1), 29-46.

29. Sharma, P., Kaur, H., Kehinde, B. A., Chhikara, N., Sharma, D., & Panghal, A. (2021). Food-derived anticancer peptides: a review. International Journal of Peptide Research and Therapeutics, 27(1), 55–70.

30. Shoombuatong, W., Schaduangrat, N., & Nantasenamat, C. (2018). Unraveling the bioactivity of anticancer peptides as deduced from machine learning. EXCLI Journal, 17, 734.

31. Singh, A., Kaushik, R., Mishra, A., Shanker, A., & Jayaram, B. (2016). ProTSAV: a protein tertiary structure analysis and validation server. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1864(1), 11–19.

32. Suarez-Jimenez, G.-M., Burgos-Hernandez, A., & Ezquerra-Brauer, J.-M. (2012). Bioactive peptides and depsipeptides with anticancer potential: Sources from marine animals. Marine Drugs, 10(5), 963–986.

33. Tyagi, A., Tuknait, A., Anand, P., Gupta, S., Sharma, M., Mathur, D., … Raghava, G. P. S. (2015). CancerPPD: a database of anticancer peptides and proteins. Nucleic Acids Research, 43(D1), D837–D843.

34. Variyar, P. S., Limaye, A., & Sharma, A. (2004). Radiation-induced enhancement of antioxidant contents of soybean (Glycine max Merrill). Journal of Agricultural and Food Chemistry, 52(11), 3385–3388.

35. Vijayakumar, S., & Ptv, L. (2015). ACPP: a web server for prediction and design of anti-cancer peptides. International Journal of Peptide Research and Therapeutics, 21(1), 99–106.

36. Vuister, G. W., Fogh, R. H., Hendrickx, P., Doreleijers, J. F., & Gutmanas, A. (2014). An overview of tools for the validation of protein NMR structures. Journal of Biomolecular NMR, 58(4), 259–285.

37. Vuyyuri, S. B., Shidal, C., & Davis, K. R. (2018). Development of the plant-derived peptide lunasin as an anticancer agent. Current Opinion in Pharmacology, 41, 27–33.

38. Yang, Q.-Z., Wang, C., Lang, L., Zhou, Y., Wang, H., & Shang, D.-J. (2013). Design of potent, non-toxic anticancer peptides based on the structure of the antimicrobial peptide, temporin-1CEa. Archives of Pharmacal Research, 36(11), 1302–1310.